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(54) **Title: MULTIVALENT PNEUMOCOCCAL VACCINES**

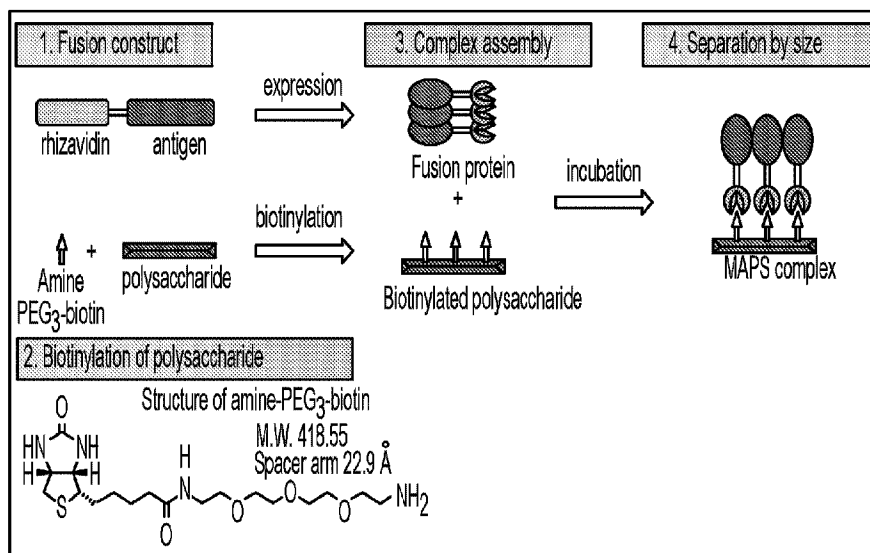


FIG. 1

(57) **Abrégé/Abstract:**
Technologies for the prevention and/or treatment of pneumococcal infections.

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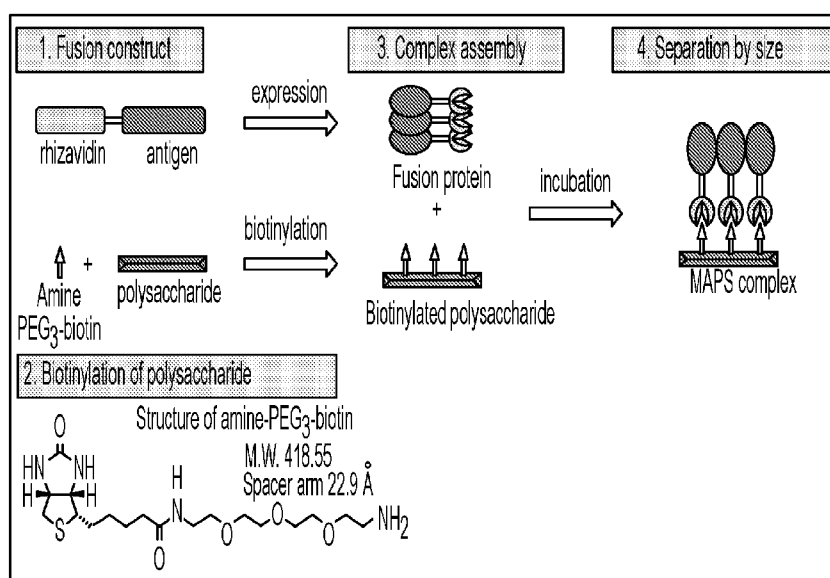


FIG. 1

(57) Abstract: Technologies for the prevention and/or treatment of pneumococcal infections.

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JUMBO APPLICATIONS/PATENTS

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MULTIVALENT PNEUMOCOCCAL VACCINES

Cross-Reference to Related Applications

[0001] The present application claims the benefit of U.S. Provisional Application No. 63/242,487 filed on September 9, 2021 and U.S. Provisional Application No. 63/353,014 filed on June 16, 2022, the contents of each of which are hereby incorporated herein in their entireties.

Background

[0002] *Streptococcus pneumoniae* remains a leading cause of serious illness, including bacteremia, sepsis, meningitis and pneumonia, among children and adults worldwide. Morbidity and mortality among infants, young children, the elderly and subjects who have certain underlying medical conditions is high.

[0003] *S. pneumoniae* is a Gram-positive encapsulated coccus that colonizes the nasopharynx in about 5-10% of healthy adults and 20-40% of healthy children. Normal colonization becomes infectious when *S. pneumoniae* is carried into the Eustachian tubes, nasal sinuses, lungs, bloodstream, meninges, joint spaces, bones and peritoneal cavity. *S. pneumoniae* infection is the most frequent cause of bacteremia, pneumonia, meningitis, sinusitis and acute otitis media [CDC, 2010].

[0004] Pneumococcal disease can be invasive or non-invasive. The most common form of non-invasive disease, non-bacteremic pneumococcal pneumonia, remains one of the most frequent causes for pneumonia hospitalizations. Invasive pneumococcal disease (IPD) is defined as *S. pneumoniae* isolated from a normally sterile site (e.g., cerebrospinal fluid, blood, joint fluid, pleural fluid or peritoneal fluid). The highest incidence of IPD is found at the extremes of age – in elderly adults and in young children younger than 2 years of age. In the U.S., prior to advent of the first pneumococcal vaccine, *S. pneumoniae* caused approximately 17,000 cases of invasive disease each year among children younger than 5 years of age, including 700 cases of meningitis and 200 deaths [CDC, 2000]. The highest morbidity and mortality rates have been reported in developing countries, but the disease burden is also considerable in industrialized countries.

[0005] *S. pneumoniae* has several virulence factors that enable the organism to evade the immune system. Examples include a polysaccharide capsule that prevents phagocytosis by host immune cells, proteases that inhibit complement-mediated opsonization, and proteins that cause lysis of host cells. In the polysaccharide capsule, the presence of complex polysaccharides forms the basis for dividing pneumococci into different serotypes. To date, close to 100 serotypes of *S. pneumoniae* have been identified.

[0006] Two vaccines for *S. pneumoniae* are currently available in the U.S.; PCV13 and PPSV23. PCV13 cannot confer protection against most of the known serotypes of *S. pneumoniae*. While PPSV23 includes polysaccharide components of more serotypes of *S. pneumoniae* than PCV13, it induces an immune response that is neither long-lasting nor anamnestic upon subsequent challenge. PPSV23 protects adults and the elderly against invasive pneumococcal disease; however, no consistent effect has been observed in the prevention of pneumonia [Gruber et al, 2008].

[0007] Thus, there is a medical need for a vaccine that provides T-cell dependent immunity against a broad range of serotypes of *S. pneumoniae*.

Summary

[0008] The present disclosure addresses the lack of suitable technologies for the prevention and/or treatment of pneumococcal infection. Among other things, the present disclosure addresses challenges in providing vaccines with sufficient immunogenicity to protect against invasive pneumococcal disease and pneumonia, by inducing a T- and B-cell response providing immunity against a broad range of *S. pneumoniae* serotypes including those serotypes not included in the vaccine.

[0009] The present Applicant has previously described that a MAPS multivalent pneumococcal vaccines (*e.g.*, in some embodiments, comprising 24-valent pneumococcal polysaccharide serotypes; see, for example, WO2020/056202) can be remarkably useful for prevention and/or treatment of pneumococcal infection when administered to subjects. Attributes of such MAPS multivalent vaccines addressed certain problems associated with standard *S. pneumoniae* vaccines such as PCV13 and PPSV23. For example, in some embodiments, a MAPS

platform provides various advantages including, *e.g.*, high affinity (dissociation constant [KD] $\approx 10^{-15}\text{M}$), non-covalent binding between biotin and rhizavidin, a biotin-binding protein that has no significant predicted homology with human proteins. Among other things, such a MAPS multivalent pneumococcal vaccine could induce a T- and B-cell immune response against a broad range of *S. pneumoniae* serotypes; among other things, such a MAPS multivalent pneumococcal vaccine could induce immune response against *S. pneumoniae* serotypes that not included in the vaccine.

[0010] Among other things, the present disclosure provides compositions and methods for prevention and/or treatment of pneumococcal infections in patient populations in need thereof. In some embodiments, a set of pneumococcal polysaccharide serotypes to be included in compositions described herein are curated and selected based on a variety of considerations including, *e.g.*, characteristics of diseases (*e.g.*, epidemiology, prevalence, worldwide coverage, etc.), and/or compatibility and immunogenicity of various components in a single composition. In some embodiments, the present disclosure provides a further surprising insight that certain pneumococcal polysaccharide serotypes can induce a stronger immune response when they are non-covalently associated with a certain polypeptide antigen. For example, in some embodiments, certain pneumococcal polysaccharide serotypes (*e.g.*, serotypes 1, 6B, 9V, 15B, 22F, 23A, 23B, 23F, and 33F) were demonstrated to induce a stronger immune response when they are non-covalently associated with a SPP2 fusion protein as described herein, as compared to the immune response observed when those serotypes were non-covalently associated with a CP1 fusion protein as described herein. In some embodiments, certain pneumococcal polysaccharide serotypes (*e.g.*, serotypes 2, 6A, 10A, 18C and 20B) were demonstrated to induce a stronger immune response when they are non-covalently associated with a CP1 fusion protein as described herein, as compared to the immune response observed when those serotypes were non-covalently associated with a SPP2 fusion protein as described herein.

[0011] In some embodiments, the present disclosure, among other things, provides compositions of SPP2 fusion polypeptides comprising a biotin-binding protein and *S. pneumoniae* polypeptide antigens that do not induce hemolysis and hemagglutination at a detectable level. In particular, the present disclosure provides a surprising insight that the relative position of *S. pneumoniae* polypeptide antigens SP0435 and PdT(G294P) in a fusion protein can

unexpectedly impact the activity of the fusion protein. Reversing the order of SP0435 and PdT(G294P) in a fusion protein, *i.e.*, positioning SP0435 to the C-terminus of PdT(G294P) in a fusion protein, unexpectedly abolished both hemolysis and hemagglutination.

[0012] In some embodiments, the present disclosure, among other things, provides an insight that immunization with SPP2 fusion protein and *S. pneumoniae* capsular polysaccharide (CPS) of a serotype can synergistically contribute to protection against invasive pneumococcal disease (IPD) by the same serotype. In some embodiments, the serotype is of serotype 3. In some embodiments, the SPP2 fusion protein is in a MAPS complex. In some embodiments, the CPS is in a MAPS complex or in a glycoconjugate (*e.g.*, PCV13). In some embodiments, immunization with a combination of SPP2 fusion protein and CPS of a serotype can increase protection (*e.g.*, as measured by percent survival) against IPD by the serotype relative to a reference without the combination of SPP2 fusion protein and CPS of the serotype. In some embodiments, the protection is increased by at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, or at least 10-fold relative to a reference. In some embodiments, such a reference is SPP2 fusion protein without CPS of the serotype. In some embodiments, such a reference is CPS of the serotype (*e.g.*, in a MAPS complex or glycoconjugate (*e.g.*, PCV13)) without SPP2 fusion protein.

[0013] One aspect of the present disclosure provides a multivalent pneumococcal immunogenic composition (*e.g.*, vaccine). In some embodiments, such an immunogenic composition (*e.g.*, vaccine) comprises one or more species of an immunogenic complexes, wherein the immunogenic complex of at least one of the species comprises: (a) a biotinylated polysaccharide antigen; and (b) a fusion protein comprising: (i) a biotin-binding moiety; and (ii) at least one polypeptide antigen; wherein the biotinylated polysaccharide antigen is non-covalently associated with the biotin-binding moiety of the fusion protein to form an immunogenic complex.

[0014] In some embodiments, where more than one species of an immunogenic complex is included in an immunogenic composition (*e.g.*, vaccine), one or more polypeptide antigens can be utilized. In some embodiments, at least one polypeptide antigen in an immunogenic complex comprises: a pneumolysin (Ply) polypeptide or variant or antigenic fragment thereof; or an SP0435 polypeptide, or variant or antigenic fragment thereof. In some

embodiments, at least one polypeptide antigen in an immunogenic complex comprises: (a) a first polypeptide antigen comprising a Ply polypeptide or variant or antigenic fragment thereof; and (b) a second polypeptide antigen comprising an SP0435 polypeptide, or variant or antigenic fragment thereof. In some embodiments, at least one polypeptide antigen in an immunogenic complex comprises: an SP1500 polypeptide or variant or antigenic fragment thereof; or an SP0785 polypeptide or variant or antigenic fragment thereof. In some embodiments, at least one polypeptide antigen in an immunogenic complex comprises: (a) a first polypeptide antigen comprising an SP1500 polypeptide or variant or antigenic fragment thereof; and (b) a second polypeptide antigen comprising an SP0785 polypeptide or variant or antigenic fragment thereof.

[0015] In some embodiments where more than one species of an immunogenic complex is included in an immunogenic composition (*e.g.*, vaccine), one or more biotinylated polysaccharide antigens can be utilized. In some embodiments, at least one biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae*. In some embodiments, a biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48.

[0016] In some embodiments, a biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, or 34) of serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.

[0017] In some embodiments, a biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, or 33) of serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.

[0018] In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising: (a) a plurality of first biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48; and (b) a plurality of first fusion proteins, each first fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof; wherein each of the plurality of first biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of first fusion proteins to form an immunogenic complex.

Additionally or alternatively, in some embodiments, immunogenic complexes in an immunogenic composition (*e.g.*, vaccine) described herein comprise (a) a plurality of second biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48; and (b) a plurality of second fusion proteins, each second fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100%

identical to SEQ ID NO: 12 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof; wherein each of the plurality of second biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of second fusion proteins to form an immunogenic complex.

[0019] In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising: (a) a plurality of first biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38; and (b) a plurality of first fusion proteins, each first fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof; wherein each of the plurality of first biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of first fusion proteins to form an immunogenic complex. Additionally or alternatively, in some embodiments, immunogenic complexes in an immunogenic composition (*e.g.*, vaccine) described herein comprise (a) a plurality of second biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38; and (b) a plurality of second fusion proteins, each second fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least

99%, at least 99.5%, or 100% identical to SEQ ID NO: 12 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof; wherein each of the plurality of second biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of second fusion proteins to form an immunogenic complex.

[0020] In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising: (a) a plurality of first biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of each of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38; and (b) a plurality of first fusion proteins, each first fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof; wherein each of the plurality of first biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of first fusion proteins to form an immunogenic complex. Additionally or alternatively, in some embodiments, immunogenic complexes in an immunogenic composition (*e.g.*, vaccine) described herein comprise (a) a plurality of second biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of each of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38; and (b) a plurality of second fusion proteins, each second fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at

least 99.5%, or 100% identical to SEQ ID NO: 12 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof; wherein each of the plurality of second biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of second fusion proteins to form an immunogenic complex.

[0021] In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising: (a) a plurality of first biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38; and (b) a plurality of first fusion proteins, each first fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof; wherein each of the plurality of first biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of first fusion proteins to form an immunogenic complex. Additionally or alternatively, in some embodiments, immunogenic complexes in an immunogenic composition (*e.g.*, vaccine) described herein comprise (a) a plurality of second biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38; and (b) a plurality of second fusion proteins, each second fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at

least 99.5%, or 100% identical to SEQ ID NO: 12 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof; wherein each of the plurality of second biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of second fusion proteins to form an immunogenic complex.

[0022] In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising: (a) a plurality of first biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of each of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38; and (b) a plurality of first fusion proteins, each first fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof; wherein each of the plurality of first biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of first fusion proteins to form an immunogenic complex. Additionally or alternatively, in some embodiments, immunogenic complexes in an immunogenic composition (*e.g.*, vaccine) described herein comprise (a) a plurality of second biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of each of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38; and (b) a plurality of second fusion proteins, each second fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at

least 99.5%, or 100% identical to SEQ ID NO: 12 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof; wherein each of the plurality of second biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of second fusion proteins to form an immunogenic complex.

[0023] In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a biotin-binding moiety, wherein the biotin-binding moiety is a polypeptide that has or comprises an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 1 or a biotin-binding fragment thereof; or a polypeptide that has or comprises an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 57, or a biotin-binding fragment thereof. In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a fusion protein that is or comprises an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 26 or SEQ ID NO: 61. In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a fusion protein CP1 as described herein.

[0024] In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a biotin-binding moiety, wherein the biotin-binding moiety is a polypeptide that has or comprises an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 1 or a biotin-binding fragment thereof; or a polypeptide that has or comprises an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 57, or a biotin-binding fragment thereof. In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a fusion protein that is or comprises an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least

94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 19 or SEQ ID NO: 58. In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a fusion protein SPP2 as described herein.

[0025] In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising one or more species of immunogenic complexes, wherein a species refers to complexes comprising a polysaccharide antigen of a single *Streptococcus pneumoniae* serotype (*e.g.*, ones described herein). In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising two or more species of immunogenic complexes, wherein a species refers to complexes comprising a polysaccharide antigen of a single *Streptococcus pneumoniae* serotype (*e.g.*, ones described herein). In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising thirty or more species of immunogenic complexes, wherein a species refers to complexes comprising a polysaccharide antigen of a single *Streptococcus pneumoniae* serotype (*e.g.*, ones described herein). In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising thirty one or more species of immunogenic complexes, wherein a species refers to complexes comprising a polysaccharide antigen of a single *Streptococcus pneumoniae* serotype (*e.g.*, ones described herein). In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising thirty two or more species of immunogenic complexes, wherein a species refers to complexes comprising a polysaccharide antigen of a single *Streptococcus pneumoniae* serotype (*e.g.*, ones described herein).

[0026] In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising thirty or more species of immunogenic complexes. In some embodiments, each species is a population of complexes comprising a polysaccharide antigen of a distinct *Streptococcus pneumoniae* serotype (*e.g.*, ones described herein).

[0027] In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising thirty three or more species of immunogenic complexes. In some embodiments, each species is a population of complexes comprising a

polysaccharide antigen of a distinct *Streptococcus pneumoniae* serotype (e.g., ones described herein).

[0028] In some embodiments, an immunogenic composition (e.g., vaccine) comprises a plurality of immunogenic complexes comprising thirty four or more species of immunogenic complexes. In some embodiments, each species is a population of complexes comprising a polysaccharide antigen of a distinct *Streptococcus pneumoniae* serotype (e.g., ones described herein).

[0029] In some embodiments, an immunogenic composition (e.g., vaccine) comprises a plurality of immunogenic complexes comprising: a first species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6C non-covalently complexed with a biotin-binding moiety of a first fusion protein, wherein each first fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof; a second species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7C non-covalently complexed with the biotin binding moiety of the first fusion protein; a third species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15A non-covalently complexed with the biotin binding moiety of the first fusion protein; a fourth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 16F non-covalently complexed with the biotin binding moiety of the first fusion protein; a fifth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23A non-covalently complexed with the biotin binding moiety of the first fusion protein; a sixth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23B non-covalently complexed with the biotin binding moiety of the first fusion protein; a seventh species of immunogenic

complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 24F non-covalently complexed with the biotin binding moiety of the first fusion protein; an eighth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 31 non-covalently complexed with the biotin binding moiety of the first fusion protein; a ninth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 35B non-covalently complexed with the biotin binding moiety of the first fusion protein; a tenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 38 non-covalently complexed with the biotin binding moiety of the first fusion protein; an eleventh species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 1 non-covalently complexed with a biotin binding moiety of a second fusion protein, wherein each second fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 12 or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof; a twelfth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 2 non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirteenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 3 non-covalently complexed with the biotin binding moiety of the second fusion protein; a fourteenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 4 non-covalently complexed with the biotin binding moiety of the second fusion protein; a fifteenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 5 non-covalently complexed with the biotin binding moiety of the second fusion protein; a sixteenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6A non-covalently complexed with the biotin

binding moiety of the second fusion protein; a seventeenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6B non-covalently complexed with the biotin binding moiety of the second fusion protein; an eighteenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7F non-covalently complexed with the biotin binding moiety of the second fusion protein; a nineteenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 8 non-covalently complexed with the biotin binding moiety of the second fusion protein; a twentieth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9N non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-first species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9V non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-second species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 10A non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-third species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 11A non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-fourth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 12F non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-fifth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 14 non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-sixth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15B non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-seventh species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 17F non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-eighth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 18C non-covalently complexed with the biotin binding moiety of the

second fusion protein; a twenty-ninth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19A non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirtieth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19F non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirty-first species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 20B non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirty-second species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 22F non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirty-third species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23F non-covalently complexed with the biotin binding moiety of the second fusion protein; and a thirty-fourth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 33F non-covalently complexed with the biotin binding moiety of the second fusion protein.

[0030] In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising: a first species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 1 non-covalently complexed with a biotin-binding moiety of a first fusion protein, wherein each first fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof; a second species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 4 non-covalently complexed with the biotin binding moiety of the first fusion protein; a third species of immunogenic complex comprising a biotinylated polysaccharide antigen of

Streptococcus pneumoniae serotype 6A non-covalently complexed with the biotin binding moiety of the first fusion protein; a fourth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6B non-covalently complexed with the biotin binding moiety of the first fusion protein; a fifth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9V non-covalently complexed with the biotin binding moiety of the first fusion protein; a sixth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15B non-covalently complexed with the biotin binding moiety of the first fusion protein; a seventh species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 18C non-covalently complexed with the biotin binding moiety of the first fusion protein; an eighth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19A non-covalently complexed with the biotin binding moiety of the first fusion protein; a ninth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23F non-covalently complexed with the biotin binding moiety of the first fusion protein; a tenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 33F non-covalently complexed with the biotin binding moiety of the first fusion protein; an eleventh species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 2 non-covalently complexed with a biotin binding moiety of a second fusion protein, wherein each second fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 12 or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof; a twelfth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 3 non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirteenth species of

immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 5 non-covalently complexed with the biotin binding moiety of the second fusion protein; a fourteenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6C non-covalently complexed with the biotin binding moiety of the second fusion protein; a fifteenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7C non-covalently complexed with the biotin binding moiety of the second fusion protein; a sixteenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7F non-covalently complexed with the biotin binding moiety of the second fusion protein; a seventeenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 8 non-covalently complexed with the biotin binding moiety of the second fusion protein; an eighteenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9N non-covalently complexed with the biotin binding moiety of the second fusion protein; a nineteenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 10A non-covalently complexed with the biotin binding moiety of the second fusion protein; a twentieth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 11A non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-first species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 12F non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-second species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 14 non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-third species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15A non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-fourth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 16F non-covalently complexed with the biotin binding moiety of the second fusion protein; ; a twenty-fifth species of immunogenic complex comprising a

biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 17F non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-sixth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19F non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-seventh species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 20B non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-eighth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 22F non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-ninth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23A non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirtieth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23B non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirty-first species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 24F non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirty-second species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 31 non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirty-third species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 35B non-covalently complexed with the biotin binding moiety of the second fusion protein; and a thirty-fourth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 38 non-covalently complexed with the biotin binding moiety of the second fusion protein.

[0031] In some embodiments, an immunogenic composition (e.g., vaccine) comprises a plurality of immunogenic complexes comprising: a first species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 1 non-covalently complexed with a biotin-binding moiety of a first fusion protein, wherein each first fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising

an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof; a second species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 4 non-covalently complexed with the biotin binding moiety of the first fusion protein; a third species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6A non-covalently complexed with the biotin binding moiety of the first fusion protein; a fourth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6B non-covalently complexed with the biotin binding moiety of the first fusion protein; a fifth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9V non-covalently complexed with the biotin binding moiety of the first fusion protein; a sixth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15B non-covalently complexed with the biotin binding moiety of the first fusion protein; a seventh species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 18C non-covalently complexed with the biotin binding moiety of the first fusion protein; an eighth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19A non-covalently complexed with the biotin binding moiety of the first fusion protein; a ninth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23F non-covalently complexed with the biotin binding moiety of the first fusion protein; a tenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 33F non-covalently complexed with the biotin binding moiety of the first fusion protein; an eleventh species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 2 non-covalently complexed with a biotin binding moiety of a second fusion protein, wherein each second fusion protein comprises

(a) a biotin-binding moiety; (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 12 or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof; a twelfth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 3 non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirteenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 5 non-covalently complexed with the biotin binding moiety of the second fusion protein; a fourteenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6C non-covalently complexed with the biotin binding moiety of the second fusion protein; a fifteenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7C non-covalently complexed with the biotin binding moiety of the second fusion protein; a sixteenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7F non-covalently complexed with the biotin binding moiety of the second fusion protein; a seventeenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 8 non-covalently complexed with the biotin binding moiety of the second fusion protein; an eighteenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9N non-covalently complexed with the biotin binding moiety of the second fusion protein; a nineteenth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 10A non-covalently complexed with the biotin binding moiety of the second fusion protein; a twentieth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 11A non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-first species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 12F non-covalently complexed

with the biotin binding moiety of the second fusion protein; a twenty-second species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 14 non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-third species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15A non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-fourth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 17F non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-fifth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19F non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-sixth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 20B non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-seventh species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 22F non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-eighth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23A non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-ninth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23B non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirtieth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 24F non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirty-first species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 31 non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirty-second species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 35B non-covalently complexed with the biotin binding moiety of the second fusion protein; and a thirty-third species of immunogenic complex comprising a

biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 38 non-covalently complexed with the biotin binding moiety of the second fusion protein.

[0032] In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising two or more species of immunogenic complexes, wherein each species of immunogenic complexes contributes a stoichiometrically equal ratio, by weight, of the polysaccharide antigen.

[0033] In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising two or more species of immunogenic complexes, wherein at least one species of immunogenic complex contributes a stoichiometrically different ratio, by weight, of the polysaccharide antigen.

[0034] In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising two or more species of immunogenic complexes, wherein different species of immunogenic complexes contribute a stoichiometrically different ratio, by weight, of the polysaccharide antigen.

[0035] In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a fusion protein comprising a biotin-binding moiety, wherein the biotin-binding moiety is a polypeptide comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 1 or a biotin-binding fragment thereof; or a polypeptide comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 57, or a biotin-binding fragment thereof.

[0036] Another aspect of the present disclosure provides an immunogenic complex comprising a fusion protein comprising a biotin-binding moiety of rhizavidin and one or more *Streptococcus pneumoniae* polypeptide antigens. In some embodiments, a biotin-binding moiety is a polypeptide comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 1 or a biotin-binding fragment thereof; or a

polypeptide comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 57, or a biotin-binding fragment thereof.

[0037] In some embodiments, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* non-covalently associated with a fusion protein described herein. In some embodiments, an immunogenic complex comprises a biotinylated polysaccharide antigen, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48.

[0038] In some embodiments, an immunogenic complex comprises a biotinylated polysaccharide antigen, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.

[0039] In some embodiments, an immunogenic complex comprises a biotinylated polysaccharide antigen, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.

[0040] In some embodiments, an immunogenic complex comprises at least one polypeptide antigen, wherein the at least one polypeptide antigen comprises a pneumolysin (Ply) polypeptide or variant or antigenic fragment thereof; an SP0435 polypeptide or variant or antigenic fragment thereof; or a combination thereof. In some embodiments, an immunogenic complex comprises at least one *Streptococcus pneumoniae* polypeptide antigen, wherein the at

least one polypeptide antigen comprises: (a) a first polypeptide antigen comprising a pneumolysin (Ply) polypeptide, or variant or antigenic fragment thereof; and (b) a second polypeptide antigen comprising an SP0435 polypeptide or variant or antigenic fragment thereof. In some embodiments, an immunogenic complex comprises at least one polypeptide antigen, wherein the at least one polypeptide antigen comprises: (a) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and (b) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof.

[0041] In some embodiments, an immunogenic complex comprises at least one polypeptide antigen, wherein the at least one polypeptide antigen comprises an SP1500 polypeptide or variant or antigenic fragment thereof; an SP0785 polypeptide or variant or antigenic fragment thereof; or a combination thereof. In some embodiments, an immunogenic complex comprises at least one polypeptide antigen, wherein the at least one polypeptide antigen comprises: (a) a first polypeptide antigen comprising an SP1500 polypeptide or variant or antigenic fragment thereof; and (b) a second polypeptide antigen comprising an SP0785 polypeptide or variant or antigenic fragment thereof. In some embodiments, an immunogenic complex comprises at least one polypeptide antigen, wherein the at least one polypeptide antigen comprises: (a) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% identical to SEQ ID NO: 12 or variant or an antigenic fragment thereof; and (b) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 10 or variant or an antigenic fragment thereof.

[0042] In some embodiments, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 1 non-covalently associated with a fusion protein, wherein the fusion protein comprises (a) a biotin-binding moiety; (b) a first

polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or a variant or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or a variant or an antigenic fragment thereof.

[0043] In some embodiments, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6B non-covalently associated with a fusion protein, wherein the fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or a variant or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or a variant or an antigenic fragment thereof.

[0044] In some embodiments, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9V non-covalently associated with a fusion protein, wherein the fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or a variant or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or a variant or an antigenic fragment thereof.

[0045] In some embodiments, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15B non-covalently associated with a fusion protein, wherein the fusion protein comprises (a) a biotin-binding moiety; (b) a first

polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or a variant or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or a variant or an antigenic fragment thereof.

[0046] In some embodiments, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 22F non-covalently associated with a fusion protein, wherein the fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or a variant or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or a variant or an antigenic fragment thereof.

[0047] In some embodiments, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23A non-covalently associated with a fusion protein, wherein the fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or a variant or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or a variant or an antigenic fragment thereof.

[0048] In some embodiments, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23B non-covalently associated with a fusion protein, wherein the fusion protein comprises (a) a biotin-binding moiety; (b) a first

polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or a variant or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or a variant or an antigenic fragment thereof.

[0049] In some embodiments, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23F non-covalently associated with a fusion protein, wherein the fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or a variant or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or a variant or an antigenic fragment thereof.

[0050] In some embodiments, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 33F non-covalently associated with a fusion protein, wherein the fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or a variant or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or a variant or an antigenic fragment thereof.

[0051] In some embodiments, an immunogenic complex comprises a biotin-binding moiety, wherein the biotin-binding moiety is a polypeptide comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at

least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 1 or a biotin-binding fragment thereof; or a polypeptide comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 57, or a biotin-binding fragment thereof.

[0052] In some embodiments, an immunogenic complex comprises a fusion protein (*e.g.*, ones described herein) and a polysaccharide antigen (*e.g.*, ones described herein), wherein the ratio of fusion protein to polysaccharide antigen in the complex is about 1:1, about 2:1, about 3:1, about 4:1, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, or about 10:1 by weight. In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises one or more immunogenic complexes disclosed herein.

[0053] In some aspects, pharmaceutical compositions comprising immunogenic composition (*e.g.*, vaccine) compositions are disclosed herein. In some embodiments, a pharmaceutical composition comprises an immunogenic composition (*e.g.*, vaccine) or immunogenic composition (*e.g.*, vaccine) composition disclosed herein, and a pharmaceutically acceptable carrier. In some embodiments, a pharmaceutical composition comprises an immunogenic complex disclosed herein, and a pharmaceutically acceptable carrier. In some embodiments, a pharmaceutical composition disclosed herein comprises one or more adjuvants. In some embodiments, the one or more adjuvants is or comprises a co-stimulation factor.

[0054] In some embodiments, the one or more adjuvants are selected from the group consisting of aluminum phosphate, aluminum hydroxide, and phosphated aluminum hydroxide. In some embodiments, wherein the one or more adjuvants is or comprises aluminum phosphate. In some embodiments, a pharmaceutical composition disclosed herein is formulated for injection.

[0055] In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein induces an immune response. In some embodiments, the immune response comprises an innate immune response. In some embodiments, the immune response comprises an antibody or B cell response. In some embodiments, the immune response comprises a T cell response. In some embodiments, the immune response comprises a CD4⁺ T

cell response (*e.g.*, T_H1, T_H2, or T_H17 response), a CD8⁺ T cell response, a CD4⁺ and CD8⁺ T cell response, or a CD4⁻/CD8⁻ T cell response. In some embodiments, the immune response comprises (i) an antibody or B cell response, and (ii) a T cell response. In some embodiments, the immune response comprises (i) an antibody or B cell response, (ii) a T cell response, and (iii) an innate immune response. In some embodiments, the immune response is to at least one polysaccharide antigen or at least one polypeptide antigen of a fusion protein. In some embodiments, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) a T cell response to at least one polypeptide of a fusion protein. In some embodiments, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) a CD4⁺ T cell response (*e.g.*, T_H1, T_H2, or T_H17 response), a CD8⁺ T cell response, a CD4⁺ and CD8⁺ T cell response, or a CD4⁻/CD8⁻ T cell response to at least one polypeptide of a fusion protein. In some embodiments, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) an antibody or B cell response to at least one polypeptide of a fusion protein. In some embodiments, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) an antibody or B cell response and a T cell response, to at least one polypeptide of a fusion protein. In some embodiments, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) an antibody or B cell response, and a CD4⁺ T cell response, including a T_H1, T_H2, or T_H17 response, a CD8⁺ T cell response, a CD4⁺ and CD8⁺ T cell response, or a CD4⁻/CD8⁻ T cell response, to at least one polypeptide of a fusion protein.

[0056] In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein induces an opsonic/bactericidal response against one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such an opsonic/bactericidal response may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such an opsonic/bactericidal response may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include

polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such an opsonic/bactericidal response may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, an opsonic/bactericidal response can include production of antibodies that opsonize one or more serotypes of *Streptococcus pneumoniae*, which leads to killing of such serotype(s) by one or more immune pathways. In some embodiments, such an opsonic/bactericidal response can be determined by an opsonophagocytic assay (OPA) or a concentrated opsonophagocytic assay (COPA) known in the art and/or as described herein.

[0057] In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein inhibits transmission of one or more serotypes of *Streptococcus pneumoniae* from the subject to another subject. In some embodiments, such inhibition of transmission may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition of transmission may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition of transmission may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0058] In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein reduces or inhibits replication, or reduces bacterial load, of one or

more serotypes of *Streptococcus pneumoniae*. In some embodiments, such reduction or inhibition of replication, or reduction of bacterial load, may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction or inhibition of replication, or reduction of bacterial load, may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction or inhibition of replication, or reduction of bacterial load, may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0059] In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein inhibits or reduces the rate of occurrence of Invasive Pneumococcal Disease (IPD) associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such inhibition of IPD or reduction in the rate of occurrence of IPD may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition of IPD or reduction in the rate of occurrence of IPD may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition of IPD or reduction in the rate of occurrence of IPD may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5,

6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein reduces the severity of Invasive Pneumococcal Disease (IPD) associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such reduction in the severity of IPD may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of IPD may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of IPD may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0060] In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein inhibits or reduces the rate of occurrence of bacteremia, sepsis, and/or meningitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such inhibition, or reduction in the rate of occurrence, of bacteremia, sepsis, and/or meningitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition, or reduction in the rate of occurrence, of bacteremia, sepsis, and/or meningitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some

embodiments, such inhibition, or reduction in the rate of occurrence, of bacteremia, sepsis, and/or meningitis may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein reduces the severity of bacteremia, sepsis, and/or meningitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such reduction in the severity of bacteremia, sepsis, and/or meningitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of bacteremia, sepsis, and/or meningitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of bacteremia, sepsis, and/or meningitis may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0061] In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein inhibits or reduces the rate of occurrence of organ damage associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such inhibition, or reduction in the rate of occurrence, of organ damage may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In

some embodiments, such inhibition, or reduction in the rate of occurrence, of organ damage may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition, or reduction in the rate of occurrence, of organ damage may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein reduces the severity of organ damage associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such reduction in the severity of organ damage may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of organ damage may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of organ damage may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0062] In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein inhibits or reduces the rate of occurrence of pneumonia associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such inhibition, or reduction in the rate of occurrence, of pneumonia may be directed against one

or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition, or reduction in the rate of occurrence, of pneumonia may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition, or reduction in the rate of occurrence, of pneumonia may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein reduces the severity of pneumonia associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such reduction in the severity of pneumonia may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of pneumonia may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of pneumonia may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0063] In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein inhibits or reduces the rate of occurrence of otitis media associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such inhibition, or reduction in the rate of occurrence, of otitis media may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition, or reduction in the rate of occurrence, of otitis media may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition, or reduction in the rate of occurrence, of otitis media may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein reduces the severity of otitis media associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such reduction in the severity of otitis media may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of otitis media may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of otitis media may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least

one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0064] In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein inhibits or reduces the rate of occurrence of sinusitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such inhibition, or reduction in the rate of occurrence, of sinusitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition, or reduction in the rate of occurrence, of sinusitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition, or reduction in the rate of occurrence, of sinusitis may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein reduces the severity of sinusitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such reduction in the severity of sinusitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of sinusitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such

reduction in the severity of sinusitis may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0065] In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein reduces or inhibits colonization by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such reduction or inhibition of colonization may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction or inhibition of colonization may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction or inhibition of colonization may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0066] In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein reduces or inhibits colonization of mucosal surface(s) by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such reduction or inhibition of colonization of mucosal surface(s) may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction or inhibition of colonization of mucosal surface(s) may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical

composition described herein does not include polysaccharide(s) present in at least one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction or inhibition of colonization of mucosal surface(s) may be directed against two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0067] In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein reduces or inhibits colonization of the nasopharynx by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such reduction or inhibition of colonization of nasopharynx may be directed against one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction or inhibition of colonization of nasopharynx may be directed against one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction or inhibition of colonization of nasopharynx may be directed against two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0068] In some embodiments, upon administration to a subject, a pharmaceutical composition disclosed herein inhibits or reduces asymptomatic infection by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such inhibition or reduction of asymptomatic infection may be directed against one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described

herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition or reduction of asymptomatic infection may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition or reduction of asymptomatic infection may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0069] Some aspects of the present disclosure provide a method of making an immunogenic composition (*e.g.*, vaccine), the method comprising non-covalently complexing a plurality of first biotinylated polysaccharide antigens with a plurality of a first fusion protein comprising at least one polypeptide antigen selected from: a pneumolysin polypeptide or variant or antigenic fragment thereof; an SP0435 polypeptide or antigenic fragment thereof; or a combination thereof. In some embodiments, the method further comprises non-covalently complexing a plurality of second biotinylated polysaccharide antigens with a plurality of a second fusion protein comprising at least one polypeptide antigen selected from: an SP1500 polypeptide or antigenic fragment thereof; an SP0785 polypeptide or antigenic fragment thereof; or a combination thereof. In some embodiments, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48. In some embodiments, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A,

12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some embodiments, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from each of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some embodiments, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some embodiments, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from each of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some embodiments, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some embodiments, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from each of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some embodiments, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some embodiments, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from each of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.

[0070] In some embodiments, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 6C, 7C, 15A, 16F, 23A, 23B, 24F, 31, 35B, and 38. In some embodiments, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus*

pneumoniae having a serotype selected from each of 6C, 7C, 15A, 16F, 23A, 23B, 24F, 31, 35B, and 38. In some embodiments, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F. In some embodiments, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from each of 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F. In some embodiments, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 4, 6A, 6B, 9V, 15B, 18C, 19A, 23F, and 33F. In some embodiments, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from each of 1, 4, 6A, 6B, 9V, 15B, 18C, 19A, 23F, and 33F. In some embodiments, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 2, 3, 5, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 16F, 17F, 19F, 20B, 22F, 23A, 23B, 24F, 31, 35B, and 38. In some embodiments, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from each of 2, 3, 5, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 16F, 17F, 19F, 20B, 22F, 23A, 23B, 24F, 31, 35B, and 38. In some embodiments, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 4, 6A, 6B, 9V, 15B, 18C, 19A, 23F, and 33F. In some embodiments, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from each of 1, 4, 6A, 6B, 9V, 15B, 18C, 19A, 23F, and 33F. In some embodiments, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 2, 3, 5, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 17F, 19F, 20B, 22F, 23A, 23B, 24F, 31, 35B, and 38. In some embodiments, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from each of 2, 3, 5, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 17F, 19F, 20B, 22F, 23A, 23B, 24F, 31, 35B, and 38.

[0071] Some embodiments provide for a method of immunizing a subject against *Streptococcus pneumoniae* infection and/or colonization, the method comprising administering to the subject an immunologically effective amount of an immunogenic composition (*e.g.*, vaccine) disclosed herein. In some embodiments, a method of immunizing a subject against *Streptococcus pneumoniae* infection and/or colonization, comprises administering to a subject an immunologically effective amount of an immunogenic complex disclosed herein. In some embodiments, a method of immunizing a subject against *Streptococcus pneumoniae* infection and/or colonization, comprises administering to a subject an immunologically effective amount of a pharmaceutical composition disclosed herein.

[0072] In some embodiments, administration of the immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition induces an immune response. In some embodiments, the immune response comprises an innate immune response. In some embodiments, the immune response comprises an antibody or B cell response. In some embodiments, the immune response comprises a T cell response. In some embodiments, the immune response comprises a CD4⁺ T cell response (*e.g.*, T_H1, T_H2, or T_H17 response), a CD8⁺ T cell response, a CD4⁺ and CD8⁺ T cell response, or a CD4⁻/CD8⁻ T cell response. In some embodiments, the immune response comprises (i) an antibody or B cell response and (ii) a T cell response. In some embodiments, the immune response comprises (i) an antibody or B cell response, (ii) a T cell response, and (iii) an innate immune response. In some embodiments, the immune response is to at least one polysaccharide antigen or at least one polypeptide of a fusion protein. In some embodiments, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) a T cell response to at least one polypeptide of a fusion protein. In some embodiments, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) a CD4⁺ T cell response (*e.g.*, T_H1, T_H2, or T_H17 response), a CD8⁺ T cell response, a CD4⁺ and CD8⁺ T cell response, or a CD4⁻/CD8⁻ T cell response to at least one polypeptide of a fusion protein. In some embodiments, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) an antibody or B cell response to at least one polypeptide of a fusion protein. In some embodiments, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) an antibody or B cell response, and a T

cell response, to at least one polypeptide of a fusion protein. In some embodiments, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) an antibody or B cell response, and a CD4⁺ T cell response, including Th1, Th2, or Th17 response, or a CD8⁺ T cell response, a CD4⁺ and CD8⁺ T cell response, or a CD4⁻/CD8⁻ T cell response, to at least one polypeptide of a fusion protein.

[0073] In some embodiments, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) induces an opsonic/bactericidal response against one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such an opsonic/bactericidal response may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such an opsonic/bactericidal response may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such an opsonic/bactericidal response may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones as described and/or utilized herein) inhibits transmission of one or more serotypes of *Streptococcus pneumoniae* from the subject to another subject. In some embodiments, such inhibition of transmission may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described

herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition of transmission may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition of transmission may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0074] In some embodiments, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) inhibits or reduces the rate of occurrence of Invasive Pneumococcal Disease (IPD) associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such inhibition, or reduction in the rate of occurrence, of IPD may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition, or reduction in the rate of occurrence, of IPD may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition, or reduction in the rate of occurrence, of IPD may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*,

1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) reduces the severity of Invasive Pneumococcal Disease (IPD) associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such reduction in the severity of IPD may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of IPD may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of IPD may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0075] In some embodiments, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) inhibits or reduces the rate of occurrence of bacteremia, sepsis, and/or meningitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such inhibition, or reduction in rate of occurrence, of bacteremia, sepsis, and/or meningitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such

serotype(s). In some embodiments, such inhibition, or reduction in rate of occurrence, of bacteremia, sepsis, and/or meningitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition, or reduction in rate of occurrence, of bacteremia, sepsis, and/or meningitis may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) reduces the severity of bacteremia, sepsis, and/or meningitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such reduction in the severity of bacteremia, sepsis, and/or meningitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of bacteremia, sepsis, and/or meningitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of bacteremia, sepsis, and/or meningitis may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such

serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0076] In some embodiments, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) inhibits or reduces the rate of occurrence of organ damage associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such inhibition, or reduction in the rate of occurrence, of organ damage may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition, or reduction in the rate of occurrence, of organ damage may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition, or reduction in the rate of occurrence, of organ damage may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) reduces the severity of organ damage associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such reduction in the severity of organ damage may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such

serotype(s). In some embodiments, such reduction in the severity of organ damage may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of organ damage may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0077] In some embodiments, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) inhibits or reduces the rate of occurrence of pneumonia associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such inhibition, or reduction in the rate of occurrence, of pneumonia may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition, or reduction in the rate of occurrence, of pneumonia may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition, or reduction in the rate of occurrence, of pneumonia may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such

serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) reduces the severity of pneumonia associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such reduction in the severity of pneumonia may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of pneumonia may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of pneumonia may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0078] In some embodiments, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) inhibits or reduces the rate of occurrence of otitis media associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such inhibition, or reduction in the rate of occurrence, of otitis media may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition, or

reduction in the rate of occurrence, of otitis media may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition, or reduction in the rate of occurrence, of otitis media may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) reduces the severity of otitis media associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such reduction in the severity of otitis media may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of otitis media may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of otitis media may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include

polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0079] In some embodiments, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) inhibits or reduces the rate of occurrence of sinusitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such inhibition, or reduction in the rate of occurrence, of sinusitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition, or reduction in the rate of occurrence, of sinusitis be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such inhibition, or reduction in the rate of occurrence, of sinusitis may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) reduces the severity of sinusitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such reduction in the severity of sinusitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of sinusitis may be directed

against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction in the severity of sinusitis may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0080] In some embodiments, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) reduces or inhibits colonization by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such reduction or inhibition of colonization may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction or inhibition of colonization may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction or inhibition of colonization may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, administration of an

immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) reduces or inhibits colonization of mucosal surface(s) by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such reduction or inhibition of colonization of mucosal surface(s) may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction or inhibition of colonization of mucosal surface(s) may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction or inhibition of colonization of mucosal surface(s) may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) reduces or inhibits colonization of nasopharynx by one or more serotypes of *Streptococcus pneumoniae*. In some embodiments, such reduction or inhibition of colonization of nasopharynx may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction or inhibition of colonization of nasopharynx may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic

complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some embodiments, such reduction or inhibition of colonization of nasopharynx may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0081] In some embodiments of the methods disclosed herein, the *Streptococcus pneumoniae* has a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48. In some embodiments, the *Streptococcus pneumoniae* has a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some embodiments, the *Streptococcus pneumoniae* has a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.

[0082] In some embodiments, the subject is immunized against *Streptococcus pneumoniae* infection and/or colonization with one dose of an immunogenic composition (*e.g.*, vaccine). In some embodiments, the subject is immunized against *Streptococcus pneumoniae* infection and/or colonization with two doses of an immunogenic composition (*e.g.*, vaccine). In some embodiments, the subject is immunized against *Streptococcus pneumoniae* infection and/or colonization with three doses of an immunogenic composition (*e.g.*, vaccine). In some embodiments, the subject is immunized against *Streptococcus pneumoniae* infection and/or colonization with four doses of an immunogenic composition (*e.g.*, vaccine).

Brief Description of the Drawings

[0083] The present teachings described herein will be more fully understood from the following description of various illustrative embodiments, when read together with the accompanying drawings. It should be understood that the drawings described below are for illustration purposes only and are not intended to limit the scope of the present teachings in any way.

[0084] Figure 1 is a schematic representation of an exemplary Multiple Antigen Presenting System (MAPS). In the exemplary embodiment shown, MAPS immunogenic complexes comprise one or more polypeptide antigens fused to the biotin-binding protein rhizavidin, or a biotin-binding domain or biotin-binding fragment thereof, and a biotinylated antigenic polysaccharide. In this figure, each MAPS complex is formed between one or more fusion proteins and a biotinylated polysaccharide by non-covalent binding of a truncated rhizavidin to biotin.

[0085] Figure 2A is a schematic of an exemplary mutant pneumolysin (Ply) polypeptide. Such an exemplary mutant Ply polypeptide comprises a Ply polypeptide (*e.g.*, amino acids 2-470 of a full-length *S. pneumoniae* Ply polypeptide) comprising mutations G294P, D385N, C428G, and W433F, denoted as PdT(G294P). In some embodiments, a mutant Ply polypeptide (*e.g.*, a PdT(G294P) polypeptide) may further comprise a detection or purification tag (*e.g.*, a His tag).

[0086] Figure 2B is a schematic of an exemplary SPP2 fusion protein. Such an exemplary SPP2 fusion protein comprises a biotin-binding protein, such as *e.g.*, a truncated rhizavidin protein (*e.g.*, amino acids 45-179 of a wild-type rhizavidin protein, denoted as Rhavi), a first linker (*e.g.*, a GGGGSSS (SEQ ID NO: 30) linker), a pneumolysin (Ply) polypeptide (*e.g.*, amino acids 2-470 of a full-length *S. pneumoniae* Ply polypeptide comprising mutations G294P, D385N, C428G, and W433F, denoted as PdT(G294P)), a second linker (*e.g.*, a GGGGSSS (SEQ ID NO: 30) linker), and a SP0435 polypeptide (*e.g.*, amino acids 62-185 of a full length *S. pneumoniae* SP0435 polypeptide). In some embodiments, a SPP2 fusion protein may further comprise a detection or purification tag (*e.g.*, a His tag). For a GGGGSSS (SEQ ID NO: 30) linker, the SSS amino acid sequence can be from the Sac I site on a pET21/24 plasmid, with the

GGGG (SEQ ID NO: 54) amino acid sequence added to create a flexible linker with minimal steric hindrance. Alternatively, the GGGGSSS (SEQ ID NO: 30) linker can be synthesized.

[0087] Figure 3 is a schematic of a plasmid construct composed of the pET-24a(+) vector containing a sequence encoding an exemplary SPP2 fusion protein.

[0088] Figure 4 is a model depicting the tertiary structure of an exemplary pneumolysin polypeptide containing the mutations G294P, D385N, C428G, and W433F (denoted as PdT(G294P)).

[0089] Figure 5A illustrates the hemolytic activity of pneumolysin, pneumolysin plus SPP2 fusion protein, a PdT(G294P) polypeptide, and fusion proteins (Precursor 1, Precursor 2, and SPP2) as measured using a rabbit red blood cell hemolysis assay. No hemolysis was observed for PdT(G294P), Precursor 2, or SPP2.

[0090] Figure 5B illustrates the hemolytic activity, as measured using a rabbit red blood cell hemolysis assay, of fusion protein SPP2 in MAPS complexes with *Streptococcus pneumoniae* polysaccharide SP1 (1.SPP2 MAPS), and of pneumolysin prepared from standard 0.5 mg/mL stock (Pneumolysin-1), pneumolysin prepared from diluted 0.1 mg/mL stock (Pneumolysin-2), and pneumolysin prepared from diluted 0.1 mg/mL stock, then subjected to one freeze-thaw cycle (Pneumolysin-2-F/T). No hemolysis was observed for 1.SPP2 MAPS.

[0091] Figure 6A illustrates hemagglutination activity of fusion proteins (Precursor 1, Precursor 2, and SPP2), as measured using a rabbit red blood cell assay. Precursor 2 wells denoted by the dashed line show evidence of hemagglutination. No hemagglutination was observed in Precursor 1 or SPP2 wells.

[0092] Figure 6B illustrates hemagglutination activity of pneumolysin, pneumolysin plus fusion protein SPP2, a PdT(G294P) polypeptide, and fusion proteins (Precursor 1, Precursor 2, and SPP2), as measured using a rabbit red blood cell assay. Precursor 2 wells denoted by the dashed lines show evidence of hemagglutination. In this experiment, Precursor 1 wells may show a small amount of hemagglutination. No hemagglutination was observed in PdT(G294P) or SPP2 wells.

[0093] Figure 6C illustrates hemagglutination activity, as measured using a rabbit red blood cell assay, of fusion protein SPP2 in MAPS complexes with *Streptococcus pneumoniae* polysaccharide PS1 (1.SPP2 MAPS), and of pneumolysin prepared from standard 0.5 mg/mL stock (Pneumolysin-1), pneumolysin prepared from diluted 0.1 mg/mL stock (Pneumolysin-2), and pneumolysin prepared from diluted 0.1 mg/mL stock, then subjected to one freeze-thaw cycle (Pneumolysin-2-F/T). No hemagglutination was observed in 1.SPP2 MAPS wells.

[0094] Figure 7 is a schematic of an exemplary CP1 fusion protein. Such an exemplary CP1 fusion protein comprises a biotin-binding protein such as, *e.g.*, a truncated rhizavidin protein (*e.g.*, amino acids 45-179 of a wild-type rhizavidin protein), a first linker (*e.g.*, a GGGGSSS (SEQ ID NO: 31) linker), a SP1500 polypeptide (*e.g.*, amino acids 27-278 of a full-length *S. pneumoniae* SP1500 polypeptide), a second linker (*e.g.*, the amino acid sequence AAA (SEQ ID NO: 31)), and a SP0785 polypeptide (*e.g.*, amino acids 33-399 of a full length *S. pneumoniae* SP0785 polypeptide). In some embodiments, a CP1 fusion protein may further comprise a detectable or purification tag (*e.g.*, His tag). The amino acid sequence AAA can be from the Not I site on a pET21/24 plasmid, or synthesized. For a GGGGSSS (SEQ ID NO: 30) linker, the SSS amino acid sequence can be from the Sac I site on a pET21/24 plasmid, with the GGGG (SEQ ID NO: 54) amino acid sequence added to create a flexible linker with minimal steric hindrance. Alternatively, the GGGGSSS (SEQ ID NO: 30) linker can be synthesized.

[0095] Figure 8 are flow-charts depicting an exemplary upstream (upper panel) and downstream (lower panel) manufacturing process for fusion proteins as described herein (*e.g.*, CP1 and SPP2). An exemplary SPP2 polypeptide is a fusion protein comprising a truncated rhizavidin [amino acids 45-179 of a full-length rhizavidin protein], a first linker (*e.g.*, a GGGGSSS linker), a pneumolysin (Ply) polypeptide, a second linker (*e.g.*, a GGGGSSS linker), and a SP0435 polypeptide. An exemplary CP1 polypeptide is a fusion protein comprising a truncated rhizavidin [amino acids 45-179 of a full-length rhizavidin protein], a first linker (*e.g.*, a GGGGSSS linker), a SP1500 polypeptide, a second linker (*e.g.*, the amino acid sequence AAA), and a SP0785 polypeptide. SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis; TFF: tangential flow filtration; DS: drug substance.

[0096] Figure 9 is a table showing exemplary structures of *S. pneumoniae* antigenic polysaccharides of serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, and 35B.

[0097] Figure 10 is a flow-chart depicting an exemplary manufacturing process for MAPS immunogenic complexes, comprising biotinylation of antigenic polysaccharides and assembly with fusion protein(s) as described herein (e.g., CP1 or SPP2). MAPS: Multiple Antigen Presenting System.

[0098] Figure 11 is a flow-chart depicting exemplary manufacturing processes for a MAPS vaccine. For example, such exemplary processes can be used to manufacture a MAPS34 vaccine, comprising MAPS immunogenic complexes that comprise capsular polysaccharides from 34 different *S. pneumoniae* serotypes. DMAP: 4-Dimethylaminopyridine; MAPS: Multiple Antigen Presenting System; PS: polysaccharide. † In-process tests

[0099] Figure 12 is a schematic depicting various multi-valent vaccine candidates with different combinations of *S. pneumoniae* polysaccharides (serotypes) with antigenic fusion proteins CP1 and SPP2. The immunization group (A-H) corresponding to each vaccine candidate is indicated. While the figure illustrates certain multivalent vaccine candidates in a MAPS format as described herein, in some embodiments, such multivalent vaccine candidates can be provided in a conjugated format where a *S. pneumoniae* polysaccharide is covalently conjugated to an antigenic polypeptide and/or a fusion protein as described herein (e.g., CP1 and/or SPP2).

[0100] Figure 13 shows representative IgG levels against CP1 fusion protein in P0, P1 and P2 rabbit sera from each of rabbit immunization groups A-H. Each dot on the graph represents one rabbit. Results are expressed in $\mu\text{g/ml}$ with 95% confidence intervals on the graph and tabulated as geometric means ($\mu\text{g/ml}$) below the graph.

[0101] Figure 14 shows representative IgG levels against the PdT(G294P) component of SPP2 fusion protein in P0, P1 and P2 rabbit sera from each of immunization groups A-H. Each dot on the graph represents one rabbit. Results are expressed in $\mu\text{g/ml}$ with 95% confidence intervals on the graph and tabulated as geometric means ($\mu\text{g/ml}$) below the graph.

[0102] Figure 15 shows representative IgG levels against the SP0435 component of SPP2 fusion protein in P0, P1 and P2 rabbit sera from each of immunization groups A-H. Each dot on the graph represents one rabbit. Results are expressed in $\mu\text{g/ml}$ with 95% confidence intervals on the graph and tabulated as geometric means ($\mu\text{g/ml}$) below the graph.

[0103] Figure 16 shows representative half-maximal inhibitory concentration (IC₅₀) of neutralizing antibodies against the hemolytic activity of native pneumolysin, in P0, P1 and P2 rabbit sera from each of immunization groups C-G. Each dot on the graph represents one rabbit. Results are expressed as the IC₅₀ (serum dilution) with 95% confidence intervals on the graph and tabulated as geometric means (IC₅₀) below the graph.

[0104] Figure 17A shows representative IgG levels against 20 capsular polysaccharide (CPS) serotypes in P2 rabbit sera from each of immunization groups A-E and G. Results are expressed as IgG level in arbitrary units (a.u.) for comparison across the immunization groups.

[0105] Figure 17B shows representative functional antibody (OPA) levels against 20 capsular polysaccharide (CPS) serotypes in P2 rabbit sera from each of immunization groups A-E and G. Results are expressed as OPA titers for comparison across the immunization groups.

[0106] Figure 18 shows relative antibody titers against capsular polysaccharides of various serotypes for both MAPS-34 all-on-CP1 (immunization group B, gray dot) and MAPS-34 all-on-SPP2 (immunization group F, black dot) compared to a baseline of MAPS-23 all-on-CP1 (immunization group A, dotted line) in P2 rabbit sera. The upper graph shows results for 13 serotypes common to MAPS-23, MAPS-34 and PCV13. The lower graph shows results for 10 additional serotypes common to MAPS-23 and MAPS-34. Geometric mean titer (GMT) ratio and 95% confidence intervals are shown on each graph.

[0107] Figure 19 shows relative antibody titers against capsular polysaccharides of various serotypes for MAPS-34 all-on-SPP2 (immunization group F) compared to a baseline of MAPS-34 all-on-CP1 (immunization group B, dotted line) in P2 rabbit sera. The upper graph shows results for the first 17 serotypes of MAPS-34, and the lower graph shows results for the next 17 serotypes, as shown graphically in Figure 12. Geometric mean titer (GMT) ratio and 95% confidence intervals are shown on the graph.

[0108] Figure 20 shows relative antibody titers against capsular polysaccharides of various serotypes for MAPS-34 on CP1 and SPP2 (immunization group C) compared to a baseline of MAPS-34 all-on-CP1 (immunization group B, dotted line) in P2 rabbit sera. The upper graph shows results for the first 17 serotypes of MAPS-34, and the lower graph shows results for the next 17 serotypes, as shown graphically in Figure 12. Geometric mean titer (GMT) ratio and 95% confidence intervals are shown on the graph. For MAPS-34 on CP1 and SPP2 (immunization group C), the boxed subset of polysaccharides are present in MAPS complexes on SPP2.

[0109] Figure 21 shows relative antibody titers against capsular polysaccharides of various serotypes for both MAPS-34 on CP1 and SPP2 (Combo #1, immunization group D, gray dot) and MAPS-34 on SPP2 and CP1 (Combo #2, immunization group E, black dot) compared to a baseline of MAPS-34 all-on-CP1 (immunization group B, dotted line) in P2 rabbit sera. The upper graph shows results for the first 17 serotypes of MAPS-34, and the lower graph shows results for the next 17 serotypes, as shown graphically in Figure 12. Geometric mean titer (GMT) ratio and 95% confidence intervals are shown on the graph.

[0110] Figure 22 shows relative antibody titers against capsular polysaccharides of various serotypes for MAPS-34 on CP1 and SPP2 (Combo #1, immunization group D) compared to MAPS-34 on SPP2 and CP1 (Combo #2, immunization group E) in P2 rabbit sera. The upper graph shows results comparing Combo #2 (on SPP2) to Combo #1 (on CP1) for the first 17 serotypes of MAPS-34, as shown graphically in Figure 12. The lower graph shows results comparing Combo #1 (on SPP2) to Combo #2 (on CP1) for the next 17 serotypes of MAPS-34, as shown graphically in Figure 12. Geometric mean titer (GMT) ratio and 95% confidence intervals are shown on the graph.

[0111] Figure 23 shows relative antibody titers against capsular polysaccharides of various serotypes for MAPS-34 on CP1 and SPP2 (immunization group C), MAPS-34 on CP1 and SPP2 (Combo #1, immunization group D), MAPS-34 on SPP2 and CP1 (Combo #2, immunization group E), and MAPS-33 on CP1 and SPP2 (immunization group G) compared to a baseline of MAPS-34 all-on-CP1 (immunization group B). The upper graph shows results for the first 17 serotypes of MAPS-34 and the lower graph shows results for the next 17 serotypes. Geometric mean titer (GMT) ratio and 95% confidence intervals are shown on the graph.

[0112] Figure 24 is based on the same data as Figure 23, but shows only the comparison of MAPS-34 on CP1 and SPP2 (immunization group C) and MAPS-33 on CP1 and SPP2 (immunization group G) compared to a baseline of MAPS-34 all-on-CP1 (immunization group B) in P2 rabbit sera. The upper graph shows results for the first 17 serotypes of MAPS-34 and the lower graph shows results for the next 17 serotypes. Geometric mean titer (GMT) ratio and 95% confidence intervals are shown on the graph. The star symbol denotes polysaccharides that are presented in MAPS complexes comprising SPP2 for immunization group C (below the graph) or immunization group G (above the graph).

[0113] Figure 25 shows total IgG levels against SP1500-SP0785 and PdT(G294P) for mice immunized with vaccines comprising isolated proteins SPP2, or SPP2 plus CP1, or with vaccines comprising SPP2 plus CP1 both in MAPS complexes with *Streptococcus pneumoniae* polysaccharide PS1 (PPS1). Mice immunized with rhizavidin polypeptide (Rhavi) and PCV13 are shown as controls. Each dot on the graph represents an individual mouse. Geometric mean titer and 95% confidence intervals are shown on the graph.

[0114] Figure 26 shows percent survival at indicated time points for groups of mice immunized with vaccines comprising isolated proteins SPP2, or SPP2 plus CP1, or with vaccines comprising SPP2 plus CP1 both in MAPS complexes with *Streptococcus pneumoniae* polysaccharide PS1, and challenged with *S. pneumoniae* strain AR003. Mice immunized with Rhavi and Prevnar 13 (PCV13) are shown as negative and positive controls, respectively.

[0115] Figure 27 shows total IgG levels against SP1500-SP0785 and PdT(G294P) for mice immunized with vaccines comprising CP1, SPP2, or CP1 plus SPP2, all in MAPS complexes with *Streptococcus pneumoniae* polysaccharide PS1 (PPS1). Mice immunized with Rhavi and PCV13 are shown as controls. Each dot on the graph represents an individual mouse. Geometric mean titer and 95% confidence intervals are shown on the graph.

[0116] Figure 28A shows the percent survival at indicated time points for groups of mice immunized with vaccines comprising CP1, SPP2, or CP1 plus SPP2, all in MAPS complexes with *Streptococcus pneumoniae* polysaccharide PS1, and challenged with *S. pneumoniae* strain AR003. Mice immunized with Rhavi and Prevnar 13 (PCV13) are shown as negative and positive controls, respectively.

[0117] Figure 28B shows the same results as in Figure 28A but separating survival curves for the immunization groups onto different graphs.

[0118] Figure 29 shows percent survival at the indicated time points for groups of mice passively immunized with P2 sera from rabbits immunized with MAPS34 vaccines, and challenged with high dose *S. pneumoniae* strain AR003. Mice passively immunized with saline or with P2 sera from rabbits immunized with Prevnar 13 (PCV13) are shown as controls.

[0119] Figure 30 shows killing activity of representative P2 sera from rabbits immunized with MAPS-34 on CP1 and SPP2 (Combo #1, immunization group D), MAPS-34 on SPP2 and CP1 (Combo #2, immunization group E), and PCV13 (immunization group H). Killing activity was measured by an opsonophagocytic assay (OPA) against *S. pneumoniae* AR003. Results are expressed as % killing activity relative to serum dilution.

[0120] Figure 31 shows representative half-maximal inhibitory concentration (IC₅₀) of pneumolysin neutralizing antibodies (Ply neutralization (IC₅₀)) in P2 sera from rabbits immunized with SPP2 fusion protein, graphed against anti-PdT(G294P) IgG titers (μg/mL). Results of two studies were combined (n=5 and n=3).

[0121] Figure 32 shows the percent survival at indicated time points for the indicated groups of immunized mice, challenged with 1.0×10^7 CFU of *S. pneumoniae* strain AR003 (serotype 3) per mouse. Rhavi: primed and boosted with Rhavi. PCV13: primed with Prevnar 13 (PCV13). SPP2: primed and boosted with SPP2 fusion protein. 1.SPP2+PCV13: primed and boosted with SPP2 fusion protein in MAPS complexes with *S. pneumoniae* capsular polysaccharide 1 (CPS1) (denoted “1.SPP2”), and re-boosted with PCV13.

[0122] Figure 33 shows the percent survival at indicated time points for the indicated groups of immunized mice, challenged with 6.7×10^6 CFU of *S. pneumoniae* strain AR003 (serotype 3). Rhavi: primed and boosted with Rhavi. PCV13: primed with Prevnar 13 (PCV13). 1.SPP2+3.CP1: primed and boosted with SPP2 fusion protein in MAPS complexes with *S. pneumoniae* capsular polysaccharide 1 (CPS1) (denoted “1.SPP2”), and re-boosted with CP1 fusion protein in MAPS complexes with *S. pneumoniae* capsular polysaccharide 3 (CPS3) (denoted “3.CP1”).

[0123] Figures 34A-34C show immunogenicity and functional antibody responses against antigens relevant to the *S. pneumoniae* strain AR003 (serotype 3) sepsis model. Figure 34A shows representative results for IgG titers against serotype 3 capsular polysaccharide (CPS3) in P0, P1 and P2 rabbit sera of immunization Groups D and E shown in Figure 12 as Combo #1 and Combo #2, respectively. Each point on the graph represents serum from one rabbit. Horizontal bars are the geometric mean concentration of the groups and error bars are 95% confidence intervals. Results are expressed in arbitrary units (a.u.) of anti-CPS3 IgG. Data presented includes a subset of data for immunogenicity against capsular polysaccharide (CPS) serotypes presented in Figure 17A. Figure 34B shows representative titers of PdT toxoid-binding IgG (anti-PdT(G294P)) in P0, P1 and P2 rabbit sera of immunization Groups D and E shown in Figure 12 as Combo #1 and Combo #2, respectively. Each point on the graph represents serum from one rabbit. Horizontal bars are the geometric mean concentration of the groups and error bars are 95% confidence intervals. Results are expressed in $\mu\text{g/ml}$ of anti-PdT(G294P) IgG. Data presented is a subset of data for immunogenicity against PdT(G294P) presented in Figure 14. Figure 34C shows representative half-maximal inhibitory concentration (IC50) of pneumolysin neutralizing antibodies against the hemolytic activity of native pneumolysin in P0, P1 and P2 rabbit sera of immunization Groups D and E shown in Figure 12 as Combo #1 and Combo #2, respectively. Each point on the graph represents serum from one rabbit. Horizontal bars are the geometric mean IC50 of the groups and error bars are 95% confidence intervals. Results are expressed as the IC50 (serum dilution). Data presented is a subset of data for functional responses against PdT(G294P) presented in Figure 16.

[0124] Figures 35A and 35B show representative half-maximal inhibitory concentration (IC50) of pneumolysin neutralizing antibodies against the hemolytic activity of native pneumolysin, in sera from rabbits immunized with non-His-tagged SPP2 (Figure 35A) or PdT(G294P) (Figure 35B). Each dot on the graph represents one rabbit. Results are expressed as the IC50 (serum dilution) with 95% confidence intervals on the graphs and tabulated as geometric means (IC50) below each graph.

[0125] Figures 36A and 36B show representative survival curves, up to 24 hours after dosing, for groups of mice receiving the indicated amounts of pneumolysin (Ply), SPP2 fusion protein alone, or SPP2 fusion protein in MAPS complexes with *Streptococcus pneumoniae*

polysaccharide SP1 (1.SPP2 MAPS) by intranasal inoculation. Figure 36A shows representative results for groups inoculated with His-tagged SPP2 and 1.SPP2 MAPS. Figure 36B shows representative results for groups inoculated with non-His-tagged SPP2 and 1.SPP2 MAPS.

[0126] Figure 37 is a schematic depicting various exemplary multi-valent vaccine candidates with different combinations of *S. pneumoniae* polysaccharides (serotypes) with antigenic fusion proteins CP1 and SPP2. The immunization group (A, B, C, and S) corresponding to each vaccine candidate is indicated. While the figure illustrates these multivalent vaccine candidates in a MAPS format as described herein, in some embodiments, such multivalent vaccine candidates can be provided in a conjugated format where a *S. pneumoniae* polysaccharide is conjugated to an antigenic polypeptide or a fusion protein as described herein (e.g., CP1 and/or SPP2).

Certain Definitions

[0127] In this application, unless otherwise clear from context, (i) the term “a” may be understood to mean “at least one”; (ii) the term “or” may be understood to mean “and/or”; (iii) the terms “comprising” and “including” may be understood to encompass itemized components or steps whether presented by themselves or together with one or more additional components or steps; and (iv) the terms “about” and “approximately” may be understood to permit standard variation as would be understood by those of ordinary skill in the art; and (v) where ranges are provided, endpoints are included.

[0128] **About:** The term “about”, when used herein in reference to a value, refers to a value that is similar, in context to the referenced value. In general, those skilled in the art, familiar with the context, will appreciate the relevant degree of variance encompassed by “about” in that context. For example, in some embodiments, the term “about” may encompass a range of values that within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less of the referred value.

[0129] **Administration:** As used herein, the term “administration” typically refers to the administration of a composition to a subject or system to achieve delivery of an agent that is, or is included in, the composition. Those of ordinary skill in the art will be aware of a variety of

routes that may, in appropriate circumstances, be utilized for administration to a subject, for example a human. For example, in some embodiments, administration may be ocular, oral, parenteral, topical, etc. In some particular embodiments, administration may be bronchial (*e.g.*, by bronchial instillation), buccal, dermal (which may be or comprise, for example, one or more of topical to the dermis, intradermal, interdermal, transdermal, etc.), enteral, intra-arterial, intradermal, intragastrical, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, within a specific organ (*e.g.*, intrahepatic), mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (*e.g.*, by intratracheal instillation), vaginal, vitreal, etc. In some embodiments, administration may involve only a single dose. In some embodiments, administration may involve application of a fixed number of doses. In some embodiments, administration may involve dosing that is intermittent (*e.g.*, a plurality of doses separated in time) and/or periodic (*e.g.*, individual doses separated by a common period of time) dosing. In some embodiments, administration may involve continuous dosing (*e.g.*, perfusion) for at least a selected period of time.

[0130] **Agent:** In general, the term “agent”, as used herein, may be used to refer to a compound or entity of any chemical class including, for example, a polypeptide, nucleic acid, saccharide, lipid, small molecule, metal, or combination or complex thereof. In appropriate circumstances, as will be clear from context to those skilled in the art, the term may be utilized to refer to an entity that is or comprises a cell or organism, or a fraction, extract, or component thereof. Alternatively, or additionally, as context will make clear, the term may be used to refer to a natural product in that it is found in and/or is obtained from nature. In some instances, again as will be clear from context, the term may be used to refer to one or more entities that is man-made in that it is designed, engineered, and/or produced through action of the hand of man and/or is not found in nature. In some embodiments, an agent may be utilized in isolated or pure form; in some embodiments, an agent may be utilized in crude form. In some embodiments, potential agents may be provided as collections or libraries, for example that may be screened to identify or characterize active agents within them. In some cases, the term “agent” may refer to a compound or entity that is or comprises a polymer; in some cases, the term may refer to a compound or entity that comprises one or more polymeric moieties. In some embodiments, the term “agent” may refer to a compound or entity that is not a polymer and/or is substantially free

of any polymer and/or of one or more particular polymeric moieties. In some embodiments, the term may refer to a compound or entity that lacks or is substantially free of any polymeric moiety.

[0131] Amino acid: In its broadest sense, the term “amino acid”, as used herein, refers to any compound and/or substance that can be incorporated into a polypeptide chain, *e.g.*, through formation of one or more peptide bonds. In some embodiments, an amino acid has the general structure $\text{H}_2\text{N}-\text{C}(\text{H})(\text{R})-\text{COOH}$. In some embodiments, an amino acid is a naturally-occurring amino acid. In some embodiments, an amino acid is a non-natural amino acid; in some embodiments, an amino acid is a D-amino acid; in some embodiments, an amino acid is an L-amino acid. “Standard amino acid” refers to any of the twenty standard L-amino acids commonly found in naturally occurring peptides. “Non-standard amino acid” refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. In some embodiments, an amino acid, including a carboxy- and/or amino-terminal amino acid in a polypeptide, can contain a structural modification as compared with the general structure above. For example, in some embodiments, an amino acid may be modified by methylation, amidation, acetylation, pegylation, glycosylation, phosphorylation, and/or substitution (*e.g.*, of the amino group, the carboxylic acid group, one or more protons, and/or the hydroxyl group) as compared with the general structure. In some embodiments, such modification may, for example, alter the circulating half-life of a polypeptide containing the modified amino acid as compared with one containing an otherwise identical unmodified amino acid. In some embodiments, such modification does not significantly alter a relevant activity of a polypeptide containing the modified amino acid, as compared with one containing an otherwise identical unmodified amino acid. As will be clear from context, in some embodiments, the term “amino acid” may be used to refer to a free amino acid; in some embodiments, the term “amino acid” may be used to refer to an amino acid residue of a polypeptide.

[0132] Antibody: As used herein, the term “antibody” refers to a polypeptide that includes canonical immunoglobulin sequence elements sufficient to confer specific binding to a particular target antigen. As is known in the art, intact antibodies as produced in nature are approximately 150 kDa tetrameric agents comprised of two identical heavy chain polypeptides

(about 50 kDa each) and two identical light chain polypeptides (about 25 kDa each) that associate with each other into what is commonly referred to as a “Y-shaped” structure. Each heavy chain is comprised of at least four domains (each about 110 amino acids long)— an amino-terminal variable (VH) domain (located at the tips of the Y structure), followed by three constant domains: CH1, CH2, and the carboxy-terminal CH3 (located at the base of the Y’s stem). A short region, known as the “switch”, connects the heavy chain variable and constant regions. The “hinge” connects CH2 and CH3 domains to the rest of the antibody. Two disulfide bonds in this hinge region connect the two heavy chain polypeptides to one another in an intact antibody. Each light chain is comprised of two domains – an amino-terminal variable (VL) domain, followed by a carboxy-terminal constant (CL) domain, separated from one another by another “switch”. Intact antibody tetramers are comprised of two heavy chain-light chain dimers in which the heavy and light chains are linked to one another by a single disulfide bond; two other disulfide bonds connect the heavy chain hinge regions to one another, so that the dimers are connected to one another and the tetramer is formed. Naturally-produced antibodies are also glycosylated, typically on the CH2 domain. Each domain in a natural antibody has a structure characterized by an “immunoglobulin fold” formed from two beta sheets (*e.g.*, 3-, 4-, or 5-stranded sheets) packed against each other in a compressed antiparallel beta barrel. Each variable domain contains three hypervariable loops known as “complement determining regions” (CDR1, CDR2, and CDR3) and four somewhat invariant “framework” regions (FR1, FR2, FR3, and FR4). When natural antibodies fold, the FR regions form the beta sheets that provide the structural framework for the domains, and the CDR loop regions from both the heavy and light chains are brought together in three-dimensional space so that they create a single hypervariable antigen binding site located at the tip of the Y structure. The Fc region of naturally-occurring antibodies binds to elements of the complement system, and also to receptors on effector cells, including for example effector cells that mediate cytotoxicity. As is known in the art, affinity and/or other binding attributes of Fc regions for Fc receptors can be modulated through glycosylation or other modification. In some embodiments, antibodies produced and/or utilized in accordance with the present disclosure include glycosylated Fc domains, including Fc domains with modified or engineered such glycosylation. For purposes of the present disclosure, in certain embodiments, any polypeptide or complex of polypeptides that includes sufficient

immunoglobulin domain sequences as found in natural antibodies can be referred to and/or used as an “antibody”, whether such polypeptide is naturally produced (*e.g.*, generated by an organism reacting to an antigen), or produced by recombinant engineering, chemical synthesis, or other artificial system or methodology. In some embodiments, an antibody is polyclonal; in some embodiments, an antibody is monoclonal. In some embodiments, an antibody has constant region sequences that are characteristic of mouse, rabbit, primate, or human antibodies. In some embodiments, antibody sequence elements are humanized, primatized, chimeric, etc., as is known in the art. Moreover, the term “antibody” as used herein, can refer in appropriate embodiments (unless otherwise stated or clear from context) to any of the art-known or developed constructs or formats for utilizing antibody structural and functional features in alternative presentation. For example, embodiments, an antibody utilized in accordance with the present disclosure is in a format selected from, but not limited to, intact IgA, IgG, IgE or IgM antibodies; bi- or multi- specific antibodies (*e.g.*, Zybodies[®], etc.); antibody fragments such as Fab fragments, Fab’ fragments, F(ab’)2 fragments, Fd’ fragments, Fd fragments, and isolated CDRs or sets thereof; single chain Fvs; polypeptide-Fc fusions; single domain antibodies (*e.g.*, shark single domain antibodies such as IgNAR or fragments thereof); cameloid antibodies; masked antibodies (*e.g.*, Probodies[®]); Small Modular ImmunoPharmaceuticals (“SMIPs[™]”); single chain or Tandem diabodies (TandAb[®]); VHHs; Anticalins[®]; Nanobodies[®] minibodies; BiTE[®]s; ankyrin repeat proteins or DARPINS[®]; Avimers[®]; DARTs; TCR-like antibodies; Adnectins[®]; Affilins[®]; Trans-bodies[®]; Affibodies[®]; TrimerX[®]; MicroProteins; Fynomers[®], Centyrins[®], and KALBITOR[®]s. In some embodiments, an antibody may lack a covalent modification (*e.g.*, attachment of a glycan) that it would have if produced naturally. In some embodiments, an antibody may contain a covalent modification (*e.g.*, attachment of a glycan, a payload [*e.g.*, a detectable moiety, a therapeutic moiety, a catalytic moiety, etc.], or other pendant group [*e.g.*, poly-ethylene glycol, etc.]).

[0133] Antigen: The term “antigen”, as used herein, refers to (i) an agent that induces an immune response; and/or (ii) an agent that binds to a T cell receptor (*e.g.*, when presented by an MHC molecule) or to an antibody. In some embodiments, an antigen induces a humoral response (*e.g.*, including production of antigen-specific antibodies); in some embodiments, an antigen induces a cellular response (*e.g.*, involving T cells whose receptors specifically interact

with the antigen). In some embodiments, an antigen induces a humoral response and a cellular response. In some embodiments, an antigen binds to an antibody and may or may not induce a particular physiological response in an organism. In general, an antigen may be or include any chemical entity such as, for example, a small molecule, a nucleic acid, a polypeptide, a carbohydrate, a lipid, a polymer (in some embodiments, other than a biologic polymer (*e.g.*, other than a nucleic acid or amino acid polymer)), etc. In some embodiments, an antigen is or comprises a polypeptide. In some embodiments, an antigen is or comprises a polysaccharide. Those of ordinary skill in the art will appreciate that, in general, an antigen may be provided in isolated or pure form, or alternatively may be provided in crude form (*e.g.*, together with other materials, for example in an extract such as a cellular extract or other relatively crude preparation of an antigen-containing source). In some embodiments, antigens utilized in accordance with the present disclosure are provided in a crude form. In some embodiments, an antigen is a recombinant antigen. In some embodiments, an antigen is a polypeptide or a polysaccharide that, upon administration to a subject, induces a specific and/or clinically relevant immune response to such polypeptide or polysaccharide. In some embodiments, an antigen is selected to induce a specific and/or clinically relevant immune response to such polypeptide or polysaccharide.

[0134] Associated with: Two entities are “associated” with one another, as that term is used herein, if the presence, level and/or form of one is correlated with that of the other. In some embodiments, two or more entities are physically “associated” with one another if they interact, directly or indirectly, so that they are and/or remain in physical proximity with one another. In some embodiments, two or more entities that are physically associated with one another are covalently linked to one another. In some embodiments, two or more entities that are physically associated with one another are not covalently linked to one another but are non-covalently associated, for example by means of affinity interactions, electrostatic interactions, hydrogen bonds, van der Waals interaction, hydrophobic interactions, magnetism, and combinations thereof.

[0135] Binding: It will be understood that the term “binding”, as used herein, typically refers to a non-covalent association between or among two or more entities. “Direct” binding involves physical contact between entities or moieties; indirect binding involves physical

interaction by way of physical contact with one or more intermediate entities. Binding between two or more entities can typically be assessed in any of a variety of contexts – including where interacting entities or moieties are studied in isolation or in the context of more complex systems (*e.g.*, while covalently or otherwise associated with a carrier entity and/or in a biological system or cell).

[0136] Carrier protein: As used herein, the term “carrier protein” refers to a protein or peptide that is coupled, or complexed, or otherwise associated with a hapten (*e.g.*, a small peptide or lipid) or less immunogenic antigen (*e.g.*, a polysaccharide) and that induces or improves an immune response to such a coupled, or complexed, or otherwise associated hapten (*e.g.*, a small peptide or lipid) or less immunogenic antigen (*e.g.*, a polysaccharide). In some embodiments, such an immune response is or comprises a response to a hapten or less immunogenic antigen that is coupled, or complexed, or otherwise associated with such a carrier protein. In some embodiments, such an immune response is or comprises a response to both a carrier protein and a hapten or less immunogenic antigen that is coupled, or complexed, or otherwise associated with such a carrier protein. In some embodiments, no significant immune response to a carrier protein itself occurs. In some embodiments, immune response to a carrier protein may be detected; in some embodiments, immune response to such a carrier protein is strong. In some embodiments, a carrier protein is coupled, or complexed, or otherwise associated with one or more other molecules.

[0137] Colonization: As used herein, the term “colonization” generally refers to the ability of a microbe to grow at a target site or surface. For example, the terms “colonization” refers to the ability of a microbe (*e.g.*, a bacterium) to grow at an anatomical site (*e.g.*, a mucosal membrane, gastrointestinal tract, injury site, organ, *etc.*) of a host.

[0138] Combination therapy: As used herein, the term “combination therapy” refers to those situations in which a subject is exposed to two or more therapeutic regimens (*e.g.*, two or more therapeutic agents). In some embodiments, the two or more regimens may be administered simultaneously; in some embodiments, such regimens may be administered sequentially (*e.g.*, all “doses” of a first regimen are administered prior to administration of any doses of a second regimen); in some embodiments, such agents are administered in overlapping dosing regimens. In some embodiments, “administration” of combination therapy may involve administration of

one or more agent(s) or modality(ies) to a subject receiving the other agent(s) or modality(ies) in the combination. For clarity, combination therapy does not require that individual agents be administered together in a single composition (or even necessarily at the same time), although in some embodiments, two or more agents, or active moieties thereof, may be administered together in a combination composition, or even in a combination compound (*e.g.*, as part of a single chemical complex or covalent entity).

[0139] Derivative: As used herein, the term “derivative”, or grammatical equivalents thereof, refers to a structural analogue of a reference substance. That is, a “derivative” is a substance that shows significant structural similarity with the reference substance, for example sharing a core or consensus structure, but also differs in certain discrete ways. Such a substance would be said to be “derived from” said reference substance. In some embodiments, a derivative is a substance that can be generated from the reference substance by chemical manipulation. In some embodiments, a derivative is a substance that can be generated through performance of a synthetic process substantially similar to (*e.g.*, sharing a plurality of steps with) one that generates the reference substance.

[0140] Domain: The term “domain” as used herein refers to a section or portion of an entity. In some embodiments, a “domain” is associated with a particular structural and/or functional feature of the entity so that, when the domain is physically separated from the rest of its parent entity, it substantially or entirely retains the particular structural and/or functional feature. Alternatively or additionally, a domain may be or include a portion of an entity that, when separated from that (parent) entity and linked with a different (recipient) entity, substantially retains and/or imparts on the recipient entity one or more structural and/or functional features that characterized it in the parent entity. In some embodiments, a domain is a section or portion of a molecule (*e.g.*, a small molecule, carbohydrate, lipid, nucleic acid, or polypeptide). In some embodiments, a domain is a section of a polypeptide; in some such embodiments, a domain is characterized by a particular structural element (*e.g.*, a particular amino acid sequence or sequence motif, α -helix character, β -sheet character, coiled-coil character, random coil character, etc.), and/or by a particular functional feature (*e.g.*, binding activity, enzymatic activity, folding activity, signaling activity, etc.).

[0141] Dosage form or unit dosage form: Those skilled in the art will appreciate that the term “dosage form” may be used to refer to a physically discrete unit of an active agent (*e.g.*, a therapeutic or diagnostic agent) for administration to a subject. Typically, each such unit contains a predetermined quantity of active agent. In some embodiments, such quantity is a unit dosage amount (or a whole fraction thereof) appropriate for administration in accordance with a dosing regimen that has been determined to correlate with a desired or beneficial outcome when administered to a relevant population (*i.e.*, with a therapeutic dosing regimen). Those of ordinary skill in the art appreciate that the total amount of a therapeutic composition or agent administered to a particular subject is determined by one or more attending physicians and may involve administration of multiple dosage forms.

[0142] Dosing regimen: Those skilled in the art will appreciate that the term “dosing regimen” may be used to refer to a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic agent has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which is separated in time from other doses. In some embodiments, individual doses are separated from one another by a time period of the same length; in some embodiments, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, all doses within a dosing regimen are of the same unit dose amount. In some embodiments, different doses within a dosing regimen are of different amounts. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount different from the first dose amount. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount same as the first dose amount. In some embodiments, a dosing regimen is correlated with a desired or beneficial outcome when administered across a relevant population (*i.e.*, is a therapeutic dosing regimen).

[0143] Fragment: A “fragment” of a material or entity as described herein has a structure that includes a discrete portion of the whole, but lacks one or more moieties found in the whole. In some embodiments, a fragment consists of such a discrete portion. In some embodiments, a fragment includes a discrete portion of the whole which discrete portion shares

one or more functional characteristics found in the whole. In some embodiments, a fragment consists of such a discrete portion. In some embodiments, a fragment consists of or comprises a characteristic structural element or moiety found in the whole. In some embodiments, a fragment of a polymer, *e.g.*, a polypeptide or a polysaccharide, comprises or consists of at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500 or more monomeric units (*e.g.*, residues) as found in the whole polymer. In some embodiments, a polymer fragment comprises or consists of at least about 5%, 10%, 15%, 20%, 25%, 30%, 25%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more of the monomeric units (*e.g.*, residues) found in the whole polymer. The whole material or entity may, in some embodiments, be referred to as the “parent” of the whole.

[0144] Homology: As used herein, the term “homology” refers to the overall relatedness between polymeric molecules, *e.g.*, between nucleic acid molecules (*e.g.*, DNA molecules and/or RNA molecules) and/or between polypeptide molecules. In some embodiments, polymeric molecules are considered to be “homologous” to one another if their sequences are at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical. In some embodiments, polymeric molecules are considered to be “homologous” to one another if their sequences are at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% similar (*e.g.*, containing residues with related chemical properties at corresponding positions). For example, as is well known by those of ordinary skill in the art, certain amino acids are typically classified as similar to one another as “hydrophobic” or “hydrophilic” amino acids, and/or as having “polar” or “non-polar” side chains. Substitution of one amino acid for another of the same type may often be considered a “homologous” substitution.

[0145] Identity: As used herein, the term “identity” refers to the overall relatedness between polymeric molecules, *e.g.*, between nucleic acid molecules (*e.g.*, DNA molecules and/or

RNA molecules) and/or between polypeptide molecules. In some embodiments, polymeric molecules are considered to be “substantially identical” to one another if their sequences are at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical. Calculation of the percent identity of two nucleic acid or polypeptide sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second sequence for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or substantially 100% of the length of a reference sequence. The nucleotides at corresponding positions are then compared. When a position in the first sequence is occupied by the same residue (*e.g.*, nucleotide or amino acid) as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the percent identity between two nucleotide sequences can be determined using the algorithm of Meyers and Miller, 1989, which has been incorporated into the ALIGN program (version 2.0). In some exemplary embodiments, nucleic acid sequence comparisons made with the ALIGN program use a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity between two nucleotide sequences can, alternatively, be determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix.

[0146] Improve, increase, inhibit or reduce: As used herein, the terms “improve”, “increase”, “inhibit”, “reduce”, or grammatical equivalents thereof, indicate values that are relative to a baseline or other reference measurement. In some embodiments, an appropriate reference measurement may be or comprise a measurement in a particular system (*e.g.*, in a

single subject) under otherwise comparable conditions absent presence of (*e.g.*, prior to and/or after) a particular agent or treatment, or in presence of an appropriate comparable reference agent. In some embodiments, an appropriate reference measurement may be or comprise a measurement in comparable system known or expected to respond in a particular way, in presence of the relevant agent or treatment.

[0147] Immunologically effective amount or immunologically effective dose: As used herein, “immunologically effective amount” or “immunologically effective dose” refers to an amount of an antigenic or immunogenic substance, *e.g.*, an antigen, immunogen, immunogenic complex, immunogenic composition, vaccine, or pharmaceutical composition, which when administered to a subject, either in a single dose or as part of a series of doses, that is sufficient to enhance a subject’s own immune response against a subsequent exposure to a pathogen. In some embodiments, the pathogen is *S. pneumoniae*. In some embodiments, the immune response is against one or more different serotypes of *S. pneumoniae*. In some embodiments, the immune response is against two or more different serotypes of *S. pneumoniae*. In some embodiments, the immune response is against nine or more different serotypes of *S. pneumoniae*. In some embodiments, the immune response is against thirteen or more different serotypes of *S. pneumoniae*. In some embodiments, the immune response is against fifteen or more different serotypes of *S. pneumoniae*. In some embodiments, the immune response is against twenty-three or more different serotypes of *S. pneumoniae*. In some embodiments, the immune response is against twenty-four or more different serotypes of *S. pneumoniae*. An immunologically effective amount may vary based on the subject to be treated, the species of the subject, the degree of immune response desired to induce, etc. In some embodiments, an immunologically effective amount is sufficient for treatment or protection of a subject having or at risk of having disease. In some embodiments, an immunologically effective amount refers to a non-toxic but sufficient amount that can be an amount to treat, attenuate, or prevent infection and/or disease (*e.g.*, bacterial infection, pneumococcal infection, bacterial colonization, pneumococcal colonization, complications associated with bacterial infection, complications associated with pneumococcal infection, etc.) in any subject. In some embodiments, an immunologically effective amount is sufficient to induce an immunoprotective response upon administration to a subject.

[0148] Immunoprotective response or protective response: As used herein, “immunoprotective response” or “protective response” refers to an immune response that mediates antigen or immunogen-induced immunological memory. In some embodiments, an immunoprotective response is induced by the administration of a substance, *e.g.*, an antigen, immunogen, immunogenic complex, immunogenic composition, vaccine, or pharmaceutical composition to a subject. In some embodiments, immunoprotection involves one or more of active immune surveillance, a more rapid and effective response upon immune activation as compared to a response observed in a naïve subject, efficient clearance of the activating agent or pathogen, followed by rapid resolution of inflammation. In some embodiments, an immunoprotective response is an adaptive immune response. In some embodiments, an immunoprotective response is sufficient to protect an immunized subject from productive infection by a particular pathogen or pathogens to which a vaccine is directed (*e.g.*, *S. pneumoniae* infection).

[0149] Immunization: As used herein, “immunization”, or grammatical equivalents thereof, refers to a process of inducing an immune response to an infectious organism or agent in a subject (“active immunization”), or alternatively, providing immune system components against an infectious organism or agent to a subject (“passive immunization”). In some embodiments, immunization involves the administration of one or more antigens, immunogens, immunogenic complexes, vaccines, immune molecules such as antibodies, immune sera, immune cells such as T cells or B cells, or pharmaceutical compositions to a subject. In some embodiments, immunization is performed by administering an immunologically effective amount of a substance, *e.g.*, an antigen, immunogen, immunogenic complex, immunogenic composition, vaccine, immune molecule such as an antibody, immune serum, immune cell such as a T cell or B cell, or pharmaceutical composition to a subject. In some embodiments, immunization results in an immunoprotective response in the subject. In some embodiments, active immunization is performed by administering to a subject an antigenic or immunogenic substance, *e.g.*, an antigen, immunogen, immunogenic complex, vaccine, or pharmaceutical composition. In some embodiments, passive immunization is performed by administering to a subject an immune system component, *e.g.*, an immune molecule such as an antibody, immune serum, or immune cell such as a T cell or B cell.

[0150] Isolated: As used herein, the term “isolated”, or grammatical equivalents thereof, refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) designed, produced, prepared, and/or manufactured by the hand of man. Isolated substances and/or entities may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of the other components with which they were initially associated. In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is "pure" if it is substantially free of other components. In some embodiments, as will be understood by those skilled in the art, a substance may still be considered "isolated" or even "pure", after having been combined with certain other components such as, for example, one or more carriers or excipients (*e.g.*, buffer, solvent, water, etc.); in such embodiments, percent isolation or purity of the substance is calculated without including such carriers or excipients. To give but one example, in some embodiments, a biological polymer such as a polypeptide or polysaccharide that occurs in nature is considered to be "isolated" when, a) by virtue of its origin or source of derivation is not associated with some or all of the components that accompany it in its native state in nature; b) it is substantially free of other polypeptides or nucleic acids of the same species from the species that produces it in nature; c) is expressed by or is otherwise in association with components from a cell or other expression system that is not of the species that produces it in nature. Thus, for instance, in some embodiments, a polypeptide or polysaccharide that is chemically synthesized or is synthesized in a cellular system different from that which produces it in nature is considered to be an "isolated" polypeptide or polysaccharide. Alternatively or additionally, in some embodiments, a polypeptide or polysaccharide that has been subjected to one or more purification techniques may be considered to be an "isolated" polypeptide or polysaccharide to the extent that it has been separated from other components a) with which it is associated in nature; and/or b) with which it was associated when initially produced.

[0151] Linker: As used herein, the term “linker” is used to refer to an entity that connects two or more elements to form a multi-element agent. For example, those of ordinary skill in the art appreciate that a polypeptide whose structure includes two or more functional or organizational domains often includes a stretch of amino acids between such domains that links them to one another. In some embodiments, a polypeptide comprising a linker element has an overall structure of the general form S1-L-S2, wherein S1 and S2 may be the same or different and represent two domains associated with one another by the linker (L). In some embodiments, a polypeptide linker is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more amino acids in length. In some embodiments, a linker is characterized in that it tends not to adopt a rigid three-dimensional structure, but rather provides flexibility to the polypeptide. A variety of different linker elements that can appropriately be used when engineering polypeptides (*e.g.*, fusion polypeptides) are known in the art (Holliger et al, 1993; Poljak, 1994).

[0152] Non-inferior: As used herein, the term “non-inferior” in the context of evaluating a test pharmaceutical composition refers to a test pharmaceutical composition that is (*e.g.*, in terms of immunogenicity and/or functional antibody titer generated by the test composition) at least as effective as a reference composition. In some embodiments, non-inferiority is demonstrated when the lower bound of the 95% confidence interval (CI) for the geometric mean titer (GMT) ratio of a test pharmaceutical composition over a reference composition is at least greater than or equal to 0.4 or above, including, *e.g.*, at least 0.5, at least 0.6, at least 0.7, at least 0.8, at least 0.9, at least 0.95, at least 0.98, at least 1.0, or higher. For example, in some embodiments, an immunogenic composition (*e.g.*, vaccine) described herein is non-inferior to a reference vaccine (*e.g.*, PCV13, PCV20, or PPSV23) when the lower bound of the 95% confidence interval (CI) for the geometric mean titer (GMT) ratio of the immunogenic composition (*e.g.*, vaccine) over the reference vaccine is at least greater than or equal to 0.4 or above, including, *e.g.*, at least 0.5, at least 0.6, at least 0.7, at least 0.8, at least 0.9, at least 0.95, at least 0.98, at least 1.0, or higher. In some embodiments, an immunogenic composition (*e.g.*, vaccine) described herein is non-inferior to a reference vaccine (*e.g.*, PCV13, PCV20, or PPSV23) when the lower bound of the 95% confidence interval (CI) for the geometric mean titer (GMT) ratio of the immunogenic composition (*e.g.*, vaccine) over the reference vaccine is at

least greater than or equal to 0.95 or above, including, *e.g.*, at least 0.96, at least 0.97, at least 0.98, at least 0.99, at least 1.0, at least 1.1, at least 1.3, at least 1.5, or higher. In some embodiments, an immunogenic composition (*e.g.*, vaccine) described herein is non-inferior to a reference vaccine (*e.g.*, PCV13, PCV20, or PPSV23) when the seroconversion rates, or percentages of vaccine recipients with immune responses, are above a pre-defined threshold, *e.g.*, the lower bound of the 95% confidence interval for the difference between the percentage of subjects who seroconvert, following immunization with an immunogenic composition (*e.g.*, vaccine) described herein or immunization with the reference vaccine (*e.g.*, PCV13, PCV20, or PPSV23), is greater than -0.10.

[0153] Pharmaceutical composition: As used herein, the term “pharmaceutical composition” refers to a composition in which an active agent is formulated together with one or more pharmaceutically acceptable carriers. In some embodiments, the active agent is present in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, a pharmaceutical composition may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, *e.g.*, those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as, for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces.

[0154] Pharmaceutically acceptable: As used herein, the term “pharmaceutically acceptable” applied to the carrier, diluent, or excipient used to formulate a composition as disclosed herein means that the carrier, diluent, or excipient must be compatible with the other ingredients of the composition and not deleterious to the recipient thereof.

[0155] Polysaccharide: The term “polysaccharide” as used herein refers to a polymeric carbohydrate molecule composed of long chains of monosaccharide units bound together by

glycosidic, phosphodiester, or other linkages and on hydrolysis give the constituent monosaccharides or oligosaccharides. Polysaccharides range in structure from linear to highly branched. Examples include storage polysaccharides such as starch and glycogen, structural polysaccharides such as cellulose and chitin and microbial polysaccharides, and antigenic polysaccharides found in microorganisms including, but not limited to, capsular polysaccharides (CPS), O polysaccharides (OPS), core O polysaccharides (COPS), and lipopolysaccharides (LPS).

[0156] Polypeptide: The term “polypeptide”, as used herein, generally has its art-recognized meaning of a polymer of at least three amino acids, *e.g.*, linked to each other by peptide bonds. Those of ordinary skill in the art will appreciate that the term “polypeptide” is intended to be sufficiently general as to encompass not only polypeptides having a complete sequence recited herein, but also to encompass polypeptides that represent functional fragments (*i.e.*, fragments retaining at least one activity) of such complete polypeptides. Moreover, those of ordinary skill in the art understand that protein sequences generally tolerate some substitution without destroying activity. Thus, any polypeptide that retains activity and shares at least about 30-40% overall sequence identity, often greater than about 50%, 60%, 70%, or 80%, and further usually including at least one region of much higher identity, often greater than 90% or even 95%, 96%, 97%, 98%, or 99% in one or more highly conserved regions, usually encompassing at least 3-4 and often up to 20 or more amino acids, with another polypeptide of the same class, is encompassed within the relevant term “polypeptide” as used herein. Polypeptides may contain L-amino acids, D-amino acids, or both and may contain any of a variety of amino acid modifications or analogs known in the art. Useful modifications include, *e.g.*, terminal acetylation, amidation, methylation, etc. In some embodiments, proteins may comprise natural amino acids, non-natural amino acids, synthetic amino acids, and combinations thereof.

[0157] Prevention: The term “prevent” or “prevention”, as used herein in connection with a disease, disorder, and/or medical condition, refers to reducing the risk of developing the disease, disorder and/or condition, and/or a delay of onset, and/or reduction in frequency and/or severity of one or more characteristics or symptoms of a particular disease, disorder or condition. In some embodiments, prevention is assessed on a population basis such that an agent is considered to “prevent” a particular disease, disorder or condition if a statistically significant

decrease in the development, frequency, and/or intensity of one or more symptoms of the disease, disorder or condition is observed in a population susceptible to the disease, disorder, or condition. In some embodiments, prevention may be considered complete when onset of a disease, disorder or condition has been delayed for a pre-defined period of time.

[0158] Protein: As used herein, the term “protein” encompasses a polypeptide. Proteins may include moieties other than amino acids (*e.g.*, may be glycoproteins, proteoglycans, etc.) and/or may be otherwise processed or modified. Those of ordinary skill in the art will appreciate that a “protein” can be a complete polypeptide chain as produced by a cell (with or without a signal sequence), or can be a characteristic portion thereof. Those of ordinary skill will appreciate that a protein can sometimes include more than one polypeptide chain, for example linked by one or more disulfide bonds or associated by other means. Polypeptides may contain L-amino acids, D-amino acids, or both and may contain any of a variety of amino acid modifications or analogs known in the art. Useful modifications include, *e.g.*, terminal acetylation, amidation, methylation, etc. In some embodiments, proteins may comprise natural amino acids, non-natural amino acids, synthetic amino acids, and combinations thereof. The term “peptide” is generally used to refer to a polypeptide having a length of less than about 100 amino acids, less than about 50 amino acids, less than 20 amino acids, or less than 10 amino acids. In some embodiments, proteins are antibodies, antibody fragments, biologically active portions thereof, and/or characteristic portions thereof.

[0159] Recombinant: As used herein, the term “recombinant” is intended to refer to polypeptides that are designed, engineered, prepared, expressed, created, manufactured, and/or isolated by recombinant means, such as polypeptides expressed using a recombinant expression vector transfected into a host cell; polypeptides isolated from a recombinant, combinatorial human polypeptide library; polypeptides isolated from an animal (*e.g.*, a mouse, rabbit, sheep, fish, etc.) that is transgenic for or otherwise has been manipulated to express a gene or genes, or gene components that encode and/or direct expression of the polypeptide or one or more component(s), portion(s), element(s), or domain(s) thereof; and/or polypeptides prepared, expressed, created or isolated by any other means that involves splicing or ligating selected nucleic acid sequence elements to one another, chemically synthesizing selected sequence elements, and/or otherwise generating a nucleic acid that encodes and/or directs expression of the

polypeptide or one or more component(s), portion(s), element(s), or domain(s) thereof. In some embodiments, one or more of such selected sequence elements is found in nature. In some embodiments, one or more of such selected sequence elements is designed in silico. In some embodiments, one or more such selected sequence elements results from mutagenesis (*e.g.*, *in vivo* or *in vitro*) of a known sequence element, *e.g.*, from a natural or synthetic source such as, for example, in the germline of a source organism of interest (*e.g.*, of a human, a mouse, etc.).

[0160] Reference: As used herein, the term “reference” describes a standard or control relative to which a comparison is performed. For example, in some embodiments, an agent, animal, subject, population, sample, sequence or value of interest is compared with a reference or control agent, animal, subject, population, sample, sequence or value. In some embodiments, a reference or control is tested and/or determined substantially simultaneously with the testing or determination of interest. In some embodiments, a reference or control is a historical reference or control, optionally embodied in a tangible medium. Typically, as would be understood by those skilled in the art, a reference or control is determined or characterized under comparable conditions or circumstances to those under assessment. Those skilled in the art will appreciate when sufficient similarities are present to justify reliance on and/or comparison to a particular possible reference or control.

[0161] Response: As used herein, a “response” to treatment may refer to any beneficial alteration in a subject’s condition that occurs as a result of or correlates with treatment. Such alteration may include stabilization of the condition (*e.g.*, prevention of deterioration that would have taken place in the absence of the treatment), amelioration of symptoms of the condition, and/or improvement in the prospects for cure of the condition, etc. It may refer to a subject’s response or to a tumor’s response. Subject or tumor response may be measured according to a wide variety of criteria, including clinical criteria and objective criteria. Techniques for assessing response include, but are not limited to, clinical examination, positron emission tomography, chest X-ray CT scan, MRI, ultrasound, endoscopy, laparoscopy, presence or level of biomarkers in a sample obtained from a subject, cytology, and/or histology. The exact response criteria can be selected in any appropriate manner, provided that when comparing groups of subjects and/or tumors, the groups to be compared are assessed based on the same or

comparable criteria for determining response rate. One of ordinary skill in the art will be able to select appropriate criteria.

[0162] Risk: As will be understood from context, “risk” of a disease, disorder, and/or condition refers to a likelihood that a particular subject will develop the disease, disorder, and/or condition. In some embodiments, risk is expressed as a percentage. In some embodiments, risk is from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90 up to 100%. In some embodiments, risk is expressed as a risk relative to a risk associated with a reference sample or group of reference samples. In some embodiments, a reference sample or group of reference samples have a known risk of a disease, disorder, condition and/or event. In some embodiments, a reference sample or group of reference samples are from subjects comparable to a particular subject. In some embodiments, relative risk is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more.

[0163] Serotype: As used herein, the term “serotype”, also referred to as a serovar, refers to a distinct variation within a species of bacteria or virus or among immune cells of different subjects. These microorganisms, viruses, or cells are classified together based on their cell surface antigens, allowing the epidemiologic classification of organisms to the sub-species level. A group of serovars with common antigens may be referred to as a serogroup or sometimes serocomplex.

[0164] Species: As used herein, the term “species” refers to a distinct immunogenic complex comprising (i) a biotinylated polysaccharide antigen and (ii) a fusion protein comprising a biotin-binding moiety and one or more *Streptococcus pneumoniae* polypeptide antigens. In some embodiments, a distinct species can differ in one or more of (i) a biotinylated polysaccharide antigen and (ii) a fusion protein comprising a biotin-binding moiety and one or more *Streptococcus pneumoniae* polypeptide antigens.

[0165] Subject: As used herein, the term “subject” refers an organism, typically a mammal (*e.g.*, a human, in some embodiments including prenatal human forms). In some embodiments, a subject is suffering from a relevant disease, disorder or condition. In some embodiments, a subject is susceptible to a disease, disorder, or condition. In some embodiments, a subject displays one or more symptoms or characteristics of a disease, disorder or condition. In some embodiments, a subject does not display any symptom or characteristic of a disease,

disorder, or condition. In some embodiments, a subject is someone with one or more features characteristic of susceptibility to or risk of a disease, disorder, or condition. In some embodiments, a subject is a patient. In some embodiments, a subject is an subject to whom diagnosis and/or therapy is and/or has been administered.

[0166] Superior: As used herein, the term “superior” in the context of evaluating a test pharmaceutical composition refers to a test pharmaceutical composition that performs (*e.g.*, in terms of immunogenicity and/or functional antibody titer generated by the test composition) better than a reference composition. In some embodiments, superiority is demonstrated when the upper bound of the 95% confidence interval (CI) for the geometric mean titer (GMT) ratio of a test pharmaceutical composition over a reference composition is at least 1.3 or above, including, *e.g.*, at least 1.4, at least 1.5, at least 2, at least 2.5, at least 3, at least 4, or higher. For example, in some embodiments, an immunogenic composition (*e.g.*, vaccine) described herein is superior to a reference vaccine (*e.g.*, PCV13, PCV20, or PPSV23) when the upper bound of the 95% confidence interval (CI) for the geometric mean titer (GMT) ratio of the immunogenic composition (*e.g.*, vaccine) over the reference vaccine is at least 1.3 or above, including, *e.g.*, at least 1.4, at least 1.5, at least 2, at least 2.5, at least 3, at least 4, or higher. In some embodiments, an immunogenic composition (*e.g.*, vaccine) described herein is superior to a reference vaccine (*e.g.*, PCV13, PCV20, or PPSV23) when the two-sided 95% confidence interval (CI) for the geometric mean titer (GMT) ratio of the immunogenic composition (*e.g.*, vaccine) over the reference vaccine excludes zero.

[0167] Susceptible to: A subject who is “susceptible to” a disease, disorder, or condition is at risk for developing the disease, disorder, or condition. In some embodiments, a subject who is susceptible to a disease, disorder, or condition does not display any symptoms of the disease, disorder, or condition. In some embodiments, a subject who is susceptible to a disease, disorder, or condition has not been diagnosed with the disease, disorder, and/or condition. In some embodiments, a subject who is susceptible to a disease, disorder, or condition is a subject who has been exposed to conditions associated with development of the disease, disorder, or condition. In some embodiments, a risk of developing a disease, disorder, and/or condition is a population-based risk (*e.g.*, family members of subjects suffering from the disease, disorder, or condition).

[0168] Symptoms are reduced: As used herein, “symptoms are reduced” when one or more symptoms of a particular disease, disorder or condition is reduced in magnitude (*e.g.*, intensity, severity, etc.) and/or frequency, *e.g.*, to a statistically and/or clinically significant or relevant level. For purposes of clarity, a delay in the onset of a particular symptom is considered one form of reducing the frequency of that symptom.

[0169] Treatment: As used herein, the term “treatment” (also “treat” or “treating”) refers to any administration of a therapy that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of, and/or reduces incidence of one or more symptoms, features, and/or causes of a particular disease, disorder, and/or condition. In some embodiments, such treatment may be of a subject who does not exhibit signs of the relevant disease, disorder and/or condition and/or of a subject who exhibits only early signs of the disease, disorder, and/or condition. Alternatively or additionally, such treatment may be of a subject who exhibits one or more established signs of the relevant disease, disorder and/or condition. In some embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, and/or condition. In some embodiments, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, and/or condition.

[0170] Vaccination: As used herein, the term “vaccination” refers to the administration of a composition intended to generate an immune response, for example to a disease-causing agent. For the purposes of the present disclosure, vaccination can be administered before, during, and/or after exposure to a disease-causing agent, and in certain embodiments, before, during, and/or shortly after exposure to the agent. In some embodiments, vaccination includes multiple administrations, appropriately spaced in time, of a vaccinating composition. In some embodiments, vaccination initiates immunization.

[0171] Variant: As used herein in the context of molecules, *e.g.*, nucleic acids, proteins, or small molecules, the term “variant” refers to a molecule that shows significant structural identity with a reference molecule but differs structurally from the reference molecule, *e.g.*, in the presence or absence or in the level of one or more chemical moieties as compared to the reference entity. In some embodiments, a variant also differs functionally from its reference molecule. In general, whether a particular molecule is properly considered to be a “variant” of a

reference molecule is based on its degree of structural identity with the reference molecule. As will be appreciated by those skilled in the art, any biological or chemical reference molecule has certain characteristic structural elements. A variant, by definition, is a distinct molecule that shares one or more such characteristic structural elements but differs in at least one aspect from the reference molecule. In some embodiments, a variant polypeptide or nucleic acid may differ from a reference polypeptide or nucleic acid as a result of one or more differences in amino acid or nucleotide sequence and/or one or more differences in chemical moieties (*e.g.*, carbohydrates, lipids, phosphate groups) that are covalently components of the polypeptide or nucleic acid (*e.g.*, that are attached to the polypeptide or nucleic acid backbone). In some embodiments, a variant polypeptide or nucleic acid shows an overall sequence identity with a reference polypeptide or nucleic acid that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, or 99%. In some embodiments, a variant polypeptide or nucleic acid does not share at least one characteristic sequence element with a reference polypeptide or nucleic acid. In some embodiments, a reference polypeptide or nucleic acid has one or more biological activities. In some embodiments, a variant polypeptide or nucleic acid shares one or more of the biological activities of the reference polypeptide or nucleic acid. In some embodiments, a variant polypeptide or nucleic acid lacks one or more of the biological activities of the reference polypeptide or nucleic acid. In some embodiments, a variant polypeptide or nucleic acid shows a reduced level of one or more biological activities as compared to the reference polypeptide or nucleic acid. In some embodiments, a polypeptide or nucleic acid of interest is considered to be a “variant” of a reference polypeptide or nucleic acid if it has an amino acid or nucleotide sequence that is identical to that of the reference but for a small number of sequence alterations at particular positions. Typically, fewer than about 20%, about 15%, about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, or about 2% of the residues in a variant are substituted, inserted, or deleted, as compared to the reference. In some embodiments, a variant polypeptide or nucleic acid comprises about 10, about 9, about 8, about 7, about 6, about 5, about 4, about 3, about 2, or about 1 substituted residues as compared to a reference. Often, a variant polypeptide or nucleic acid comprises a very small number (*e.g.*, fewer than about 5, about 4, about 3, about 2, or about 1) number of substituted, inserted, or deleted, functional residues (*i.e.*, residues that participate in a particular biological activity) relative to the

reference. In some embodiments, a variant polypeptide or nucleic acid comprises not more than about 5, about 4, about 3, about 2, or about 1 addition or deletion, and, in some embodiments, comprises no additions or deletions, as compared to the reference. In some embodiments, a variant polypeptide or nucleic acid comprises fewer than about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 10, about 9, about 8, about 7, about 6, and commonly fewer than about 5, about 4, about 3, or about 2 additions or deletions as compared to the reference. In some embodiments, a variant polypeptide or nucleic acid comprises fewer than about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 10, about 9, about 8, about 7, about 6, and commonly fewer than about 5, about 4, about 3, or about 2 modifications (*e.g.*, substitutions, additions or deletions) at the N-terminus portion, as compared to the reference. In some embodiments, a variant polypeptide or nucleic acid comprises fewer than about 25, about 20, about 19, about 18, about 17, about 16, about 15, about 14, about 13, about 10, about 9, about 8, about 7, about 6, and commonly fewer than about 5, about 4, about 3, or about 2 modifications (*e.g.*, substitutions, additions or deletions) at the C-terminus portion, as compared to the reference. In some embodiments, a reference polypeptide or nucleic acid is one found in nature.

Detailed Description of Certain Embodiments

[0172] The present disclosure relates, generally, to compositions, systems, and methods that include novel complexed proteins and polysaccharides, *e.g.*, immunogenic compositions (*e.g.*, vaccines) of complexed proteins and polysaccharides. Such complexes can be used, *e.g.*, to induce and/or increase an immunoprotective response in subjects at risk of or suffering from pneumococcal infection.

[0173] Previous attempts at preparing a vaccine to protect against pneumococcal infection have had limited efficacy.

[0174] Wyeth's first polyvalent polysaccharide conjugate vaccine, Prevnar 7 (PCV7), was licensed in 2000 in the United States and Europe. GlaxoSmithKline's 10-valent polysaccharide conjugate vaccine Synflorix (PCV10) was licensed in Europe, but not the United States, in 2009. Synflorix was not licensed in the United States because clinical trials did not demonstrate non-inferiority in infants compared to Prevnar 7; also, despite coverage of three additional serotypes compared to Prevnar 7, Synflorix did not formally have coverage of the clinically important *S. pneumoniae* serotype 19A. Also, Synflorix had originally been designed as an 11-valent polysaccharide conjugate vaccine (PCV11), but one *S. pneumoniae* serotype (serotype 3) was removed when clinical trials suggested no efficacy against this serotype, despite immunogenicity.

[0175] Merck's polyvalent polysaccharide conjugate vaccine PCV-15 is still in clinical trials. PCV15 has undergone clinical evaluation in adults, where it appears to be non-inferior to Prevnar 13 in adults. PCV15 also appeared to be non-inferior in toddlers, but then failed to meet non-inferiority in infants, so was returned to formulation. The newly-formulated PCV15 is now being evaluated in adults and infants.

[0176] A first protein vaccine based on PspA was evaluated by Sanofi Pasteur. An early clinical trial (published in 2000, PMID: 10699322) was a Phase 1 trial with 5-125 µg recombinant PspA (rPspA), which was associated with a clear rise in antibody levels, antibodies that were reactive with heterologous rPspA molecules, and increased binding of post-immune sera to 37 pneumococcal strains expressing a variety of PspA clades and capsular serotypes.

Some concerns were raised over a region of PspA that has homology with cardiac myosin, and the program was terminated by Sanofi Pasteur.

[0177] Another protein vaccine based on a combination of the three proteins, PhtD, pneumolysoid, and PcpA, was evaluated by Sanofi Pasteur from 2011 to 2014. The vaccine passed Phase 1 in adults and was then tested in toddlers (12-13 months) and infants (6 weeks). A study in Bangladesh showed immunogenicity; however, no evidence of impact on *S. pneumoniae* carriage was reported (presented at ISPPD 2014; the study was not powered to detect a small difference in prevalence of carriage). The program was terminated by Sanofi Pasteur.

[0178] A protein vaccine based on the combination of PhtD and pneumolysoid was evaluated by GlaxoSmithKline from 2012 to 2016. The vaccine was deemed safe and immunogenic in all ages (adults, toddlers, infants). However, in two further infant trials conducted in The Gambia and the Navajo Nation in the United States, there was no evidence of clinical efficacy (no impact on *S. pneumoniae* carriage in The Gambia, or on otitis media in the Navajo Nation). The program was terminated by GlaxoSmithKline.

[0179] A protein vaccine GEN-004, based on a combination of three proteins, SP0148, SP1912 and SP2108 adjuvanted with aluminum hydroxide, was developed and evaluated by Genocera Biosciences. GEN-004 was shown to be safe and immunogenic in adults in Phase 1. However, a Phase 2 trial of intentional pneumococcal challenge, conducted in the UK, showed no significant effect on *S. pneumoniae* carriage. The GEN-004 development program was suspended in 2015.

[0180] A live attenuated, engineered *Salmonella* vaccine was developed at Arizona State University. Mutant *Salmonella typhi* strains engineered to express PspA were evaluated in a Phase 1 clinical trial in adults (PMID: 23916987) from 2009 to 2011. Three different strains were evaluated. No significant adverse events were reported, but one patient had a positive blood culture for *Salmonella typhi*. No subjects shed vaccine in stool. There were no differences in pre- or post-vaccination ELISA or ELISPOT results between groups. The vaccine was deemed safe but non-immunogenic, therefore not efficacious, and development was stopped.

[0181] There are two *S. pneumoniae* vaccines currently available in the U.S. Wyeth-Pfizer's second polyvalent polysaccharide conjugate vaccine, Prevnar 13 (PCV13), was licensed

in 2010 in the U.S. and Europe. PCV13 has been approved for the prevention of IPD caused by the 13 serotypes contained in the vaccine in children and for the prevention of pneumonia and IPD in adults. In this vaccine, covalent conjugation of saccharides from 13 pneumococcal serotypes to a diphtheria toxoid mutant (CRM197) protein creates saccharide-protein conjugates, which are capable of inducing a T cell-dependent immune response against one or more of the 13 pneumococcal serotypes represented by the saccharides. [PREVNAR 13 prescribing information, 2017].

[0182] PCV13 did not formally meet non-inferiority criteria compared to PCV7 and was not immunogenic against several serotypes. However, the inclusion of *S. pneumoniae* serotype 19A assisted with licensure of PCV13, since serotype 19A had emerged between 2000-2010 as a major cause of morbidity and mortality and was associated with antibiotic resistance. While infections with *S. pneumoniae* of multidrug-resistant serotypes contained in PCV13 appeared to decrease after approval of this vaccine, an increase of infections with multidrug-resistant serotypes 35B, 23A, 23B and 15B, which are just a few of the close to 100 known serotypes of *S. pneumoniae* not included in PCV13, was noted. Also, PCV13 was reported to have marginal activity against serotype 3, as its prevalence persists in the population [Richter et al, 2014].

[0183] The second vaccine, PPSV23, is a 23-valent polysaccharide vaccine developed by Merck, and is indicated for the prevention of pneumococcal disease in adults greater than 50 years of age, or in persons greater than 2 years of age at increased risk of pneumococcal disease. It is composed of purified capsular polysaccharides from 23 pneumococcal serotypes. While this vaccine has the potential to protect against more serotypes when compared to PCV13, it does not provide protection against the emerging serotypes 35B, 23A and 23B. In addition, PPSV23 elicits a T cell-independent polysaccharide immune response that stimulates mature B-lymphocytes, but not T-lymphocytes. Thus, this vaccine induces an immune response that is neither long-lasting nor anamnestic upon subsequent challenge. PPSV23 is not effective against colonization. In addition, polysaccharide-type vaccines are not used in infants and children less than 2 years of age, because these children respond poorly to T cell-independent antigens [PNEUMOVAX 23 prescribing information, 2017; CDC, 2010]. Data suggest that PPSV23 may protect adults and the elderly against IPD; however, no consistent effect has been observed in the prevention of pneumonia [Gruber et al, 2008].

[0184] The presently disclosed novel complexed proteins and polysaccharides, *e.g.*, immunogenic compositions (*e.g.*, vaccines) of complexed proteins and polysaccharides, and combinations thereof represent a substantial advance over the previous attempts at *S. pneumoniae* vaccine development and over the currently available options for immunizing patients against pneumococcal infection. Such novel complexes and immunogenic compositions (*e.g.*, vaccines) can be used, *e.g.*, to induce and/or increase an immunoprotective response in subjects, such as those at risk of or suffering from pneumococcal infection.

Immunogenic Complexes

[0185] The present disclosure encompasses immunogenic complexes that include one or more polysaccharides and/or polypeptides of *S. pneumoniae*.

[0186] In some embodiments, immunogenic complexes are, or are based on, Multiple Antigen Presenting System (MAPS) complexes. Aspects of the MAPS platform have been previously described in WO2012/155007, the contents of which are herein incorporated by reference in their entirety, and are shown schematically in Figure 1. See also Zhang *et al.*, 2013.

[0187] As described herein, immunogenic complexes of the disclosure include one or more antigenic polypeptides non-covalently complexed with one or more antigenic polysaccharides. In some embodiments, one or more antigenic polypeptides are complexed via affinity interaction with one or more antigenic polysaccharides. In some embodiments, immunogenic complexes of the disclosure include one or more antigenic polypeptides non-covalently complexed with one or more antigenic polysaccharides using one or more affinity molecule/complementary affinity molecule pairs. In some embodiments, an immunogenic complex includes (i) a first affinity molecule described herein conjugated to one or more antigenic polysaccharides, and (ii) a fusion protein that is or comprises a complementary affinity molecule described herein and a polypeptide. In some embodiments, an immunogenic complex includes (i) a plurality of a first affinity molecule described herein conjugated to one or more antigenic polysaccharides, and (ii) a fusion protein that is or comprises a complementary affinity molecule described herein and a polypeptide. Upon association of the first affinity molecule and

the complementary affinity molecule, the one or more antigenic polypeptides are non-covalently complexed to the one or more antigenic polysaccharides.

[0188] In some embodiments, one or more antigenic polypeptides are complexed via affinity interaction with one antigenic polysaccharide. In some embodiments, immunogenic complexes of the disclosure include one or more antigenic polypeptides non-covalently complexed with one antigenic polysaccharide using one affinity molecule/complementary affinity molecule pair. In some embodiments, immunogenic complexes of the disclosure include one or more antigenic polypeptides non-covalently complexed with one antigenic polysaccharide using one or more affinity molecule/complementary affinity molecule pairs. In some embodiments, each of the affinity molecule/complementary affinity molecule pairs is the same, *e.g.*, biotin/biotin-binding moiety pairs. In some embodiments, an immunogenic complex includes (i) a first affinity molecule described herein conjugated to one antigenic polysaccharide, and (ii) a fusion protein that is or comprises a complementary affinity molecule described herein and a polypeptide. In some embodiments, an immunogenic complex includes (i) a plurality of a first affinity molecule described herein conjugated to one antigenic polysaccharide, and (ii) a fusion protein that is or comprises a complementary affinity molecule described herein and a polypeptide. Upon association of the first affinity molecule and the complementary affinity molecule, the one or more antigenic polypeptides are non-covalently complexed to the one antigenic polysaccharide.

[0189] In some embodiments, the affinity molecule/complementary affinity molecule pair is selected from one or more of biotin/biotin-binding moiety, antibody/antigen, enzyme/substrate, receptor/ligand, metal/metal-binding protein, carbohydrate/carbohydrate binding protein, lipid/lipid-binding protein, and His tag/His tag-binding molecule.

[0190] In some embodiments, the first affinity molecule is biotin (or a derivative or fragment thereof), and the complementary affinity molecule is a moiety, *e.g.*, a biotin-binding protein, or a biotin-binding domain or biotin-binding fragment thereof. In some embodiments, the biotin-binding moiety is rhizavidin, avidin, streptavidin, bradavidin, tamavidin, lentiavidin, zebavidin, NeutrAvidin, CaptAvidin™, or a biotin-binding domain or biotin-binding fragment thereof, or a combination thereof. In some embodiments, the biotin-binding moiety is a dimer, *e.g.*, a non-covalent dimer. In some embodiments, the biotin-binding moiety is rhizavidin, or a

biotin-binding domain or biotin-binding fragment thereof. In some embodiments, the biotin-binding moiety is or comprises a polypeptide of SEQ ID NO: 1, or a biotin-binding domain or biotin-binding fragment thereof. In some embodiments, the biotin-binding moiety is or comprises a polypeptide that comprises an amino acid sequence that is at least 80% (including, e.g., at least 90%, at least 95%, at least 98%, at least 99%, and 100%) identical to SEQ ID NO: 1, or a biotin-binding domain or biotin-binding fragment thereof. In some embodiments, the biotin-binding moiety is or comprises a polypeptide of SEQ ID NO: 2 or SEQ ID NO: 57, or a biotin-binding domain or biotin-binding fragment thereof. In some embodiments, the biotin-binding moiety is or comprises a polypeptide that comprises an amino acid sequence that is at least 80% (including, e.g., at least 90%, at least 95%, at least 98%, at least 99%, 100%) identical to SEQ ID NO: 2 or SEQ ID NO: 57, or a biotin-binding domain or biotin-binding fragment thereof.

[0191] In some embodiments, the one or more antigenic polysaccharides are, or are derived from Gram-negative bacteria and/or Gram-positive bacteria. In some embodiments, one or more bacterial antigenic polysaccharides are, or are derived from *S. pneumoniae*. In some embodiments, one or more antigenic polysaccharides are, or are derived from one or more pathogens. In some embodiments, one or more antigenic polysaccharides are, or are derived from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 serotypes or strains of a pathogen. In some embodiments, one or more antigenic polysaccharides are, or are derived from more than 35 serotypes or strains of a pathogen, e.g., 36, 37, 38, 39, 40, 45, 50, 55, or 60 serotypes or strains. In some embodiments, one or more antigenic polysaccharides are, or are derived from more than 70, 80, 90, or 100 serotypes or strains of a pathogen.

[0192] In some embodiments, the one or more antigenic polysaccharides comprise one or more affinity molecules conjugated to the antigenic polysaccharides. In some embodiments, the one or more affinity molecules comprise biotin or biotin derivatives.

[0193] In some embodiments, the antigenic polysaccharides comprise a plurality of affinity molecules conjugated to the antigenic polysaccharides. In some embodiments, the affinity molecules comprise biotin or biotin derivatives.

[0194] In some embodiments, one or more antigenic polypeptides are covalently linked (*e.g.*, fused) to a complementary affinity molecule described herein. In some embodiments, a fusion protein comprises one or more antigenic polypeptides and a complementary affinity molecule disclosed herein. In some embodiments, the complementary affinity molecule is or comprises a biotin-binding moiety. In some embodiments, the biotin-binding moiety is a dimer, *e.g.*, a non-covalent dimer. In some embodiments, the biotin-binding moiety comprises rhizavidin or a biotin-binding portion thereof.

[0195] In some embodiments, antigenic polysaccharides and/or antigenic polypeptides that may be included in immunogenic complexes are recombinantly or synthetically produced. In some embodiments, antigenic polysaccharides and/or antigenic polypeptides that may be included in immunogenic complexes are isolated and/or derived from natural sources. In some embodiments, antigenic polysaccharides and/or antigenic polypeptides that may be included in immunogenic complexes are isolated from bacterial cells. Exemplary polysaccharides and/or polypeptides are described below.

Antigenic Polypeptides

[0196] In some embodiments, an immunogenic complex described herein comprises one or more polypeptide antigens. In some embodiments, a polypeptide antigen is a bacterial polypeptide, a fungal polypeptide, and/or a viral polypeptide. In some embodiments, a polypeptide antigen is a polypeptide of, or derived from *S. pneumoniae*. In some embodiments, the one or more polypeptide antigen is a polypeptide of, or derived from, a pathogen other than *S. pneumoniae*. In some embodiments, the one or more polypeptide antigens comprise (i) a polypeptide of, or derived from, *S. pneumoniae*, and (ii) a polypeptide of, or derived from, a pathogen other than *S. pneumoniae*. In some embodiments, an immunogenic complex includes one or more of the following *S. pneumoniae* antigenic polypeptides, or portions thereof.

Pneumolysin Polypeptides

[0197] Pneumolysin (Ply) is a *S. pneumoniae* protein toxin. In some embodiments, Ply polypeptide is a cholesterol-dependent toxin of the thiol-activated cytolysin family. In some embodiments, a Ply polypeptide is or comprises a full-length Ply polypeptide. For example, in some embodiments, a full-length Ply polypeptide has 470 amino acids (53 kDa) and is

represented by the amino acid sequence as set forth in SEQ ID NO: 3. In some embodiments, a Ply polypeptide includes a portion of a Ply polypeptide (*e.g.*, a portion of a Ply polypeptide of SEQ ID NO: 3, which portion includes at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, 400, 450 or more contiguous amino acids of SEQ ID NO: 3). In some embodiments, a portion of an Ply polypeptide corresponds to a protein having amino acids 2-470 of the amino acid sequence set forth in SEQ ID NO: 3. In some embodiments, a Ply polypeptide contains one or more amino acid alterations (*e.g.*, deletion, substitution, and/or insertion) from a naturally-occurring wild-type Ply polypeptide sequence. For example, a Ply polypeptide may contain an amino acid sequence that is at least 60% or more (*e.g.*, at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 3 or a portion thereof (*e.g.*, at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, 400, 450 or more consecutive amino acids of the sequence shown in SEQ ID NO: 3). Alternatively, a Ply polypeptide may contain a portion (*e.g.*, at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, 400, 450 or more consecutive amino acids) of a sequence that is at least 60% or more (*e.g.*, at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 3. In some embodiments, a nucleotide sequence encoding a Ply polypeptide is provided herein as SEQ ID NO: 13.

[0198] Pneumolysins are exotoxins produced by bacteria that can cause hemolytic activity and complement activation. While highly immunogenic, their use in vaccines is limited because they cause lysis of red blood cells. Accordingly, in another aspect, provided herein are variants of *S. pneumoniae* pneumolysin (Ply), its fusion construct with a biotin-binding protein, and its uses. In some embodiments, such variants, designated herein as mutant Ply or “mPly” are substantially non-hemolytic. As used herein, the phrase “substantially non-hemolytic” means the ability of lysing red blood cells being reduced by at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or up to 100%, as compared to that of an equivalent concentration of a reference Ply (*e.g.*, a wild-type Ply). In some embodiments, hemolytic activity of substantially non-hemolytic Ply is at least 5%, at least 10%, at least 15%, at

least 20%, at least 20%, at least 30%, at least 30%, at least 35%, least 40 %, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% lower than an equivalent concentration of a reference Ply (*e.g.*, a wild-type Ply). In some embodiments, the substantially non-hemolytic Ply has no detectable hemolytic activity. The term “wild-type Ply” is accorded the usual definition associated with such phrase, *e.g.*, in some embodiments, a naturally occurring Ply (*e.g.*, a Ply that is naturally secreted by a capable bacterial source). In some embodiments, a wild-type Ply protein is represented by the amino acid sequence as set forth in SEQ ID NO: 3.

[0199] In some embodiments, a mutant Ply (*e.g.*, non-hemolytic Ply) comprises a wild-type Ply amino acid sequence (*e.g.*, an amino acid sequence as set forth in SEQ ID NO: 3) or an antigenic fragment thereof, with one or more amino acid substitutions. In some embodiments, a mutant Ply (*e.g.*, non-hemolytic Ply) comprises a wild-type Ply amino acid sequence (*e.g.*, an amino acid sequence as set forth in SEQ ID NO: 3) or an antigenic fragment thereof, with one or more of the following amino acid substitutions: residue D385 substituted with N; residue C428 substituted with G, and residue W433 substituted with F. See, for example, Berry et al., “Effect of defined point mutations in the pneumolysin gene on the virulence of *Streptococcus pneumoniae*”. Infect Immune 1995 63(5):1969-1974). In some embodiments, a mutant Ply (*e.g.*, non-hemolytic Ply) carrying the amino acid substitutions D385N, C428G, and W433F is referred to as PdT. In some embodiments, a PdT is or comprises the amino acid sequence as set forth in SEQ ID NO: 4.

[0200] In some embodiments, a mutant Ply (*e.g.*, non-hemolytic Ply) comprises a wild-type Ply amino acid sequence (*e.g.*, an amino acid sequence as set forth in SEQ ID NO: 3) or an antigenic fragment thereof, with G294 substituted with P (See, *e.g.*, Oloo *et al.*, “Structure-guided antigen engineering yields pneumolysin mutants suitable for vaccination against pneumococcal disease”. J Biol Chem. 2011 286(14):12133-12140). In some embodiments, a mutant Ply (*e.g.*, non-hemolytic Ply) comprises a wild-type Ply amino acid sequence (*e.g.*, an amino acid sequence as set forth in SEQ ID NO: 3) or an antigenic fragment thereof, with all of the following amino acid substitutions: residue D385 substituted with N; residue C428 substituted with G; residue W433 substituted with F; and G294 substituted with P. In some embodiments, a mutant Ply (*e.g.*, non-hemolytic Ply) carrying the amino acid substitutions

G294P, D385N, C428G, and W433F is referred to as PdT(G294P). In some embodiments, a PdT(G294P) is or comprises the amino acid sequence as set forth in SEQ ID NO: 5.

[0201] In some embodiments, a mutant Ply (*e.g.*, non-hemolytic Ply) is a portion of a PdT(G294P) polypeptide (*e.g.*, a portion of the PdT(G294P) polypeptide of SEQ ID NO: 5, which portion includes at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, 400, 450 or more contiguous amino acids of SEQ ID NO: 5. In some embodiments, such a portion of PdT(G294P) polypeptide include the four amino acid substitutions described herein. In some embodiments, a portion of a PdT(G294P) polypeptide corresponds to a protein having amino acids 2-470 of the amino acid sequence set forth in SEQ ID NO: 5. In some embodiments, a mutant Ply (*e.g.*, non-hemolytic Ply) contains one or more amino acid alterations (*e.g.*, deletion, substitution, and/or insertion) from the PdT(G294P) polypeptide sequence of SEQ ID NO: 5. For example, a mutant Ply (*e.g.*, non-hemolytic Ply) may contain an amino acid sequence that is at least 60% or more (*e.g.*, at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 5 or a portion thereof (*e.g.*, at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, 400, 450 or more consecutive amino acids of the sequence shown in SEQ ID NO: 5). Alternatively, a mutant Ply (*e.g.*, non-hemolytic Ply) may contain a portion (*e.g.*, at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, 400, 450 or more consecutive amino acids) of a sequence that is at least 60% or more (*e.g.*, at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 5. In some embodiments, a mutant Ply (*e.g.*, non-hemolytic Ply) may comprises no more than 25 (including, *e.g.*, no more than 20, no more than 15, no more than 10, no more than 9, no more than 8, no more than 7, no more than 6, no more than 5, no more than 4, no more than 3, no more than 2) amino acid modifications (*e.g.*, deletion, substitution, and/or insertion) within the sequence of SEQ ID NO: 5 or a portion thereof as described herein. In some embodiments, such amino acid modifications may be present in the N-terminal portion and/or C-terminal portion.

SP0435 Polypeptides

[0202] SP0435 is a *S. pneumoniae* protein. In some embodiments, an SP0435 polypeptide is an elongation factor P. In some embodiments, an SP0435 polypeptide is or

comprises a full-length SP0435 polypeptide. For example, in some embodiments, a full-length SP0435 polypeptide has 186 amino acids (20 kDa) and is represented by the amino acid sequence as set forth in SEQ ID NO: 7. Without wishing to be bound by a particular theory, amino acids 1-61 of SEQ ID NO: 7 are predicted to be a dimerization domain of an SP0435 polypeptide (amino acids 1-61 of the full-length protein). Accordingly, in some embodiments, a SP0435 polypeptide may exclude such a dimerization domain, for example in some embodiments, to minimize or avoid the possibility of crosslinking or interference with a rhizavidin moiety. In some embodiments, an SP0435 polypeptide includes a portion of an SP0435 polypeptide (*e.g.*, a portion of the SP0435 polypeptide of SEQ ID NO: 7, which portion includes at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, or more contiguous amino acids of SEQ ID NO: 7). In some embodiments, a portion of an SP0435 polypeptide corresponds to a protein having amino acids 62-185 of the amino acid sequence set forth in SEQ ID NO: 7. In some embodiments, an SP0435 polypeptide contains one or more amino acid alterations (*e.g.*, deletion, substitution, and/or insertion) from a naturally-occurring wild-type SP0435 polypeptide sequence. For example, an SP0435 polypeptide may contain an amino acid sequence that is at least 60% or more (*e.g.*, at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 7 or a portion thereof (*e.g.*, at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 45, 50, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, or more consecutive amino acids of the sequence shown in SEQ ID NO: 7). Alternatively, an SP0435 polypeptide may contain a portion (*e.g.*, at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 45, 50, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, or more consecutive amino acids) of a sequence that is at least 60% or more (*e.g.*, at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 7. In some embodiments, a nucleotide sequence encoding an SP0435 polypeptide is provided herein as SEQ ID NO: 16. In some embodiments, an SP0434 polypeptide may comprises no more than 25 (including, *e.g.*, no more than 20, no more than 15, no more than 10, no more than 9, no more than 8, no more than 7, no more than 6, no more than 5, no more than 4, no more than 3, no more than 2) amino acid modifications (*e.g.*, deletion, substitution, and/or insertion) within the sequence of SEQ ID NO: 7 or a portion thereof as described herein. In some embodiments, such amino acid modifications may be present in the N-terminal portion and/or C-terminal portion. In

some embodiments, an SPN0435 polypeptide is one described in WO 2011/112906, the entire content of which is incorporated herein by reference for the purposes described herein.

SP0785 Polypeptides

[0203] SP0785 is a conserved hypothetical *S. pneumoniae* protein, for example, in some embodiments as described in WO 2014/124228, the entire content of which is incorporated herein by reference for the purposes described herein. In some embodiments, an SP0785 polypeptide is an efflux transporter protein conserved across *S. pneumoniae* strains. In some embodiments, an SP0785 polypeptide is or comprises a full-length SP0785 polypeptide. For example, in some embodiments, a full-length SP0785 polypeptide has 399 amino acids (38 kDa) and is represented by the amino acid sequence as set forth in SEQ ID NO: 9. Without wishing to be bound by a particular theory, amino acids 1-32 of SEQ ID NO: 9 are predicted to be a signal sequence and transmembrane domain of an SP0785 polypeptide (amino acids 1-32 of the full-length protein). Accordingly, in some embodiments, an SP0785 polypeptide may exclude such a signal sequence and transmembrane domain. In some embodiments, an SP0785 polypeptide includes a portion of an SP0785 polypeptide (*e.g.*, a portion of the SP0785 polypeptide of SEQ ID NO: 9, which portion includes at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, or more contiguous amino acids of SEQ ID NO:10). In some embodiments, a portion of an SP0785 polypeptide corresponds to a protein having amino acids 33-399 of the amino acid sequence set forth in SEQ ID NO: 9. In some embodiments, an SP0785 polypeptide contains one or more amino acid alterations (*e.g.*, deletion, substitution, and/or insertion) from a naturally-occurring wild-type SP0785 polypeptide sequence. For example, an SP0785 polypeptide may contain an amino acid sequence that is at least 60% or more (*e.g.*, at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 9 or a portion thereof (*e.g.*, at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 45, 50, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, or more consecutive amino acids of the sequence shown in SEQ ID NO: 9). Alternatively, an SP0785 polypeptide may contain a portion (*e.g.*, at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 45, 50, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, 300, 350, or 400 consecutive amino acids) of a sequence that is at least 60% or more (*e.g.*, at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 9. In some

embodiments, a nucleotide sequence encoding an SP0785 polypeptide is provided herein as SEQ ID NO: 17. In some embodiments, an SP0785 polypeptide may comprises no more than 25 (including, *e.g.*, no more than 20, no more than 15, no more than 10, no more than 9, no more than 8, no more than 7, no more than 6, no more than 5, no more than 4, no more than 3, no more than 2) amino acid modifications (*e.g.*, deletion, substitution, and/or insertion) within the sequence of SEQ ID NO: 9 or a portion thereof as described herein. In some embodiments, such amino acid modifications may be present in the N-terminal portion and/or C-terminal portion.

SP1500 Polypeptides

[0204] SP1500 is a *S. pneumoniae* protein, for example, in some embodiments as described in WO 2014/124228, the entire content of which is incorporated herein by reference for the purposes described herein. In some embodiments, an SP1500 polypeptide is an Amino Acid ABC Transporter, amino acid-binding polypeptide conserved across *S. pneumoniae* strains. In some embodiments, an SP1500 polypeptide is or comprises a full-length SP1500 polypeptide. For example, in some embodiments, a full-length SP1500 polypeptide has 278 amino acids (28 kDa) and is represented by the amino acid sequence as set forth in SEQ ID NO: 11. Without wishing to be bound by a particular theory, amino acids 1-26 of SEQ ID NO: 11 are predicted to be a signal sequence of an SP1500 polypeptide (amino acids 1-26 of the full-length protein). Accordingly, in some embodiments, a SP1500 polypeptide may exclude such a signal sequence. In some embodiments, an SP1500 polypeptide includes a portion of an SP1500 polypeptide (*e.g.*, a portion of the SP1500 polypeptide of SEQ ID NO: 11, which portion includes at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, or more contiguous amino acids of SEQ ID NO: 11). In some embodiments, a portion of an SP1500 polypeptide corresponds to a protein having amino acids 27-278 of the amino acid sequence set forth in SEQ ID NO: 11. In some embodiments, an SP1500 polypeptide contains one or more amino acid alterations (*e.g.*, deletion, substitution, and/or insertion) from a naturally-occurring wild-type SP1500 polypeptide sequence. For example, an SP1500 polypeptide may contain an amino acid sequence that is at least 60% or more (*e.g.*, at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 11 or a portion thereof (*e.g.*, at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 45, 50, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, or more consecutive amino acids of the sequence shown in SEQ ID

NO: 11). Alternatively, an SP1500 polypeptide may contain a portion (*e.g.*, at least 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 45, 50, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, 200, 250, or more consecutive amino acids) of a sequence that is at least 60% or more (*e.g.*, at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 11. In some embodiments, a nucleotide sequence encoding an SP1500 polypeptide is provided herein as SEQ ID NO: 18. In some embodiments, an SP1500 polypeptide may comprises no more than 25 (including, *e.g.*, no more than 20, no more than 15, no more than 10, no more than 9, no more than 8, no more than 7, no more than 6, no more than 5, no more than 4, no more than 3, no more than 2) amino acid modifications (*e.g.*, deletion, substitution, and/or insertion) within the sequence of SEQ ID NO: 11 or a portion thereof as described herein. In some embodiments, such amino acid modifications may be present in the N-terminal portion and/or C-terminal portion.

[0205] In some embodiments, nucleic acid sequences encoding a pneumolysin polypeptide (SEQ ID NO: 3), an SP0435 polypeptide (SEQ ID NO: 7), and an SPP2 polypeptide (SEQ ID NO: 19 or SEQ ID NO: 58) are provided as SEQ ID NO: 13, SEQ ID NO: 16, SEQ ID NO: 27, and SEQ ID NO: 62, respectively. In some embodiments, nucleic acid sequences encoding an SP0785 polypeptide (SEQ ID NO: 9), an SP1500 polypeptide (SEQ ID NO: 11), and a CP1 polypeptide (SEQ ID NO: 26 or SEQ ID NO: 61) are provided as SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 29, and SEQ ID NO: 64, respectively. Due to degeneracy in the genetic code, those of ordinary skill in the art would understand that other DNA sequences (including codon-optimized sequences) could encode these polypeptides, as well as the others disclosed herein.

Fusion Proteins that Include Antigenic Polypeptides

[0206] Antigenic polypeptides described herein can be part of a fusion protein. For example, in some embodiments, an immunogenic complex described herein comprises a fusion protein that is or comprises a complementary affinity molecule and one or more antigenic polypeptides described herein. In some embodiments, a fusion protein comprises two antigenic polypeptides described herein. In some embodiments, a fusion protein comprises three antigenic

polypeptides described herein. In some embodiments, a fusion protein comprises four antigenic polypeptides described herein. In some embodiments, a fusion protein comprises one or more antigenic polypeptides encoded by a nucleic acid comprising a sequence encoding the antigenic polypeptide described herein. In some embodiments, a fusion protein of the immunogenic complex has carrier properties. In some embodiments, a fusion protein of the immunogenic complex has antigenic properties. In some embodiments, a fusion protein of the immunogenic complex has carrier properties and antigenic properties.

[0207] In some embodiments, a fusion protein comprises one or more antigenic polypeptides of *S. pneumoniae* having an amino acid sequence comprising any of SEQ ID NOs: 3-12, or antigenic fragments thereof. In some embodiments, a fusion protein comprises two antigenic polypeptides having an amino acid sequence comprising any of SEQ ID NOs: 3-12, or antigenic fragments thereof. In some embodiments, a fusion protein comprises (i) two antigenic polypeptides having an amino acid sequence comprising any of SEQ ID NOs: 3-12, or antigenic fragments thereof, and (ii) a biotin-binding moiety comprising SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 57, or biotin-binding fragments thereof. In some such embodiments, at least one antigenic polypeptide is or comprises a Ply polypeptide (*e.g.*, any one of SEQ ID NOs: 3-6). In some such embodiments, at least one antigenic polypeptide is or comprises an SP0435 polypeptide (*e.g.*, SEQ ID NOs: 7 or 8). In some such embodiments, at least one antigenic polypeptide is or comprises an SP0785 polypeptide (*e.g.*, SEQ ID NOs: 9 or 10). In some such embodiments, at least one antigenic polypeptide is or comprises an SP1500 polypeptide (*e.g.*, SEQ ID NOs: 11 and 12).

[0208] In some embodiments, a fusion protein comprises one or more polypeptides homologous to the *S. pneumoniae* polypeptides described herein, *e.g.*, a Ply polypeptide, an SP0435 polypeptide, an SP0785 polypeptide or an SP1500 polypeptide isolated from different serotypes of *S. pneumoniae*. Individual serotypes of *S. pneumoniae* contain numerous mutations relative to each other, and some of these result in different protein sequences between the different serotypes. One of skill in the art may readily substitute an amino acid sequence, or a portion thereof, with the homologous amino acid sequence from a different *S. pneumoniae* serotype. In some embodiments, antigenic polypeptides have at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100%

identity to the polypeptides of any of SEQ ID NOs: 3-12, or antigenic fragments thereof.

Serotypic variation may be used to design such variants of the antigenic polypeptides described herein.

[0209] In some embodiments, fusion proteins described herein comprise one or more fragments of one or more polypeptides described herein, *e.g.*, biotin-binding fragments of rhizavidin, a Ply polypeptide or antigenic fragments thereof, an SP0435 polypeptide or antigenic fragments thereof, a SP0785 polypeptide or antigenic fragments thereof (*e.g.*, with or without a signal sequence), or a SP1500 polypeptide or antigenic fragments thereof (*e.g.*, with or without a signal sequence). In some embodiments, fusion proteins described herein comprise truncation mutants that are close in size to the polypeptides with amino acid sequences of SEQ ID NOs: 1-12 and 57. For example, they may lack at most one, two, three, four, five, ten, or twenty amino acids from one or both termini (referring to component polypeptides in a fusion protein). In some embodiments, a fragment is a truncated fragment of any of SEQ ID NOs: 1-12 and 57 lacking 1-5, 1-10, or 1-20 amino acid residues from the N-terminus, C-terminus, or both, of any one of SEQ ID NOs: 1-12 and 57. In some embodiments, a fragment is a truncated fragment of any of SEQ ID NOs: 1-12 and 57 lacking 1-10 amino acid residues from the N-terminus, C-terminus, or both, of any one of SEQ ID NOs: 1-12 and 57. For instance, a fragment may lack 10 amino acid residues at both the N-terminus and C-terminus of any one of SEQ ID NOs: 1-12 and 57, resulting in a protein lacking 20 amino acid residues. Internal deletions, *e.g.*, of 1-10, 11-20, 21-30, or 31-40 amino acids, are also contemplated.

[0210] In some embodiments, a fusion protein comprises an N-terminal polypeptide and a C-terminal polypeptide. In some embodiments, one or both of the N-terminal polypeptide and the C-terminal polypeptide is an antigenic polypeptide, for example, a polypeptide having an amino acid sequence comprising one or more of SEQ ID NOs: 3-12, or an antigenic fragment or variant thereof. In some embodiments, one or both of the N-terminal polypeptide and the C-terminal polypeptide is a biotin-binding moiety, for example a polypeptide having an amino acid sequence comprising SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 57, or a biotin-binding fragment thereof. In some embodiments, one of the N-terminal polypeptide or the C-terminal polypeptide is a biotin-binding moiety, *e.g.*, a polypeptide having an amino acid sequence comprising SEQ ID NO: 1, SEQ ID NO: 2, or SEQ ID NO: 57, or a biotin-binding fragment

thereof, and the other terminal polypeptide is an antigenic polypeptide, *e.g.*, a polypeptide having an amino acid sequence comprising one or more of SEQ ID NOs: 3-12, or an antigenic fragment or variant thereof.

[0211] In some embodiments, the N-terminal polypeptide and the C-terminal polypeptide are directly bound to each other. In some embodiments, the N-terminal polypeptide and the C-terminal polypeptide are linked via a linker peptide. The length and/or amino acids of a linker, when present, can be adjusted to obtain a more flexible, semi-rigid, or rigid linker. In some embodiments, a linker can be a GS-enriched linker. In some embodiments, a linker can be an A-enriched linker. A linker can generally be from 1-40, such as 3-10 or 10-30 and specifically 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids in length. Exemplary flexible peptide linkers are shown as SEQ ID NOs: 30-33. In some embodiments, the fusion protein comprises one linker. In some embodiments, the fusion protein comprises two linkers. In some embodiments, the one or two linkers are of SEQ ID NO: 30 (GGGGSSS). In some embodiments, the fusion protein comprises SEQ ID NO: 30 (GGGGSSS). In some embodiments, the one or two linkers are selected from SEQ ID NO: 30 (GGGGSSS) and SEQ ID NO: 31 (AAA). In some embodiments, the fusion protein comprises a linker of SEQ ID NO: 30 (GGGGSSS) and SEQ ID NO: 31 (AAA). In some embodiments, the fusion protein comprises an amino acid sequence AAA residual from a Not I restriction site. In some embodiments, the fusion protein comprises a linker of SEQ ID NO: 30 (GGGGSSS) and an amino acid sequence AAA (SEQ ID NO: 31) residual from a Not I restriction site.

[0212] In some embodiments, the present disclosure provides fusion proteins with at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% sequence identity to a fusion protein having an amino acid sequence of any one of SEQ ID NOs: 19-26 and 58-61. In some embodiments, a fusion protein is or includes an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to SEQ ID NO: 19 or SEQ ID NO: 58. In some embodiments, a fusion protein is or includes an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to SPP2. In some embodiments, a fusion protein is or includes an amino acid sequence having at least 80%, at least

85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to SEQ ID NO: 26 or SEQ ID NO: 61. In some embodiments, a fusion protein is or includes an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to CP1.

[0213] In some embodiments, a fusion protein described herein comprises an antigenic fragment of a fusion protein having an amino acid sequence of any one of SEQ ID NOs: 19-26 and 58-61. For example, a fusion protein may lack at most one, two three, four, five, ten, or twenty amino acids from the N-terminus, C-terminus, or both, of any one of SEQ ID NOs: 19-26 and 58-61. In some embodiments, the same number of residues is removed from the N-terminus and the C-terminus, while in other embodiments, a different number of residues is removed from the N-terminus compared to the C-terminus. In some embodiments, a fusion protein is or includes an antigenic fragment of SEQ ID NO: 19 or SEQ ID NO: 58. In some embodiments, a fusion protein is or includes an antigenic fragment of SPP2. In some embodiments, a fusion protein is or includes an antigenic fragment of SEQ ID NO: 26 or SEQ ID NO: 61. In some embodiments, a fusion protein is or includes an antigenic fragment of CP1.

[0214] In some embodiments, a fusion protein described herein comprises a biotin-binding moiety. In some embodiments, the fusion protein comprises a biotin-binding moiety, and one or more polypeptide antigens. In some embodiments, the fusion protein comprises a biotin-binding moiety and two or more polypeptide antigens. As used herein, a “biotin-binding moiety” refers to a biotin-binding polypeptide or protein, a biotin-binding fragment thereof, or a biotin-binding domain thereof. In some embodiments, the biotin-binding moiety of a fusion protein comprises rhizavidin or a biotin-binding fragment thereof, which in some embodiments as further described in WO 2012/155053, the contents of which are herein incorporated by reference in their entirety for the purposes described herein.

[0215] In some embodiments, a fusion protein described herein comprises a biotin-binding moiety that is or comprises a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 1 (rhizavidin), or biotin-binding fragment thereof. In some embodiments, a fusion protein comprises a biotin-binding moiety that is or comprises a

polypeptide having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO: 2 (amino acids 45-179 of rhizavidin, denoted Rhavi) or SEQ ID NO: 57, or biotin-binding fragment thereof.

[0216] In some embodiments, a fusion protein comprises a pneumolysin polypeptide as described herein. For example, in some embodiments, a fusion protein comprises a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 6 (amino acids 2-470 of *S. pneumoniae* PdT(G294P) polypeptide), or an antigenic fragment thereof. In some embodiments, a pneumolysin polypeptide described herein may be truncated from its N-terminal portion and/or C-terminal portion.

[0217] In some embodiments, a fusion protein comprises an SPN0435 polypeptide. For example, in some embodiments, a fusion protein comprises a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 8 (amino acids 62-185 of *S. pneumoniae* SP0435 polypeptide), or an antigenic fragment thereof. In some embodiments, an SPN0435 polypeptide described herein may be truncated from its N-terminal portion and/or C-terminal portion.

[0218] In some embodiments, a fusion protein comprises an SP0785 polypeptide. For example, in some embodiments, a fusion protein comprises a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 10 (amino acids 33-399 of *S. pneumoniae* SP0785 polypeptide), or an antigenic fragment thereof. In some embodiments, an SPN0785 polypeptide described herein may be truncated from its N-terminal portion and/or C-terminal portion.

[0219] In some embodiments, a fusion protein comprises an SP1500 polypeptide. For example, in some embodiments, a fusion protein comprises a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 12 (amino acids 27-278 of *S. pneumoniae* SP1500 polypeptide), or an antigenic fragment thereof.

In some embodiments, an SPN1500 polypeptide described herein may be truncated from its N-terminal portion and/or C-terminal portion.

[0220] In some embodiments, a fusion protein described herein comprises each of: (a) a biotin-binding moiety that is or comprises a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 1 (rhizavidin), or biotin-binding fragment thereof; (b) a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 6 (amino acids 2-470 of *S. pneumoniae* PdT(G294P) polypeptide), or an antigenic fragment thereof; and (c) a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 8 (amino acids 62-185 of *S. pneumoniae* SP0435 polypeptide) or an antigenic fragment thereof. In some embodiments, the fusion protein further comprises one or more linkers. In some embodiments, the one or more linkers are SEQ ID NO: 30 (GGGGSSS).

[0221] In some embodiments, a fusion protein described herein comprises each of: (a) a biotin-binding moiety that is or comprises a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 1 (rhizavidin), or biotin-binding fragment thereof; (b) a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 10 (amino acids 33-399 of *S. pneumoniae* SP0785 polypeptide), or an antigenic fragment thereof; and (c) a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 12 (amino acids 27-278 of *S. pneumoniae* SP1500 polypeptide) or an antigenic fragment thereof. In some embodiments, the fusion protein further comprises one or more linkers. In some embodiments, the one or more linkers are selected from SEQ ID NO: 30 (GGGGSSS) and SEQ ID NO: 31 (AAA). In some embodiments, the fusion protein comprises an amino acid sequence AAA residual from a Not I restriction site. In some embodiments, the fusion protein comprises a

linker of SEQ ID NO: 30 (GGGGSSS) and an amino acid sequence AAA residual from a Not I restriction site.

[0222] In some embodiments, a fusion protein described herein comprises each of: (a) a biotin-binding moiety that is or comprises a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 2 (amino acids 45-179 of rhizavidin, denoted Rhavi) or SEQ ID NO: 57, or biotin-binding fragment thereof; (b) a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 6 (amino acids 2-470 of *S. pneumoniae* PdT(G294P) polypeptide) or an antigenic fragment thereof; and (c) a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 8 (amino acids 62-185 of *S. pneumoniae* SP0435 polypeptide) or an antigenic fragment thereof. In some embodiments, the fusion protein further comprises one or more linkers. In some embodiments, the one or more linkers are SEQ ID NO: 30 (GGGGSSS). In some embodiments, a fusion protein described herein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the sequence SEQ ID NO: 19 or SEQ ID NO: 58. In some embodiments, the fusion protein comprises the amino acid sequence SEQ ID NO: 19 or SEQ ID NO: 58. In some embodiments, the fusion protein consists of the amino acid sequence SEQ ID NO: 19 (SPP2) or SEQ ID NO: 58.

[0223] In some embodiments, a fusion protein described herein comprises each of: (a) a biotin-binding moiety that is or comprises a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 2 (amino acids 45-179 of rhizavidin, denoted Rhavi) or SEQ ID NO: 57, or biotin-binding fragment thereof; (b) a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 10 (amino acids 33-399 of *S. pneumoniae* SP0785 polypeptide) or an antigenic fragment

thereof; and (c) a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 12 (amino acids 27-278 of *S. pneumoniae* SP1500 polypeptide) or an antigenic fragment thereof. In some embodiments, the fusion protein further comprises one or more linkers. In some embodiments, the one or more linkers are selected from SEQ ID NO: 30 (GGGGSSS) and SEQ ID NO: 31 (AAA). In some embodiments, the fusion protein comprises an amino acid sequence AAA residual from a Not I restriction site. In some embodiments, the fusion protein comprises a linker of SEQ ID NO: 30 (GGGGSSS) and an amino acid sequence AAA residual from a Not I restriction site. In some embodiments, a fusion protein described herein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the sequence SEQ ID NO: 26 or SEQ ID NO: 61. In some embodiments, the fusion protein comprises the amino acid sequence SEQ ID NO: 26 or SEQ ID NO: 61. In some embodiments, the fusion protein consists of the amino acid sequence SEQ ID NO: 26 (CP1) or SEQ ID NO: 61.

[0224] In some embodiments, a fusion protein described herein includes a variant or fragment of a polypeptide having an amino acid sequence of SEQ ID NOs: 1-12 or 57. In some embodiments, a fusion protein described herein includes a polypeptide encoded by a variant or fragment of a gene having a nucleic acid sequence of SEQ ID NOs: 1-12 or 57. In some embodiments, a fragment included in a fusion protein described herein is close in size to a full-length polypeptide or a polypeptide having an amino acid sequence of SEQ ID NOs: 1-12 or 57. For example, they may lack at most one, two, three, four, five, ten, twenty, or thirty amino acids from one or both termini. In some embodiments, the fragment is 25-50 amino acids in length, or 50-100, or 100-150, or 150-200, or 200-250, or 250-300, or 300-350 amino acids in length. In some embodiments, the fragments result from processing, or partial processing, of signal sequences by an expression host, *e.g.* *E. coli*, an insect cell line (*e.g.*, the baculovirus expression system), or a mammalian (*e.g.*, human or Chinese Hamster Ovary) cell line. The fragments described above or sub-fragments thereof (*e.g.*, fragments of 8-50, 8-30, or 8-20 amino acid residues) preferably have one of the biological activities described below, such as increasing the

amount of IL-17 released by at least 1.5 fold or 2 fold or more (*e.g.*, either as an absolute measure or relative to a control protein).

Linker or Spacer

[0225] In some embodiments, the fusion protein of the immunogenic complex comprises one or more linkers. In some embodiments, a linker is or comprises one or more amino acids. In some embodiments, a fusion protein comprises an antigenic polypeptide joined to a biotin-binding moiety by a linker. In some embodiments, a fusion protein comprises a first antigenic polypeptide, a second antigenic polypeptide, a biotin-binding moiety, and at least one linker. In some embodiments, the first antigenic polypeptide and the second antigenic polypeptide are joined by a linker. In some embodiments, the first antigenic polypeptide or the second antigenic polypeptide are joined to the biotin-binding moiety by a linker. In some embodiments, the first antigenic polypeptide and the second antigenic polypeptide are joined by a first linker; and the first antigenic polypeptide or the second antigenic polypeptide are joined to the biotin-binding moiety by a second linker.

[0226] In some embodiments, a fusion protein described herein comprises a first linker comprising the amino acid sequence of SEQ ID NO: 30 (GGGGSSS), and a second linker comprising the amino acid sequence of SEQ ID NO: 30 (GGGGSSS). In some embodiments, a fusion protein described herein comprises a first linker comprising the amino acid sequence of SEQ ID NO: 30 (GGGGSSS), and a second linker comprising the amino acid sequence of SEQ ID NO: 31 (AAA).

[0227] In some embodiments, a linker interposes a structure between two protein moieties. In some embodiments, the structure is or comprises an α -helix. In some embodiments, the structure is or comprises a β -strand. In some embodiments, the structure is or comprises a coil/bend. In some embodiments, the structure is or comprises a turn. In some embodiments, a linker decreases steric hindrance between two protein moieties joined by the linker. In some embodiments, a linker decreases unfavorable interactions between two protein moieties joined by the linker. In some embodiments, a linker comprises a mixture of glycine and serine residues. In some embodiments, the linker may additionally comprise threonine, proline, and/or alanine residues. In some embodiment, a linker is hydrophilic. In some embodiments, a linker is

hydrophobic. In some embodiments, a linker increases the stability of the fusion protein containing the linker.

[0228] In some embodiments, a linker does not interfere with the folding of an antigenic polypeptide to which it is joined. In some embodiments, a linker does not interfere with the antigenicity of an antigenic polypeptide to which it is joined. In some embodiments, a linker does not reduce the antigenicity of an antigenic polypeptide to which it is joined. In some embodiments, a linker does not eliminate the antigenicity of an antigenic polypeptide to which it is joined. In some embodiments, the effect of the linker is determined by comparing the polypeptide with the polypeptide joined to the linker.

[0229] In some embodiments, a linker does not interfere with the folding of a biotin-binding moiety to which it is joined. In some embodiments, a linker does not interfere with the biotin-binding ability of a biotin-binding moiety to which it is joined. In some embodiments, a linker does not reduce the biotin-binding ability of a biotin-binding moiety to which it is joined. In some embodiments, a linker does not eliminate the biotin-binding ability of a biotin-binding moiety to which it is joined. In some embodiments, the effect of the linker is determined by comparing the biotin-binding moiety with the biotin-binding moiety joined to the linker.

[0230] In some embodiments, a linker is not antigenic. In some embodiments, a linker does not elicit a T cell response. In some embodiments, a linker does not elicit a B cell response. In some embodiments, a linker does not induce a T cell or a B cell response.

[0231] In some embodiments, a linker comprises two or more amino acids. In some embodiments, a linker may be 3-100, 5-100, 10-100, 20-100, 30-100, 40-100, 50-100, 60-100, 70-100, 80-100, 90-100, 5-55, 10-50, 10-45, 10-40, 10-35, 10-30, 10-25, 10-20, 10-15, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, or 2-3 amino acids in length. In some embodiments, a linker comprises between 10-100, 10-90, 10-80, 10-70, 10-60, 10-50, 10-40, 10-30, 10-20, 10-15 amino acids. In some embodiments, the linker comprises at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95 amino acids. In some embodiments, a linker is or comprises a peptidyl linker comprising 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids.

[0232] In some embodiments, a linker is a flexible linker. Flexible linkers may be useful

for joining domains that require a certain degree of movement or interaction and may include small, non-polar (*e.g.*, Gly) or polar (*e.g.*, Ser or Thr) amino acids. Incorporation of Ser or Thr can also maintain the stability of the linker in aqueous solutions by forming hydrogen bonds with water molecules, and therefore reduce unfavorable interactions between the linker and the protein moieties. In some embodiments, a linker comprises small non-polar (*e.g.* Gly) or polar (*e.g.* Ser or Thr) amino acids. In some embodiments, a linker is a Gly-Ser linker.

[0233] In some embodiments, a linker is or comprises an amino acid sequence of GGGGSSS (SEQ ID NO: 30). In some embodiments, a linker is or comprises a sequence of (GGGGS)_n (SEQ ID NO: 32), where n represents the number of repeating GGGGS units and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more. In some embodiments, a polypeptide linker may have an amino acid sequence that is or comprises GGGGSGGGGSGGGGS (SEQ ID NO: 34) (*i.e.*, (GGGGS)₃) or GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 35) (*i.e.*, (GGGGS)₆). In some embodiments, a linker comprises one or more of Gly, Ser, Thr, Ala, Lys, and Glu. In some embodiments, a linker is or comprises KESGSVSSEQLAQFRSLD (SEQ ID NO: 36). In some embodiments, a linker is or comprises EGKSSGSGSESKST (SEQ ID NO: 37). In some embodiments, a linker is or comprises (Gly)_n (SEQ ID NO: 38) where n represents the number of repeating Gly residues and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more. In some embodiments, a linker is or comprises GGG. In some embodiments, a linker is or comprises (Gly)₆ (SEQ ID NO: 33). In some embodiments, a linker is or comprises (Gly)₈ (SEQ ID NO: 39). In some embodiments, a linker is or comprises GSAGSAAGSGEF (SEQ ID NO: 40). In some embodiments, a linker is or comprises an amino acid sequence of AAA (SEQ ID NO: 31). In some embodiments, such a linker may be synthesized, or derived from amino acid residues from a restriction site (*e.g.*, a Not I restriction site).

[0234] In some embodiments, a linker is a rigid linker. Rigid linkers are useful to keep a fixed distance between domains and to maintain their independent functions. Rigid linkers may also be useful when a spatial separation of the domains is critical to preserve the stability or bioactivity of one or more components in the fusion. In some embodiments, a linker is or comprises (EAAAK)_n (SEQ ID NO: 41) where n represents the number of repeating EAAAK units and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more. In

some embodiments, a linker is or comprises $A(EAAAK)_nA$, (SEQ ID NO: 42) where n represents the number of repeating EAAAK units and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more. In some embodiments, a linker is or comprises $A(EAAAK)_nA$, where n represents the number of repeating EAAAK units and is 2, 3, 4, or 5. In some embodiments, a linker is or comprises $A(EAAAK)_4ALEA(EAAAK)_4A$ (SEQ ID NO: 43). In some embodiments, a linker is or comprises $[A(EAAAK)_nA]_m$, (SEQ ID NO: 44) wherein n is 2, 3, or 4 and m is 1 or 2. In some embodiments, a linker is or comprises AEAAAKEAAKA (SEQ ID NO: 45).

[0235] In some embodiments, a linker is or comprises $(X-Pro)_n$ (SEQ ID NO:46), with X designating any amino acid, where n represents the number of repeating $X-Pro$ units and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more. In some embodiments, a linker is or comprises $(Ala-Pro)_n$ (SEQ ID NO: 47), where n represents the number of repeating $Ala-Pro$ units and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more. In some embodiments, a linker is or comprises $(Ala-Pro)_n$, where n represents the number of repeating $Ala-Pro$ units and is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17.

[0236] In some embodiments, a linker is or comprises $(Lys-Pro)_n$ (SEQ ID NO:48), where n represents the number of repeating $Lys-Pro$ units and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more. In some embodiments, a linker is or comprises $(Glu-Pro)_n$ (SEQ ID NO: 49), where n represents the number of repeating $Glu-Pro$ units and is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30 or more. In some embodiments, a linker is or comprises $(Ala-Pro)_7$ (SEQ ID NO: 50).

[0237] In some embodiments, a linker is or comprises GAPGGGGGAAAAAGGGGGGAP (GAG linker, SEQ ID NO: 51). In some embodiments, a linker is or comprises GAPGGGGGAAAAAGGGGGGAPGGGGGAAAAAGGGGGGAP (GAG2 linker, SEQ ID NO: 52). In some embodiments, a linker is or comprises GAPGGGGGAAAAAGGGGGGAPGGGGGAAAAAGGGGGGAPGGGGGAAAAAGGGGGGAP (GAG3 linker, SEQ ID NO: 53).

[0238] Suitable linkers or spacers also include those having an amino acid sequence at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or more homologous or identical to the above exemplary linkers. In some embodiments, the linker comprises a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the sequence of SEQ ID NO: 30 (GGGGSSS).

[0239] Additional linkers suitable for use with some embodiments may be found in U.S. Patent Publication No. 2012/0232021, filed on March 2, 2012, and [Chen, 2013] the disclosures of which is hereby incorporated by reference in their entireties.

Tags

[0240] In some embodiments, a fusion protein of the immunogenic complex may comprise one or more tags, *e.g.*, a histidine tag. A tag may be N-terminal or C-terminal. For instance, tags may be added to a polypeptide (via additions or modifications on the encoding DNA sequence) to facilitate purification, detection, solubility, or confer other desirable characteristics on the protein. In some embodiments, a tag may be a peptide, oligopeptide, or polypeptide that may be used in affinity purification. In some embodiments, a tag is, comprises, or is derived from one or more of polyhistidine (His), Glutathione S-transferase (GST), tandem affinity purification (TAP), FLAG, myc, human influenza hemagglutinin (HA), maltose binding protein (MBP), vesicular Stomatitis viral glycoprotein (VSV-G), thioredoxin, V5, avidin, streptavidin, biotin carboxyl carrier protein (BCCP), Calmodulin, Nus, S tags, lipoprotein D, and galactosidase. In some embodiments, a His tag is or comprises an amino acid sequence of H_n, wherein n is an integer between 2 and 10. Exemplary His tags include HHHHHH (SEQ ID NO: 55) and MSYYHHHHHH (SEQ ID NO: 56). In other embodiments, a fusion protein described herein is free of tags such as protein purification tags, and is purified by a method not relying on affinity for a purification tag. In some embodiments, a fusion protein described herein comprises no more than 1, 2, 3, 4, 5, 10, or 20 additional amino acids on one or both termini of a polypeptide of amino acid sequence of any of SEQ ID NOs: 1-12 or 57 or fusion protein of amino acid sequence of any of SEQ ID NOs: 19-26 or 58-61.

[0241] In some embodiments, a fusion protein described herein may contain a membrane translocating sequence (MTS), for example, in some embodiments, to facilitate introduction of the fusion protein into a mammalian cell and subsequent stimulation of the cell-mediated immune response. Exemplary membrane translocating sequences include, but are not limited to the hydrophobic region in the signal sequence of Kaposi fibroblast growth factor, the MTS of a synuclein, the third helix of the Antennapedia homeodomain, SN50, integrin 3 h-region, HIV Tat, pAntp, PR-39, abaecin, apidaecin, Bac5, Bac7, P. berghei CS protein, and those MTSS described in U.S. Patent Nos. 6,248, 558, 6,432,680 and 6,248,558.

Complementary Affinity Molecules

[0242] In some embodiments, a complementary affinity molecule comprises a biotin-binding moiety. In some embodiments, a fusion protein of the immunogenic complex comprises a biotin-binding moiety, and one or more polypeptide antigens. In some embodiments, a fusion protein comprises a biotin-binding moiety and two or more polypeptide antigens. As used herein, a “biotin-binding moiety” refers to a biotin-binding protein, a biotin-binding fragment thereof, or a biotin-binding domain thereof.

[0243] In some embodiments, MAPS complexes disclosed herein utilize the high affinity (dissociation constant [KD] $\approx 10^{-15}$ M) non-covalent binding between biotin and rhizavidin, a biotin-binding protein that has no significant predicted homology with human proteins. Rhizavidin, a naturally occurring dimeric protein in the avidin protein family, was first discovered in *Rhizobium etli*, a symbiotic bacterium of the common bean. Rhizavidin has only a 22% amino acid identity with chicken avidin, a protein commonly found in eggs, but with high conservation of amino acid residues involved in biotin binding. No cross-reactivity to rhizavidin is observed in human serum samples obtained from subjects exposed to avidin [Helppolainen et al, 2007], suggesting that rhizavidin antibodies may not cross-react with chicken avidin. Biotin conjugates have been used in several clinical applications without any reported adverse events [Buller et al, 2014; Paty et al, 2010; Lazzeri et al, 2004].

[0244] In some embodiments, a biotin-binding moiety that is useful in accordance with the present disclosure comprises rhizavidin or a biotin-binding domain or biotin-binding fragment thereof, for example, in some embodiments as further described in WO 2012/155053,

the contents of which are herein incorporated by reference in their entirety for the purposes described herein. In some embodiments, a biotin-binding moiety is or comprises a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to rhizavidin, or a biotin-binding domain or biotin-binding fragment thereof. In some embodiments, a biotin-binding moiety that is useful in accordance with the present disclosure comprises a polypeptide of SEQ ID NO: 1 or a biotin-binding domain or biotin-binding fragment thereof. In some embodiments, a biotin-binding moiety is or comprises a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, 100% identity to the sequence of SEQ ID NO: 1, or biotin-binding domain or biotin-binding fragment thereof. In some embodiments, a biotin-binding moiety that is useful in accordance with the present disclosure comprises a polypeptide of SEQ ID NO: 2 or SEQ ID NO: 57, or a biotin-binding domain or biotin-binding fragment thereof. In some embodiments, a biotin-binding moiety is or comprises a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the sequence of SEQ ID NO: 2 or SEQ ID NO: 57, or biotin-binding domain or biotin-binding fragment thereof.

Fusion Protein SPP2

[0245] In some embodiments, a fusion protein described herein is or comprises a complementary affinity molecule described herein (*e.g.*, a biotin-binding moiety described herein), and one or more polypeptides of or derived from *S. pneumoniae*. In some embodiments, a provided fusion protein is a SPP2 described herein. In some embodiments, a SPP2 fusion protein is a fusion protein comprising a biotin-binding moiety (*e.g.*, a biotin-binding protein), a non-hemolytic pneumolysin polypeptide comprising mutations at amino acid residues 294, 385, 428, and 433 of wild-type *Streptococcus pneumoniae* pneumolysin or an antigenic fragment thereof; and (iii) a *S. pneumoniae* elongation factor P (SP0435) polypeptide or an antigenic fragment thereof. In some embodiments, a biotin-binding moiety is or comprises a rhizavidin polypeptide or a biotin-binding portion thereof. In some embodiments, a fusion protein comprises a complementary affinity molecule described herein (*e.g.*, a biotin-binding moiety described herein) and a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at

least 99.5%, or 100% identity to the sequence of SEQ ID NO: 6 (amino acids 2-470 of *S. pneumoniae* PdT(G294P)) or an antigenic variant or fragment thereof and a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 8 (amino acids 62-185 of *S. pneumoniae* SP0435) or an antigenic variant or fragment thereof. In some embodiments, the fusion protein comprises a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 19 or SEQ ID NO: 58. In some embodiments, the fusion protein is or comprises a complementary affinity molecule described herein (*e.g.*, a biotin-binding moiety described herein), a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to pneumolysin or an antigenic variant or fragment thereof, and a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SP0435 or an antigenic variant or fragment thereof. In some embodiments, the fusion protein is or comprises a complementary affinity molecule described herein (*e.g.*, a biotin-binding moiety described herein), a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to a non-hemolytic pneumolysin comprising mutations at amino acid residues 294, 385, 428, and 433 of wild-type *S. pneumoniae* pneumolysin or an antigenic variant or fragment thereof, and a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SP0435 or an antigenic variant or fragment thereof.

[0246] In some embodiments, a fusion protein described herein comprises a complementary affinity molecule described herein (*e.g.*, a biotin-binding moiety described herein) and a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 6 (amino acids 2-470 of *S. pneumoniae* PdT(G294P)) or an antigenic variant or fragment thereof. In some embodiments, a fusion protein comprises a complementary affinity molecule described herein (*e.g.*, a biotin-binding moiety described herein) and a polypeptide comprising an amino acid sequence having at least

80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 8 (amino acids 62-185 of *S. pneumoniae* SP0435) or an antigenic variant or fragment thereof.

[0247] In some embodiments, a fusion protein described herein comprises a biotin-binding moiety that is or comprises a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the sequence of SEQ ID NO: 1 (rhizavidin), or biotin-binding fragment thereof. In some embodiments, a fusion protein comprises a biotin-binding moiety that is or comprises a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the sequence of SEQ ID NO: 2 (amino acids 45-179 of rhizavidin) or SEQ ID NO: 57, or biotin-binding fragment thereof. In some embodiments, a fusion protein comprises a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 6 (amino acids 2-470 of *S. pneumoniae* PdT(G294P)) or an antigenic variant or fragment thereof. In some embodiments, the fusion protein comprises a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 8 (amino acids 62-185 of *S. pneumoniae* SP0435) or an antigenic variant or fragment thereof.

[0248] In some embodiments, a fusion protein described herein comprises each of: (a) a biotin-binding moiety that is or comprises a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the sequence of SEQ ID NO: 1 (rhizavidin), or biotin-binding fragment thereof; (b) a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 6 (amino acids 2-470 of *S. pneumoniae* PdT(G294P)) or an antigenic variant or fragment thereof; and (c) a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 8

(amino acids 62-185 of *S. pneumoniae* SP0435) or an antigenic variant or fragment thereof. In some embodiments, such a fusion protein further comprises one or more linkers.

[0249] In some embodiments, a fusion protein described herein comprises each of: (a) a biotin-binding moiety that is or comprises a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the sequence of SEQ ID NO: 2 (amino acids 45-179 of rhizavidin) or SEQ ID NO: 57, or biotin-binding fragment thereof; (b) a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 6 (amino acids 2-470 of *S. pneumoniae* PdT(G294P)) or an antigenic variant or fragment thereof; and (c) a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 8 (amino acids 62-185 of *S. pneumoniae* SP0435) or an antigenic variant or fragment thereof. In some embodiments, such a fusion protein further comprises one or more linkers.

[0250] In some embodiments, a fusion protein described herein is or comprises a SPP2 fusion protein. In some embodiments, a SPP2 fusion protein is a fusion protein comprising, from N-terminus to C-terminus, a biotin-binding moiety (*e.g.*, a biotin-binding protein), a first peptide linker, a pneumolysin (Ply) polypeptide described herein or an antigenic variant or fragment thereof; a second peptide linker, and an SPN0435 polypeptide described herein or an antigenic variant or fragment thereof. In some such embodiments, a biotin-binding moiety is or comprises rhizavidin or a biotin-binding portion thereof. In some embodiments, a SPP2 fusion protein comprises, from N-terminus to C-terminus, (a) a biotin-binding moiety that is or comprises a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the sequence of SEQ ID NO: 1 (rhizavidin) or to the sequence of SEQ ID NO: 2 (amino acids 45-179 of rhizavidin) or SEQ ID NO: 57, or biotin-binding fragment thereof; (b) a first polypeptide linker comprising the amino acid sequence of SEQ ID NO: 30 (GGGGSSS); (c) an pneumolysin (Ply) polypeptide described herein; (d) a second polypeptide linker comprising the amino acid sequence of SEQ ID NO: 30 (GGGGSSS); and (e) an SP0435 polypeptide described herein. In some embodiments, a SPP2

fusion protein comprises, from N-terminus to C-terminus, (a) a biotin-binding moiety that is or comprises the polypeptide of SEQ ID NO: 1 (rhizavidin), SEQ ID NO: 2 (amino acids 45-179 of rhizavidin), or SEQ ID NO: 57, or biotin-binding fragment thereof; (b) a first polypeptide linker comprising the amino acid sequence of SEQ ID NO: 30 (GGGGSSS); (c) an pneumolysin (Ply) polypeptide described herein; (d) a second polypeptide linker comprising the amino acid sequence of SEQ ID NO: 30 (GGGGSSS); and (e) an SP0435 polypeptide described herein. In some embodiments, such a SPP2 fusion protein may further comprise a detection or purification tag, *e.g.*, a His tag. In some embodiments, a SPP2 fusion protein comprising a His tag is referred to as SPP2-H. In some such embodiments, a SPP2 fusion protein comprises an SP0435 polypeptide (*e.g.*, ones described herein) between a biotin-binding moiety and a Ply polypeptide (*e.g.*, ones described herein). In some embodiments, a SPP2 fusion protein may comprise a Ply polypeptide between a biotin-binding moiety and a SP0435 polypeptide. In some embodiments, a Ply polypeptide included in a fusion protein described herein is or comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 6 (amino acids 2-470 of *S. pneumoniae* PdT(G294P)) or an antigenic variant or fragment thereof. In some embodiments, an SP0435 polypeptide included in a fusion protein described herein is or comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 8 (amino acids 62-185 of *S. pneumoniae* SP0435) or an antigenic variant or fragment thereof. In some embodiments, a fusion protein described herein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence SEQ ID NO: 19 or SEQ ID NO: 58. In some embodiments, a fusion protein described herein comprises the amino acid sequence SEQ ID NO: 19 or SEQ ID NO: 58. In some embodiments, a fusion protein described herein consists of the amino acid sequence SEQ ID NO: 19 or SEQ ID NO: 58.

Fusion Protein CPI

[0251] In some embodiments, a fusion protein described herein is or comprises a complementary affinity molecule described herein (*e.g.*, a biotin-binding moiety described

herein), and one or more polypeptides of or derived from *S. pneumoniae*. In some embodiments, a fusion protein described herein is CP1, further described in the International Patent Publication No. WO 2020/056127, the contents of which are incorporated herein by reference in their entirety for the purposes described herein. In some embodiments, a fusion protein comprises a complementary affinity molecule described herein (*e.g.*, a biotin-binding moiety described herein) and a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 10 (amino acids 33-399 of *S. pneumoniae* SP0785) or an antigenic variant or fragment thereof and a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 12 (amino acids 27-278 of *S. pneumoniae* SP1500) or an antigenic variant or fragment thereof. In some embodiments, a fusion protein comprises a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 26 or SEQ ID NO: 61. In some embodiments, a fusion protein is or comprises a complementary affinity molecule described herein (*e.g.*, a biotin-binding moiety described herein), a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SP0785 or an antigenic variant or fragment thereof, and a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SP1500 or an antigenic variant or fragment thereof.

[0252] In some embodiments, a fusion protein described herein comprises a complementary affinity molecule described herein (*e.g.*, a biotin-binding moiety described herein) and a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 10 (amino acids 33-399 of *S. pneumoniae* SP0785) or an antigenic variant or fragment thereof. In some embodiments, a fusion protein comprises a complementary affinity molecule described herein (*e.g.*, a biotin-binding moiety described herein) and a polypeptide comprising an amino acid sequence having at least 80%, at

least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 12 (amino acids 27-278 of *S. pneumoniae* SP1500) or an antigenic variant or fragment thereof.

[0253] In some embodiments, a fusion protein described herein comprises a biotin-binding moiety that is or comprises a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the sequence of SEQ ID NO: 1 (rhizavidin), or biotin-binding fragment thereof. In some embodiments, a fusion protein comprises a biotin-binding moiety that is or comprises a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the sequence of SEQ ID NO: 2 (amino acids 45-179 of rhizavidin) or SEQ ID NO: 57, or biotin-binding fragment thereof. In some embodiments, a fusion protein comprises a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 10 (amino acids 33-399 of *S. pneumoniae* SP0785) or an antigenic variant or fragment thereof. In some embodiments, a fusion protein comprises a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 12 (amino acids 27-278 of *S. pneumoniae* SP1500) or an antigenic variant or fragment thereof.

[0254] In some embodiments, a fusion protein described herein comprises each of: (a) a biotin-binding moiety that is or comprises a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the sequence of SEQ ID NO: 1 (rhizavidin), or biotin-binding fragment thereof; (b) a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 10 (amino acids 33-399 of *S. pneumoniae* SP0785) or an antigenic variant or fragment thereof; and (c) a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 12 (amino

acids 27-278 of *S. pneumoniae* SP1500) or an antigenic variant or fragment thereof. In some embodiments, such a fusion protein further comprises one or more linkers.

[0255] In some embodiments, a fusion protein described herein comprises each of: (a) a biotin-binding moiety that is or comprises a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the sequence of SEQ ID NO: 2 (amino acids 45-179 of rhizavidin) or SEQ ID NO: 57, or biotin-binding fragment thereof; (b) a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 10 (amino acids 33-399 of *S. pneumoniae* SP0785) or an antigenic variant or fragment thereof; and (c) a polypeptide comprising an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 12 (amino acids 27-278 of *S. pneumoniae* SP1500) or an antigenic variant or fragment thereof. In some embodiments, such a fusion protein further comprises one or more linkers. In some embodiments, a fusion protein described herein is or comprises a CP1 fusion protein. In some embodiments, a CP1 fusion protein comprises (a) a biotin-binding moiety that is or comprises a polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to the sequence of SEQ ID NO: 1 (rhizavidin) or to the sequence of SEQ ID NO: 2 (amino acids 45-179 of rhizavidin) or SEQ ID NO: 57, or biotin-binding fragment thereof; (b) a first polypeptide linker comprising the amino acid sequence of SEQ ID NO: 30 (GGGGSSS); (c) an SP0785 polypeptide described herein; (d) a second polypeptide linker comprising the amino acid sequence of SEQ ID NO: 31 (AAA); and (e) an SP1500 polypeptide described herein. In some embodiments, a CP1 fusion protein comprises, from N-terminus to C-terminus, (a) a biotin-binding moiety that is or comprises the polypeptide of SEQ ID NO: 1 (rhizavidin), SEQ ID NO: 2 (amino acids 45-179 of rhizavidin), or SEQ ID NO: 57, or biotin-binding fragment thereof; (b) a first polypeptide linker comprising the amino acid sequence of SEQ ID NO: 30 (GGGGSSS); (c) an SP1500 polypeptide described herein; (d) a second polypeptide linker comprising the amino acid sequence of SEQ ID NO: 31 (AAA); and (e) an SP0785 polypeptide described herein. In some embodiments, such a CP1 fusion protein may further comprise a detection or purification tag, *e.g.*, a His tag. In some

such embodiments, a CP1 fusion protein comprises an SP1500 polypeptide between a biotin-binding moiety and an SP0785 polypeptide. In some embodiments, a CP1 fusion protein may comprise an SP0785 polypeptide between a biotin-binding moiety and a SP1500 polypeptide. In some embodiments, an SP0785 polypeptide included in a fusion protein described herein is or comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 10 (amino acids 33-399 of *S. pneumoniae* SP0785) or an antigenic variant or fragment thereof. In some embodiments, an SP1500 polypeptide included in a fusion protein described herein is or comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence of SEQ ID NO: 12 (amino acids 27-278 of *S. pneumoniae* SP1500) or an antigenic variant or fragment thereof. In some embodiments, a fusion protein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to the sequence SEQ ID NO: 26 or SEQ ID NO: 61. In some embodiments, a fusion protein comprises the amino acid sequence SEQ ID NO: 26 or SEQ ID NO: 61. In some embodiments, a fusion protein consists of the amino acid sequence SEQ ID NO: 26 or SEQ ID NO: 61.

Carrier Proteins

[0256] In some embodiments, a multivalent pneumococcal immunogenic composition (e.g., vaccine) as described herein comprises one or more carrier proteins. In some embodiments, a carrier protein is or comprises an antigenic polypeptide (e.g., ones described herein including, e.g., SP0785 as described herein, SP1500 as described herein, SP0435 as described herein, Ply as described herein or in WO 05/76696, WO 05/108580, or WO 10/71986, etc.) and/or a fusion protein that includes one or more antigenic polypeptides (e.g., ones described herein including, e.g., CP1 as described herein, SPP2 as described herein, etc.). Additional exemplary carrier proteins include, but are not limited to PspA as described in WO 19/167008; CRM197 as described in WO 14/92378; variants of CRM197 such as eCRM197 as disclosed in WO 22/178015; Diphtheria toxoid; variants of Diphtheria toxoid as described in WO18/156465;

Tetanus toxoid as described in WO 19/152921; and Protein D as described in US5858677, and combinations thereof.

[0257] In some embodiments, a fusion protein described herein may comprise a carrier protein.

Antigenic Polysaccharides

[0258] In some embodiments, an immunogenic complex described herein includes one or more *S. pneumoniae* polysaccharides. In some embodiments, an immunogenic complex described herein includes one *S. pneumoniae* polysaccharide. Capsular polysaccharides are used to distinguish serotypes of *S. pneumoniae*. There are at least 97 distinct serotypes of *S. pneumoniae* polysaccharides, each having a different chemical structure. Serotype designations as used herein are designations according to Danish nomenclature [Kauffmann et al, 1960; Geno et al, 2015]. (N.B. Serotype 20 in U.S. nomenclature is referred to as serotype 20A in Danish nomenclature.)

[0259] In some embodiments, an immunogenic complex includes one or more *S. pneumoniae* capsular polysaccharides from, or derived from, one or more *S. pneumoniae* serotypes selected from 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48.

[0260] In some embodiments, an immunogenic complex includes one or more *S. pneumoniae* capsular polysaccharides from, or derived from, one or more *S. pneumoniae* serotypes selected from 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.

[0261] In some embodiments, an immunogenic complex does not include a *S. pneumoniae* capsular polysaccharide from, or derived from, *S. pneumoniae* serotype 3. In some embodiments, an immunogenic complex does not include a *S. pneumoniae* capsular

polysaccharide from, or derived from, *S. pneumoniae* serotype 16F. In some embodiments, an immunogenic complex does not include a *S. pneumoniae* capsular polysaccharide from, or derived from, *S. pneumoniae* serotype 31.

[0262] In some embodiments, an immunogenic complex includes one or more *S. pneumoniae* capsular polysaccharides from, or derived from, one or more *S. pneumoniae* serotypes selected from 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.

[0263] In some embodiments, an immunogenic complex includes one or more *S. pneumoniae* capsular polysaccharides from, or derived from, one or more *S. pneumoniae* serotypes selected from 1, 2, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.

Methods of Isolating and Purifying Polysaccharides

[0264] In some embodiments, the disclosure provides methods of purifying one or more polysaccharides described herein from one or more cellular components of bacteria. In some embodiments, methods comprise purifying capsular polysaccharides from one or more cellular components of bacteria.

[0265] In some embodiments, the bacteria are Gram-negative. In some embodiments, the bacteria are Gram-positive. In some embodiments, the bacteria are *S. pneumoniae*.

[0266] In some embodiments, the cellular components include protein. In some embodiments, the cellular proteins include nucleic acid. In some embodiments, the cellular components include lipids. In some embodiments, the cellular components include polysaccharides. In some embodiments, the cellular components are part of a lysate.

[0267] In some embodiments, the polysaccharide purification processes incorporate a series of ethanol precipitations, washes of crude polysaccharide preparations with ethanol, diethyl ether, and/or acetone, and drying under vacuum to furnish purified products. In some embodiments, a phenol extraction step is incorporated for polysaccharide purifications. In some embodiments, the purification process employs a CTAB (cetyltrimethyl ammonium bromide) precipitation step in addition to using ethanol and phenol precipitation steps.

[0268] Figure 9 depicts exemplary structures and chemical information for *S. pneumoniae* capsular polysaccharides included in immunogenic complexes of the present disclosure. All structures are from European Pharmacopoeia 9.0.

Methods of Biotinylating Polysaccharides

[0269] In some embodiments, the disclosure provides methods of biotinylating one or more polysaccharides described herein. In some embodiments, the method comprises reacting purified polysaccharides with 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP) for activation of hydroxyl groups in the polysaccharides followed by the addition of amine PEG biotin under conditions that result in covalent linkage of biotin to the polysaccharides. In some embodiments, the desired level of biotinylation is achieved by varying the ratio of CDAP to polysaccharide. In some embodiments, the biotinylated polysaccharides are purified by filtration to remove process residuals such as unreacted biotin, dimethylaminopyridine, acetonitrile, cyanide and unreacted glycine. In some embodiments, the level of polysaccharide biotinylation described herein is optimized to reduce the amount of accessible biotin following MAPS complexation.

Manufacture of Immunogenic Complexes

[0270] The present disclosure includes methods for manufacturing immunogenic complexes described herein. In some embodiments, a method of manufacturing immunogenic complexes comprises complexing at least one biotinylated polysaccharide with at least one biotin-binding fusion protein. In some embodiments, the fusion protein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to SEQ ID NO: 19 or SEQ ID NO: 58. In some embodiments, the fusion protein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identity to SEQ ID NO: 26 or SEQ ID NO: 61.

[0271] In some embodiments, the average (*e.g.*, the mean) protein (*e.g.*, antigenic protein) to polysaccharide ratio of a plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1, 7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or

10:1 (weight/weight [w/w]). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average protein to polysaccharide ratio of a plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions (*e.g.*, vaccines) of the present disclosure may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0272] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises a plurality of immunogenic complexes comprising a fusion protein and a capsular polysaccharide. In some embodiments, the fusion protein is a SPP2 protein. In some embodiments, the fusion protein is a CP1 protein. In some embodiments, the capsular polysaccharide is from or derived from *S. pneumoniae* having a serotype selected from 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some embodiments, the capsular polysaccharide is from or derived from *S. pneumoniae* having a serotype selected a group that includes 30, 31, 32, or 33 serotypes selected

from 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some embodiments, the capsular polysaccharide is from or derived from *S. pneumoniae* having a serotype selected a group that does not include one or more of serotypes 3, 16F, and 31. In some embodiments, the capsular polysaccharide is from or derived from *S. pneumoniae* having a serotype selected from 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some embodiments, the capsular polysaccharide is from or derived from *S. pneumoniae* having a serotype selected from 1, 2, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some embodiments, the average ratio of fusion protein to capsular polysaccharide in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1, 7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of fusion protein to capsular polysaccharide in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of fusion protein to capsular polysaccharide in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of fusion protein to capsular polysaccharide in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of fusion protein to capsular polysaccharide in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of fusion protein to capsular polysaccharide in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of fusion protein to capsular polysaccharide in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of fusion protein to capsular polysaccharide in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of fusion protein to capsular polysaccharide in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of fusion protein to capsular polysaccharide in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of fusion protein to capsular polysaccharide in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of fusion protein to capsular

polysaccharide in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions (*e.g.*, vaccines) of the present disclosure may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0273] In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1. In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1, 7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular

polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions (*e.g.*, vaccines) of the present disclosure may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0274] In some embodiments, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B. In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1, 7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some

embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions (*e.g.*, vaccines) of the present disclosure may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0275] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V. In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1, 7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of

immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions (*e.g.*, vaccines) of the present disclosure may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0276] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B. In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1, 7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide

from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions (*e.g.*, vaccines) of the present disclosure may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0277] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F. In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in

the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1, 7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions (*e.g.*,

vaccines) of the present disclosure may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0278] In some embodiments, an immunogenic composition (e.g., a vaccine) comprises a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A. In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1, 7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some

embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions (*e.g.*, vaccines) of the present disclosure may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0279] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B. In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1, 7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of

immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions (*e.g.*, vaccines) of the present disclosure may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0280] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F. In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1, 7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from

S. pneumoniae serotype 23F in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions (*e.g.*, vaccines) of the present disclosure may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0281] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F. In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1, 5:1, 5.5:1, 6:1, 6.5:1, 7:1, 7.5:1, 8:1, 8.5:1, 9:1, 9.5:1, or 10:1 (weight/weight [w/w]). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 1:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 2:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 3:1 (w/w). In some embodiments, the average ratio of

SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 4:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 5:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 6:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 7:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 8:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 9:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 10:1 (w/w). In some embodiments, the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is chosen to enhance the polysaccharide immunogenicity potential (carrier function) and/or to elicit protection against, or to inhibit, pneumococcal colonization by any pneumococcus (independent of polysaccharide serotype) through a protein-specific immune response. Immunogenic compositions (*e.g.*, vaccines) of the present disclosure may comprise mixtures of immunogenic complexes with different average protein to polysaccharide ratios.

[0282] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises:

- (1) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (2) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of

SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(3) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(4) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 16F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 16F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(5) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(6) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(7) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(8) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(9) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of

SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(10) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w); and

(34) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w).

[0283] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises:

(1) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(2) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

- (3) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (4) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (5) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (6) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (7) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (8) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (9) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

- (10) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

- (17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

- (24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 16F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 16F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

- (31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w); and
- (34) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w).

[0284] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises:

- (1) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (2) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (3) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of

SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(4) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(5) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(6) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(7) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(8) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(9) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(10) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of

SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w); and

(33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w).

[0285] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises:

(1) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(2) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(3) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(4) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

- (5) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (6) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (7) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (8) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (9) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (10) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

- (12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

- (19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 16F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 16F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

- (26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w); and

(33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w).

[0286] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises:

- (1) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (2) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (3) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (4) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (5) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);
- (6) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(7) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(8) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(9) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(10) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 16F, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 16F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w);

(33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w); and

(34) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is between about 2.5:1 and about 3.5:1 (w/w).

[0287] In some embodiments, an immunogenic composition (e.g., a vaccine) comprises:

- (1) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (2) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (3) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (4) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 16F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 16F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (5) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

- (6) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (7) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (8) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (9) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (10) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the

plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

- (23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the

plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w); and

(34) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w).

[0288] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises:

- (1) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (2) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (3) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (4) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (5) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

- (6) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (7) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (8) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (9) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (10) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the

plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

- (23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 16F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 16F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the

plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w); and

(34) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w).

[0289] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises:

- (1) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (2) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (3) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (4) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (5) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

- (6) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (7) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (8) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (9) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (10) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the

plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the

plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w); and

(33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w).

[0290] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises:

- (1) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (2) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (3) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (4) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (5) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (6) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in

the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(7) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(8) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(9) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(10) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

- (18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 16F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 16F in the

plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w); and

(33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w).

[0291] In some embodiments, an immunogenic composition (e.g., a vaccine) comprises:

(1) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

- (2) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (3) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (4) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (5) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (6) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (7) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the

plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(8) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(9) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(10) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

- (19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 16F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 16F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);
- (24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the

plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w);

(33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w); and

[0292] (34) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is approximately 2.5:1, 2.6:1, 2.7:1, 2.8:1, 2.9:1, 3:1, 3.1:1, 3.2:1, 3.3:1, 3.4:1, or 3.5:1 (w/w). In some embodiments, an immunogenic composition (e.g., a vaccine) comprises:

(1) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

- (2) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (3) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (4) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 16F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 16F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (5) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (6) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (7) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (8) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

- (9) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (10) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

- (16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

- (23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

- (30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w); and
- (34) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w).

[0293] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises:

- (1) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (2) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of

SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(3) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(4) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(5) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(6) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(7) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(8) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(9) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of

SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(10) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 16F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 16F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w); and

(34) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w).

[0294] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises:

(1) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(2) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

- (3) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (4) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (5) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (6) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (7) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (8) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (9) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

- (10) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

- (17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

- (24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

- (31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w); and
- (33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w).

[0295] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises:

- (1) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (2) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (3) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (4) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of

SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(5) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(6) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(7) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(8) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(9) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(10) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 16F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 16F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w); and

(33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w).

[0296] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises:

(1) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(2) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(3) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(4) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(5) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

- (6) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (7) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (8) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (9) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (10) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

- (13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

- (20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 16F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 16F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

- (27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);
- (33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w);

(34) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is between about 2.9:1 and about 3.1:1 (w/w).

[0297] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises:

- (1) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is about 3:1;
- (2) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is about 3:1;
- (3) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is about 3:1;
- (4) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 16F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 16F in the plurality of immunogenic complexes is about 3:1;
- (5) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is about 3:1;
- (6) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of

SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is about 3:1;

(7) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is about 3:1;

(8) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is about 3:1;

(9) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is about 3:1;

(10) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is about 3:1;

(11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is about 3:1;

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is about 3:1;

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is about 3:1;

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is about 3:1;

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is about 3:1;

(16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is about 3:1;

(17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is about 3:1;

(18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is about 3:1;

(19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is about 3:1;

(20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is about 3:1;

(21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is about 3:1;

(22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is about 3:1;

(23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is about 3:1;

(24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is about 3:1;

(25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is about 3:1;

(26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is about 3:1;

(27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is about 3:1;

(28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is about 3:1;

(29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is about 3:1;

(30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is about 3:1;

(31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is about 3:1;

(32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is about 3:1;

(33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is about 3:1; and

(34) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is about 3:1.

[0298] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises:

- (1) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is about 3:1;
- (2) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is about 3:1;
- (3) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is about 3:1;
- (4) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is about 3:1;
- (5) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is about 3:1;
- (6) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is about 3:1;

- (7) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is about 3:1;
- (8) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is about 3:1;
- (9) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is about 3:1;
- (10) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is about 3:1;
- (11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is about 3:1;
- (12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is about 3:1;
- (13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is about 3:1;

- (14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is about 3:1;
- (15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is about 3:1;
- (16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is about 3:1;
- (17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is about 3:1;
- (18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is about 3:1;
- (19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is about 3:1;
- (20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is about 3:1;

- (21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is about 3:1;
- (22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is about 3:1;
- (23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is about 3:1;
- (24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 16F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 16F in the plurality of immunogenic complexes is about 3:1;
- (25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is about 3:1;
- (26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is about 3:1;
- (27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is about 3:1;

- (28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is about 3:1;
- (29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is about 3:1;
- (30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is about 3:1;
- (31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is about 3:1;
- (32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is about 3:1;
- (33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is about 3:1; and
- (34) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is about 3:1.

[0299] In some embodiments, an immunogenic composition (e.g., a vaccine) comprises:

- (1) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is about 3:1;
- (2) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is about 3:1;
- (3) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is about 3:1;
- (4) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is about 3:1;
- (5) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is about 3:1;
- (6) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is about 3:1;
- (7) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of

SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is about 3:1;

(8) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is about 3:1;

(9) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is about 3:1;

(10) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is about 3:1;

(11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is about 3:1;

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is about 3:1;

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is about 3:1;

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is about 3:1;

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is about 3:1;

(16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is about 3:1;

(17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is about 3:1;

(18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is about 3:1;

(19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is about 3:1;

(20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is about 3:1;

(21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is about 3:1;

(22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is about 3:1;

(23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is about 3:1;

(24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is about 3:1;

(25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is about 3:1;

(26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is about 3:1;

(27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is about 3:1;

(28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is about 3:1;

(29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is about 3:1;

(30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is about 3:1;

(31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is about 3:1;

(32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is about 3:1; and

(33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is about 3:1.

[0300] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises:

(1) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is about 3:1;

- (2) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is about 3:1;
- (3) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is about 3:1;
- (4) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is about 3:1;
- (5) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is about 3:1;
- (6) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is about 3:1;
- (7) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is about 3:1;
- (8) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is about 3:1;

- (9) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is about 3:1;
- (10) a plurality of immunogenic complexes comprising a SPP2 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of SPP2 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is about 3:1;
- (11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is about 3:1;
- (12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is about 3:1;
- (13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is about 3:1;
- (14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is about 3:1;
- (15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is about 3:1;

- (16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is about 3:1;
- (17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is about 3:1;
- (18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is about 3:1;
- (19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is about 3:1;
- (20) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is about 3:1;
- (21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is about 3:1;
- (22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is about 3:1;

- (23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 16F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 16F in the plurality of immunogenic complexes is about 3:1;
- (24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is about 3:1;
- (25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is about 3:1;
- (26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is about 3:1;
- (27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is about 3:1;
- (28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is about 3:1;
- (29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is about 3:1;

- (30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is about 3:1;
- (31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is about 3:1;
- (32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is about 3:1; and
- (33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is about 3:1.

[0301] In some embodiments, an immunogenic composition (*e.g.*, a vaccine) comprises:

- (1) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 1, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 1 in the plurality of immunogenic complexes is about 3:1;
- (2) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 2, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 2 in the plurality of immunogenic complexes is about 3:1;
- (3) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 3, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 3 in the plurality of immunogenic complexes is about 3:1;

(4) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 4, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 4 in the plurality of immunogenic complexes is about 3:1;

(5) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 5, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 5 in the plurality of immunogenic complexes is about 3:1;

(6) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6A in the plurality of immunogenic complexes is about 3:1;

(7) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6B in the plurality of immunogenic complexes is about 3:1;

(8) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 6C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 6C in the plurality of immunogenic complexes is about 3:1;

(9) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7C in the plurality of immunogenic complexes is about 3:1;

(10) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 7F, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 7F in the plurality of immunogenic complexes is about 3:1;

(11) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 8, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 8 in the plurality of immunogenic complexes is about 3:1;

(12) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9N, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9N in the plurality of immunogenic complexes is about 3:1;

(13) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 9V, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 9V in the plurality of immunogenic complexes is about 3:1;

(14) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 10A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 10A in the plurality of immunogenic complexes is about 3:1;

(15) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 11A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 11A in the plurality of immunogenic complexes is about 3:1;

(16) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 12F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 12F in the plurality of immunogenic complexes is about 3:1;

(17) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 14, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 14 in the plurality of immunogenic complexes is about 3:1;

(18) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15A in the plurality of immunogenic complexes is about 3:1;

(19) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 15B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 15B in the plurality of immunogenic complexes is about 3:1;

(29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 16F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 16F in the plurality of immunogenic complexes is about 3:1;

(21) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 17F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 17F in the plurality of immunogenic complexes is about 3:1;

(22) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 18C, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 18C in the plurality of immunogenic complexes is about 3:1;

(23) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19A in the plurality of immunogenic complexes is about 3:1;

(24) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 19F, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 19F in the plurality of immunogenic complexes is about 3:1;

(25) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 20B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 20B in the plurality of immunogenic complexes is about 3:1;

(26) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 22F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 22F in the plurality of immunogenic complexes is about 3:1;

(27) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23A, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23A in the plurality of immunogenic complexes is about 3:1;

(28) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23B in the plurality of immunogenic complexes is about 3:1;

(29) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 23F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 23F in the plurality of immunogenic complexes is about 3:1;

(30) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 24F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 24F in the plurality of immunogenic complexes is about 3:1;

(31) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 31, wherein the average ratio of

CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 31 in the plurality of immunogenic complexes is about 3:1;

(32) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 33F, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 33F in the plurality of immunogenic complexes is about 3:1;

(33) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 35B, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 35B in the plurality of immunogenic complexes is about 3:1; and

(34) a plurality of immunogenic complexes comprising a CP1 protein and a capsular polysaccharide, from or derived from *S. pneumoniae* serotype 38, wherein the average ratio of CP1 protein to capsular polysaccharide from or derived from *S. pneumoniae* serotype 38 in the plurality of immunogenic complexes is about 3:1.

Immunogenic Compositions (e.g., Vaccines)

[0302] Another aspect of the disclosure provides compositions that include one or more immunogenic complexes described herein. For example, an immunogenic composition, *e.g.*, vaccine composition, can include one or more immunogenic complexes described herein. In some embodiments, such compositions can include a plurality of one type or species of immunogenic complex described herein. For example, a composition can include a population of one type or species of immunogenic complex, where all of the immunogenic complexes include the same antigenic polypeptide and the same antigenic polysaccharide. Additionally or alternatively, such compositions can include a plurality of more than one type or species of immunogenic complex described herein. For example, a composition can include populations of different types or species of immunogenic complexes. In some embodiments, a composition can include a population of a first type or species of immunogenic complex and a population of a second type or species of immunogenic complex, where the first type or species and the second

type or species of the immunogenic complex have different antigenic polypeptides and/or different antigenic polysaccharides. In some embodiments, a composition can include a population of a first type or species of immunogenic complex and a population of a second type or species of immunogenic complex, where the first type and the second type or species of the immunogenic complex include different antigenic polypeptides and different antigenic polysaccharides (*e.g.*, polysaccharides of different serotypes). In some embodiments, a composition can include a population of a first type or species of immunogenic complex and a population of a second type or species of immunogenic complex, where the first type and the second type or species of the immunogenic complex include the same antigenic polypeptide and different antigenic polysaccharides (*e.g.*, polysaccharides of different serotypes). In some embodiments, immunogenic complexes described herein are formulated into a pharmaceutical composition. In some embodiments, a pharmaceutical composition may be a vaccine. In some embodiments, a pharmaceutical composition comprises a pharmaceutically acceptable carrier. In some embodiments, a pharmaceutical composition comprises an adjuvant.

[0303] In some embodiments, an immunogenic composition comprises one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, or more) species of immunogenic complexes selected from:

- (1) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 1 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (2) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 2 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (3) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 3 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (4) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 4 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (5) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 5 capsular polysaccharide, and one or more CP1 SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (6) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (7) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6B capsular polysaccharide, and one or more or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (8) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6C capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (9) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6D capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (10) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6E capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (11) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (12) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6G capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (13) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6H capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (14) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (15) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (16) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7C capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (17) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (18) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 8 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (19) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (20) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9L capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (21) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9N capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (22) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9V capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (23) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (24) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (25) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10C capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (26) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (27) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (28) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (29) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11C capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (30) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11D capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (31) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11E capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (32) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (33) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 12A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (34) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 12B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (35) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 12F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (36) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 13 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (37) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 14 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (38) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (39) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (40) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15C capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (41) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (42) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 16A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (43) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 16F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (44) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 17A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (45) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 17F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (46) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (47) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (48) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18C capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (49) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (50) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19A capsular polysaccharide, and one or more CP1 SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (51) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (52) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19C capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (53) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (54) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 20A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (55) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 20B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (56) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 21 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (57) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 22A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (58) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 22F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (59) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (60) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (61) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (62) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 24A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (63) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 24B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (64) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 24F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (65) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 25A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (66) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 25F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (67) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 27 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (68) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 28A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (69) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 28F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (70) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 29 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (71) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 31 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (72) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 32A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (73) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 32F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (74) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (75) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (76) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33C capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (77) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33D capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (78) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33E capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (79) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (80) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 34 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (81) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 35A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (82) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 35B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (83) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 35C capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (84) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 35F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (85) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 36 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (86) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 37 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (87) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 38 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (88) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 39 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (89) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 40 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (90) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 41A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (91) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 41F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (92) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 42 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (93) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 43 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (94) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 44 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (95) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 45 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (96) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 46 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (97) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 47A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (98) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 47F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide; and
- (99) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 48 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide.

[0304] In some embodiments, an immunogenic composition comprises one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, or 34) species of immunogenic complexes selected from:

- (1) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 1 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (2) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 2 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (3) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 3 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (4) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 4 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (5) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 5 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (6) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (7) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (8) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6C capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (9) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7C capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (10) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (11) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 8 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (12) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9N capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (13) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9V capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (14) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (15) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (16) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 12F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (17) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 14 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (18) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (19) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (20) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 16F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (21) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 17F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (22) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18C capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (23) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (24) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (25) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 20B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (26) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 22F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (27) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (28) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (29) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (30) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 24F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (31) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 31 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (32) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (33) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 35B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide; and
- (34) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 38 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide.

[0305] In some embodiments, an immunogenic composition comprises one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, or 33) species of immunogenic complexes selected from:

- (1) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 1 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (2) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 2 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (3) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 3 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (4) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 4 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (5) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 5 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (6) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (7) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (8) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6C capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (9) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7C capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (10) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (11) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 8 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (12) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9N capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (13) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9V capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (14) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (15) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (16) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 12F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (17) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 14 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (18) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (19) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (20) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 17F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (21) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18C capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (22) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (23) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (24) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 20B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (25) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 22F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (26) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (27) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (28) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (29) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 24F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (30) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 31 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (31) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(32) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 35B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide; and

(33) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 38 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide.

[0306] In some embodiments, an immunogenic composition comprises one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, or 33) species of immunogenic complexes selected from:

(1) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 1 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(2) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 2 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(3) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 4 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(4) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 5 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(5) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

(6) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (7) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 6C capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (8) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7C capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (9) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 7F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (10) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 8 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (11) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9N capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (12) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 9V capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (13) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 10A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (14) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 11A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (15) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 12F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (16) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 14 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (17) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (18) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 15B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (18) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 16F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (20) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 17F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (21) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 18C capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (22) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (23) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 19F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (24) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 20B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;

- (25) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 22F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (26) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23A capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (27) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (28) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 23F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (29) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 24F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (30) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 31 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (31) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 33F capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide;
- (32) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 35B capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide; and
- (33) an immunogenic complex comprising a biotinylated *S. pneumoniae* serotype 38 capsular polysaccharide, and one or more CP1 or SPP2 fusion proteins non-covalently complexed to the biotinylated polysaccharide.

Vaccine compositions

[0307] In some embodiments, a vaccine composition is a polyvalent or multivalent vaccine. In some embodiments, the valency of a vaccine composition refers to the number of species of immunogenic complexes present in the vaccine composition. The valency of a vaccine described herein is not limiting with respect to the total antigens present in said pharmaceutical composition, immunogenic complex, or vaccine, or to the number of pathogen strains for which administration of said pharmaceutical composition, immunogenic complex, immunogenic composition, or vaccine composition may induce an immune-protective response. In a non-limiting example, a 34-valent vaccine composition may comprise more than 34 antigenic components (*e.g.*, peptide and/or polysaccharide components) and may induce an immunoprotective response against more than 34 pathogens, or pathogenic serotypes or strains.

[0308] In some embodiments, a vaccine composition comprises between 1-60 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises between 1-50 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises between 1-45 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises between 1-40 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises between 1-40 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises between 1-34 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises between 1-33 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises between 1-32, 1-31, or 1-30 species of immunogenic complexes described herein. In some embodiments, a vaccine is a polyvalent vaccine. In some embodiments, a vaccine composition comprises at least 30 (including, *e.g.*, at least 31, at least 32, at least 33, at least 34) species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 30-40 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 30-35 species of immunogenic complexes described herein.

[0309] In some embodiments, a multivalent pneumococcal composition comprises between 1-60 serotypes of *S. pneumoniae* capsular polysaccharides described herein. In some embodiments, a multivalent pneumococcal composition comprises between 1-50 serotypes of *S. pneumoniae* capsular polysaccharides described herein. In some embodiments, a multivalent pneumococcal composition comprises between 1-45 serotypes of *S. pneumoniae* capsular polysaccharides described herein. In some embodiments, a multivalent pneumococcal composition comprises between 1-40 serotypes of *S. pneumoniae* capsular polysaccharides described herein. In some embodiments, a multivalent pneumococcal composition comprises between 1-34 serotypes of *S. pneumoniae* capsular polysaccharides described herein. In some embodiments, a multivalent pneumococcal composition comprises between 1-33 serotypes of *S. pneumoniae* capsular polysaccharides described herein. In some embodiments, a multivalent pneumococcal composition comprises between 1-32, 1-31, or 1-30 serotypes of *S. pneumoniae* capsular polysaccharides described herein. In some embodiments, a multivalent pneumococcal composition comprises at least 30 (including, e.g., at least 31, at least 32, at least 33, at least 34) serotypes of *S. pneumoniae* capsular polysaccharides described herein. In some embodiments, a multivalent pneumococcal composition comprises 30-40 serotypes of *S. pneumoniae* capsular polysaccharides described herein. In some embodiments, a multivalent pneumococcal composition comprises 30-35 serotypes of *S. pneumoniae* capsular polysaccharides described herein. In some embodiments, one or more serotypes of *S. pneumoniae* capsular polysaccharides described herein in a multivalent pneumococcal composition are non-covalently associated with one or more carrier proteins, antigenic polypeptides, and/or fusion proteins described herein. In some embodiments, one or more serotypes of *S. pneumoniae* capsular polysaccharides described herein in a multivalent pneumococcal composition are covalently associated with one or more carrier proteins, antigenic polypeptides and/or fusion proteins described herein.

[0310] In some embodiments, a vaccine composition comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, or 60 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 1 species of immunogenic complex described herein. In some embodiments, a vaccine composition comprises 2 species of immunogenic complexes described

herein. In some embodiments, a vaccine composition comprises 4 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 6 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 7 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 8 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 9 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 10 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 11 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 12 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 13 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 14 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 15 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 16 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 17 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 18 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 19 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 20 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 21 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 22 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 23 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 24 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 25 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 26 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 27 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 28 species of immunogenic complexes described herein. In some embodiments, a

vaccine composition comprises 29 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 30 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 31 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 32 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 33 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 34 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 35 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 36 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 37 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 38 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 39 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 40 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 45 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 50 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 55 species of immunogenic complexes described herein. In some embodiments, a vaccine composition comprises 60 species of immunogenic complexes described herein.

[0311] In some embodiments, a vaccine composition comprises two or more species of immunogenic complexes (*e.g.*, in immunogenic compositions) in amounts such that the weight of polysaccharides in the vaccine composition from each immunogenic complex is about the same, *e.g.*, present in a w/w ratio of about 1:1. In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 0.20 μg . In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 0.25 μg . In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 0.5 μg . In some embodiments, the weight of polysaccharides in the vaccine contributed by each immunogenic complex is about 1 μg . In some embodiments, the weight of polysaccharides in the vaccine contributed by each

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Claims

We claim:

1. A vaccine comprising one or more species of immunogenic complexes, wherein the immunogenic complex of at least one of the species comprises:
 - (a) a biotinylated polysaccharide antigen; and
 - (b) a fusion protein comprising:
 - (i) a biotin-binding moiety;
 - (ii) a non-hemolytic pneumolysin (Ply) polypeptide comprising mutations at amino acid residues 294, 385, 428, and 433 of wild-type *Streptococcus pneumoniae* pneumolysin or antigenic fragment thereof; and
 - (iii) an SP0435 polypeptide or antigenic fragment thereof;
 wherein the biotinylated polysaccharide antigen is non-covalently associated with the biotin-binding moiety of the fusion protein to form an immunogenic complex.
2. The vaccine of claim 1, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae*.
3. The vaccine of claim 1 or claim 2, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48.
4. The vaccine of any one of claims 1-3, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.

5. The vaccine of any one of claims 1-3, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.
6. The vaccine of any one of claims 1-3, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from 1, 2, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.
7. The vaccine of any one of claims 1-3, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from 1, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F.
8. The vaccine of any one of claims 1-3, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from 6C, 7C, 15A, 16F, 23A, 23B, 24F, 31, 35B, and 38.
9. A vaccine comprising:
a plurality of immunogenic complexes comprising:
 - (a) a plurality of first biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48; and

(b) a plurality of first fusion proteins, wherein each first fusion protein comprises:

- (i) a biotin-binding moiety;
- (ii) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and
- (iii) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof;

wherein each of the plurality of first biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of first fusion proteins to form an immunogenic complex.

10. The vaccine of claim 9, wherein the plurality of immunogenic complexes further comprises:

(a) a plurality of second biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48; and

(b) a plurality of second fusion proteins, wherein each second fusion protein comprises:

- (i) a biotin-binding moiety;
- (ii) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least

95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 12 or an antigenic fragment thereof; and
 (iii) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof;

wherein each of the plurality of second biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of second fusion proteins to form an immunogenic complex.

11. The vaccine of claim 10, wherein each of the plurality of second fusion proteins, is or comprises an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 26 or SEQ ID NO: 61.

12. The vaccine of any one of claims 1-11, wherein the fusion protein, or each of the plurality of first fusion proteins, is or comprises an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 58, or SEQ ID NO: 59

13. A vaccine comprising a plurality of different species of immunogenic complexes, wherein the different species comprise:

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6C non-covalently complexed with a biotin binding moiety of a first fusion protein;

wherein the first fusion protein comprises:

- (a) a biotin-binding moiety;
- (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at

least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and

- (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7C non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15A non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 16F non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23A non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23B non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 24F non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 31 non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 35B non-covalently complexed with the biotin-binding moiety of the first fusion protein; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 38 non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 1 non-covalently complexed with a second fusion protein;

wherein the second fusion protein comprises:

- (a) a biotin-binding moiety;
- (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at

least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 12 or an antigenic fragment thereof; and

- (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 2 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 3 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 4 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 5 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6A non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6B non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 8 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9N non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9V non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 10A non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 11A non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 12F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 14 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15B non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 17F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 18C non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19A non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 20B non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 22F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23F non-covalently complexed with the biotin-binding moiety of the second fusion protein; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 33F non-covalently complexed with the biotin-binding moiety of the second fusion protein.

14. A vaccine comprising a plurality of different species of immunogenic complexes, wherein the different species comprise:

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 1 non-covalently complexed with a biotin binding moiety of a first fusion protein;

wherein the first fusion protein comprises:

(a) a biotin-binding moiety;

- (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and
- (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 4 non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6A non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6B non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9V non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15B non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 18C non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19A non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23F non-covalently complexed with the biotin-binding moiety of the first fusion protein; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 33F non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 2 non-covalently complexed with a second fusion protein;

wherein the second fusion protein comprises:

- (a) a biotin-binding moiety;

- (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 12 or an antigenic fragment thereof; and
- (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 3 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 5 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6C non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7C non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 8 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9N non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 10A non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 11A non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 12F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 14 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15A non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 16F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 17F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 20B non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 22F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23A non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23B non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 24F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 31 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 35B non-covalently complexed with the biotin-binding moiety of the second fusion protein; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 38 non-covalently complexed with the biotin-binding moiety of the second fusion protein.

15. A vaccine comprising a plurality of different species of immunogenic complexes, wherein the different species comprise:

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 1 non-covalently complexed with a biotin binding moiety of a fusion protein;

wherein the fusion protein comprises:

- (a) a biotin-binding moiety;
- (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 12 or an antigenic fragment thereof; and
- (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 2 non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 3 non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 4 non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 5 non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6A non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6B non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6C non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7C non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7F non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 8 non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9N non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9V non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 10A non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 11A non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 12F non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 14 non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15A non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15B non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 16F non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 17F non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 18C non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19A non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19F non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 20B non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 22F non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23A non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23B non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23F non-covalently complexed with the biotin-binding moiety of the fusion protein; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 24F non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 31 non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 33F non-covalently complexed with the biotin-binding moiety of the fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 35B non-covalently complexed with the biotin-binding moiety of the fusion protein; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 38 non-covalently complexed with the biotin-binding moiety of the fusion protein.

16. A vaccine comprising a plurality of different species of immunogenic complexes, wherein the different species comprise:

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 1 non-covalently complexed with a biotin binding moiety of a first fusion protein;

wherein the first fusion protein comprises:

- (a) a biotin-binding moiety;
- (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and
- (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at

least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 4 non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6A non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6B non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9V non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15B non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 18C non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19A non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23F non-covalently complexed with the biotin-binding moiety of the first fusion protein; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 33F non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 2 non-covalently complexed with a second fusion protein;

wherein the second fusion protein comprises:

- (a) a biotin-binding moiety;
- (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 12 or an antigenic fragment thereof; and
- (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at

least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 3 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 5 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6C non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7C non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 8 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9N non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 10A non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 11A non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 12F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 14 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15A non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 17F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 20B non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 22F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23A non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23B non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 24F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 31 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 35B non-covalently complexed with the biotin-binding moiety of the second fusion protein; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 38 non-covalently complexed with the biotin-binding moiety of the second fusion protein.

17. A vaccine comprising a plurality of different species of immunogenic complexes, wherein the different species comprise:

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 1 non-covalently complexed with a biotin binding moiety of a first fusion protein;

wherein the first fusion protein comprises:

- (a) a biotin-binding moiety;
- (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and
- (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at

least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 4 non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6A non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6B non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9V non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15B non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 18C non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19A non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23F non-covalently complexed with the biotin-binding moiety of the first fusion protein; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 33F non-covalently complexed with the biotin-binding moiety of the first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 2 non-covalently complexed with a second fusion protein;

wherein the second fusion protein comprises:

- (a) a biotin-binding moiety;
- (b) a first polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 12 or an antigenic fragment thereof; and
- (c) a second polypeptide antigen comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at

least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 5 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6C non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7C non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 8 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9N non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 10A non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 11A non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 12F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 14 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15A non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 16F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 17F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 20B non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 22F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23A non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23B non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 24F non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 31 non-covalently complexed with the biotin-binding moiety of the second fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 35B non-covalently complexed with the biotin-binding moiety of the second fusion protein; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 38 non-covalently complexed with the biotin-binding moiety of the second fusion protein.

18. The vaccine of any one of claims 1-17, wherein the biotin-binding moiety is (i) a polypeptide comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 1 or a biotin-binding fragment thereof; or (ii) a polypeptide comprising an amino acid sequence at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 57, or a biotin-binding fragment thereof.

19. A pharmaceutical composition comprising the vaccine of any one of claims 1-18, and a pharmaceutically acceptable carrier.

20. A method of making a vaccine, comprising non-covalently complexing a plurality of first biotinylated polysaccharide antigens with a plurality of first fusion proteins, wherein each first fusion protein comprises:
- (a) a biotin-binding moiety;
 - (b) a pneumolysin (Ply) polypeptide or antigenic fragment thereof; and
 - (c) an SP0435 polypeptide or antigenic fragment thereof.
21. The method of claim 20, further comprising non-covalently complexing a plurality of second biotinylated polysaccharide antigens with a plurality of second fusion proteins, wherein each second fusion protein comprises:
- (a) a biotin-binding moiety;
 - (b) an SP1500 polypeptide or antigenic fragment thereof; and
 - (c) an SP0785 polypeptide or antigenic fragment thereof.
22. A multivalent pneumococcal immunogenic composition comprising *S. pneumoniae* capsular polysaccharides from at least 25, 26, 27, 28, 29, 30, 31, 32, 33, or 34 different pneumococcal serotypes, each associated with a carrier protein.
23. The multivalent pneumococcal immunogenic composition of claim 22, wherein the *S. pneumoniae* capsular polysaccharides comprise at least 30, 31, 32, 33, or 34 capsular polysaccharides selected from *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20 (e.g., 20B), 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.
24. A method of immunizing a subject against *Streptococcus pneumoniae* infection and/or colonization comprising administering to the subject an immunologically effective amount of the vaccine of any one of claims 1-18, the pharmaceutical composition of claim 19, or the multivalent pneumococcal vaccine of claim 22 or 23.

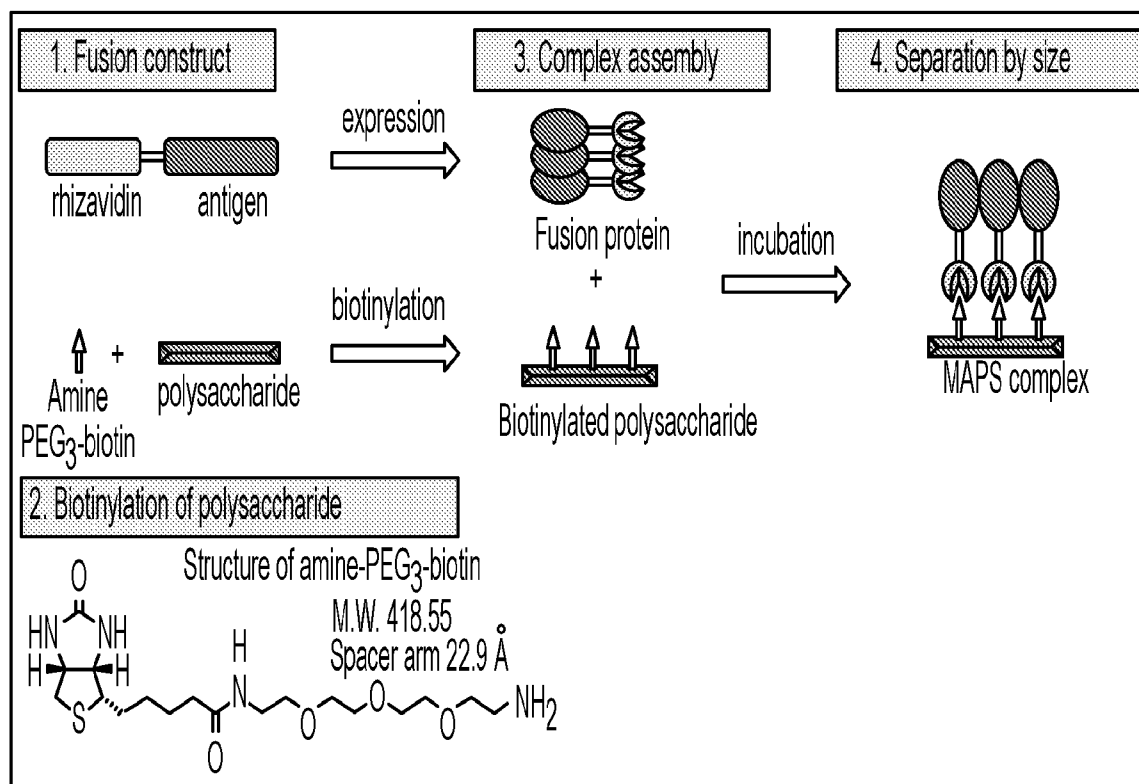


FIG. 1

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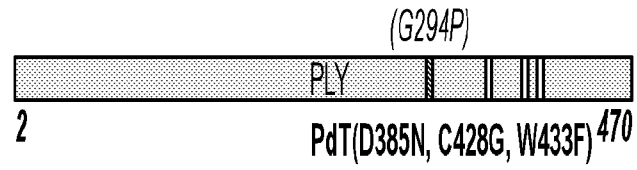


FIG. 2A

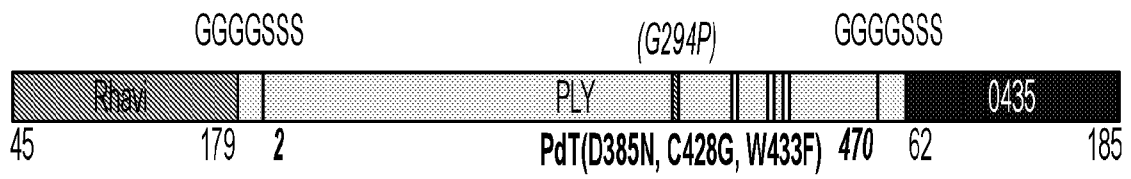


FIG. 2B

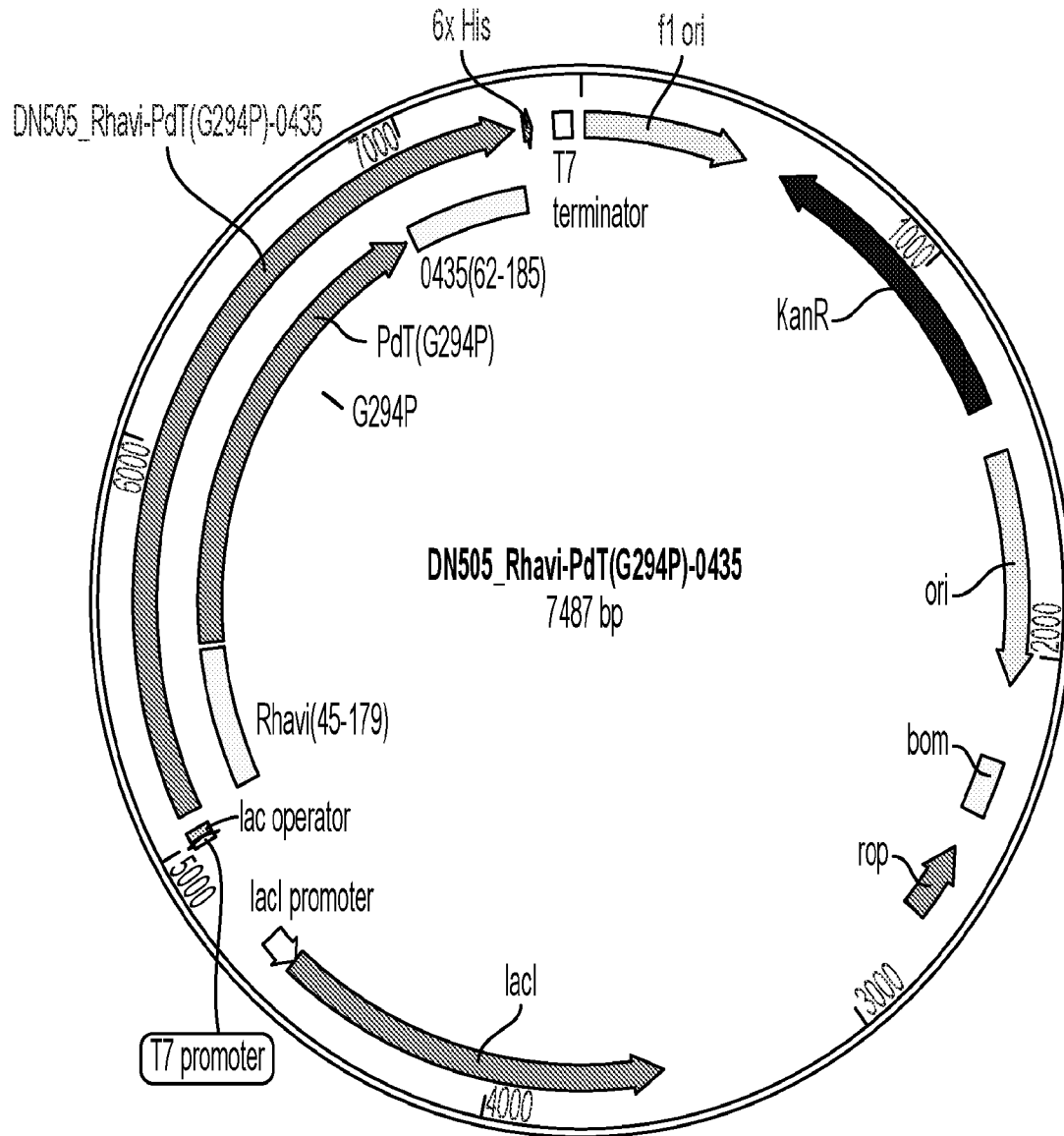


FIG. 3

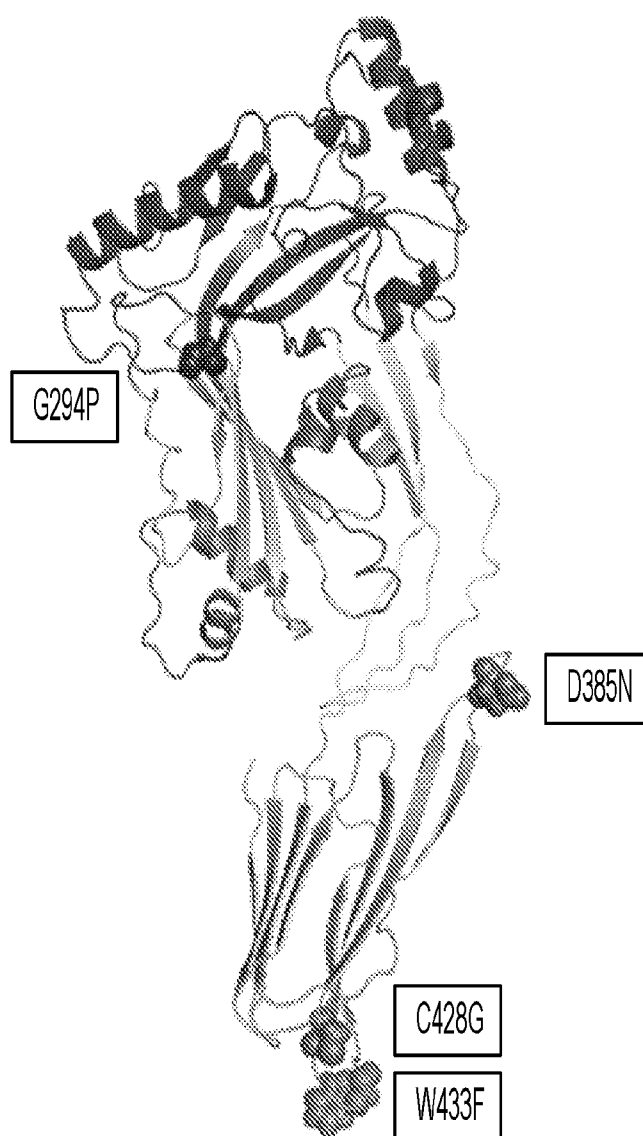


FIG. 4

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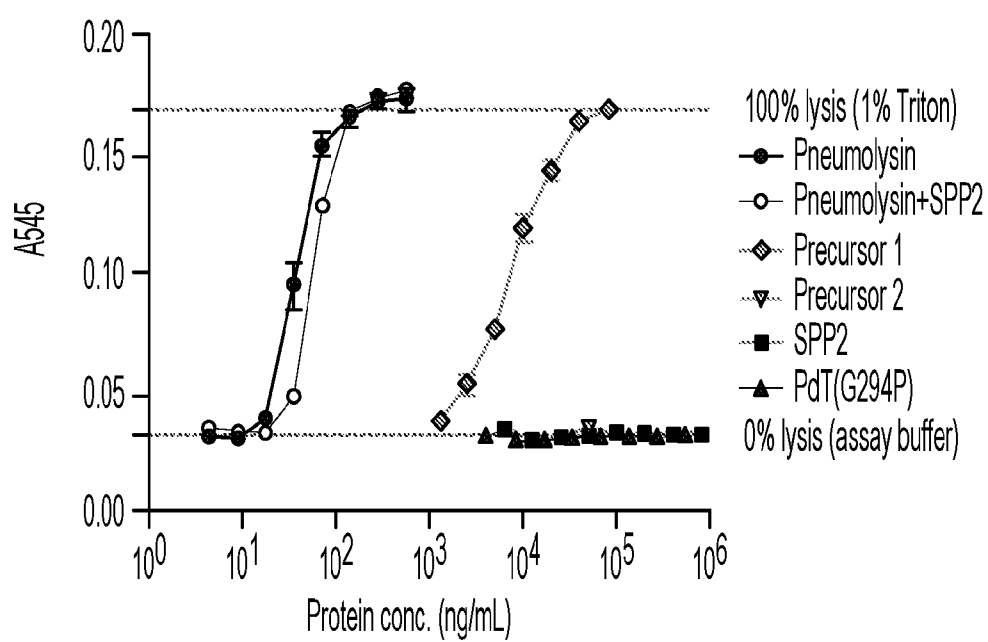


FIG. 5A

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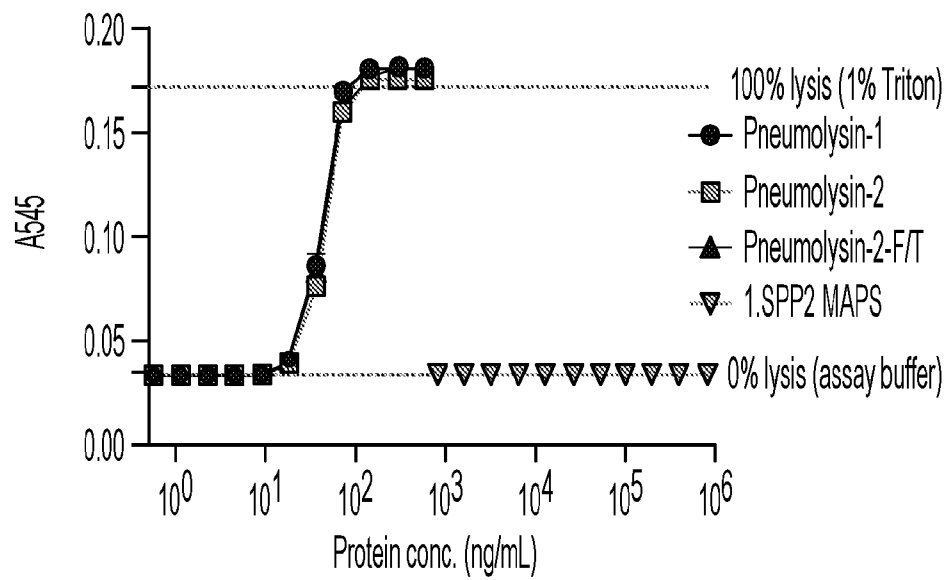


FIG. 5B

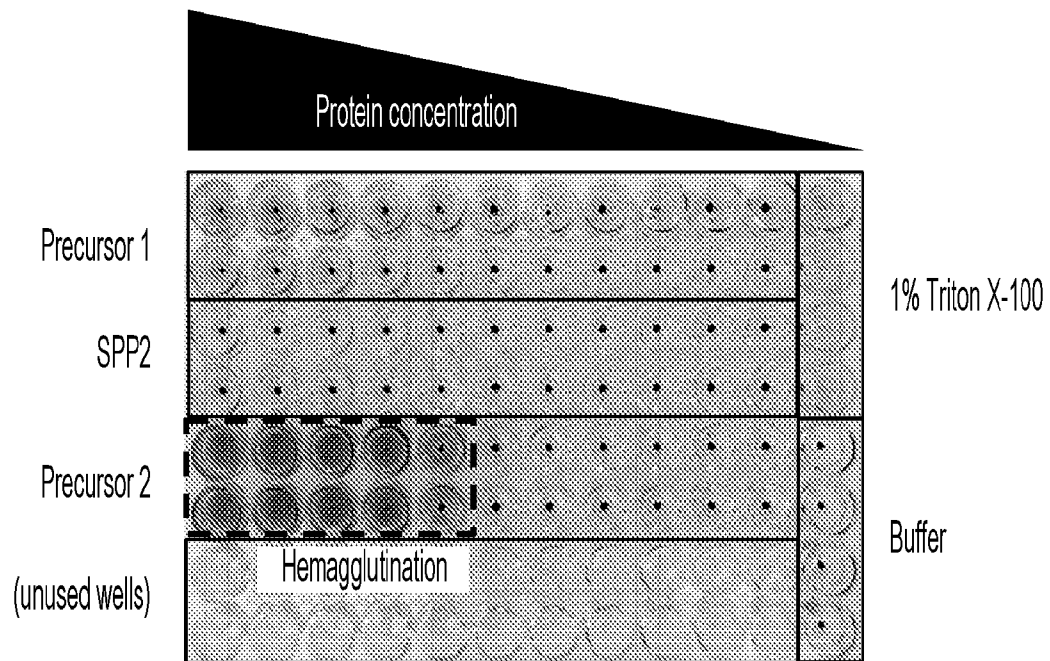


FIG. 6A

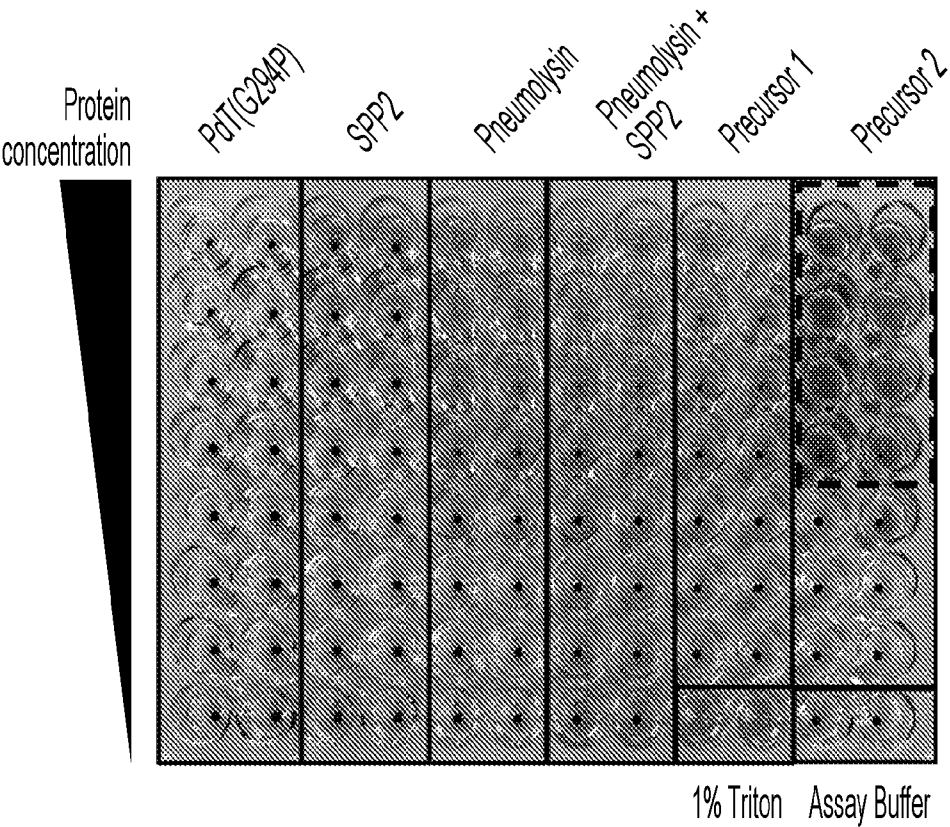


FIG. 6B

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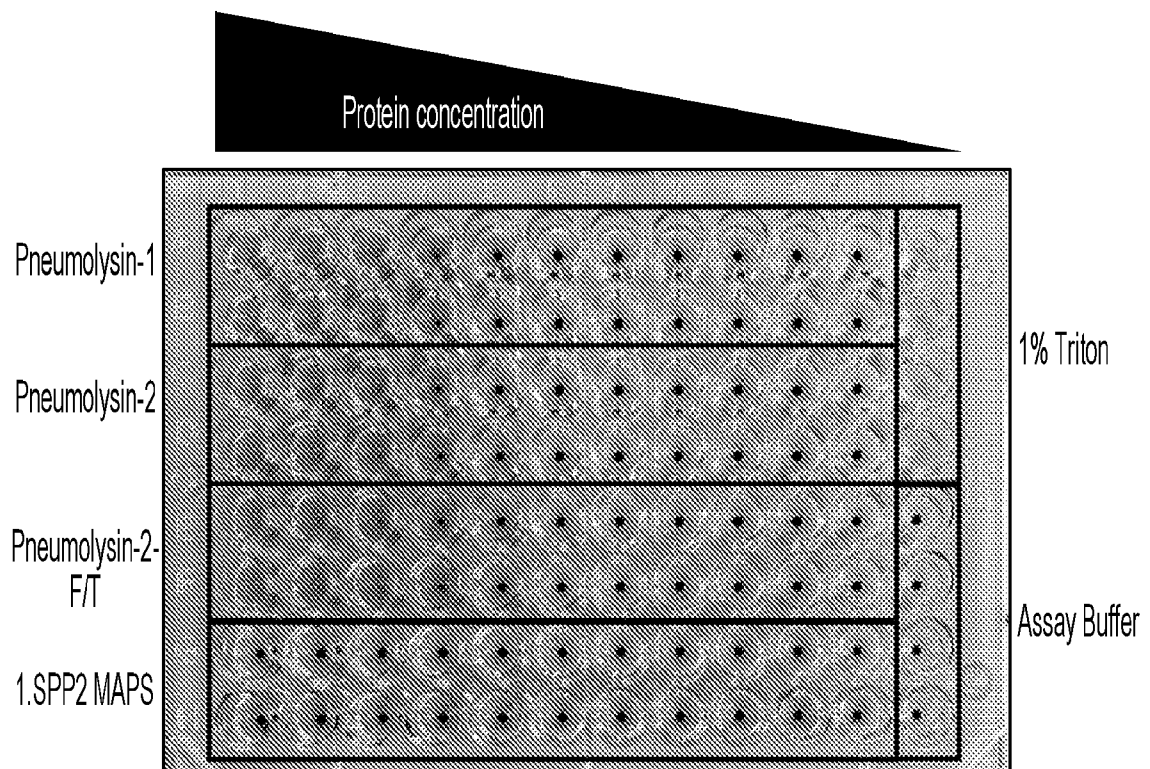
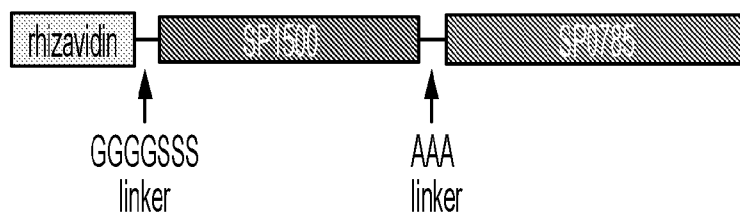


FIG. 6C



Rhizavidin

- Amino acids 45-179 of the biotin-binding protein rhizavidin (no signal peptide); 17 kDa
- No cross-reactivity in human serum samples obtained from subjects exposed to avidin

SP1500 (Amino Acid ABC Transporter, amino acid-binding protein (no signal peptide), conserved across *S. pneumonia* strains)

- Amino acids 27-278 of 278 amino acid parent (no signal peptide); 28 kDa
- No human homology

SP0785 (an efflux transporter protein conserved across *S. pneumonia* strains)

- Amino acids 33-399 of 399 amino acid parent (no signal peptide); 38 kDa
- No human homology

Linkers

- "GGGGSSS". "SSS" is from the Sac I site on PET21/24b plasmid. "GGGG" was added to create a flexible linker with minimal steric hindrance
- "AAA", the Not I site on PET21/24b plasmid.

FIG. 7

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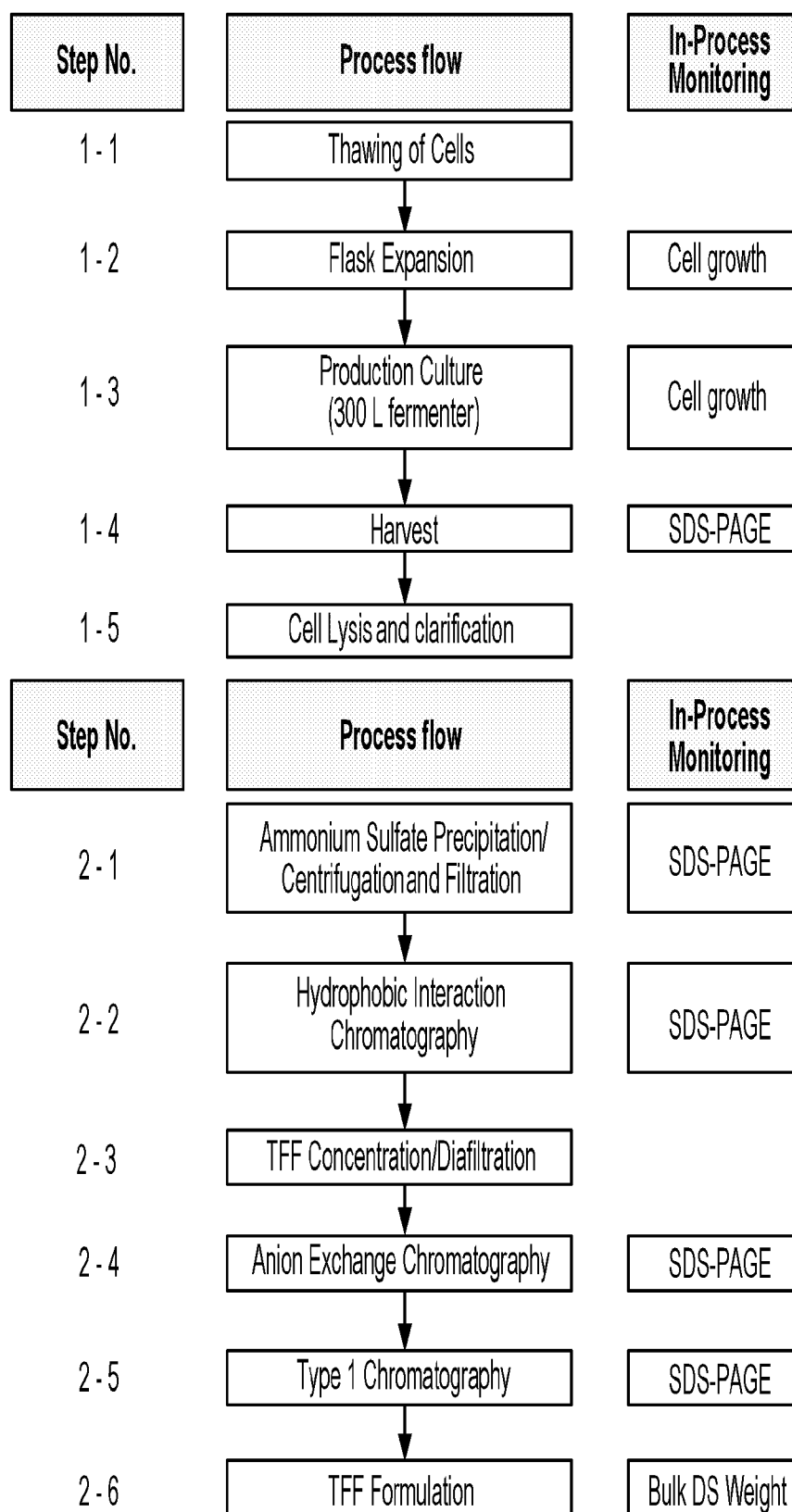


FIG. 8

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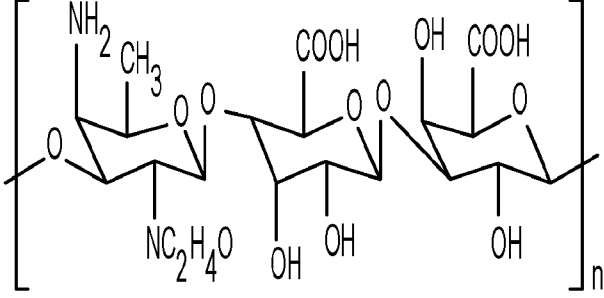
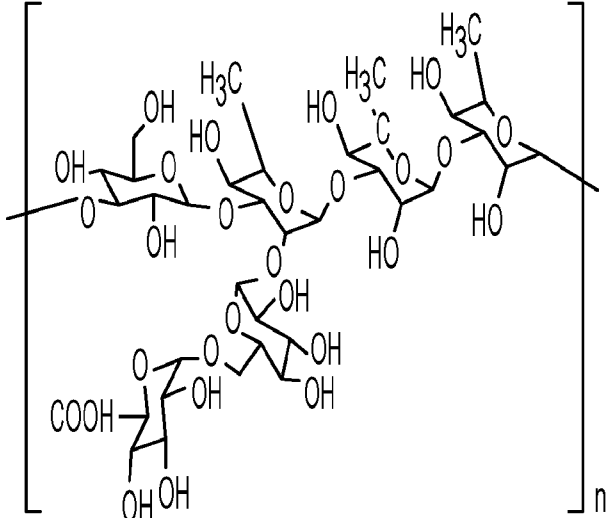
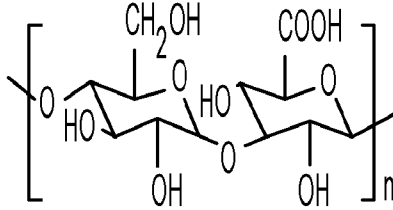
TYPE	STRUCTURE
1	 [$\rightarrow$3AATp1$\rightarrow$4D-GalpA1$\rightarrow$3D-GalpA1$\rightarrow$]n
2	 [$\rightarrow$4D-Glcp1$\rightarrow$3L-Rhap1$\rightarrow$3L-Rhap1$\rightarrow$3L-Rhap1$\rightarrow$]n D-GlcpA1$\rightarrow$6D-Glcp1$\rightarrow$2
3	 [$\rightarrow$4D-Glcp1$\rightarrow$3D-GlcpA1$\rightarrow$]n

FIG. 9

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ADDITIONAL INFORMATION	
MW (H^+):	538.165 ($C_{20}H_{30}N_2O_{15}$)
MW (Na^+):	583.136 ($C_{20}H_{29}N_2O_{15}Na_2$)
MW (Na^+ , OAc):	625.147 ($C_{22}H_{31}N_2O_{16}Na_2$)
Monosaccharides:	1 AAT, 2 D-GalA
AAT:	2-acetamido-4-amino-2,4,6-trideoxy- D-galactopyranose
Structure:	Linear
Net Charge:	-1
O-Acetate:	1
MW (H^+):	938.829 ($C_{36}H_{58}O_{28}$)
MW (Na^+):	960.811 ($C_{36}H_{57}O_{28}Na_1$)
Monosaccharides:	2 D-Glc, 3 L-Rha, 1 D-GlcA
Structure:	Branched
Net Charge:	-1
MW (H^+):	338.085 ($C_{12}H_{18}O_{11}$)
MW (Na^+):	360.067 ($C_{12}H_{17}O_{11}Na_1$)
Monosaccharides:	1 D-Glc, 1 D-GlcA
Structure:	Linear
Net Charge:	-1

FIG. 9
CONTINUED

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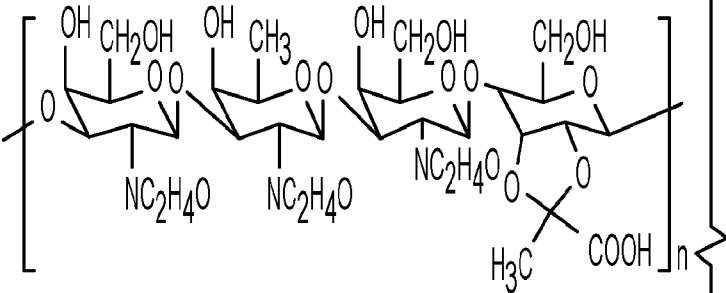
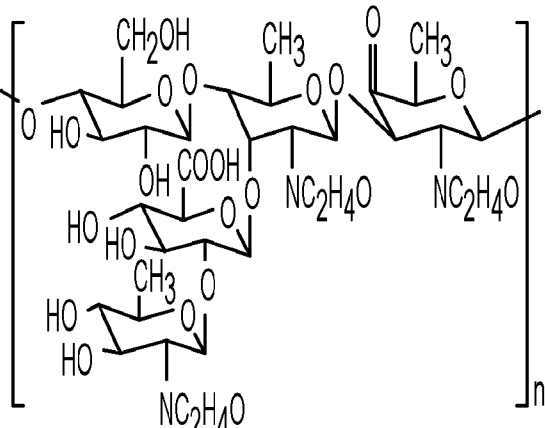
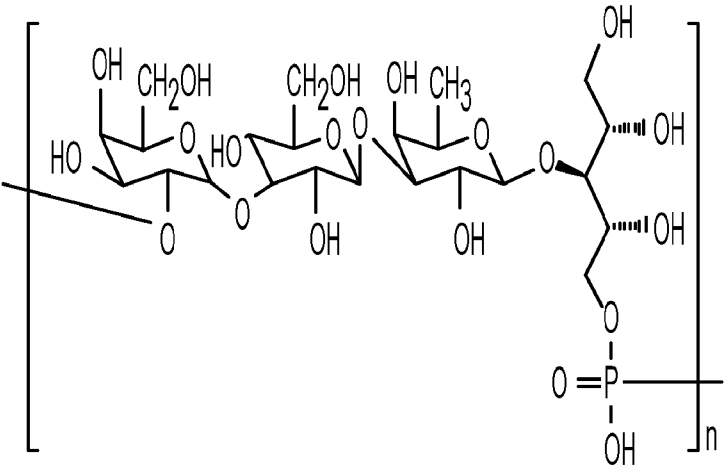
TYPE	STRUCTURE
4	 $[\rightarrow 3\text{D-ManpNac}^1 \rightarrow 3\text{L-FucpNac}^1 \rightarrow 3\text{D-GalpNac}^1 \rightarrow 4\text{D-Galp}^1 \rightarrow]$ 3L-Pyr^2
5	 $[\rightarrow 4\text{D-Glcp}^1 \rightarrow 4\text{L-FucpNac}^1 \rightarrow 3\text{D-Sugp}^1 \rightarrow]$ $\text{L-PnepNac}^1 \rightarrow 2\text{D-GlcpA}^1 \uparrow^3$
6A	 $[\rightarrow 2\text{D-Galp}^1 \rightarrow 3\text{D-Glcp}^1 \rightarrow 3\text{L-Rhap}^1 \rightarrow 4\text{D-Ribitol}^5 \rightarrow \text{PO}_4 \rightarrow]$

FIG. 9
CONTINUED

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ADDITIONAL INFORMATION	
MW (H ⁺):	825.302 (C ₃₃ H ₅₁ N ₃ O ₂₁)
MW (Na ⁺):	847.283 (C ₃₃ H ₅₀ N ₃ O ₂₁ Na ₁)
Monosaccharides:	1 D-Gal, 1 D-GalNac, 1 D-ManNac, 1 L-FucNac
Structure:	Linear
Net Charge:	-1
Pyruvate:	1
MW (H ⁺):	897.323 (C ₃₆ H ₅₅ N ₃ O ₂₃)
MW (Na ⁺):	919.305 (C ₃₆ H ₅₄ N ₃ O ₂₃ Na ₁)
Monosaccharides:	1 D-Glc, 1 L-FucNac, 1 D-GlcA, 1 D-Sug, 1 L-PneNac
D-Sug:	2-acetamido-2,6,-dideoxy-D- xylohexos-4-ulose
L-PneNac:	2-acetamido-2,6,-dideoxy-L-talose (pneumosamine)
Structure:	Branched
Net Charge:	-1
MW (H ⁺):	683.525 (C ₂₃ H ₄₀ O ₂₁ P ₁)
MW (Na ⁺):	704.507 (C ₂₃ H ₃₉ O ₂₁ P ₁ Na ₁)
Monosaccharides:	1 D-Glc, 1 D-Gal, 1 L-Rha, 1 Ribitol
Structure:	Linear
Net Charge:	-1
Phosphorous:	1 in polysaccharide backbone

FIG. 9
CONTINUED

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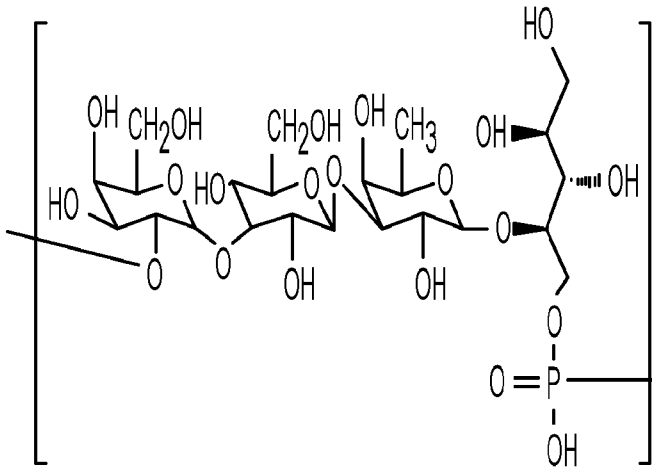
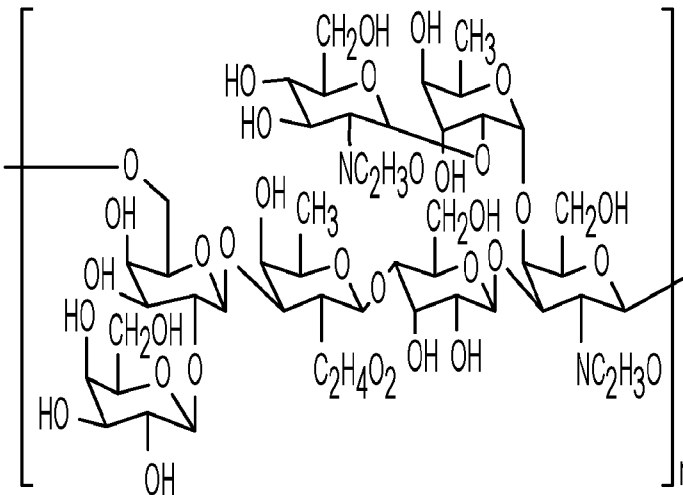
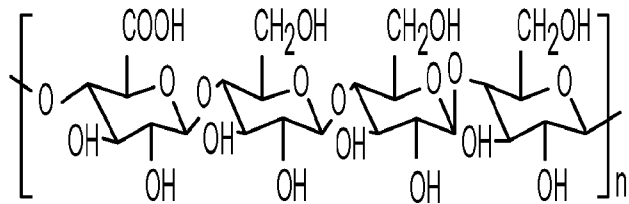
TYPE	STRUCTURE
6B	 [$\rightarrow$ 2D-Galp¹ $\rightarrow$ 3D-Glcp¹ $\rightarrow$ 3L-Rhap¹ $\rightarrow$ 4D-Ribitol⁵ $\rightarrow$ PO₄ $\rightarrow$]_n
7F	 [$\rightarrow$ 6D-Galp¹ $\rightarrow$ 3L-Rhap¹ $\rightarrow$ 4D-Glcp¹ $\rightarrow$ 3D-GalpNAC¹ $\rightarrow$]_n D-Galp¹ $\rightarrow$ 2 AcO $\rightarrow$ 2 D-GlcpNAC¹ $\rightarrow$ 2L-Rhap¹ $\rightarrow$ 4
8	 [$\rightarrow$ 4D-GlcpA¹ $\rightarrow$ 4D-Glcp¹ $\rightarrow$ 4D-Glcp¹ $\rightarrow$ 4D-Galp¹ $\rightarrow$]_n

FIG. 9
CONTINUED

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ADDITIONAL INFORMATION	
MW (H ⁺):	684.188 (C ₂₃ H ₄₁ O ₂₁ P ₁)
MW (Na ⁺):	706.170 (C ₂₃ H ₄₀ O ₂₁ P ₁ Na ₁)
Monosaccharides:	1 D-Glc, 1 D-Gal 1 L-Rha, 1 Ribitol
Structure:	Linear
Net Charge:	-1
Phosphorous:	1 in polysaccharide backbone
MW (H ⁺):	1226.444 (C ₄₈ H ₇₇ N ₂ O ₃₄)
Monosaccharides:	1 D-Glc, 2 D-Gal, 2 L-Rha, 1 D-GlcNac, 1 D-GalNac
Structure:	Branched
Net Charge:	0
O-Acetate:	1
MW (H ⁺):	662.546 (C ₂₄ H ₃₈ O ₂₁)
MW (Na ⁺):	684.528 (C ₂₄ H ₃₇ O ₂₁ Na ₁)
Monosaccharides:	2 D-Glc, 1 D-Gal, 1 D-GlcA
Structure:	Linear
Net Charge:	-1

FIG. 9
CONTINUED

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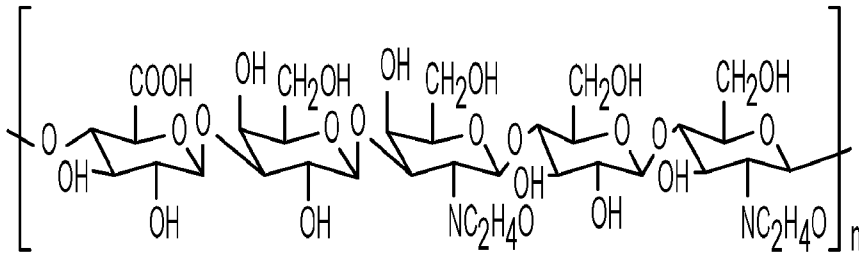
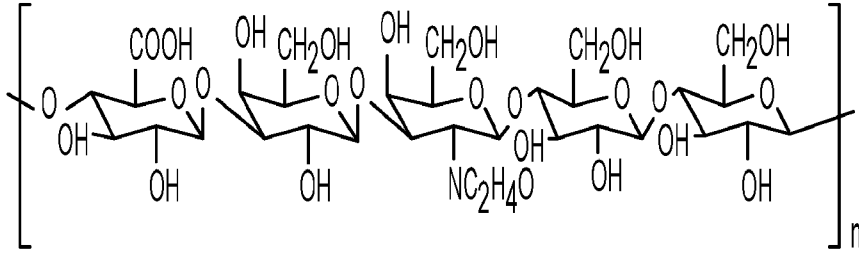
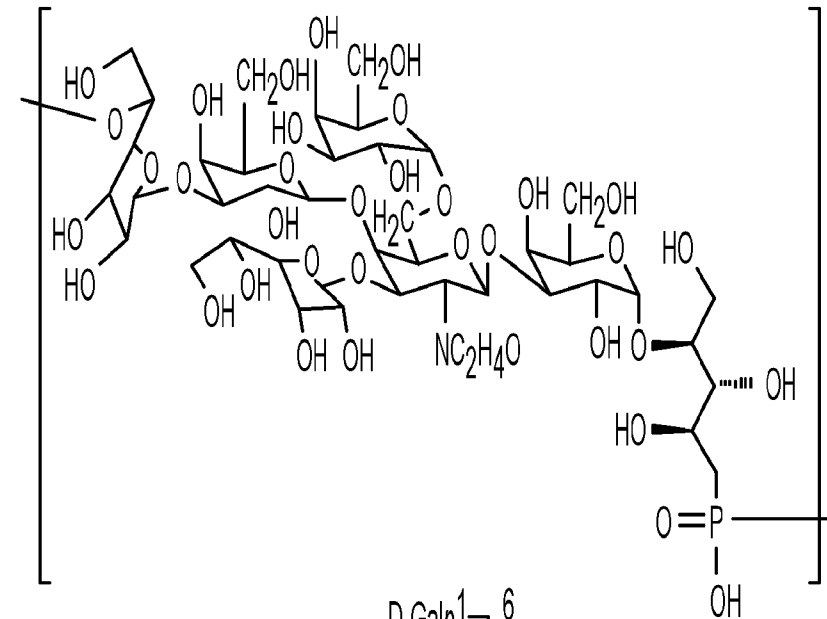
TYPE	STRUCTURE
9N	 [→ 4D-GlcpA1 → 3D-Galp1 → 3D-ManpNAc1 → 4D-Glcp1 → 4D-GlcpNAc1 →]n
9V	 [→ 4D-GlcpA1 → 3D-Galp1 → 3D-ManpNAc1 → 4D-Glcp1 → 4D-Glcp1 →]n
10A	 $[\rightarrow 5D-Galp1 \rightarrow 3D-Galp1 \rightarrow 4D-GalpNAc1 \rightarrow 3D-Galp1 \rightarrow 2D-Ribitol5 \rightarrow PO_4 \rightarrow]_n$

FIG. 9
CONTINUED

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ADDITIONAL INFORMATION	
MW (H ⁺):	906.296 (C ₃₄ H ₅₄ N ₂ O ₂₆)
MW (Na ⁺):	928.278 (C ₃₄ H ₅₃ N ₂ O ₂₆ Na ₁)
Monosaccharides:	1 D-Glc, 1 D-Gal, 1 D-GlcNac, 1 D-ManNac, 1 D-GlcA
Structure:	Linear
Net Charge:	-1
MW (H ⁺):	865.270 (C ₃₂ H ₅₁ NO ₂₆)
MW (Na ⁺):	887.252 (C ₃₂ H ₅₀ NO ₂₆ Na ₁)
MW (Na ⁺ , OAc):	971.273 (C ₃₆ H ₅₄ NO ₂₈ Na ₁)
Monosaccharides:	2 D-Glc, 1 D-Gal, 1 D-ManNac, 1 D-GlcA
Structure:	Linear
Net Charge:	-1
O-Acetate:	2
MW (H ⁺):	1242.076 (C ₄₅ H ₈₀ NO ₃₆ P ₁)
MW (Na ⁺):	1264.057 (C ₄₅ H ₇₉ NO ₃₆ P ₁ Na ₁)
Monosaccharides:	5 D-Gal, 1 D-GalNac, 1 Ribitol
Structure:	Branched
Net Charge:	-1
Phosphorous:	1 in polysaccharide backbone

FIG. 9
CONTINUED

TYPE	STRUCTURE
11A	[→ 3D-Galp¹ → 4D-Glcp¹ → 6D-Glcp¹ → 4D-Galp¹ →]_n Glycerol¹ → PO₄ ↗⁴
12F	[→ 4L-FucpNAC¹ → 3D-GalpNAC¹ → 4D-ManpNAC¹ →]_n D-Galp¹ ↗³ D-Glcp¹ → 2D-Glcp¹ ↗³
14	[→ 4D-Glcp¹ → 6D-GlcpNAC¹ → 3D-Galp¹ →]_n D-Galp¹ ↗⁴

SUBSTITUTE SHEET (RULE 26)

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ADDITIONAL INFORMATION	
MW (H ⁺):	859.692 (C ₃₀ H ₅₂ O ₂₆ P ₁)
MW (Na ⁺):	881.674 (C ₃₀ H ₅₁ O ₂₆ P ₁ Na ₁)
MW (Na ⁺ , OAc):	923.711 (C ₃₂ H ₅₃ O ₂₇ P ₁ Na ₁)
Monosaccharides:	2 D-Glc, 2 D-Gal, 1 Glycerol
Structure:	Linear
Net Charge:	-1
Phosphorous:	1 in polysaccharide sidechain
O-Acetate:	1
MW (H ⁺):	1080.000 (C ₄₂ H ₆₉ N ₃ O ₂₉)
Monosaccharides:	2 D-Glc, 1 D-Gal, 1 D-GalNac, 1 L- FucNac, 1 D-ManNacA
Structure:	Branched
Net Charge:	-1
MW (H ⁺):	689.238 (C ₂₆ H ₄₃ NO ₂₀)
Monosaccharides:	1 D-Glc, 2 D-Gal, 1 D-GlcNac
Structure:	Branched
Net Charge:	0

FIG. 9
CONTINUED

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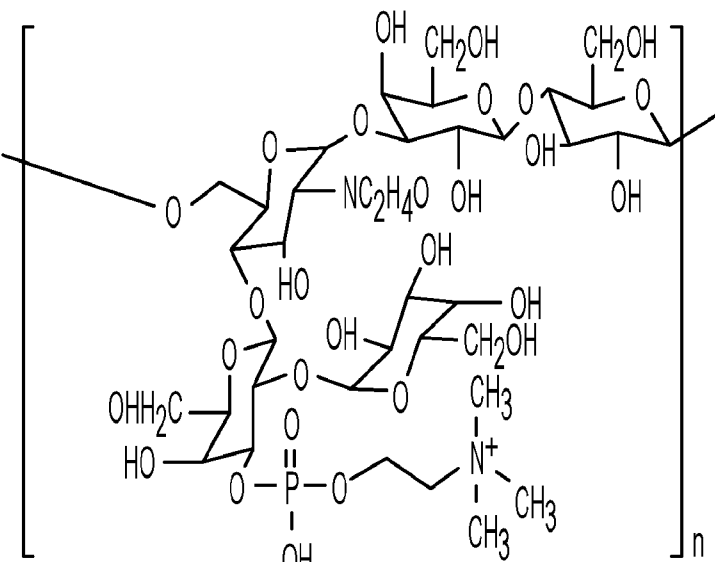
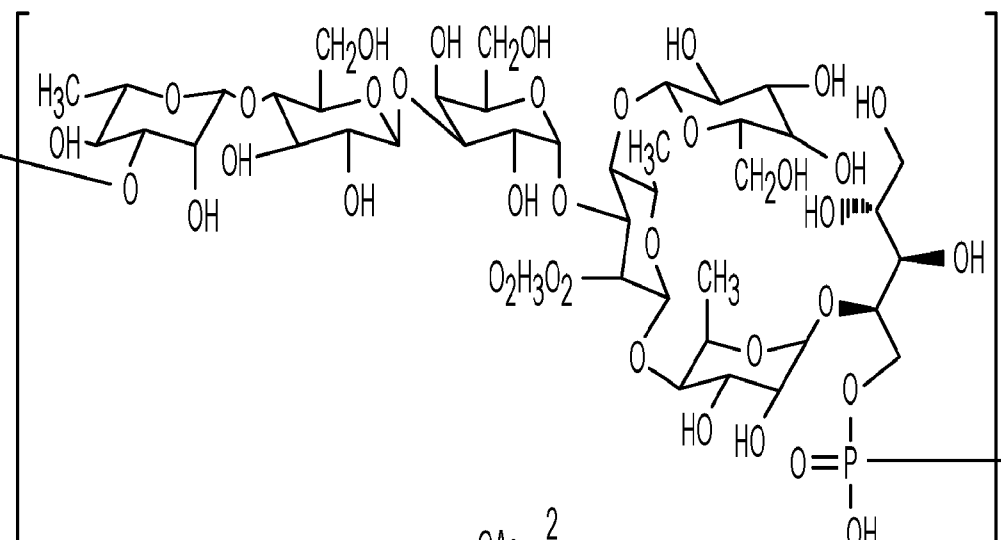
TYPE	STRUCTURE
15B	 $[\rightarrow 6\text{D-GlcpNAc}1 \rightarrow 3\text{D-Galp}1 \rightarrow 4\text{D-Glcp}1 \rightarrow]_n$ $4 \uparrow 1\text{D-Galp}2 \leftarrow 1\text{D-Galp}$ $3 \uparrow [\text{PO}_4 \rightarrow \text{CH}_2\text{CHN}(\text{CH}_3)_3]_{0.2}$
17F	 $[\rightarrow 3\text{L-Rhap}1 \rightarrow 4\text{D-Glcp}1 \rightarrow 3\text{D-Galp}1 \rightarrow 3\text{L-Rhap}1 \rightarrow 4\text{L-Rhap}1 \rightarrow 2\text{D-Arabinitol}1 \rightarrow \text{PO}_4 \rightarrow]_n$ $\text{D-Galp}1 \uparrow 4$ $\text{OAc} \rightarrow 2$

FIG. 9
CONTINUED

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ADDITIONAL INFORMATION

MW (H^+): 851.755 ($C_{32}H_{53}NO_{25}$)
 MW (H^+ , OAc): 893.792 ($C_{34}H_{55}NO_{26}$)
 MW (Na^+ , OAc, PC): 1059.927 ($C_{39}H_{68}N_2O_{29}P_1$)
 Monosaccharides: 1 D-Glc, 3 D-Gal, 1 D-GlcNac
 Structure: Branched
 Net Charge: -1
 Phosphorous: 1 in polysaccharide sidechain
 O-Acetate: 1
 Pyruvate: 1

Note: Published structure indicates 0.2 mol of phosphocholine per mol of repeat unit. Nuclear magnetic resonance analysis at Merck suggests that phosphocholine is not present.

MW (H^+): 1179.985 ($C_{43}H_{72}O_{35}P_1$)
 MW (Na^+): 1201.966 ($C_{43}H_{71}O_{35}P_1Na_1$)
 Monosaccharides: 1 D-Glc, 2 D-Gal, 3 L-Rha, 1 Arabinitol
 Structure: Branched
 Net Charge: -1
 Phosphorous: 1 in polysaccharide backbone
 O-Acetate: 1
 Pyruvate: 1

FIG. 9
 CONTINUED

SUBSTITUTE SHEET (RULE 26)

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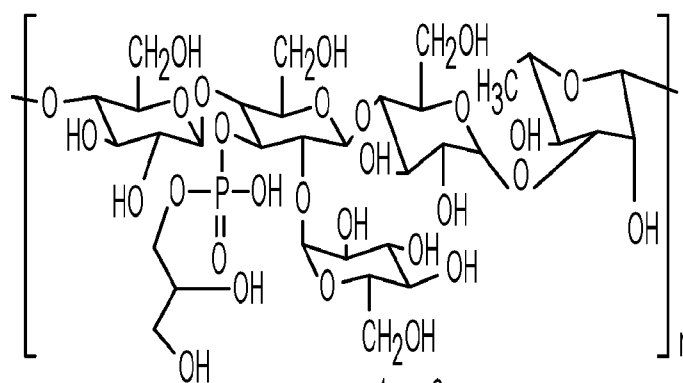
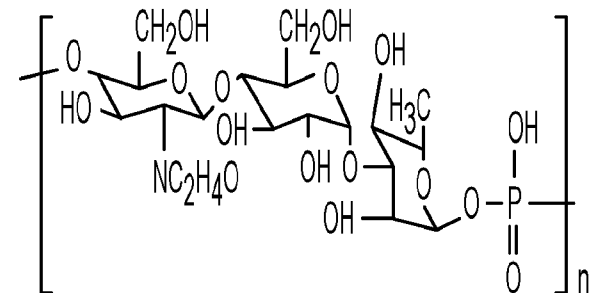
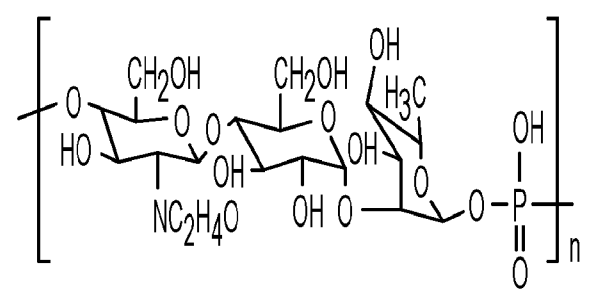
TYPE	STRUCTURE
18C	 D-Glcp¹ →² [→⁴D-Glcp¹ →⁴D-Galp¹ →⁴D-Glcp¹ →³L-Rhap¹ →]_n Glycerol¹ → PO₄ →³
19A	 [→⁴D-ManpNAc¹ →⁴D-Glcp¹ →³L-Rhap¹ → PO₄ →]_n
19F	 [→⁴D-ManpNAc¹ →⁴D-Glcp¹ →²L-Rhap¹ → PO₄ →]_n

FIG. 9
CONTINUED

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ADDITIONAL INFORMATION

MW (H⁺): 948.272 (C₃₃H₅₇O₂₉P₁)
MW (Na⁺): 970.254 (C₃₃H₅₆O₂₉P₁Na₁)
MW (Na⁺, OAc): 1012.265 (C₃₅H₅₈O₃₀P₁Na₁)
Monosaccharides: 3 D-Glc, 1 D-Gal, 1 L-Rha, 1 Glycerol
Structure: Branched
Net Charge: -1
Phosphorous: 1 in polysaccharide sidechain
O-Acetate: 1

MW (H⁺): 591.156 (C₂₀H₃₄N₁O₁₇P₁)
MW (Na⁺): 613.138 (C₂₀H₃₃N₁O₁₇P₁Na₁)
Monosaccharides: 1 D-Glc, 1 L-Rha, 1 D-ManNac
Structure: Linear
Net Charge: -1
Phosphorous: 1 in polysaccharide backbone

MW (H⁺): 591.156 (C₂₀H₃₄N₁O₁₇P₁)
MW (Na⁺): 613.138 (C₂₀H₃₃N₁O₁₇P₁Na₁)
Monosaccharides: 1 D-Glc, 1 L-Rha, 1 D-ManNac
Structure: Linear
Net Charge: -1
Phosphorous: 1 in polysaccharide backbone

FIG. 9
CONTINUED

SUBSTITUTE SHEET (RULE 26)

FIG. 9
CONTINUED

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ADDITIONAL INFORMATION

MW (H⁺): 1093.875 (C₃₈H₆₄NO₃₃P₁)
MW (Na⁺): 1115.857 (C₃₈H₆₃NO₃₃P₁Na₁)
MW (Na⁺, OAc): 1157.894 (C₄₀H₆₅NO₃₄P₁Na₁)
Monosaccharides: 3 D-Glc, 2 D-Gal, 1 D-GlcNac
Structure: Branched
Net Charge: -1
Phosphorous: 1 in polysaccharide backbone
O-Acetate: 1

MW (H⁺): 982.882 (C₃₈H₆₂O₂₉)
Monosaccharides: 2 D-Glc, 1 D-Gal, 2 L-Rha, 1 D-GlcA
Structure: Branched
Net Charge: -1
O-Acetate: 1

FIG. 9
CONTINUED

SUBSTITUTE SHEET (RULE 26)

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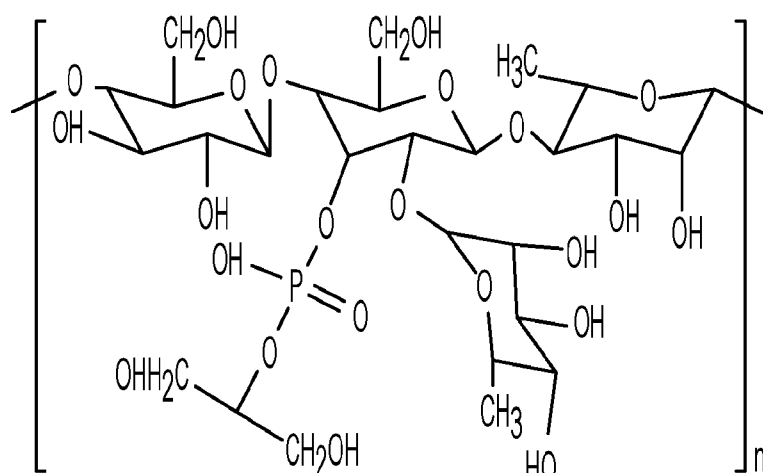
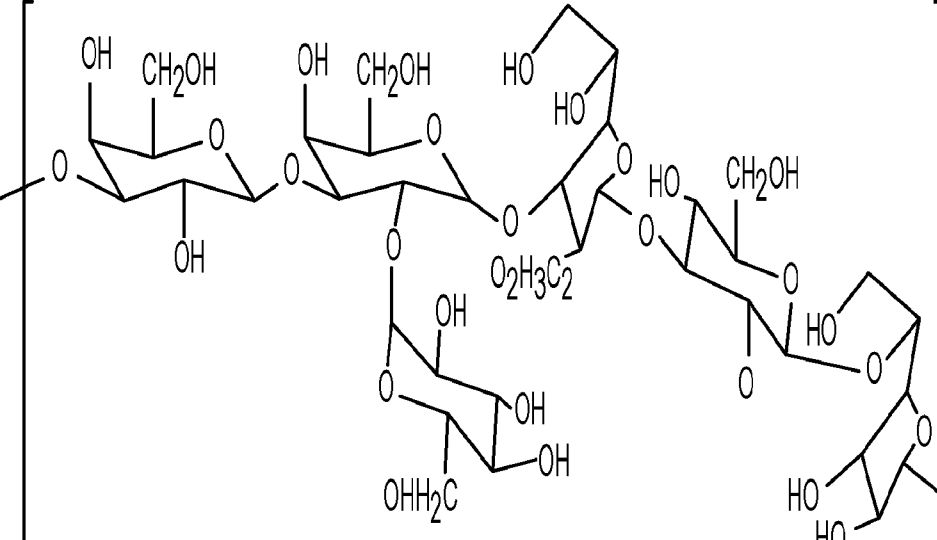
TYPE	STRUCTURE
23F	 $\begin{array}{c} \text{L-Rhap}^1 \xrightarrow{2} \\ [\rightarrow 4\text{D-Glcp}^1 \rightarrow 4\text{D-Galp}^1 \rightarrow 4\text{L-Rhap}^1 \rightarrow] \\ \text{Glycerol}^2 \rightarrow \text{PO}_4 \xrightarrow{3} \end{array}$
33F	 $\begin{array}{c} [\rightarrow 3\text{D-Galp}^1 \rightarrow 3\text{D-Galp}^1 \rightarrow 3\text{D-Galp}^1 \rightarrow 3\text{D-Glcp}^1 \rightarrow 5\text{D-Galp}^1 \rightarrow]_n \\ \text{D-Galp}^1 \xrightarrow{2} \quad \text{OAc} \xrightarrow{2} \end{array}$

FIG. 9
CONTINUED

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ADDITIONAL INFORMATION

MW (H⁺): 770.225 (C₂₇H₄₇O₂₃P₁)
MW (Na⁺): 792.207 (C₂₇H₄₆O₂₃P₁Na₁)
Monosaccharides: 1 D-Glc, 1 D-Gal, 2 L-Rha, 1 Glycerol
Structure: Branched
Net Charge: -1
Phosphorous: 1 in polysaccharide sidechain

MW (H⁺): 1014.880 (C₃₈H₆₂O₃₁)
MW (H⁺, OAc): 1056.917 (C₄₀H₆₄O₃₂)
Monosaccharides: 1 D-Glc, 5 D-Gal
Structure: Branched
Net Charge: 0
O-Acetate: 1

FIG. 9
CONTINUED

SUBSTITUTE SHEET (RULE 26)

TYPE	STRUCTURE
6C	→2)-α-D-Glcp-(1→3)-α-D-Glcp-(1→3)-α-L-Rhap-(1→3)-D-Ribitol-(5→phosphate-
7C	→ 6)-α-D-GlcpNAc-(1→2)-α-L-Rhap-(1→2)-β-L-Rhap-(1→4)-β-D-Glcp-(1→4)-α-D-GalpNAc-(1→O-P → ↑ (1-3) β-D-Ribf-(1→4)-α-L-Rhap

FIG. 9
CONTINUED

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ADDITIONAL INFORMATIONMW (H⁺): 684.53 (C₂₃H₄₁O₂₁P)MW (Na⁺): 706.51 (C₂₃H₄₀O₂₁P Na)

Composition: 2 D-Glc, 1 L-Rha, 1 Ribitol

Structure: Linear

Net Charge: -1

Phosphate: 1 in backbone

MW (H⁺): 1219.04 (C₄₅H₇₅N₂O₃₄P)MW (Na⁺): 1241.03 (C₄₅H₇₄N₂O₃₄PNa)

Composition: 1 D-Glc, 3 L-Rha, 1 D-Rib, 1 D-GlcNAc, 1 D-GalNAc

Structure: Branched

Net Charge: -1

Phosphate: 1 in backbone

FIG. 9
CONTINUED

SUBSTITUTE SHEET (RULE 26)

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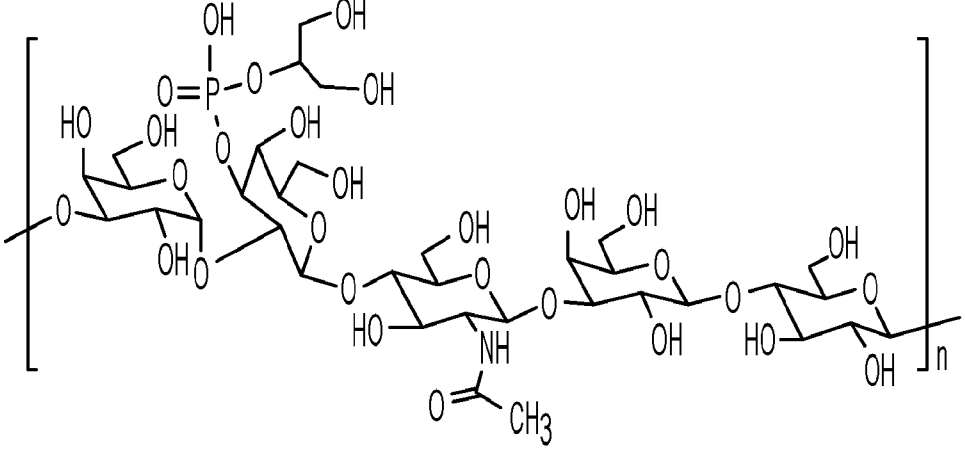
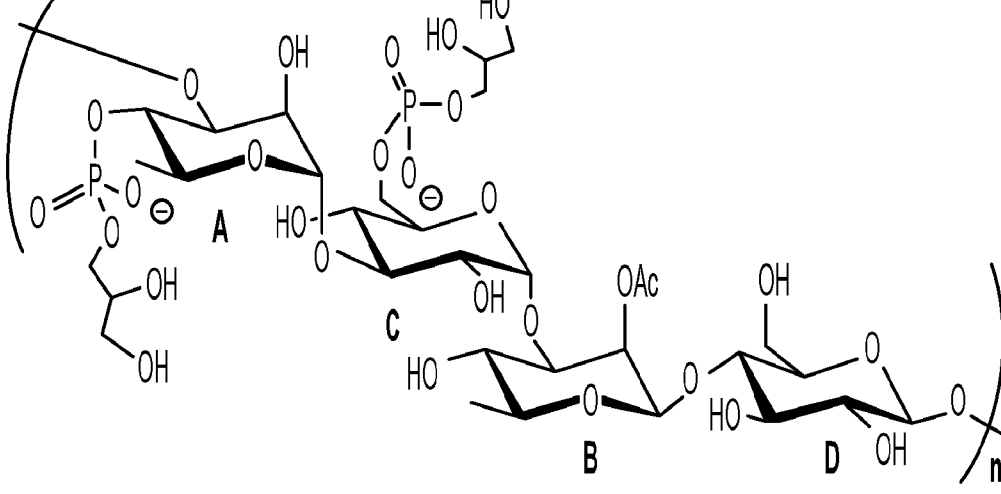
TYPE	STRUCTURE
15A	 →3)-α-D-Galp-(1→2)-β-D-Galp-(1→4)-β-D-GlcNAc-(1→3)-β-D-Galp-(1→4)-β-D-Glc-(1→ 3 ↑ Gro_{0.7}-(2→P
16F	 P-1-Gro ↓ 6 →3)-α-L-Rhap-(1→3)-α-D-Glc-(1→3)-β-L-Rhap-[2-OAc]-(1→4)-β-D-Glc-(1 4 ↑ P-1-Gro

FIG. 9
CONTINUED

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ADDITIONAL INFORMATION

MW (H^+): 1005.81 ($C_{35}H_{60}NO_{30}P$)
MW (Na^+): 1027.80 ($C_{35}H_{59}O_{30}PNa$)
Composition: 1 D-Glc, 3 D-Gal, 1 D-GlcNAc, 1 Gro
Structure: Linear
Net Charge: -1
Phosphate: 1 in branch

MW (H^+): 966.72 ($C_{32}H_{56}O_{29}P_2$)
MW (Na^+): 988.70 ($C_{32}H_{55}O_{29}P_2Na$)
MW ($2 Na^+$): 1010.68 ($C_{32}H_{54}O_{29}P_2Na_2$)
Composition: 2 D-Glc, 2 L-Rha, 2 Gro, 1 O-Ac
Structure: Linear
Net Charge: -2
Phosphate: 2 in branch

FIG. 9
CONTINUED

SUBSTITUTE SHEET (RULE 26)

[illegible]

SUBSTITUTE SHEET (RULE 26)

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ADDITIONAL INFORMATION	
MW (H ⁺):	770.62 (C ₂₇ H ₄₇ O ₂₃ P)
MW (Na ⁺):	792.60 (C ₂₇ H ₄₆ O ₂₃ PNa)
Composition:	1 D-Glc, 1 D-Gal, 2 L-Rha, 1 Gro
Structure:	Branched
Net Charge:	-1
Phosphate:	1 in branch
MW (H ⁺):	624.48 (C ₂₁ H ₃₇ O ₁₉ P)
MW (Na ⁺):	646.46 (C ₂₁ H ₃₆ O ₁₉ PNa)
Composition:	1 D-Glc, 1 D-Gal, 1 L-Rha, 1 Gro
Structure:	Linear
Net Charge:	-1
Phosphate:	1 in branch
MW (H ⁺):	1003.84 (C ₃₆ H ₆₂ NO ₂₉ P)
MW (Na ⁺):	1025.82 (C ₃₆ H ₆₁ NO ₂₉ PNa)
Composition:	1 D-Glc, 2 L-Rha, 1 D-GlcNAc, 1 D-Rib, 1 Arabinitol
Structure:	Branched
Net Charge:	-1
Phosphate:	1 in branch

FIG. 9
CONTINUED

SUBSTITUTE SHEET (RULE 26)

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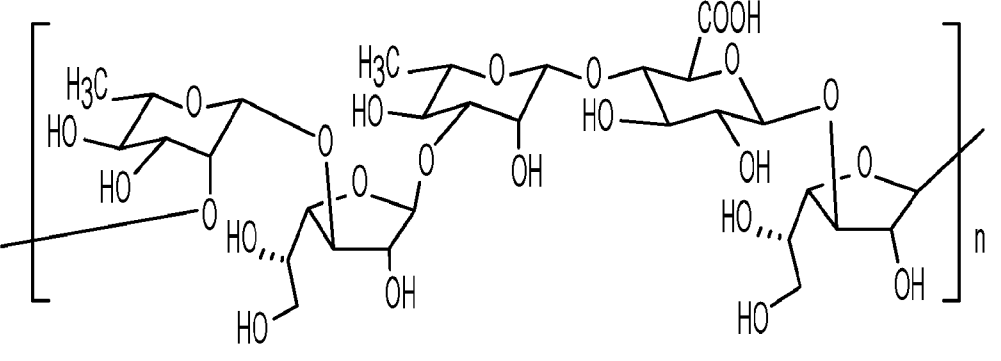
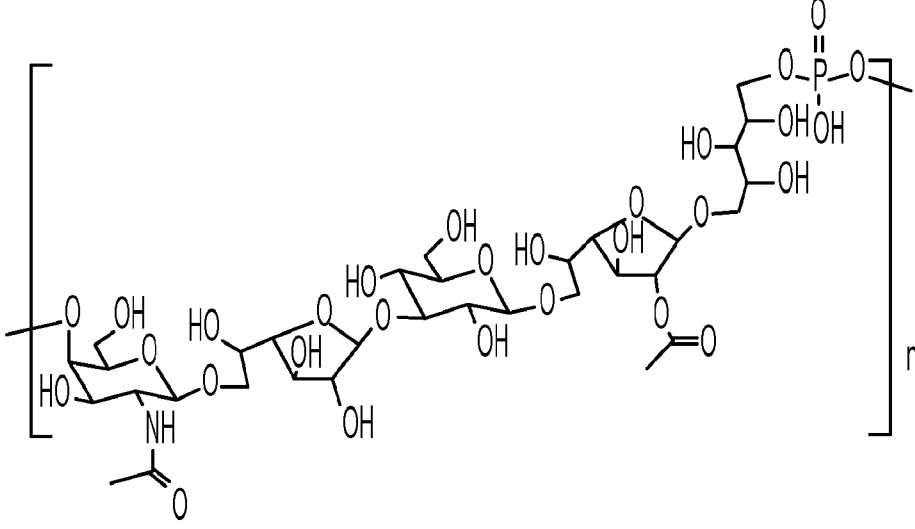
TYPE	STRUCTURE
31	 → 2) -β-L-Rhap-(1→3)-β-D-Galf-(1→3)-β-L-Rhap-(1→4)-β-D-GlcpA-(1→3)-β-D-Galf-(1→
35B	 → 4) -β-D-GalpNAc-(1→6)-β-D-Galf-(1→3)-β-D-Glcp-(1→6)-β-D-Galf2Ac0.7-(1→1)-Rib-ol-(5→P→

FIG. 9
CONTINUED

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ADDITIONAL INFORMATIONMW (H⁺): 792.69 (C₃₀H₄₈O₂₄)MW (Na⁺): 814.67 (C₃₀H₄₇O₂₄Na)

Composition: 2 D-Gal, 2 L-Rha, 1 GlcA, 3 O-Ac (80% each)

Structure: Linear

Net Charge: -1

3 O-acetyl sites not determined; each site with ~80% acetylation

MW (H⁺): 945.76 (C₃₃H₅₅NO₂₈P)MW (Na⁺): 967.74 (C₃₃H₅₅NO₂₈PNa)

Composition: 1 D-Glc, 2 D-Gal, 1 D-GalNAc, 1 Ribitol, 0.7 O-Ac

Structure: Linear

Net Charge: -1

Phosphate: 1 in backbone

FIG. 9
CONTINUED

SUBSTITUTE SHEET (RULE 26)

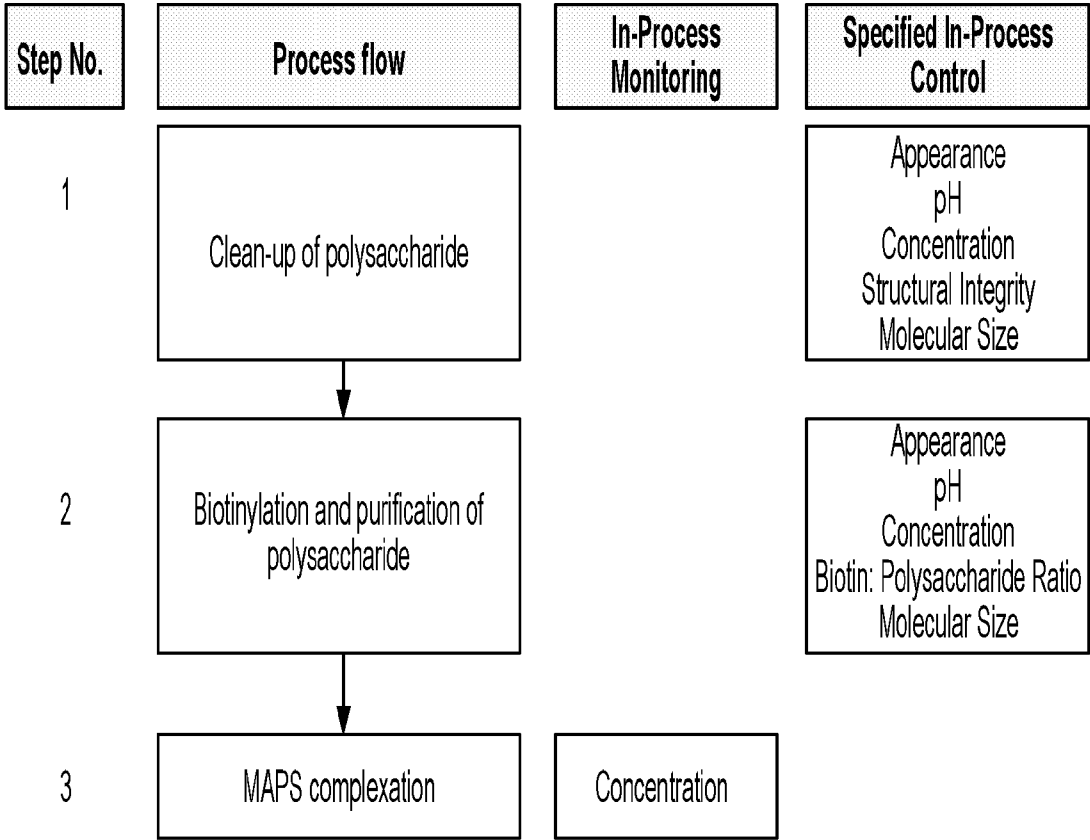


FIG. 10

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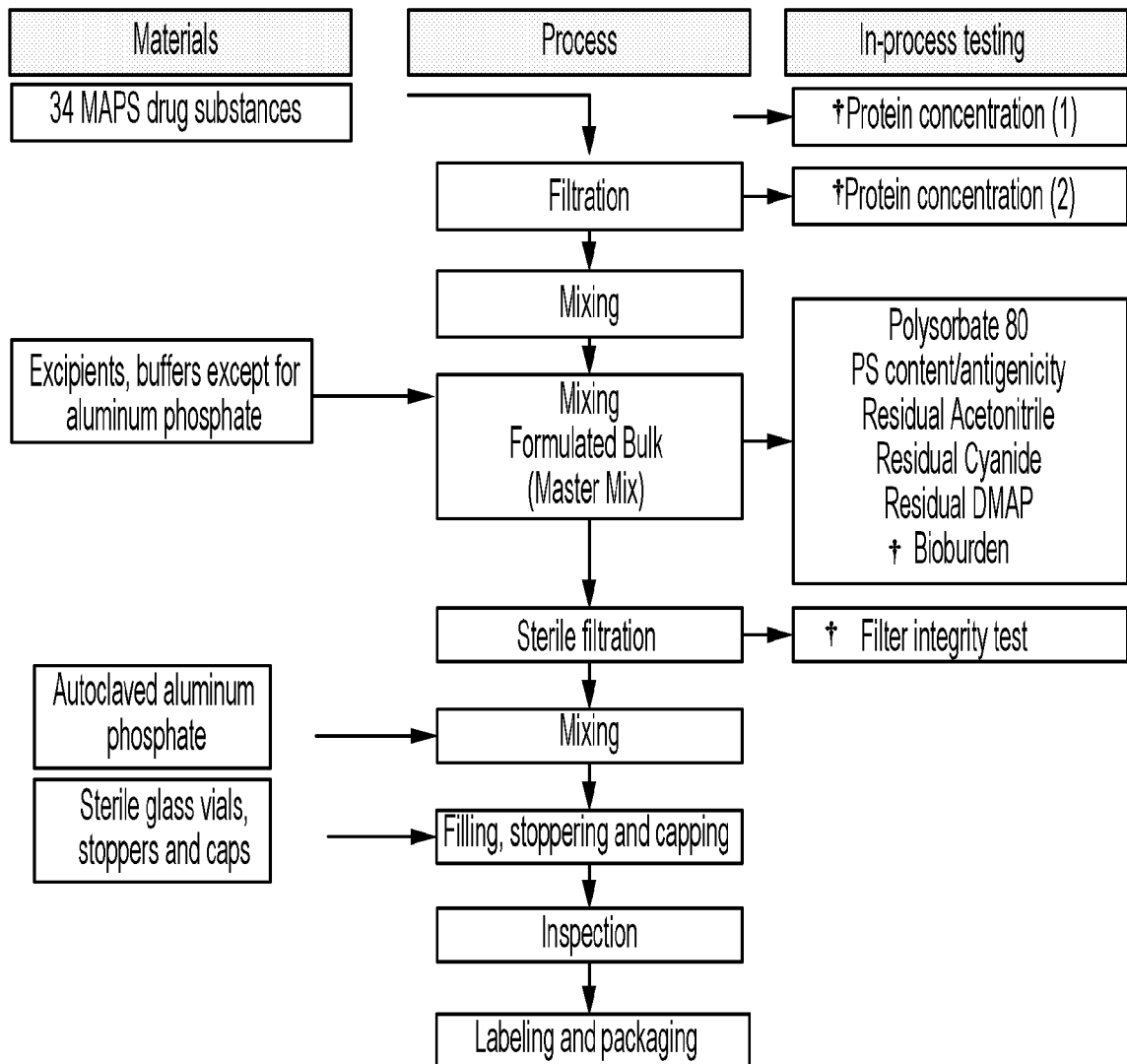
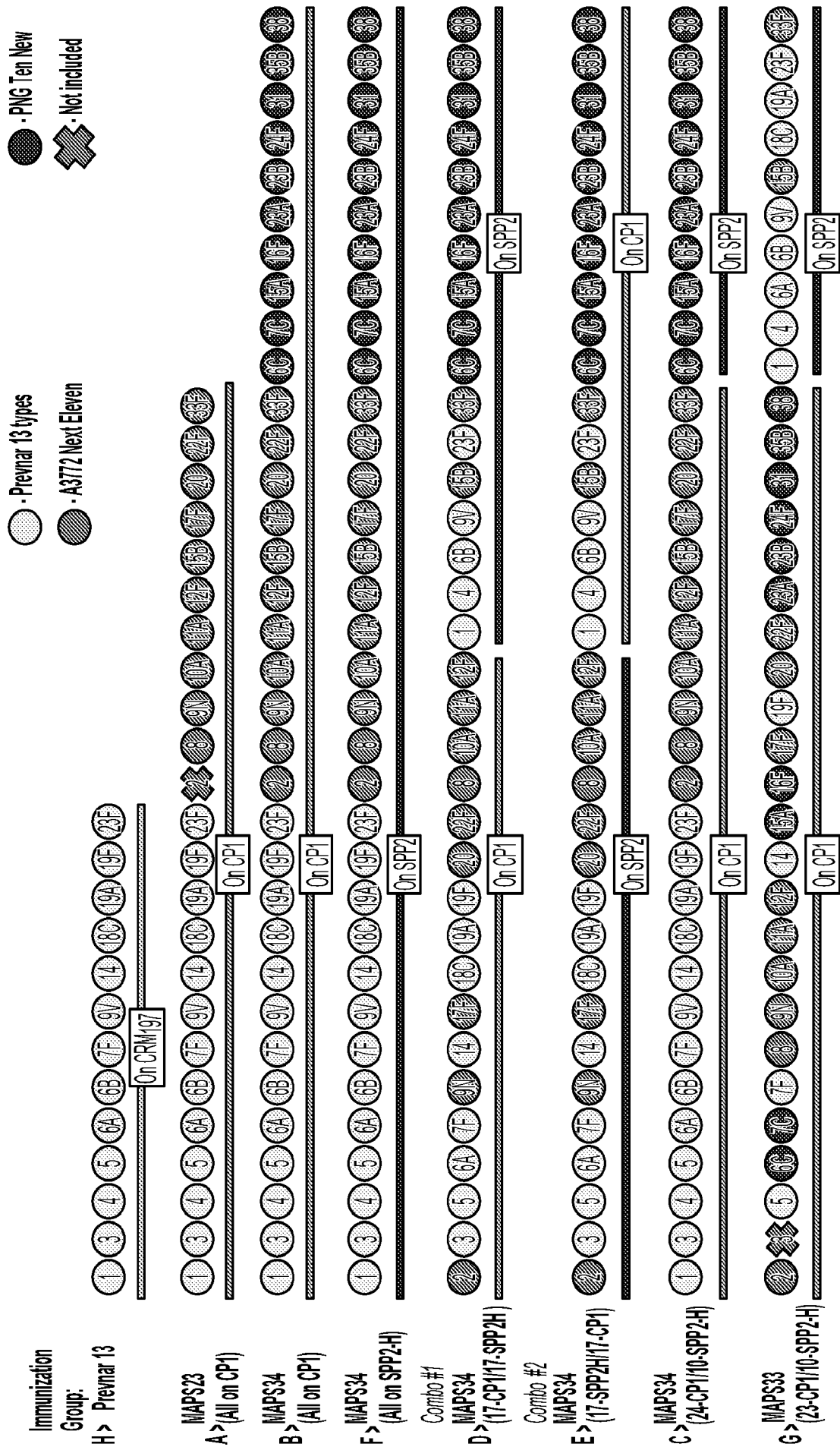
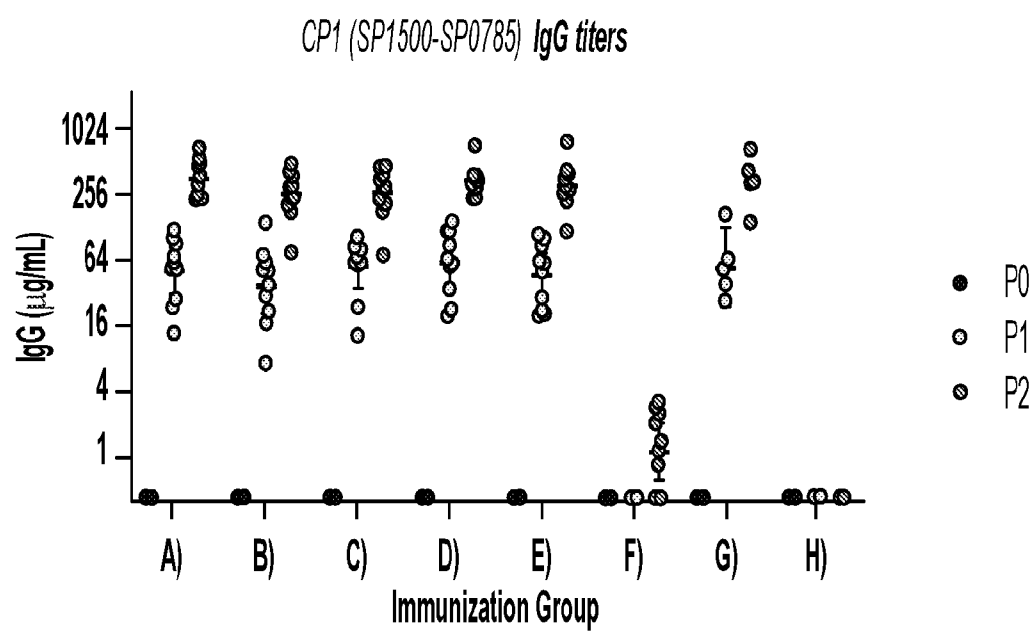


FIG. 11



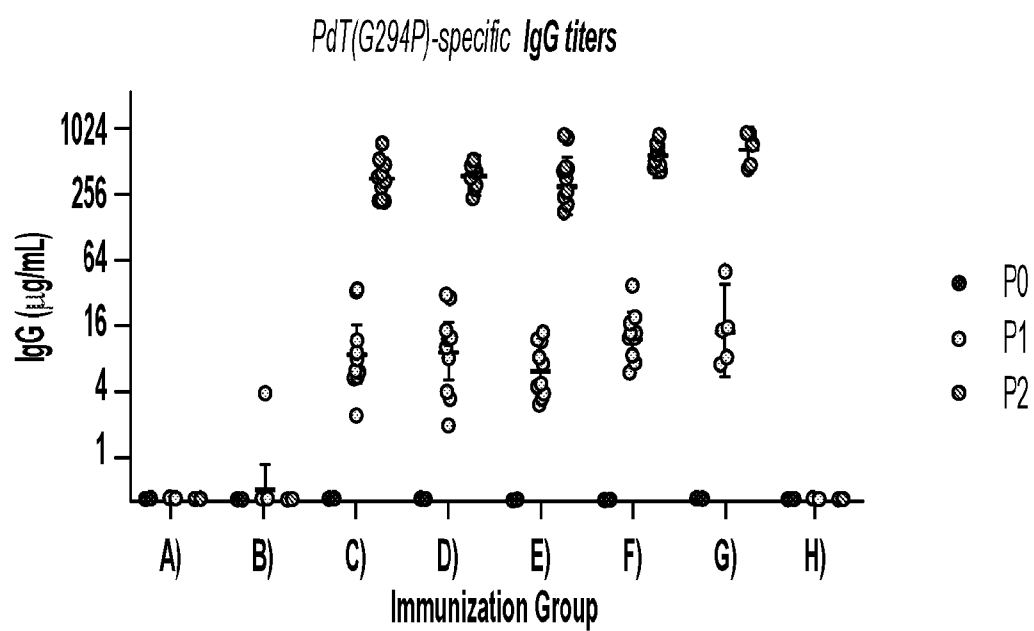
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Geo Means ($\mu\text{g/mL}$)	A	B	C	D	E	F	G	H
P1	52.3	38.0	56.9	61.7	48.0	0.4	58.4	0.4
P2	372.7	264.4	274.0	356.6	321.1	1.2	352.1	0.4

FIG. 13

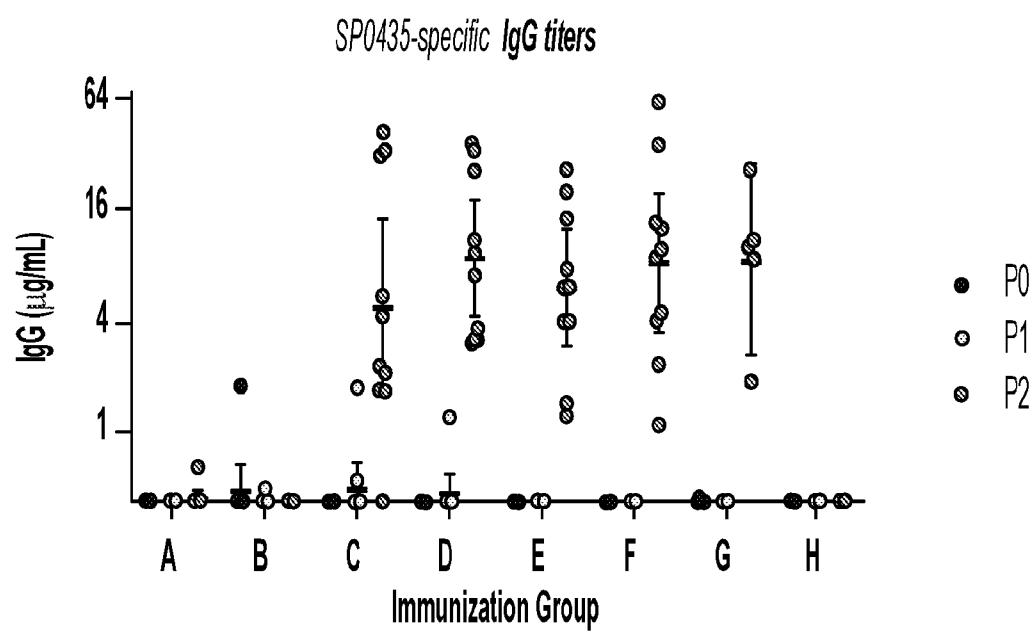
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Geo Means ($\mu\text{g/mL}$)	A	B	C	D	E	F	G	H
P1	0.4	0.5	9.1	9.6	6.5	13.5	15.1	0.4
P2	0.4	0.4	382.7	406.7	402.3	625.4	719.4	0.4

FIG. 14

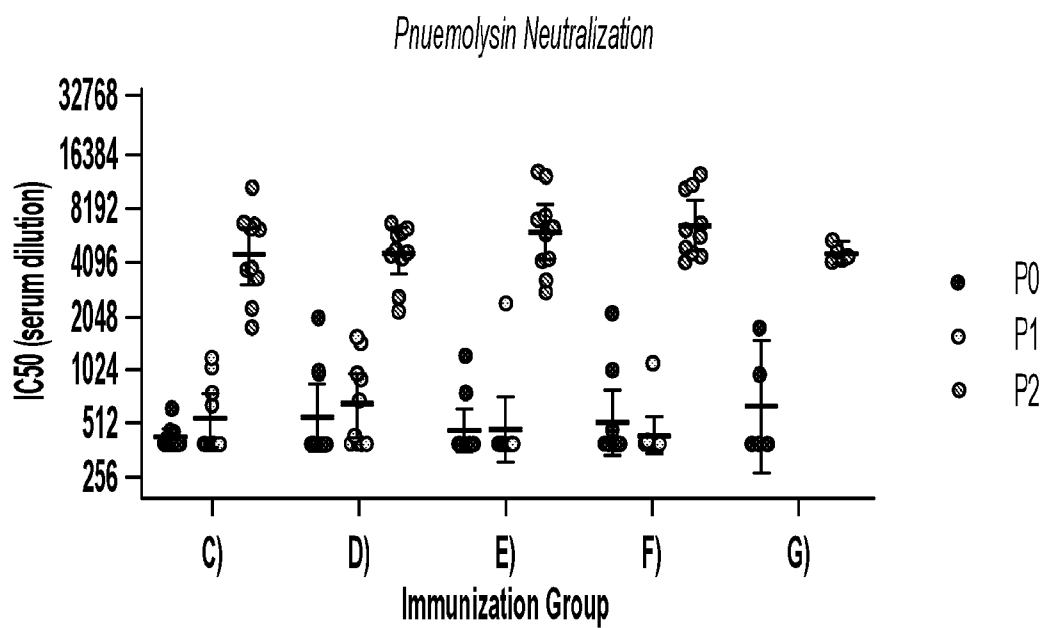
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Geo Means (ug/mL)	A	B	C	D	E	F	G	H
P1	0.42	0.43	0.50	0.47	0.42	0.42	0.42	0.42
P2	0.44	0.42	4.80	8.84	6.05	8.36	8.71	0.42

FIG. 15

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Geo Means (IC ₅₀)	A	B	C	D	E	F	G	H
P0	N/A	N/A	437	564	478	529	642	TBD
P1	N/A	N/A	552	662	479	445	TBD	TBD
P2	N/A	N/A	4514	4531	5900	6525	4529	TBD

FIG. 16

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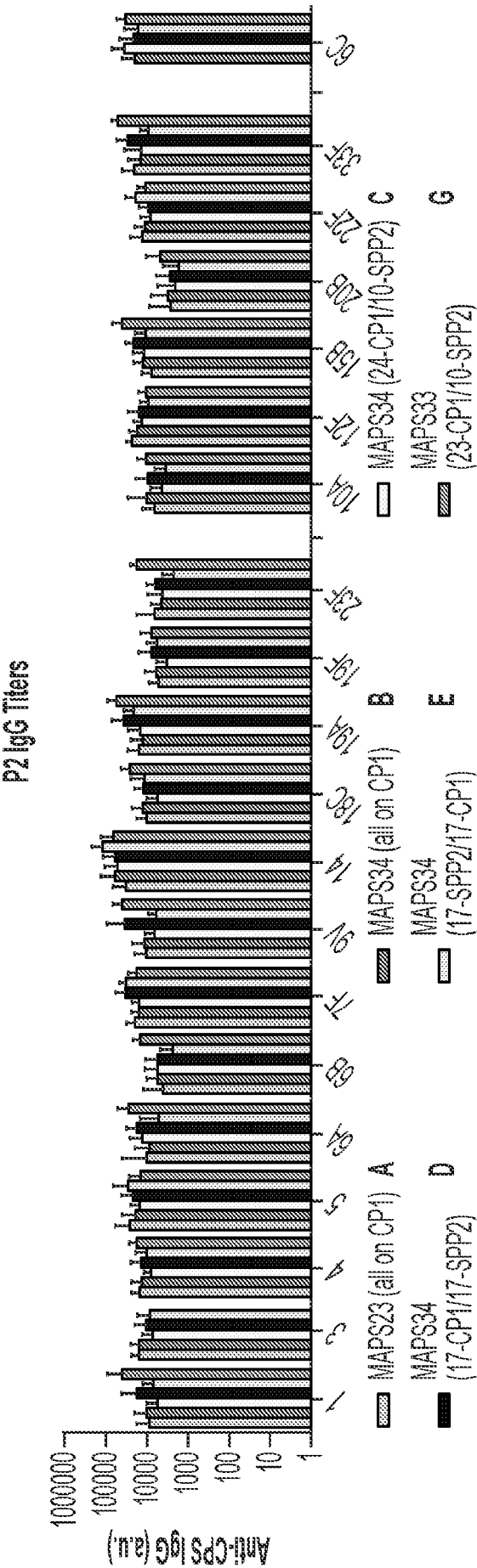


FIG. 17A

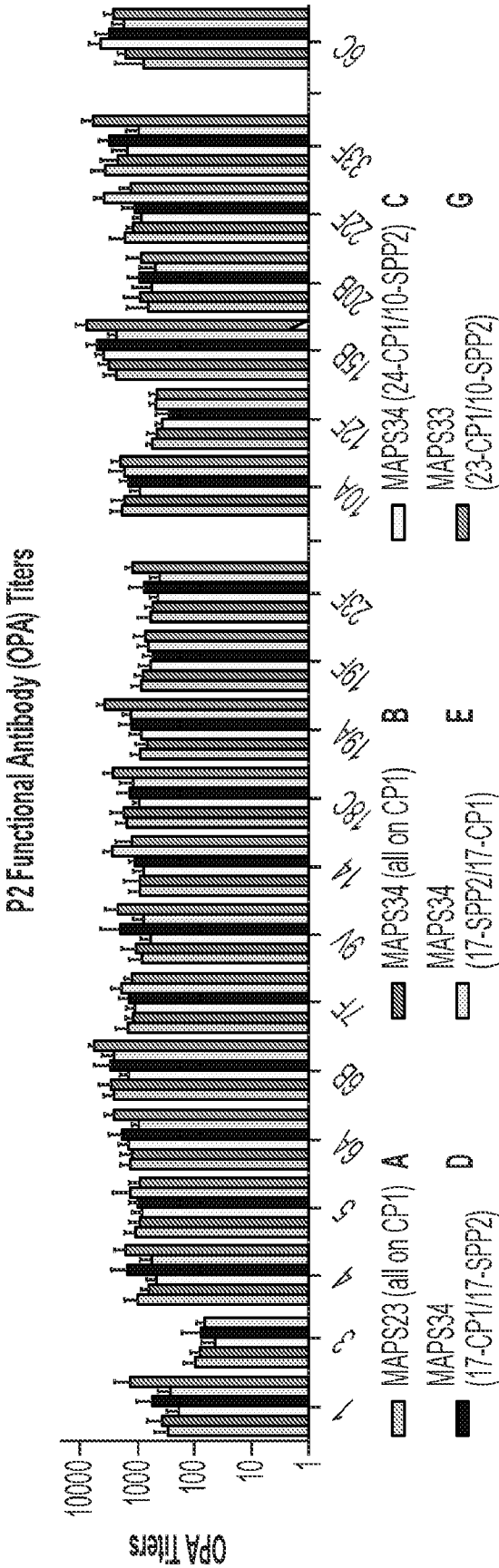


FIG. 17B

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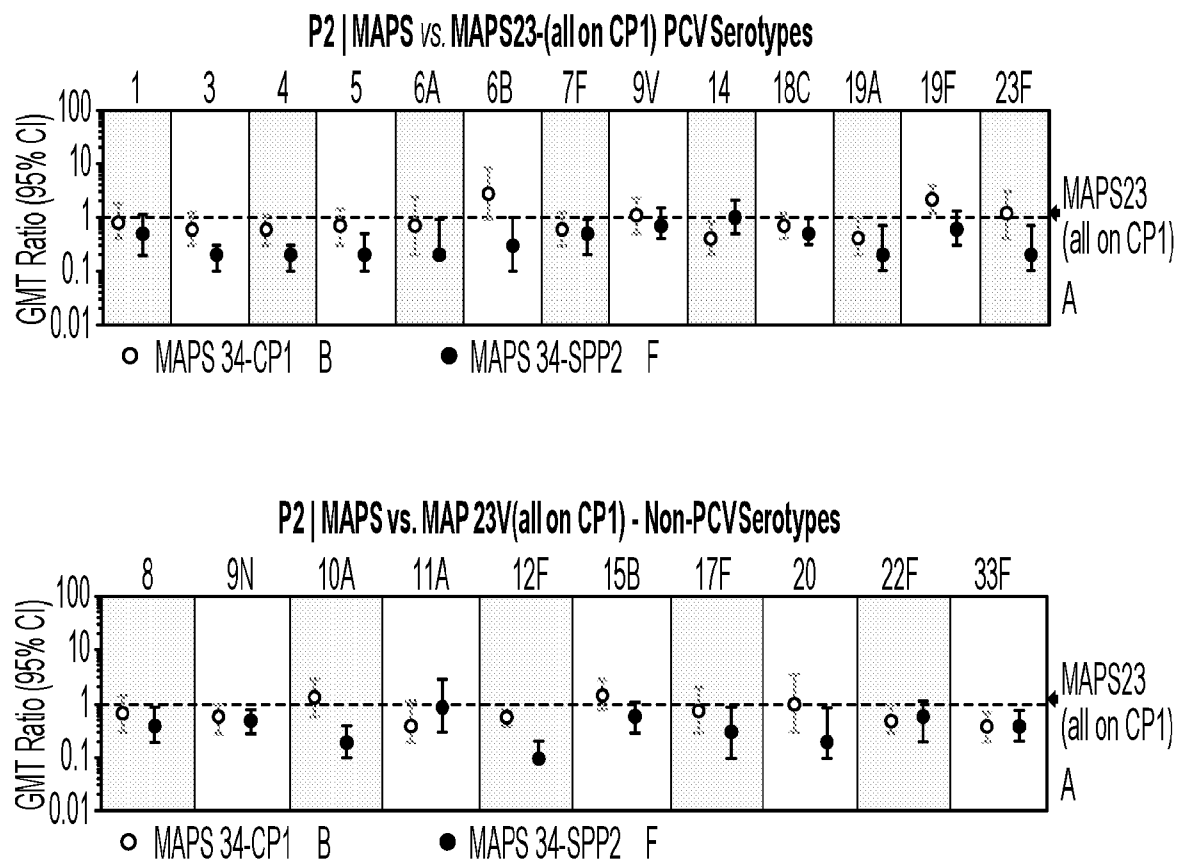


FIG. 18

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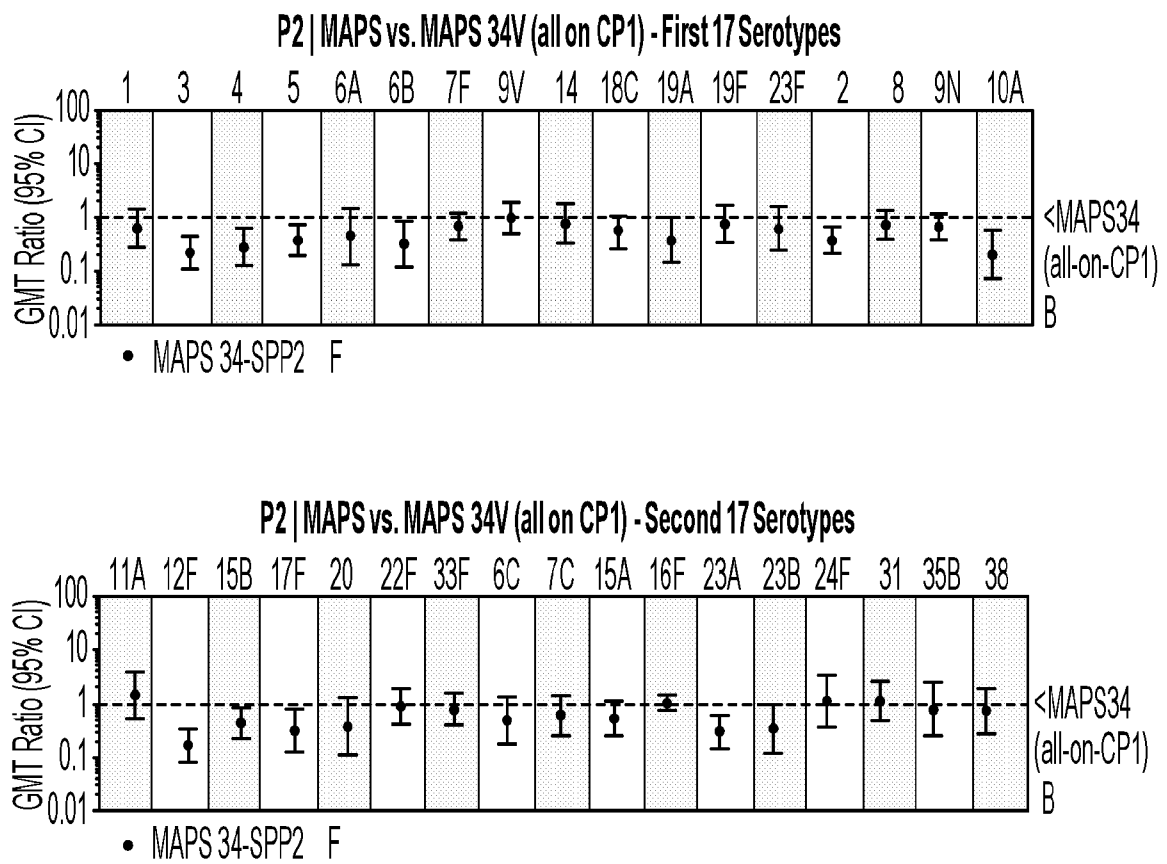


FIG. 19

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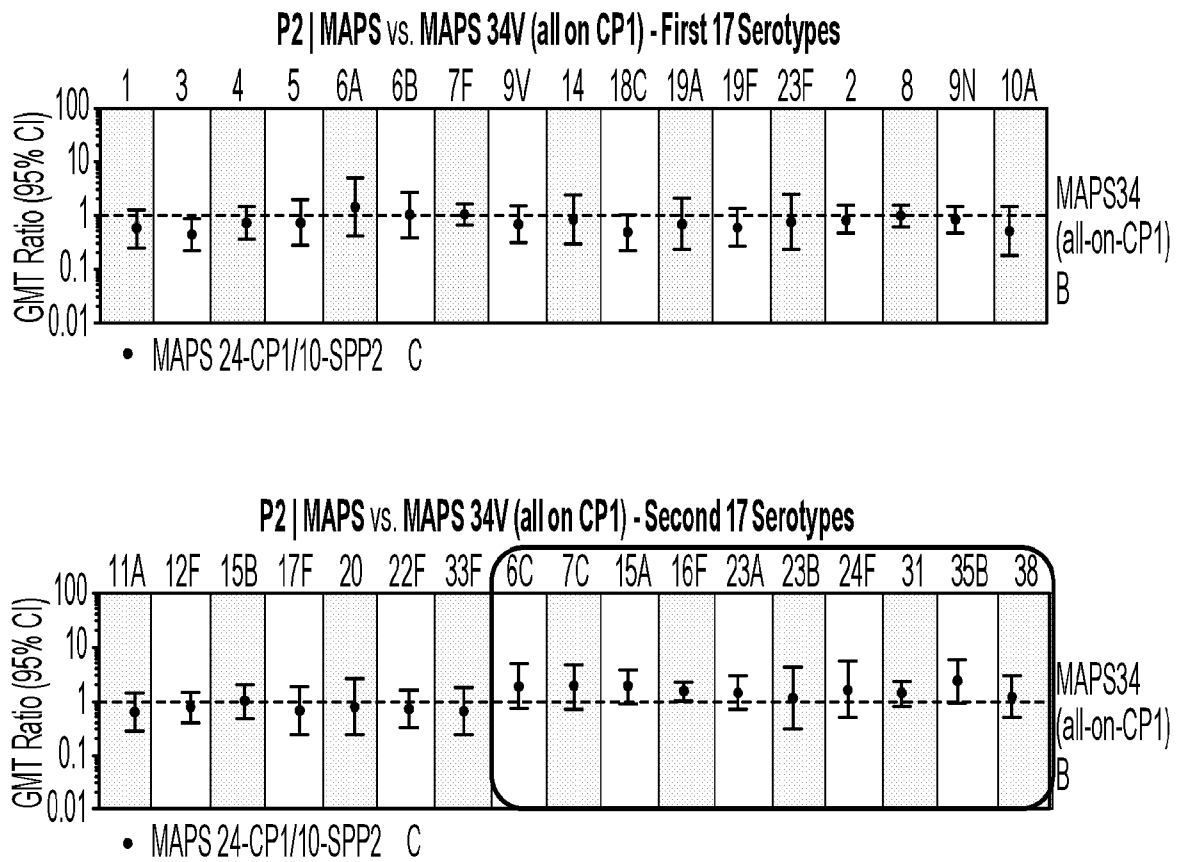


FIG. 20

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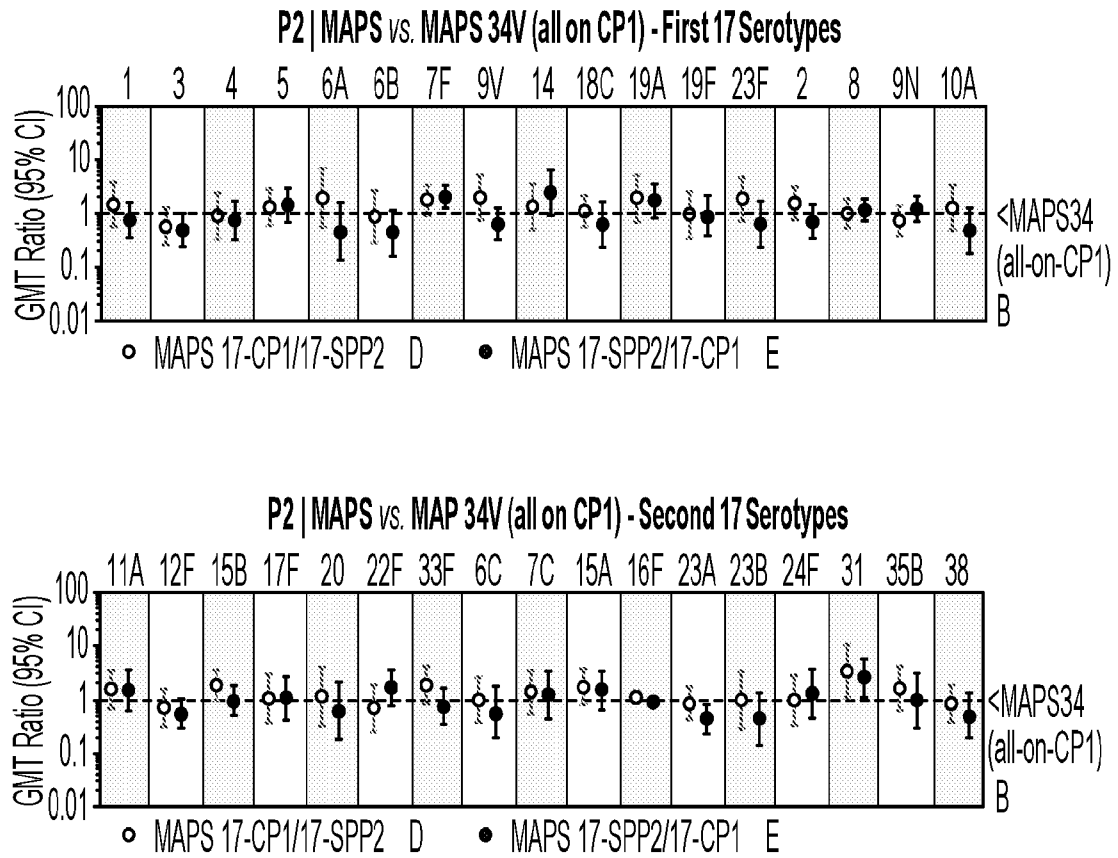


FIG. 21

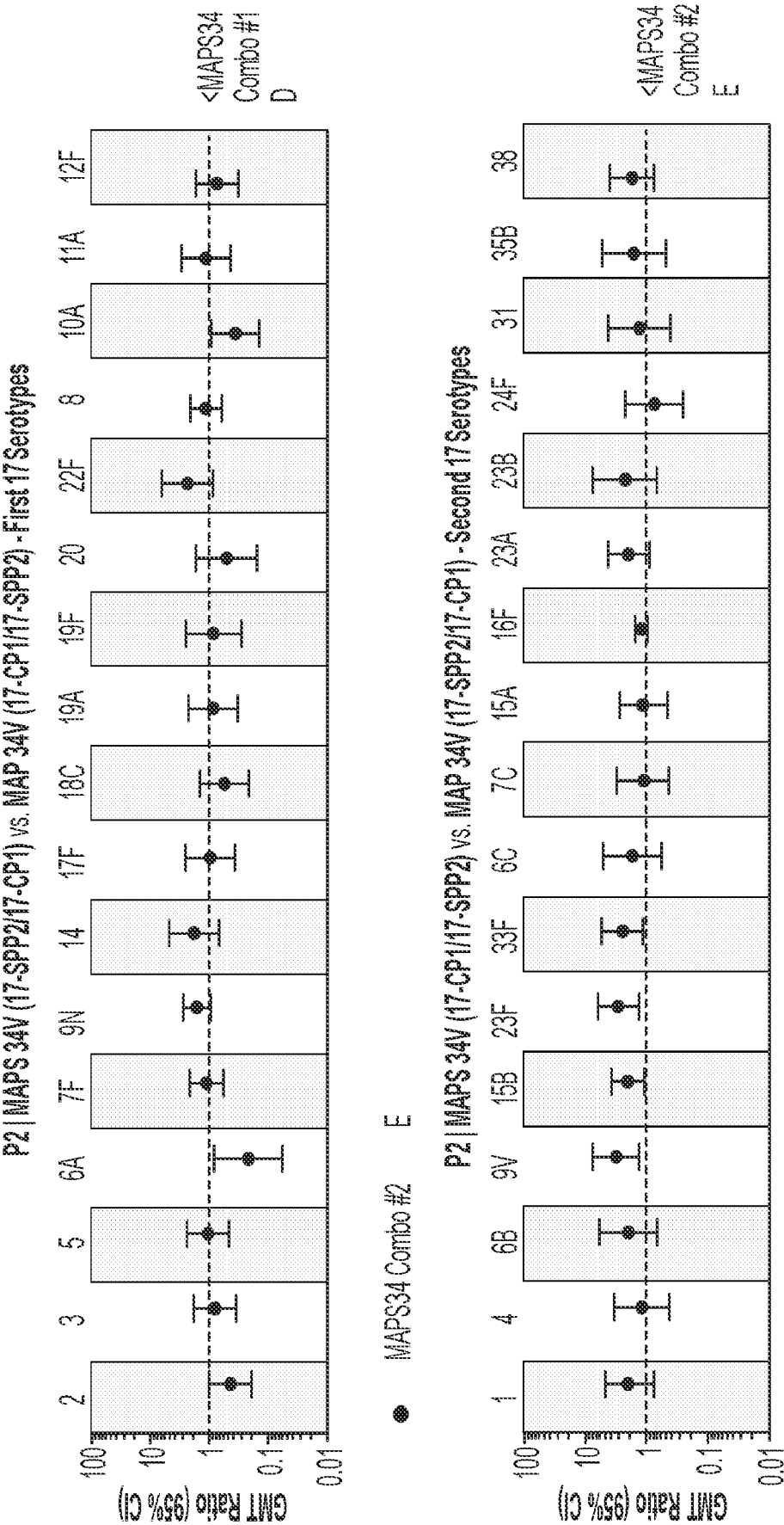


FIG. 22

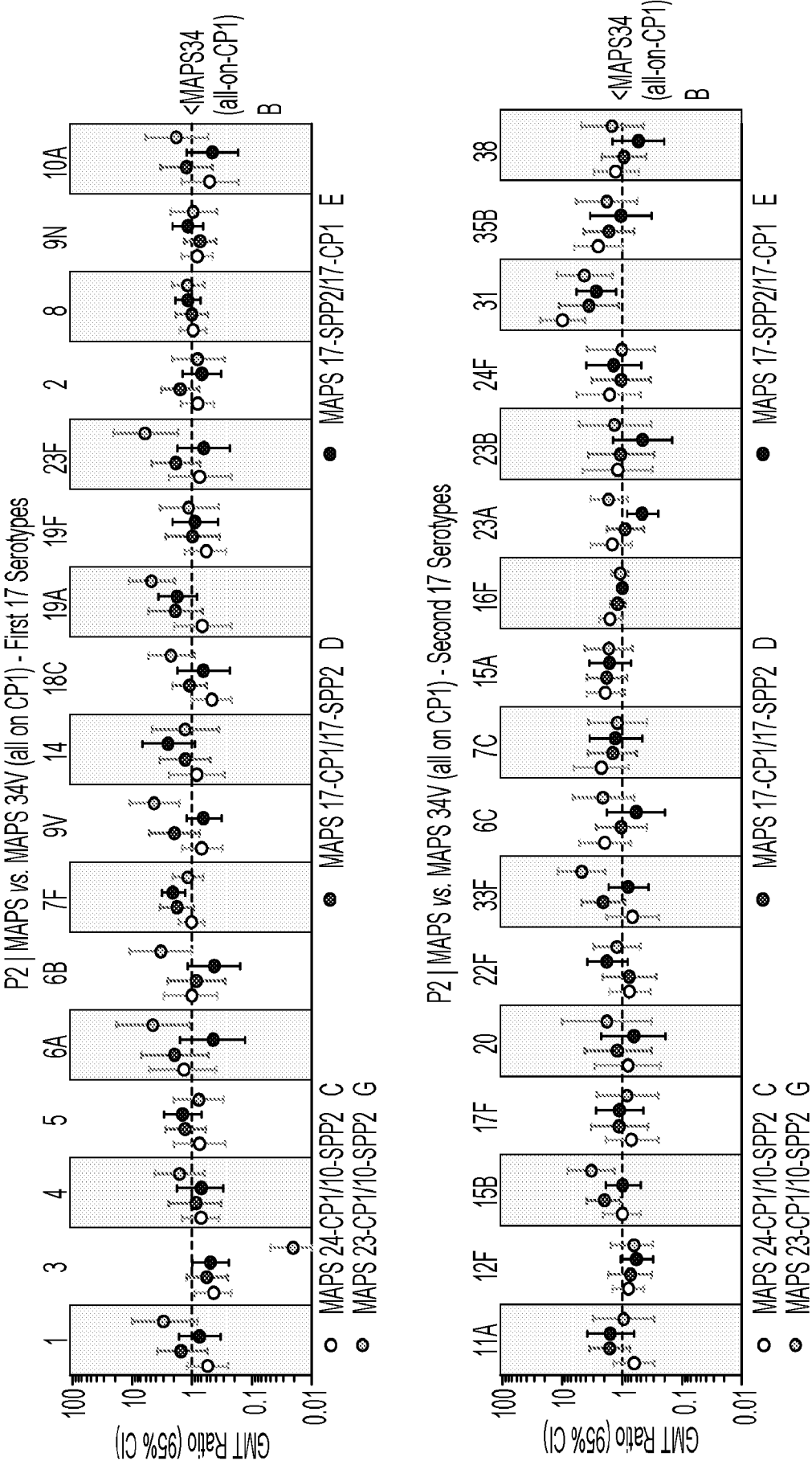


FIG. 23

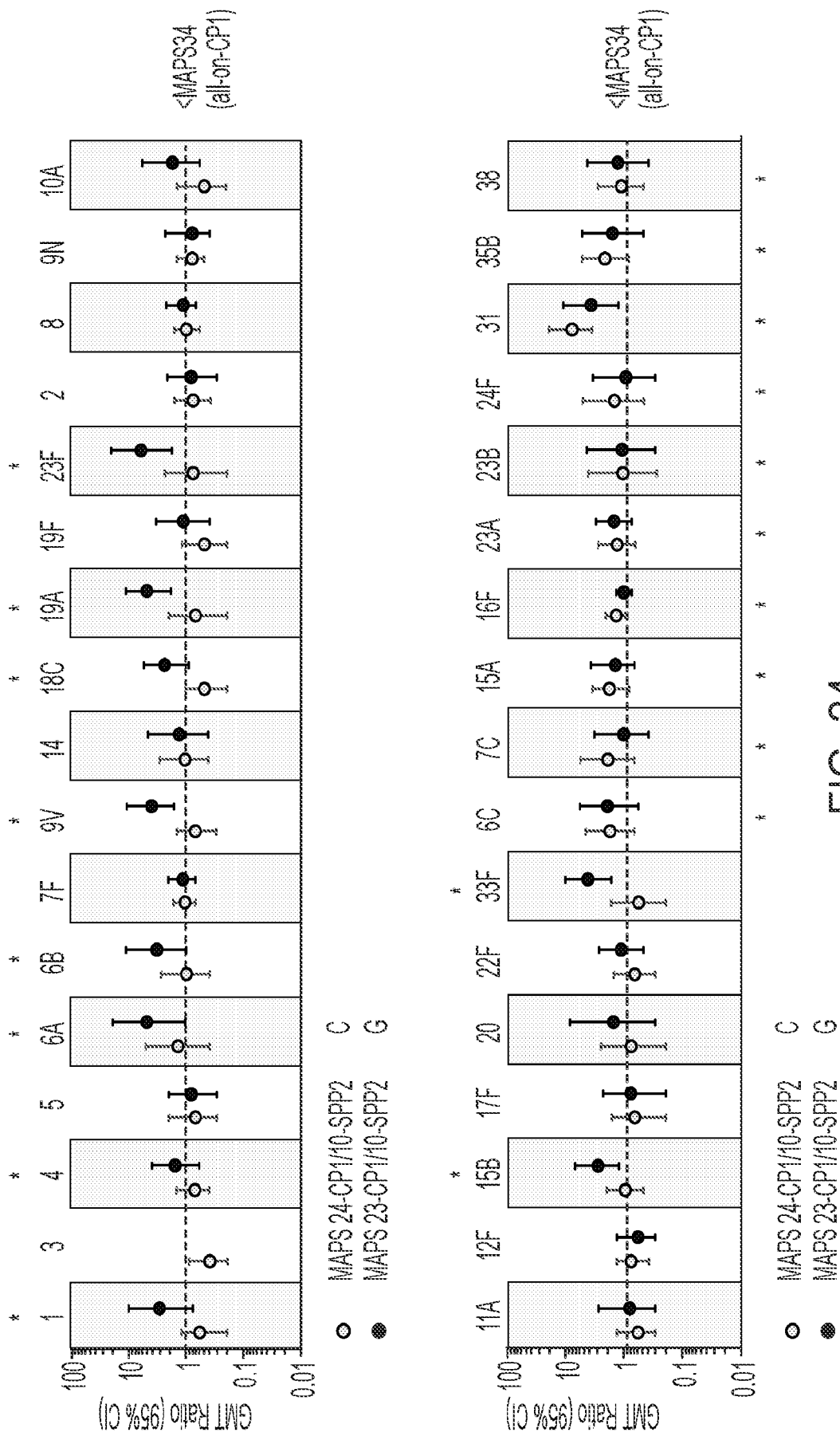


FIG. 24

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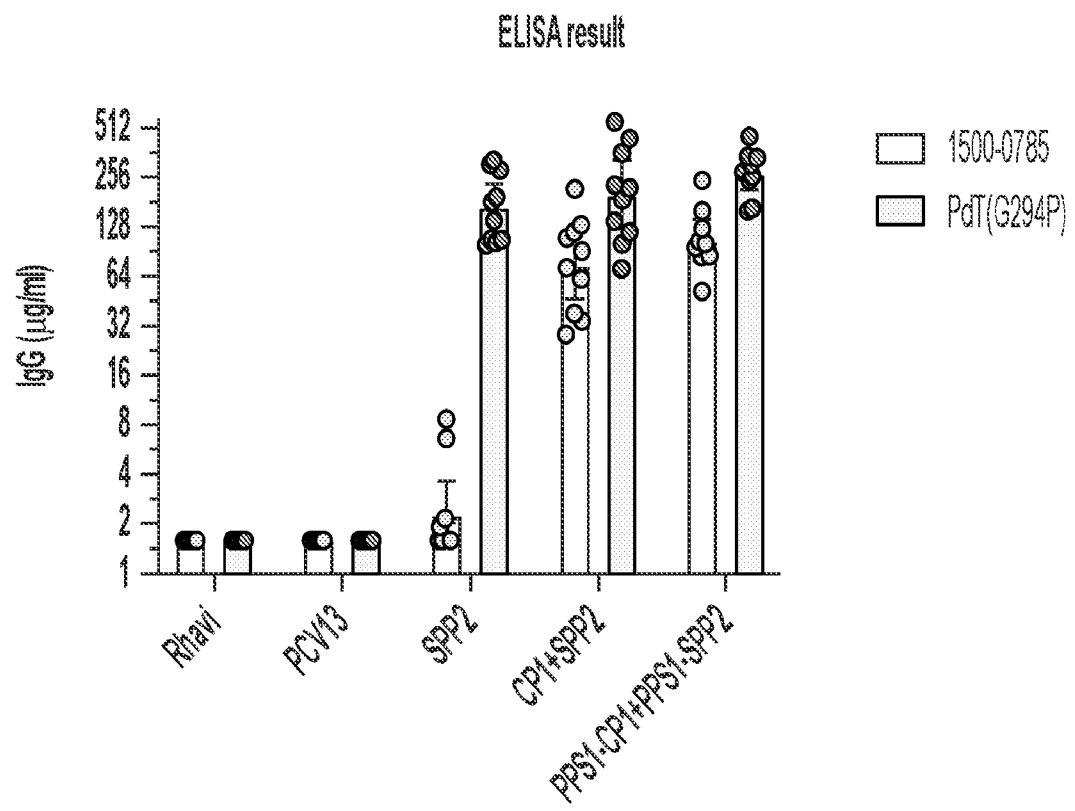


FIG. 25

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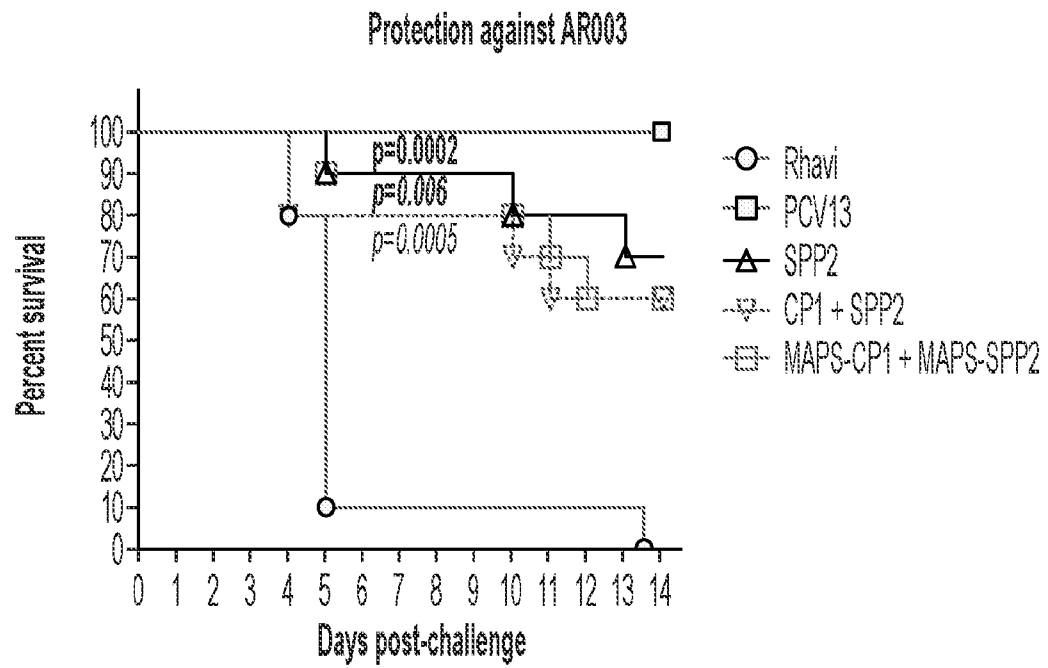


FIG. 26

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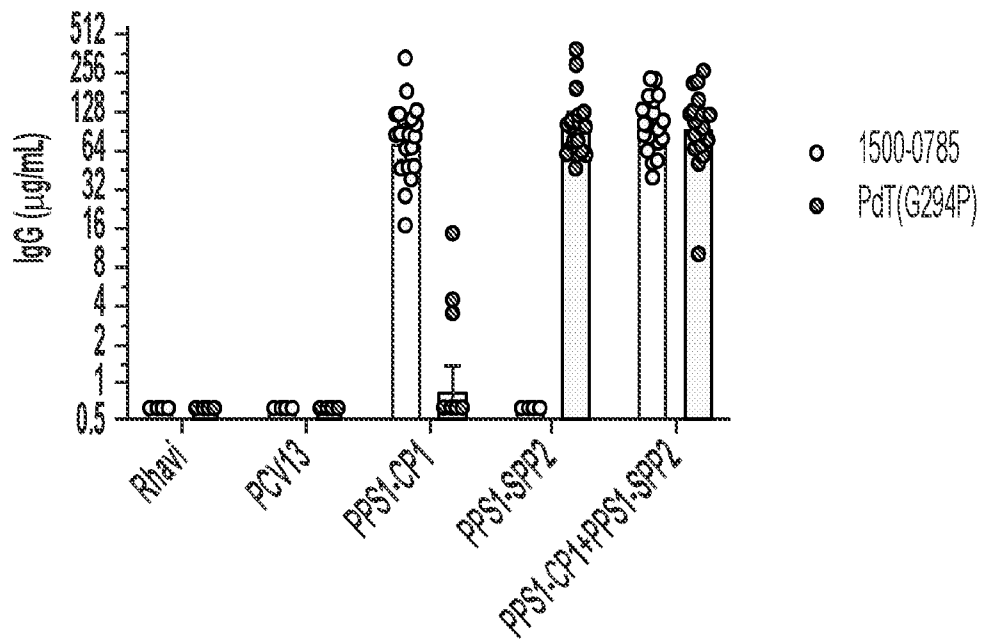


FIG. 27

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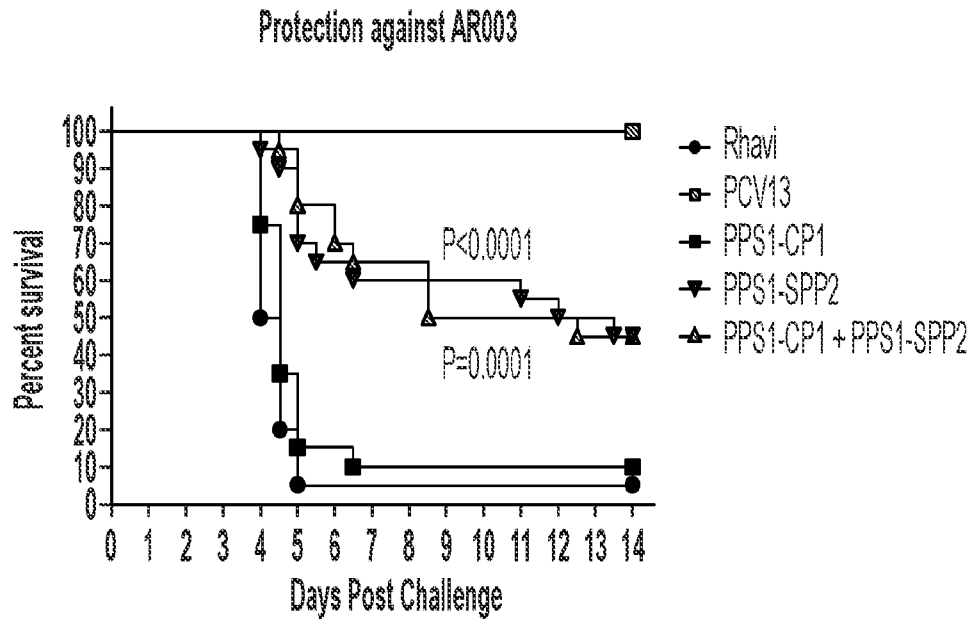


FIG. 28A

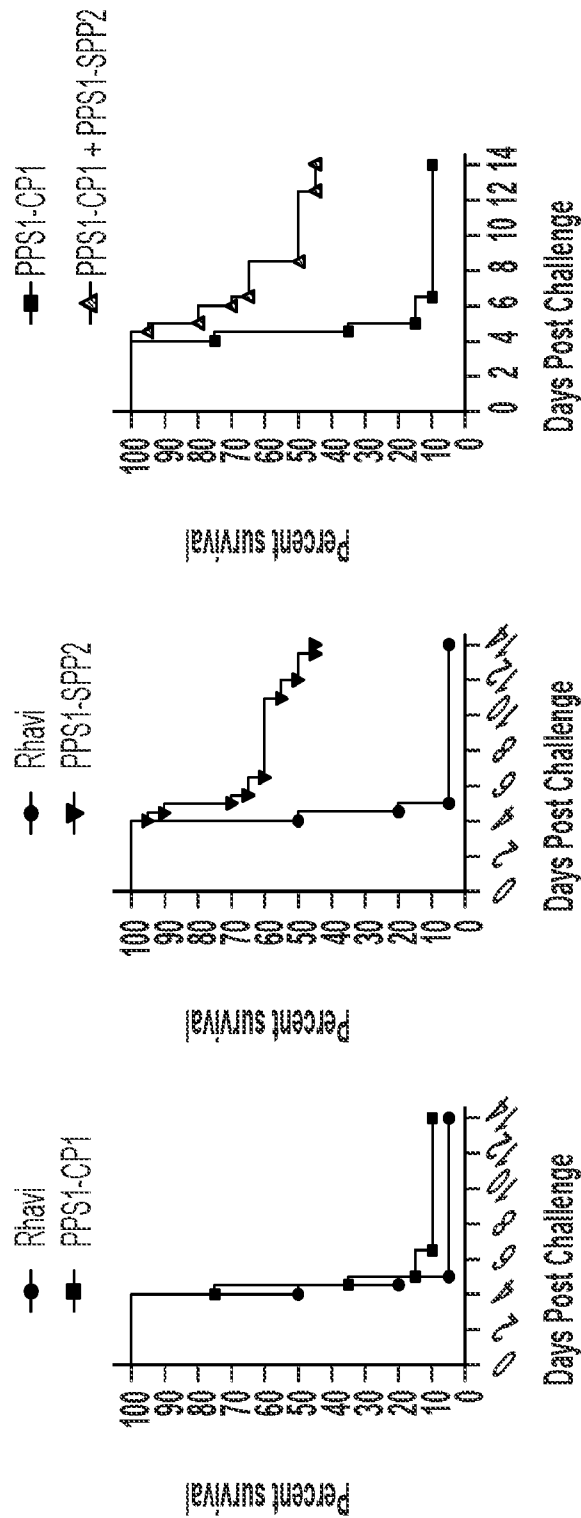


FIG. 28B

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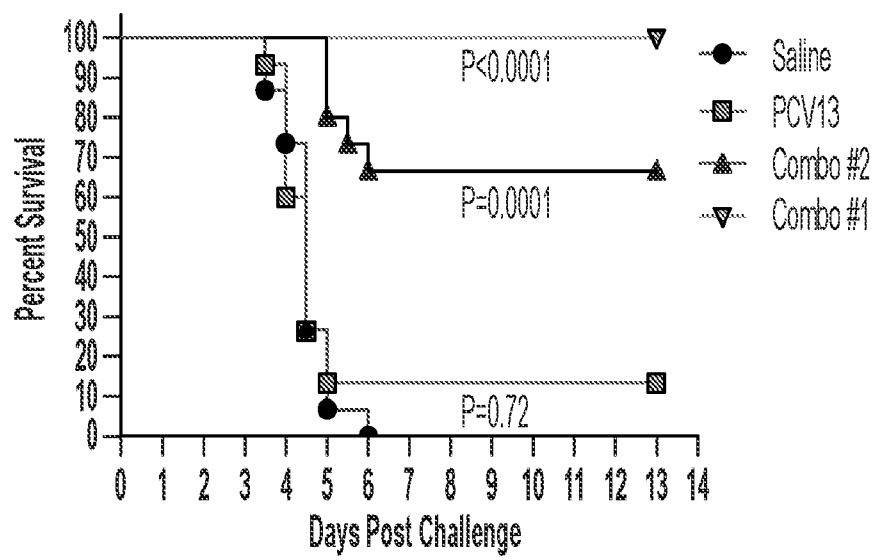


FIG. 29

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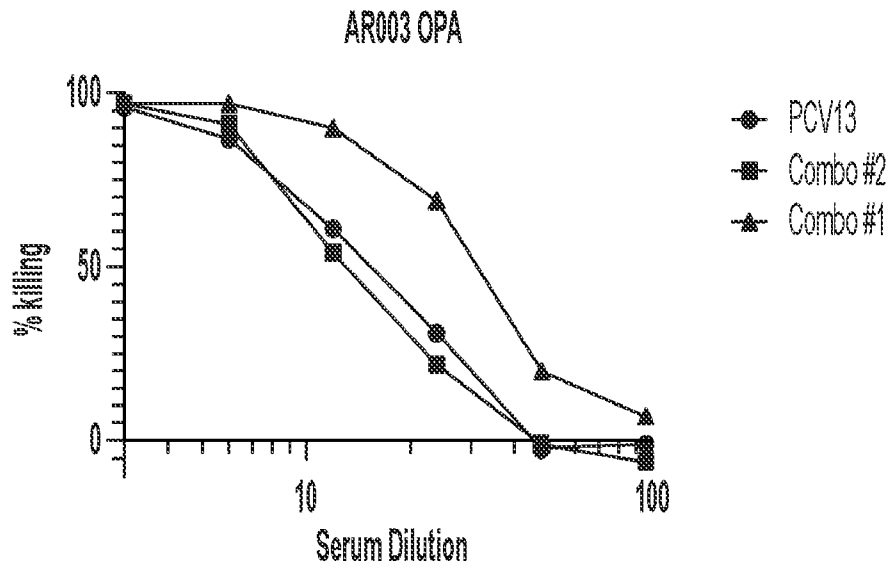


FIG. 30

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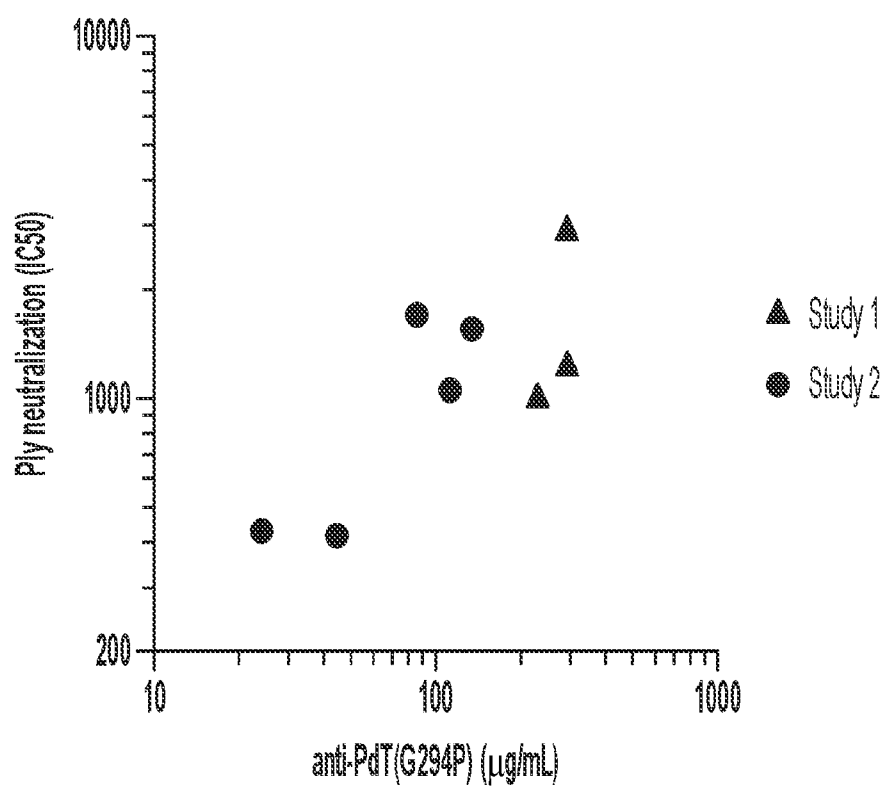


FIG. 31

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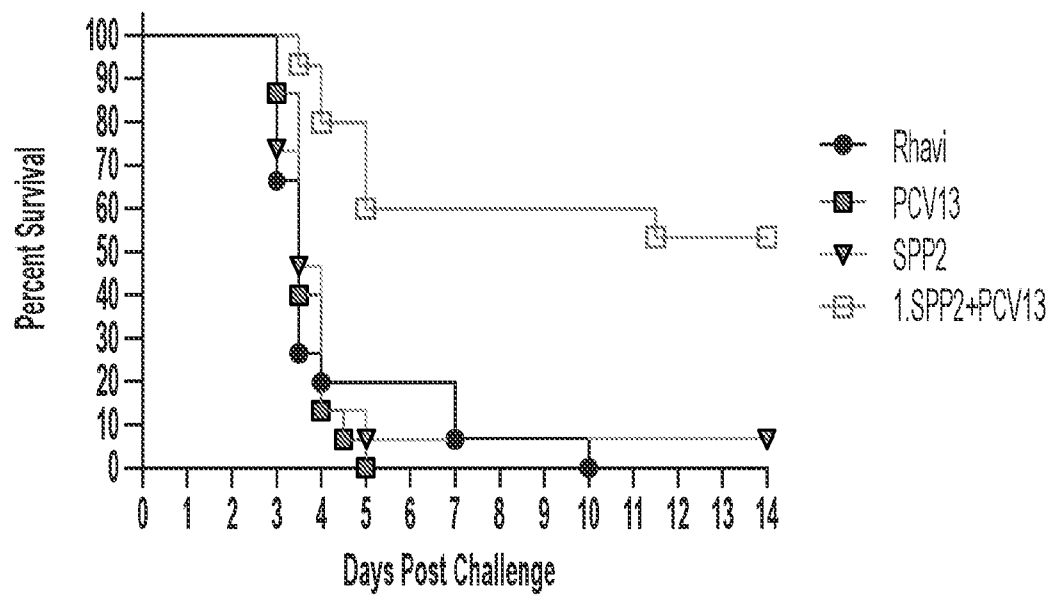


FIG. 32

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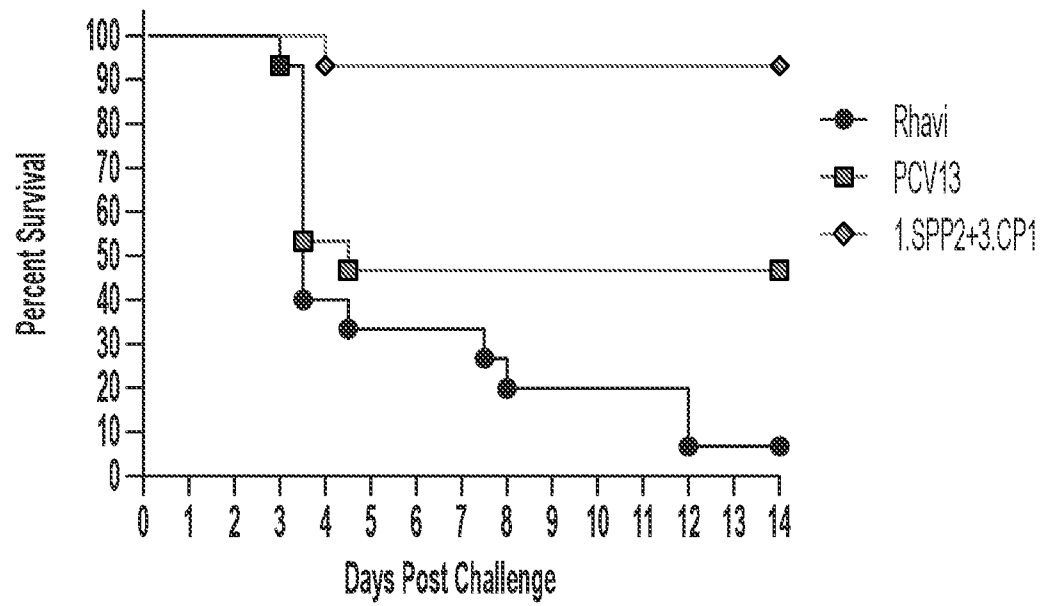


FIG. 33

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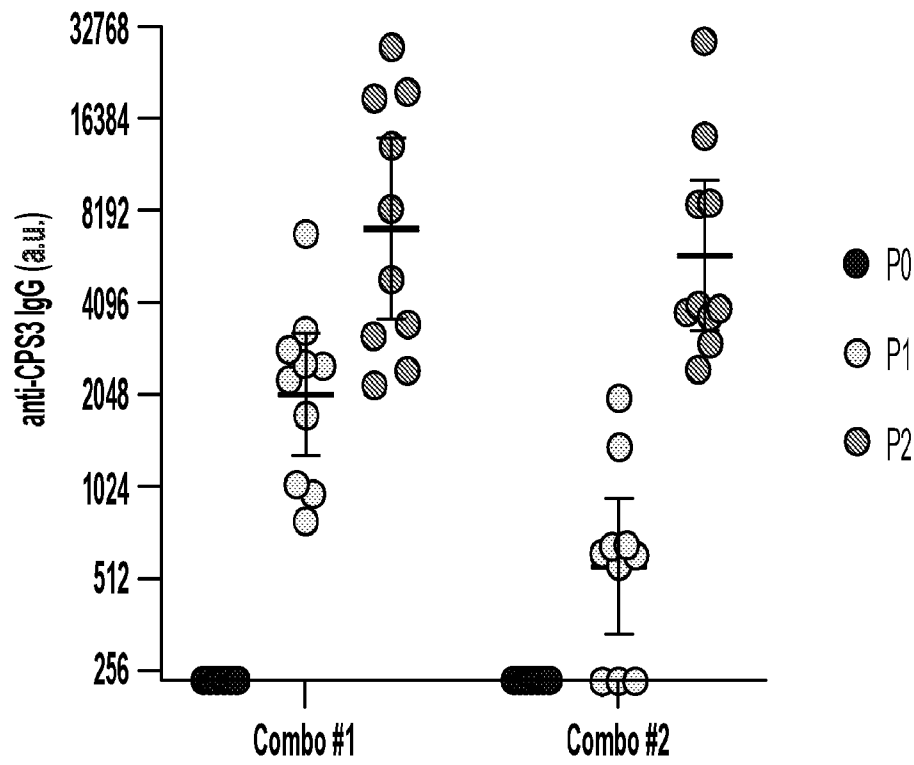


FIG. 34A

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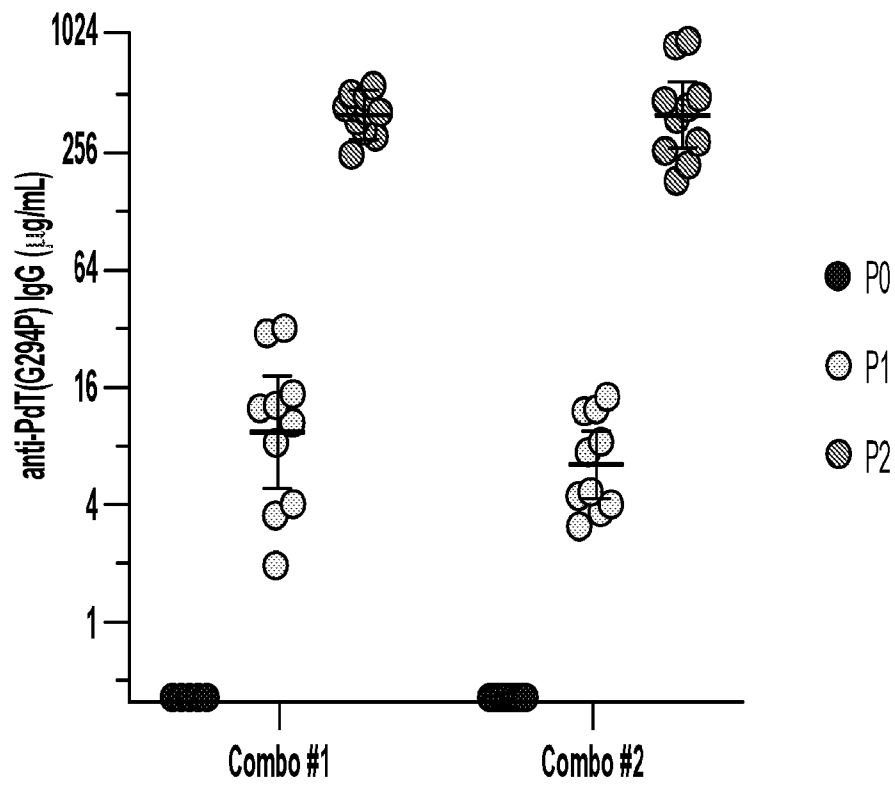


FIG. 34B

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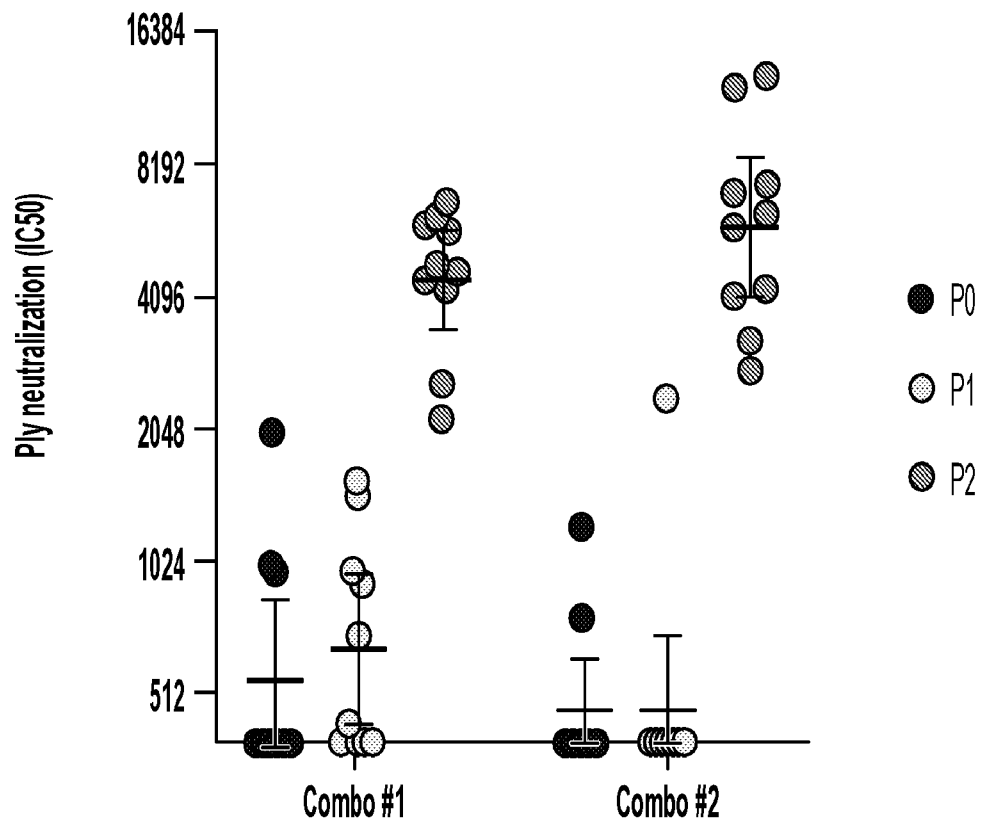
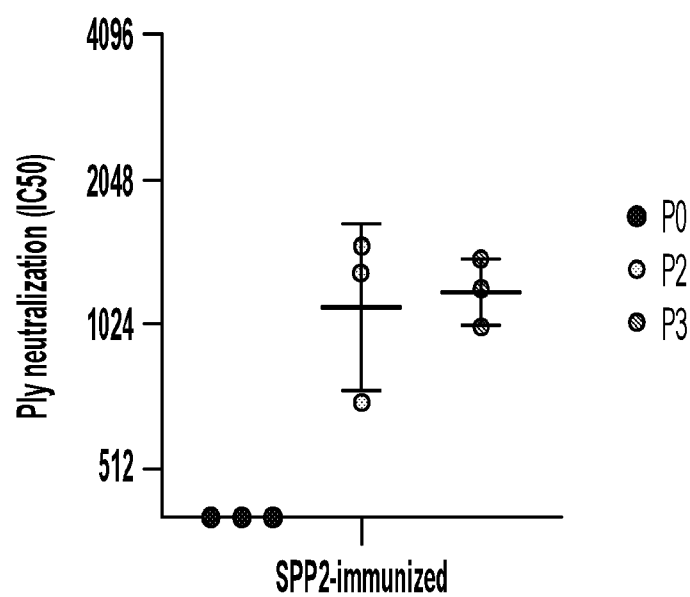


FIG. 34C

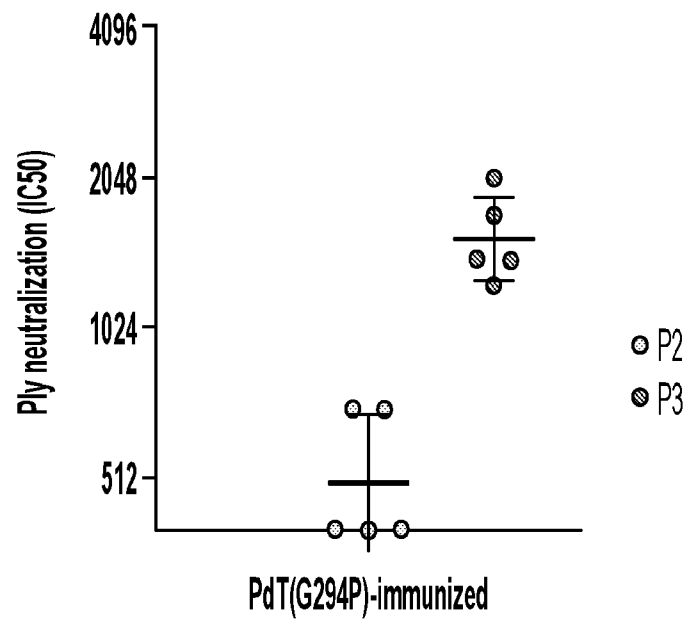
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Geometric mean IC50 (serum dilution)			
Group	P0	P2	P3
non-His-tagged SPP2	400	1109	1194

FIG. 35A

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Geometric mean IC50 (serum dilution)			
Group	P0	P2	P3
PdT(G294P)	n/a	500	1535

FIG. 35B

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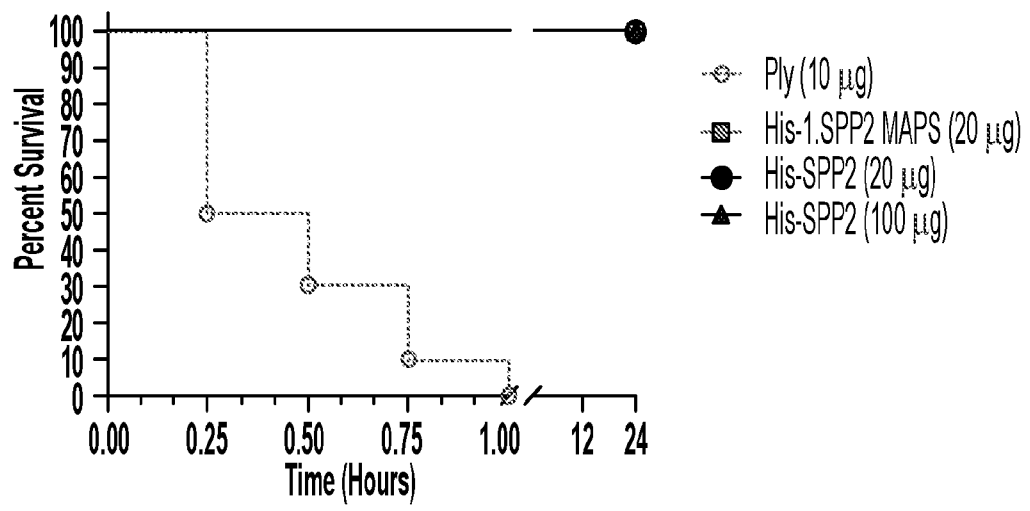


FIG. 36A

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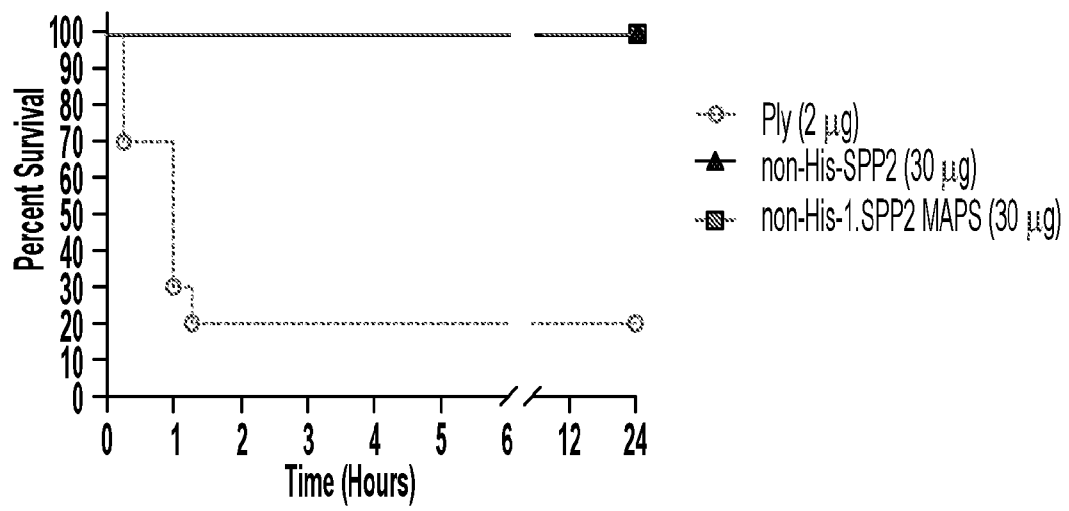


FIG. 36B

●

• Prena 13 Types

●

• A3772 Next Eleven

●

• PNG Ten New

✕

• Not included

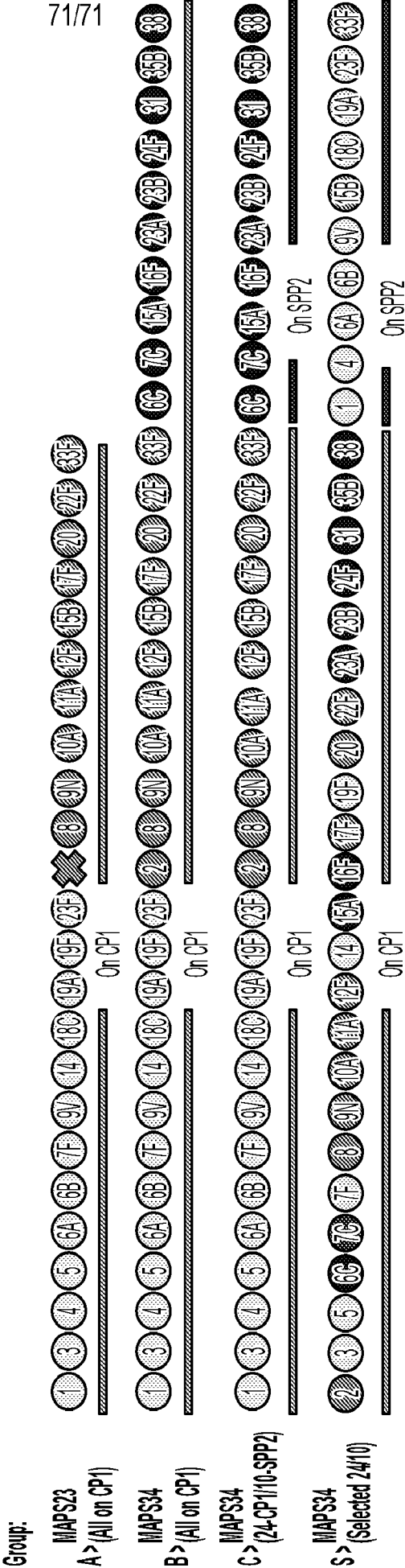


FIG. 37

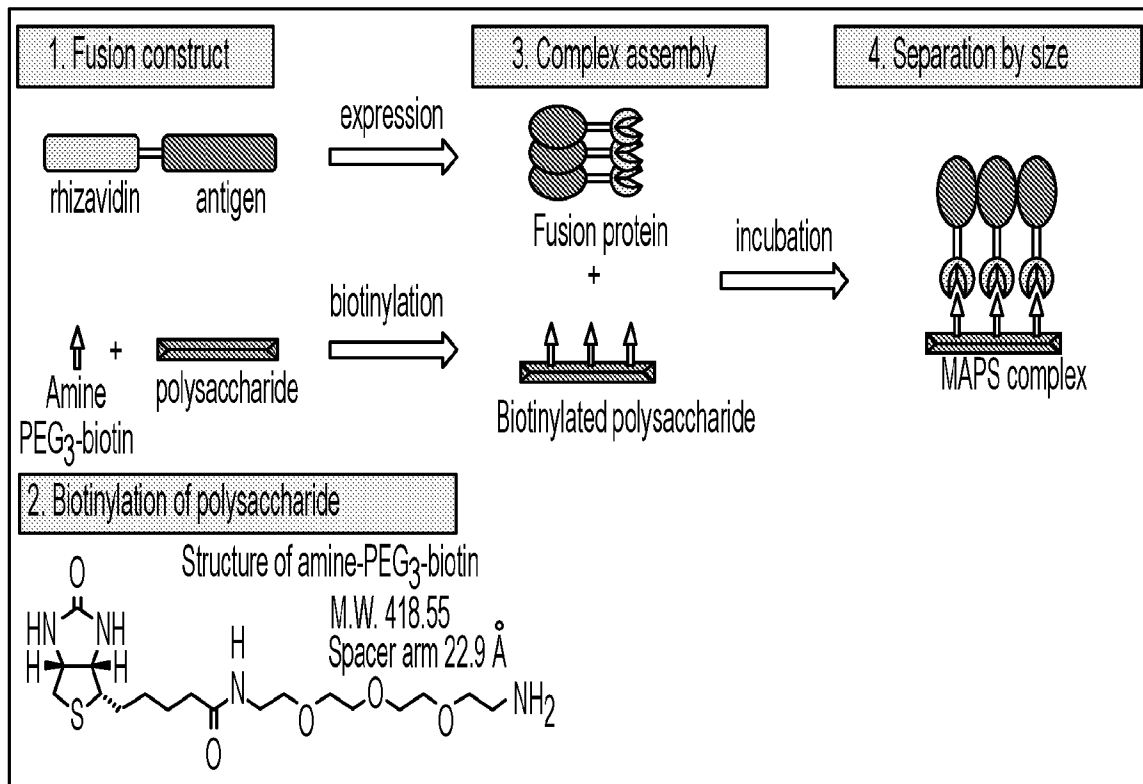


FIG. 1