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(54) Title: RSV IMMUNOGENS, ANTIBODIES AND COMPOSITIONS THEREOF

EPITOPE NSLLSLINDMPITNDQKKLSNN

NDQKKLSNN NSLLSLIND

FINAL SFNDEKKLASNETAHLPLNLEEQRSAFLSSINDDPQSANLLAEAKKLNDQAQPK

ORIGINAL KFNKEQQNAPFYELHLPLNLEEQRNAPFIQSLKDDPQSANLLAEAKKLNDQAQPK



FIG. 7

(57) Abstract: The present invention provides immunogens that protect against RSV infection. The present invention also provides antibody proteins that protect against RSV infection. Such immunogens and antibody proteins are produced based on three-dimensional models also included in the invention. One model is of a complex between motavizumab and its antibody-binding domain on RSV fusion (F) protein. A second model is of a complex between 10 IF antibody and its antibody-binding domain on RSV F protein. The immunogens disclosed herein have been modified to elicit a humoral response against RSV F protein without eliciting a significant cell-mediated response against RSV. Such immunogens can comprise a scaffold into which RSV contact residues are embedded. The present invention also includes methods that utilize the disclosed three-dimensional models to produce immunogens and antibody proteins of the present invention. Also disclosed are methods of using the disclosed immunogens, for example to protect individuals from RSV infection. Also disclosed are methods of using the disclosed antibody proteins, for example to protect individuals from RSV infection.

RSV IMMUNOGENS, ANTIBODIES AND COMPOSITIONS THEREOF

This application claims the benefit of U.S. Provisional Patent Application No. 61/253,826, filed October 21, 2009, which is hereby expressly incorporated by reference in its entirety.

FIELD

The present invention relates to novel compositions that protect individuals from respiratory syncytial virus (RSV) infection. In particular, the present invention relates to vaccines that elicit antibodies having a high affinity for the RSV fusion (F) protein. The present invention also relates to therapeutic compositions comprising antibodies having a high affinity for the RSV F protein.

BACKGROUND

Respiratory syncytial virus (RSV) is a highly contagious member of the paramyxoviridae family of viruses that causes significant worldwide morbidity and mortality each year, particularly in infants. RSV infects people repeatedly throughout life, and causes significant morbidity in healthy children and adults. The RSV fusion (F) protein (see, e.g., Lopez JA et al., 1998, J. Virol. 72, 6922-6928) and antibodies thereto, have been targets for vaccine efforts. There is currently no licensed RSV vaccine. A previous vaccine trial in the 1960s containing a formalin-inactivated RSV actually enhanced the severity of disease upon natural infection with RSV. This was thought to have occurred due to an imbalanced T-cell response and elicitation of low avidity antibodies. Since there is currently no licensed RSV vaccine, passive immunization is used to prevent RSV infection, especially in those infants with prematurity, bronchopulmonary dysplasia, or congenital heart disease. Originally, RSV-neutralizing polyclonal antibodies from pooled human sera (RESPIGAM[®]) were used (see, e.g., Groothuis JR et al., 1995, Pediatrics 95, 463-467). This treatment was followed by the development of palivizumab (SYNAGIS[®]) (see, e.g., Johnson S et al., 1997, J. Infect. Dis. 176, 1215-1224). Palivizumab was humanized from mouse antibody 1129, which binds a 24-amino acid, linear, conformational epitope on the RSV F protein (see, e.g., Beeler JA, et al., 1989, J. Virol. 63, 2941-2950; Arbiza J et al., J. Gen. Virol. 73, 2225-2234, Lopez JA et al., 1993, J. Gen. Virol. 74, 2567-2577). Palivizumab binds to the F protein and thereby neutralizes the virus. Such treatments are expensive, costing approximately \$1000 per dose. Moreover, the antibodies must be administered on a monthly basis during the

winter months, thereby adding to the cost of treatment. When administered at a dose of 15 mg/kg each month during the RSV season, palivizumab reduces RSV-related hospitalizations by 55% (see, e.g., The Impact-RSV Study Group, 1998, Pediatrics 102, 531-537). Thus, a vaccine requiring only one or two administrations would have advantages over the current preventative treatment for RSV.

SUMMARY

The present invention provides immunogens that protect against RSV infection. The present invention also provides antibody proteins that protect against RSV infection. Such immunogens and antibody proteins are produced based on three-dimensional models also included in the invention. One model is of a complex between motavizumab and its antibody-binding domain on RSV fusion (F) protein. A second model is of a complex between 101F antibody and its antibody-binding domain on RSV F protein. The immunogens disclosed herein have been modified to elicit a humoral response against RSV F protein without eliciting a significant cell-mediated response against RSV. Such immunogens can comprise a scaffold into which RSV contact residues are embedded. The present invention also includes methods that utilize the disclosed three-dimensional models to produce immunogens and antibody proteins of the present invention. Also disclosed are methods of using the disclosed immunogens, for example to protect individuals from RSV infection. Also disclosed are methods of using the disclosed antibody proteins, for example to protect individuals from RSV infection.

The present disclosure provides an RSV immunogen comprising an amino acid sequence of 1LP1_b (SEQ ID NO: 11) having from one to twenty amino acid substitutions, wherein at least one amino acid substitution is selected from the group consisting of: (a) substitution of a serine at amino acid position N-25 in SEQ ID NO:11; (b) substitution of a leucine at amino acid position I-28 in SEQ ID NO:11; (c) substitution of a serine at amino acid position Q-29 in SEQ ID NO: 11; (d) substitution of an isoleucine at amino acid position L-31 in SEQ ID NO:11; (e) substitution of an asparagine at amino acid position K-32 in SEQ ID NO:11; (f) substitution of an aspartic acid at amino acid position K-4 in SEQ ID NO:11; (g) substitution of a lysine at amino acid position Q-6 in SEQ ID NO:11; (h) substitution of a lysine at amino acid position Q-7 in SEQ ID NO:11; (i) substitution of a leucine at amino acid position N-8 in SEQ ID NO:11; (j) substitution of a serine at amino acid position F-10 in SEQ ID NO:11; and (k) substitution of an asparagine at amino acid position Y-11 in SEQ ID NO:11. Such an RSV

immunogen can comprise all of the substitutions (a) through (k). The present disclosure also provides an RSV immunogen comprising an amino acid sequence of truncated 1S2X_a (SEQ ID NO:13) having from one to twenty-five amino acid substitutions, wherein at least one amino acid substitution is selected from the group consisting of: (a) substitution of a serine at amino acid position 92 in SEQ ID NO:13; (b) substitution of a leucine at amino acid position 95 in SEQ ID NO:13; (c) substitution of a serine at amino acid position 96 in SEQ ID NO:13; (d) substitution of an isoleucine at amino acid position 98 in SEQ ID NO:13; (e) substitution of an asparagine at amino acid position 99 in SEQ ID NO:13; (f) substitution of an aspartic acid at amino acid position 100 in SEQ ID NO:13; (g) substitution of an asparagine at amino acid position 105 in SEQ ID NO:13; (h) substitution of an aspartic acid at amino acid position 106 in SEQ ID NO:13; (i) substitution of a lysine at amino acid position 108 in SEQ ID NO:13; (j) substitution of a lysine at amino acid position 109 in SEQ ID NO:13; (k) substitution of a leucine at amino acid position 110 in SEQ ID NO:13; (l) substitution of a serine at amino acid position 112 in SEQ ID NO:13; and (m) substitution of an asparagine at amino acid position 113 in SEQ ID NO:13. Such an RSV immunogen can comprise all of the substitutions (a) through (m). The present disclosure also provides a nucleic acid molecule comprising a nucleic acid sequence that encodes any of such RSV immunogens. Also provided is a recombinant molecule comprising such a nucleic acid molecule. Also provided is a recombinant cell comprising such a recombinant molecule. Also provided are uses of such nucleic acid molecules, recombinant molecules, and recombinant cells to produce any of such RSV immunogens. A recombinant molecule can also be a nucleic acid vaccine. Also provided is a composition that can include any of the RSV immunogens, nucleic acid molecules, recombinant molecules, or recombinant cells. Also provided is a vaccine comprising any of such RSV immunogens. The disclosure provides a method to elicit a neutralizing humoral immune response against RSV; such method comprises administering any of such RSV immunogens or vaccines, wherein such administration elicits a neutralizing humoral immune response against RSV. Also provided is a method to protect a patient from RSV infection; such method comprises administering to the patient any of such RSV immunogens or vaccines, wherein such administration protects the patient from RSV infection.

The disclosure provides an immunogen comprising an antibody-binding domain that binds an antibody selected from the group consisting of motavizumab and 101F

antibody, wherein the three-dimensional structure of the antibody-binding domain of the immunogen spatially corresponds to a three-dimensional structure of an antibody-binding domain of a fusion (F) peptide derived from respiratory syncytial virus (RSV) fusion (F) protein in a complex selected from the group consisting of:

5 (a) a complex between a F peptide consisting of amino acid sequence SEQ ID NO:2 and motavizumab, the complex being set forth in the three-dimensional model defined by the atomic coordinates specified in Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank accession code 3IXT; and

(b) a complex between a F peptide consisting of amino acid sequence SEQ ID
10 NO:4 and 101F antibody, the complex being set forth in the three-dimensional model defined by the atomic coordinates specified in Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank accession code 3O41;

wherein the antibody-binding domain of the immunogen comprises less than 12 consecutive amino acids from a motavizumab-binding domain or a 101F antibody-binding
15 domain of RSV F protein, and wherein the immunogen elicits a humoral immune response against RSV. In one embodiment, the antibody-binding domain of the immunogen comprises contact residues that have a spatial orientation represented by atomic coordinates that have a root mean square deviation of protein backbone atoms of less than
20 10 angstroms from the corresponding backbone atoms of contact residues in an RSV F peptide that contacts motavizumab or 101F antibody in the respective complex. In one embodiment, the immunogen comprises contact residues of the motavizumab-binding domain embedded in a protein scaffold comprising a three-dimensional structure having two alpha helices defined by atomic coordinates that have a root mean square deviation of protein backbone atoms of less than 10 angstroms when superimposed on the two alpha
25 helices of the peptide consisting of amino acid sequence SEQ ID NO:2 when complexed with motavizumab, the three-dimensional model of the complex being defined by the coordinates specified in Protein Data Bank accession code 3IXT. In one embodiment, the immunogen comprises contact residues of the 101F antibody-binding domain embedded in a protein scaffold comprising a three-dimensional structure with atomic coordinates that
30 have a root mean square deviation of protein backbone atoms of less than 10 angstroms when superimposed on the peptide consisting of amino acid sequence SEQ ID NO:4 when complexed with 101F antibody, the 3-dimensional model of the complex being defined by the coordinates specified in Protein Data Bank accession code 3O41. The present

disclosure also provides a nucleic acid molecule comprising a nucleic acid sequence that encodes any of such immunogens. Also provided is a recombinant molecule comprising such a nucleic acid molecule. Also provided is a recombinant cell comprising such a recombinant molecule. Also provided are uses of such nucleic acid molecules,

5 recombinant molecules, and recombinant cells to produce any of such immunogens. A recombinant molecule can also be a nucleic acid vaccine. Also provided is a composition that can include any of the immunogens, nucleic acid molecules, recombinant molecules, or recombinant cells. Also provided is a vaccine comprising any of such immunogens.

The disclosure provides a method to elicit a neutralizing humoral immune response
10 against RSV; such method comprises administering any of such immunogens or vaccines, wherein such administration elicits a neutralizing humoral immune response against RSV. Also provided is a method to protect a patient from RSV infection; such method comprises administering to the patient any of such immunogens or vaccines, wherein such administration protects the patient from RSV infection.

15 The disclosure provides an antibody protein comprising a heavy chain comprising SEQ ID NO:5, except that the antibody protein comprises at least one amino acid substitution selected from the group consisting of: (a) substitution of the amino acid corresponding to position 32 of SEQ ID NO:5, is a histidine or a glutamic acid; (b) substitution of the amino acid at position 35 of SEQ ID NO:5 is substituted with an
20 alanine; (c) substitution of the amino acid at position 52 of SEQ ID NO:5 is substituted with an amino acid selected from the group consisting of lysine, histidine, threonine, serine and arginine; (d) substitution of the amino acid at position 53 of SEQ ID NO:5 is substituted with a histidine or a serine; (e) substitution of the amino acid at position 54 of SEQ ID NO:5 is substituted with a phenylalanine or an arginine; (f) substitution of the
25 amino acid at position 56 of SEQ ID NO:5 is substituted with an amino acid selected from the group consisting of isoleucine, serine, glutamic acid, and aspartic acid; (g) substitution of the amino acid at position 58 of SEQ ID NO:5 is substituted with a tyrosine or a tryptophan; (h) substitution of the amino acid at position 97 of SEQ ID NO:5 is substituted with an aspartic acid, a histidine or an arginine; (i) substitution of the amino acid at
30 position 99 of SEQ ID NO:5 is substituted with aspartic acid; (j) substitution of the amino acid at position 100 of SEQ ID NO:5 is substituted with a tyrosine, a tryptophan or a histidine; and (k) substitution of the amino acid at position 100A of SEQ ID NO:5 is substituted with a serine or a threonine. The disclosure provides an antibody protein

comprising a heavy chain comprising SEQ ID NO:6, except that the antibody protein comprises at least one amino acid substitution selected from the group consisting of: (a) substitution of the amino acid at position 32 of SEQ ID NO:6 is substituted with a phenylalanine; (b) substitution of the amino acid at position 49 of SEQ ID NO:6 is substituted with a histidine or an arginine; (c) substitution of the amino acid at position 92 of SEQ ID NO:6 is substituted with a lysine; (d) substitution of the amino acid at position 94 of SEQ ID NO:6 is substituted with a histidine; and (e) substitution of the amino acid at position 96 of SEQ ID NO:6 is substituted with a histidine. The present disclosure also provides a nucleic acid molecule comprising a nucleic acid sequence that encodes any of such antibody proteins. Also provided is a recombinant molecule comprising such a nucleic acid molecule. Also provided is a recombinant cell comprising such a recombinant molecule. Also provided are uses of such nucleic acid molecules, recombinant molecules, and recombinant cells to produce any of such antibody proteins. Also provided is a composition that can include any of the antibody proteins. Also provided is a method to protect a patient from RSV infection; such method comprises administering to the patient any of such immunogens or vaccines, wherein such administration protects the patient from RSV infection.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Structural basis of motavizumab binding to its F glycoprotein epitope.

(a) RSV neutralization curves for palivizumab and motavizumab IgG as determined by a flow cytometric assay. (b) Surface representation of the Fab and ribbon representation of the peptide, viewed looking down at the CDRs. (c) Interactions between the peptide and six Fab CDRs. Side chains are shown for those residues making intermolecular interactions. Dashed lines represent hydrogen bonds. Residues in the hydrophobic patch on the heavy chain are shown with transparent surfaces. These include Trp52 and Trp53 on the right, and Ile97, Phe98 and Phe100 on the left (d) Motavizumab mutations that alter affinity to the F glycoprotein. Side chains are shown for those Fab residues that increase affinity by directly contacting the peptide (red), by altering the position of residues that directly contact the peptide (cyan), and by binding to the F glycoprotein outside the primary epitope or enhancing long-range electrostatic interactions (magenta). Fab residues in yellow decrease the affinity for the F glycoprotein but increase *in vivo* potency compared to an earlier version of motavizumab.

Figure 2. Motavizumab binding to the RSV F glycoprotein. (a) Superposition of the motavizumab-bound peptide (grey) and residues 229-252 of the PIV5 F glycoprotein structure (red). (b) Ribbon representation of the model of motavizumab Fab (green and blue) bound to the PIV5 F glycoprotein monomer (tan) via the superposition shown in a. (c) Same as b, except the entire PIV5 F glycoprotein trimer is shown (tan, green, pink). (d) Magnification of the boxed area shown in c. (e) Gel filtration elution profile and corresponding Coomassie blue-stained SDS-PAGE gel of the RSV F₀ Fd glycoprotein. (f) Gel filtration elution profile and corresponding Coomassie blue-stained SDS-PAGE gel of a mixture of RSV F₀ Fd glycoprotein and excess palivizumab Fab. Densitometric analysis of the gel yields a ratio of 2.97 Fabs per trimer.

Figure 3. Peptide electron density. Stereo image of 2F_o-F_c density contoured at 1 σ around the 24-amino acid peptide, viewed from the bound antibody. The electrostatic potential of the peptide in this orientation is shown in Figure 4 (Right).

Figure 4. Shape and electrostatic potential complementarity. (Left) Cartoon and stick representation of the peptide bound to a surface of the motavizumab Fab colored according to electrostatic potentials (negative potentials are colored red, positive potentials are colored blue). (Right) Cartoon and stick representation of the six motavizumab CDRs bound to a surface of the peptide colored according to electrostatic potentials. Heavy chain CDRs are green, and light chain CDRs are blue. The scale is from -5 to 5 kT/e. The images are related by a 180° rotation.

Figure 5. RSV/PIV5 F glycoprotein alignments. Figure 5-1 (also labeled "a") Sequence alignment. Sequence of the crystallized PIV5 F glycoprotein fragment aligned with the corresponding residues from the RSV F glycoprotein A2 strain. The sequences were first aligned using CLUSTALW2 (Larkin MA et al., 2007, Bioinformatics 23, 2947-2948) and then manually adjusted to align the furin cleavage site and disulfide bonds. Secondary structure of the PIV5 F glycoprotein as determined from the crystal structure and the PSIPRED-predicted (Bryson K et al., 2005, Nucl. Acids Res. 33, W36-38) RSV F and PIV5 F glycoprotein secondary structure is shown. The motavizumab epitope is highlighted in grey. Arrows indicate β -strands and coils indicate α -helices. Numbering corresponds to the RSV F glycoprotein. Figure 5-2 (also labeled "b") Structure alignment. The structure of the motavizumab-bound RSV F peptide was used to refine the alignment with the PIV5 F glycoprotein. Superposition of RSV F residues 254-277 with PIV5 F residues 229-252 provided an rmsd of 2.1 Å for all 24 C α atoms. This optimal

structure alignment requires a shift of 3 amino acids relative to the sequence alignment in a, which also results in a better sequence alignment for this region.

Figure 6. RSV F0 Fd cross-linking. Coomassie-stained SDS-PAGE gel of glutaraldehyde cross-linked RSV F₀ Fd proteins immunoprecipitated with motavizumab IgG.

Figure 7. Comparison of the motavizumab epitope to Protein A and the 11p1b scaffold. The 24-amino acid sequence of the motavizumab epitope on RSV F is shown on top, with the two helical regions identified in the crystal structure indicated by blue and orange cylinders. The sequence of a single domain of Protein A from *S. aureus* is shown (labeled Original), as well as the sequence of the 11p1b_003 scaffold (labeled Final). Bold residues were mutated to preserve motavizumab contact residues, while underlined residues were mutated to stabilize the conformation of the two helices. Structures of the motavizumab/peptide complex and the scaffold are shown below the sequences.

Figure 8. Recombinant immunogen structure. Expression of immunogen 11p1b_003 in HEK293 and bacterial cells yields proteins that are alpha-helical in solution and have a melting temperature that confirms the proteins are folded in solution.

DETAILED DESCRIPTION

Previous attempts at making an RSV vaccine using the RSV F protein have been unsuccessful. This was due to poor immunogenicity or a concern about eliciting T cells that could worsen disease. The inventors have now solved the crystal structures of two, antibody-binding domains of the F protein when they are bound to their respective monoclonal antibodies: motavizumab, which binds to the F protein at a domain spanning amino acids 254 to 277 (see, e.g., Wu H et al., 2007, J. Mol. Biol. 368, 652-665); and chimeric 101F antibody (also referred to as 101F or CH101 (Centocor)) that binds to the F protein at a domain spanning from amino acids 422-436 (see, e.g., Wu, S-J et al., 2007, J. Gen Virol 88, 2719-2723). Analysis of this structure has led to the identification of the contact residues in the F protein when bound to motavizumab or 101F antibody. This information allows the identification of non-RSV proteins that have a similar three-dimensional structure to the respective antibody-binding domains (referred to as scaffold proteins), which can then be modified to contain the appropriate residues that enable the modified protein to bind motavizumab or 101F. Since such a modified protein is unrelated to the RSV F protein, except for the contact residues, it can be used as an immunogen to elicit antibodies against the RSV F protein. Preferably the immunogens do not elicit a

significant cellular response against the F protein. The information gained from the three-dimensional model can also be used to design antibodies that have a high affinity for the RSV F protein, and that can be used to protect individuals from RSV infection.

Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

It should be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

It should be understood that as used herein, the term "a" entity or "an" entity refers to one or more of that entity. For example, a peptide or protein refers to one or more peptides or proteins. As such, the terms "a", "an", "one or more" and "at least one" can be used interchangeably. Similarly the terms "comprising", "including" and "having" can be used interchangeably. Moreover, as used herein, the terms about and substantially refer to a variation of less than 5% from the object of the term, and preferably less than 2%.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates, which may need to be independently confirmed.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

Except as otherwise noted, the methods and techniques of the present embodiments are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification.

5 It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments are specifically
10 embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

One embodiment of the present invention is an immunogen comprising an
15 antibody-binding domain, wherein the three-dimensional structure of the antibody-binding domain of the immunogen spatially corresponds to the three-dimensional structure of an antibody-binding domain of a peptide derived from RSV F protein, when such peptide is in a complex with an RSV neutralizing antibody that specifically binds the F protein. In one embodiment, the neutralizing antibody is palivizumab (SYNAGIS®). In another
20 embodiment, the neutralizing antibody is motavizumab. In yet another embodiment, the neutralizing antibody is 101F. In one embodiment, the neutralizing antibody can be any antibody that binds an antibody-binding domain recognized by motavizumab (i.e., an antibody-binding domain to which motavizumab binds). In one embodiment, the neutralizing antibody can be any antibody that binds an antibody-binding domain
25 recognized by 101F antibody (also referred to herein as 101F).

As used herein, a peptide derived from the RSV F protein is any peptide comprising at least a portion of SEQ ID NO:1, wherein said portion comprises an antibody-binding domain that binds palivizumab, motavizumab or 101F. Such a peptide can also be referred to as an RSV F peptide. As used herein, an antibody-binding domain
30 is a group, or cluster, of amino acids within a protein or peptide, wherein at least one of the amino acid residues in the sequence interacts directly, or indirectly (e.g., forms a bond, such as an ionic bond or salt-bridge) with at least one amino acid residue in an antibody such as palivizumab, motavizumab or 101F, such that the antibody specifically binds the

peptide. As used herein, the terms selectively, selective, specific, and the like, indicate the antibody has a greater affinity for the RSV protein or peptide, or the immunogen, than it does for proteins unrelated to the RSV F protein or peptide. More specifically, the terms selectively, selective, specific, and the like indicate that the affinity of the antibody the

5 RSV protein or peptide, or the immunogen, is statistically significantly higher than its affinity for a negative control (e.g., an unrelated protein) as measured using a standard assay (e.g., ELISA). Suitable techniques for assaying the ability of an antibody to selectively interact with the RSV protein or peptide, or the immunogen, are known to those skilled in the art. Amino acid residues that act directly or indirectly to form bonds at

10 the interface of two molecules, such as a peptide and antibody, are referred to as contact residues. Contact residues within a molecule can be contiguous, non-contiguous, or partly contiguous in the two-dimensional (linear) structure (i.e., linearly contiguous, linearly non-contiguous, or the like), but are sufficiently contiguous, or close together, in the three-dimensional structure to form an epitope (i.e., structurally contiguous).

15 In one embodiment, the peptide comprises a palivizumab-binding domain. In another embodiment, the peptide comprises a motavizumab antibody-binding domain (also referred to herein as a motavizumab antibody-binding site, motavizumab-binding domain, or motavizumab-binding site). In a preferred embodiment, the motavizumab antibody-binding site corresponds to amino acids 254-277 of SEQ ID NO:1. The amino

20 acid sequence spanning residues 254-277 is NSELLSLIND MPITNDQKKL MSNN, also denoted herein as SEQ ID NO:2.

In yet another embodiment the peptide comprises a 101F antibody-binding site (also referred to herein as a 101F antibody-binding domain). In another preferred embodiment, the 101F antibody-binding site corresponds to amino acids 422-436 of SEQ

25 ID NO:1. The amino acid sequence spanning residues 422-436 is STASNKNRGI IKTFS, also denoted herein as SEQ ID NO:3, except that the S at amino acid position 422 is replaced by a C in RSV F protein. In yet another preferred embodiment, the 101F antibody-binding site corresponds to amino acids 427-436 of SEQ ID NO:1. The amino acid sequence spanning residues 427-436 is KNRGI IKTFS, also denoted herein as SEQ

30 ID NO:4. In yet another preferred embodiment, the 101F antibody-binding site corresponds to amino acids 422-438 of SEQ ID NO:1. The amino acid sequence spanning residues 422-438 is STASNKNRGI IKTFSNG, also denoted herein as SEQ ID NO:9, except that the S at amino acid position 422 is replaced by a C in RSV F protein.

A preferred embodiment of the present invention is an immunogen comprising an antibody-binding domain, wherein the three-dimensional structure of such antibody-binding domain spatially corresponds to the three-dimensional structure of a peptide consisting of amino acids 254-277 from the RSV F protein, when such peptide is
5 complexed with motavizumab. Another preferred embodiment of the present invention is an immunogen comprising an antibody-binding domain, wherein the three-dimensional structure of such antibody-binding domain spatially corresponds to the three-dimensional structure of a peptide consisting of amino acids 427-436 from the RSV F protein, when such peptide is complexed with 101F. Another preferred embodiment of the present
10 invention is an immunogen comprising an antibody-binding domain, wherein the three-dimensional structure of such antibody-binding domain spatially corresponds to the three-dimensional structure of a peptide consisting of amino acids 427-438 from the RSV F protein, when such peptide is complexed with 101F.

As used herein, the terms spatially corresponds, spatially corresponding, and the
15 like, are used to indicate that when a three-dimensional model of a protein is superimposed on a three dimensional model of a RSV F peptide comprising a motavizumab or 101F binding domain when such peptide is in a complex with motavizumab or 101F, respectively, coordinates defining the spatial position of backbone atoms in the protein vary from coordinates defining the spatial location of analogous backbone atoms in the
20 antibody-binding domain of the RSV F peptide, when such peptide is in a complex with motavizumab, by less than about 10 angstroms. Backbone atoms are those atoms in an amino acid that form the peptide backbone, or 3-dimensional folding pattern, of the 3-dimensional model. As such, backbone atoms are those atoms that make up the base, but not the side chain, of amino acid residues in a protein (i.e., nitrogen, carbon, alpha carbon, and oxygen). Analogous backbone atoms are atoms that are in the same position within an
25 amino acid. The term spatial position refers to an object's location in three-dimensional space, as defined by X, Y and Z coordinates. One system for determining the three-dimensional structure of a protein is X-ray crystallography. It is understood by those skilled in the relevant art that three-dimensional structures of proteins are defined using
30 atomic coordinates. Thus, in one embodiment of the present invention the three-dimensional structure of the complex between the peptide consisting of amino acid sequence SEQ ID NO:2 and motavizumab is defined by the atomic coordinates recited, or specified, in Protein Data Bank (of the Research Collaboratory for Structural

Bioinformatics (RCSB) accession code 3IXT (i.e., the atomic coordinates deposited at the Protein Data Bank under accession code 3IXT; also referred to as PDB acc code 3IXT).

These coordinates were recited in Table 1 of U.S. Provisional Patent Application

No. 61/253,826, filed October 21, 2009 (US 61/253,826). In another embodiment of the

5 present invention, the three-dimensional structure of a complex between a peptide consisting of amino acid sequence SEQ ID NO:3 and 101F is defined by the atomic coordinates recited, or specified, in Protein Data Bank (of the Research Collaboratory for Structural Bioinformatics (RCSB))_accession code 3O41 (also referred to as PDB acc code 3O41). These coordinates are more highly refined atomic coordinates corresponding to
10 the atomic coordinates recited in Table 2; such refinement led to a three-dimensional structure that when superimposed on the three-dimensional structure of the complex defined by the atomic coordinates recited in Table 2 could not be distinguished visually from the latter structure; any differences were less than 0.1 angstroms. In another embodiment of the present invention, the three-dimensional structure of a complex
15 between a peptide consisting of amino acid sequence SEQ ID NO:9 and 101F is defined by the atomic coordinates specified in Protein Data Bank (of the Research Collaboratory for Structural Bioinformatics (RCSB))_accession code 3O45 (also referred to as PDB acc code 3O45).

While an immunogen of the present invention comprises an antibody binding
20 domain spatially corresponding to an antibody-binding domain from an RSV F peptide present in a complex defined by the coordinates in PDB acc code 3IXT or in PDB acc code 3O41, it should be understood that some small variance in the spatial orientation of the immunogen antibody binding domain is permissible, as long as the immunogen binds palivizumab, motavizumab, or 101F. Thus one embodiment of the present invention is an
25 immunogen comprising an antibody binding domain that has a three-dimensional structure defined by atomic coordinates having less than 10%, less than 5%, less than 3%, less than 2% or less than 1% variation from the atomic coordinates recited in PDB acc code 3IXT or in PDB acc code 3O41. Another embodiment of the present invention is an
immunogen comprising an antibody-binding domain having a three-dimensional structure
30 represented by atomic coordinates defining backbone atoms that have a root mean square deviation of less than 10 angstroms, less than 5 angstroms, less than 3 angstroms, less than 2 angstroms, or less than 1 angstrom from the backbone atoms of the antibody-binding domain of an RSV F peptide in a complex defined by the coordinates recited in PDB acc

code 3IXT or in PDB acc code 3O41. Yet another embodiment of the present invention is an immunogen comprising an antibody-binding domain having a three-dimensional structure represented by atomic coordinates defining backbone atoms, wherein each atom has a root mean square deviation of less than 0.4 angstroms, less than 0.3 angstroms, less than 0.2 angstroms, or less than 0.1 angstrom from the corresponding backbone atom in the antibody-binding domain of an RSV F peptide in a complex defined by the coordinates recited in PDB acc code 3IXT or in PDB acc code 3O41.

As disclosed herein, an immunogen of the present invention comprises an antibody-binding domain that spatially corresponds to the antibody-binding domain in the RSV F peptide when bound to motavizumab or 101F. It is preferable that an immunogen of the present invention contain little or no homology to RSV F protein sequences from outside of an antibody-binding domain. It is also preferable that the immunogen not include any contiguous sequence from RSV F protein that is of sufficient length to generate a cellular immune response. As used herein, a cellular immune response, or cell-mediated immunity, refers to a T lymphocyte immune response and the release of related cytokines and other immunomodulatory molecules in response to an antigen that contains an antigenic peptide fragment consisting of a specific sequence of about 10 amino acids. In contrast, a humoral immune response, or humoral immunity, refers to the production by B-lymphocytes of antibodies (e.g., IgG, IgM or IgA antibodies) in response to an antigen. Such antibodies preferably neutralize RSV. One embodiment of the present invention is an immunogen that elicits a humoral immune response, but not a significant cellular immune response against RSV. One embodiment of the present invention is an immunogen that elicits a humoral immune response, but not a cellular immune response against RSV.

In one embodiment, an immunogen of the present invention comprises less than 12 consecutive (also referred to herein as contiguous or adjacent) amino acids from the sequence of the RSV F protein. In one embodiment, an immunogen of the present invention comprises less than 11 consecutive amino acids from the sequence of the RSV F protein. In one embodiment, an immunogen of the present invention comprises less than 10 consecutive amino acids from the sequence of the RSV F protein. In one embodiment, an immunogen of the present invention comprises less than 9 consecutive amino acids from the sequence of the RSV F protein. In one embodiment, an immunogen of the present invention comprises less than 9, less than 8, less than 7, less than 6, less than 5,

less than 4, or less than 3 contiguous amino acids from the sequence of the RSV F protein. In one embodiment, an immunogen of the present invention does not comprise an amino acid sequence from the RSV F protein that lies outside of the antibody-binding domain and that could elicit a cellular immune response to the RSV F protein. In a preferred
5 embodiment, an immunogen of the present invention comprises less than 7, less than 6, less than 5, less than 4, or less than 3 contiguous amino acids from sequences outside of amino acids 254-277 from SEQ ID NO:1. In another preferred embodiment, an immunogen of the present invention comprises less than 7, less than 6, less than 5, less than 4, or less than 3 contiguous amino acids from outside of amino acids 427-436 from
10 SEQ ID NO:1.

As described above, antibody-binding domains contain contact residues. As used herein, a contact residue is any amino acid present in a molecule (e.g., a peptide or antibody) that interacts directly or indirectly (e.g., forms an ionic bond either directly, or indirectly through a salt bridge), with an amino acid in a second molecule (e.g., a peptide
15 or antibody), thereby resulting in formation of a complex between the two molecules. Preferably, immunogens of the present invention have contact residues capable of binding to the contact residues in motavizumab, or 101F, that are responsible for the binding of the antibody to the RSV F protein peptide. This disclosure provides immunogens of the embodiments that have contact residues capable of binding to the contact residues in
20 motavizumab that are responsible for the binding of the antibody to the RSV F protein peptide. The disclosure also provides immunogens of the embodiment that have contact residues capable of binding to the contact residues in 101F that are responsible for the binding of the antibody to the RSV F protein peptide.

One embodiment of the present invention is an immunogen comprising a
25 motavizumab-binding domain, wherein the contact residues within such motavizumab-binding domain have a spatial orientation represented by atomic coordinates defining backbone atoms, wherein each atom has a root mean square deviation of less than 10 angstroms, less than 5 angstroms, less than 2 angstroms, less than 1 angstrom, less than 0.4 angstroms, less than 0.3 angstroms, less than 0.2 angstroms, less or than 0.1 angstrom
30 from the corresponding backbone atom in the antibody-binding domain of an RSV F peptide in a complex defined by the coordinates defined by the coordinates specified in PDB acc code 3IXT. In one embodiment, an immunogen comprises a motavizumab-binding domain that comprises contact residues that have a spatial orientation represented

by atomic coordinates that have a root mean square deviation of protein backbone atoms of less than 10 angstroms from the corresponding backbone atoms of contact residues in an RSV F peptide that contacts motavizumab in a complex defined by the coordinates specified in PDB acc code 3IXT.

5 The disclosure includes an immunogen of the embodiments in which the motavizumab-binding domain from such immunogen comprises less than 15 amino acids of the motavizumab-binding domain from the RSV F peptide and in which such amino acids are in clusters of no more than 8 consecutive amino acids per cluster. The disclosure also includes an immunogen of the embodiments in which the motavizumab-binding
10 domain from such immunogen comprises less than 15 amino acids of the motavizumab-binding domain from the RSV F peptide, and in which such amino acids are in clusters of no more than 3 consecutive amino acids per cluster. Also included are immunogens in which such amino acids are in clusters of no more than 7, 6, 5, 4, 2 or 1 consecutive amino acids per cluster.

15 Another embodiment of the present invention is an immunogen comprising an 101F-binding domain, wherein the contact residues within such 101F-binding domain have a spatial orientation represented by atomic coordinates defining backbone atoms, wherein each atom has a root mean square deviation of less than 10 angstroms, less than 5 angstroms, less than 2 angstroms, less than 1 angstrom, less than 0.4 angstroms, less than
20 0.3 angstroms, less than 0.2 angstroms, or less than 0.1 angstrom from the corresponding backbone atom in the antibody-binding domain of an RSV F peptide in a complex defined by the coordinates recited in PDB acc code 3O41. In one embodiment, an immunogen comprises a 101F antibody-binding domain that comprises contact residues that have a spatial orientation represented by atomic coordinates that have a root mean square
25 deviation of protein backbone atoms of less than 10 angstroms from the corresponding backbone atoms of contact residues in an RSV F peptide that contacts 101F antibody in a complex defined by the coordinates recited in PDB acc code 3O41.

 The disclosure includes an immunogen of the embodiments in which the 101F-binding domain from such immunogen comprises no more than 10 amino acids of the
30 101F-binding domain from the RSV F peptide, and in which such amino acids are in clusters of no more than 8 consecutive amino acids per cluster. Also included are immunogens in which such amino acids are in clusters of no more than 7, 6, 5, 4, 3, 2 or 1 consecutive amino acids per cluster.

In order to produce immunogens described herein, the inventors have developed novel methods of identifying proteins that comprise regions, referred to as superpositions, that spatially correspond to an antibody-binding domain from an RSV F peptide present in a complex defined by the coordinates in PDB acc code 3IXT or in PDB acc code 3O41.

5 Such proteins are referred to as scaffolds, protein scaffolds, scaffold proteins, scaffold protein sequences, and the like. Scaffold proteins are useful for creating immunogens of the present invention in that they hold contact residues in the immunogen in the proper spatial orientation to facilitate interaction between such residues and contact residues of motavizumab, or 101F. Moreover, the selection criteria select only those proteins that

10 substantially or wholly lack immunodominant RSV epitopes that would elicit a cellular immune response. This method, which is referred to as superpositioning, comprises determining the three-dimensional structure of an epitope of interest and then computationally searching a database of known protein structures to identify those proteins that can be structurally superimposed onto the epitope of interest with minimal

15 root mean square deviation of their coordinates. Such a method can be accomplished using software such as, for example, ROSETTA. Superpositioning has been described in PCT International Publication No. WO 2008/025015 A2, published 28 February 2008, which is hereby incorporated by reference in its entirety. Once suitable scaffold proteins have been identified, they can be altered according to the methods disclosed herein. A related

20 method that can be used for the analysis of complex epitopes is referred to as double superpositioning. In this method, which is similar to superpositioning, scaffolds are scanned for structural similarity to each of the two epitope segments individually, and whenever a match is found to one of the segments, that scaffold is searched a second time for structural similarity to the other epitope segment, with the rigid body position of the

25 second epitope segment relative to the scaffold pre-determined by the superposition of the first segment to the scaffold. "Double" matches are identified if (a) one epitope segment matches a scaffold with backbone rmsd/nsup < threshold 1 and the other segment matches with backbone rmsd/nsup < threshold 2, where threshold 1 is typically 0.15 and threshold 2 is typically 0.2, or (b) if both segments are superimposed simultaneously onto the

30 scaffold and the backbone rmsd/nsup is < threshold 3, where threshold 3 is typically 0.2. In this instance, the term "backbone rmsd" is defined as the root-mean square deviation of a structural alignment computed over the backbone atoms N, CA, C, O; and "backbone rmsd/nsup" is defined as the "backbone rmsd" divided by the number of aligned residues.

One embodiment of the present invention is an immunogen in which contact residues of the motavizumab, or 101F, binding domain, are embedded in a protein scaffold that spatially corresponds to an antibody-binding domain from an RSV F peptide present in a complex defined by the coordinates in PDB acc code 3IXT or in PDB acc code 3O41, respectively. Such a protein scaffold can be identified using the three-dimensional structure of a complex described by the coordinates in PDB acc code 3IXT or in PDB acc code 3O41. As used herein, embedding of contact residues in a protein scaffold refers to positioning contact residues within the scaffold such that such contact residues form an antibody-binding domain and such that the protein scaffold retains its proper three-dimensional structure.

Analysis of the three-dimensional structure of the complex of the F peptide bound to motavizumab described by the coordinates in PDB acc cod 3IXT shows that the contact residues are embedded in a three-dimensional structure comprising two alpha helices. Thus, one embodiment of the present invention is an immunogen comprising an antibody-binding domain that binds motavizumab, wherein the contact residues in such antibody-binding domain are embedded in a protein scaffold comprising a three-dimensional structure that has two alpha helices, wherein the helices are defined by atomic coordinates defining backbone atoms, wherein each atom has a root mean square deviation of less than 10 angstroms, less than 5 angstroms, less than 2 angstroms, less than 1 angstrom, less than 0.4 angstroms, less than 0.3 angstroms, less than 0.2 angstroms, or less than 0.1 angstrom from the corresponding backbone atom in the antibody-binding domain of an RSV F peptide in a complex defined by the coordinates recited in PDB acc cod 3IXT. In one embodiment, the alpha helices consist of amino acids 2-10 and amino acids 15-23 of the F peptide.

The disclosure provides an immunogen of the embodiments in which contact residues of the motavizumab-binding domain are embedded in a protein scaffold comprising a three-dimensional structure having two alpha helices defined by atomic coordinates that have a root mean square deviation of protein backbone atoms of less than 10 angstroms when superimposed on the two alpha helices of the peptide consisting of amino acid sequence SEQ ID NO:2 when complexed with motavizumab, the three-dimensional model of such complex being defined by the coordinates specified in PDB acc cod 3IXT. The disclosure also provides an immunogen of the embodiments in which contact residues of the 101F antibody-binding domain are embedded in a protein scaffold

comprising a three-dimensional structure with atomic coordinates that have a root mean square deviation of protein backbone atoms of less than 10 angstroms when superimposed on the peptide consisting of amino acid sequence SEQ ID NO:4 when complexed with 101F antibody, the 3-dimensional model of such complex being defined by the coordinates specified in PDB acc code 3O41. The disclosure also provides an immunogen of the embodiments in which contact residues of the 101F antibody-binding domain are embedded in a protein scaffold comprising a three-dimensional structure with atomic coordinates that have a root mean square deviation of protein backbone atoms of less than 10 angstroms when superimposed on the peptide consisting of amino acid sequence SEQ ID NO:9 when complexed with 101F antibody, the 3-dimensional model of such complex being defined by the coordinates specified in PDB acc code 3O45.

As has been described, scaffold proteins are identified by their spatial similarity to the three-dimensional structure of a motavizumab or 101F binding domain. Moreover, preferable scaffold sequences do not share significant homology with the RSV F protein (i.e., they do not elicit a cellular immune response against RSV). Thus, using the techniques described herein, the inventors have now identified several proteins that can serve as scaffolds for creating motavizumab-binding and 101F-binding immunogens. Examples of such proteins include, but are not limited to, *Staphylococcus aureus* protein, *Helicobacter pylori* CagZ protein, and equine infectious anemia virus p26 protein. One embodiment is a scaffold protein having PDB (Protein Data Bank) accession code 1LP1, preferably scaffold 1LP1_b (SEQ ID NO:11). Another embodiment is a scaffold protein having PDB accession code 1S2X, preferably scaffold 1S2X_a (SEQ ID NO:12). One embodiment is a truncated 1S2X_a scaffold that has SEQ ID NO:13; this scaffold is truncated at the carboxyl terminus. Another embodiment is a scaffold protein having PDB accession code 2EIA, preferably scaffold 2EIA_a (SEQ ID NO:14).

It will be understood by those in the relevant art that while a protein scaffold may comprise a three-dimensional structure capable of holding contact residues in the correct spatial position, and since such a scaffold protein may be unrelated to the RSV F protein, the scaffold protein itself may not contain amino acids that spatially correspond to contact residues in the F protein. Consequently, an unmodified, scaffold protein may not be able to bind to motavizumab or 101F. As used herein the term unmodified scaffold protein is a scaffold protein represented by a three-dimensional model, a portion of which spatially corresponds to the antibody-binding domain of an RSV F protein in a complex defined by

the atomic coordinates in PDB acc cod 3IXT or in PDB acc cod 3O41, but which has not been altered to contain any of the contact residues present in the RSV F protein. Amino acids in the RSV F protein identified as interacting with the contact residues in motavizumab are the amino acids at positions 255, 258, 259, 261, 262, 263, 267, 268, 269, 271, 272, 273, 275 and 276 of SEQ ID NO:1. Amino acids in the RSV F protein identified as interacting with the contact residues in 101F are the amino acids at positions 427, 429, 431, 432, 433, 434, 435 and 436 of SEQ ID NO:1. Thus one embodiment of the present invention is an immunogen that comprises sequence from a scaffold protein, wherein at least one amino acid in such scaffold protein sequence spatially corresponding to a contact residue in the RSV F protein is substituted with the amino acid residue present at such spatially corresponding contact residue in the F peptide. Such an immunogen can be produced by recombinant methods and/or synthesizing a nucleic acid molecule that encodes such immunogen and expressing it to make a recombinant immunogen. Such an immunogen can be tested for efficacy by measuring the immunogen's ability to bind to its respective antibody, or to neutralize RSV, using techniques known to those skilled in the art.

One preferred embodiment of the present invention is a scaffold protein comprising at least one substitution selected from the group consisting of:

(a) substituting a serine for the amino acid spatially corresponding to the amino acid at position 2 of SEQ ID NO:2;

(b) substituting a leucine for the amino acid spatially corresponding to the amino acid at position 5 of SEQ ID NO:2;

(c) substituting a serine for the amino acid spatially corresponding to the amino acid at position 6 of SEQ ID NO:2;

(d) substituting a isoleucine for the amino acid spatially corresponding to the amino acid at position 8 of SEQ ID NO:2;

(e) substituting a asparagine for the amino acid spatially corresponding to the amino acid at position 9 of SEQ ID NO:2;

(f) substituting a aspartic acid for the amino acid spatially corresponding to the amino acid at position 10 of SEQ ID NO:2;

(g) substituting a threonine for the amino acid spatially corresponding to the amino acid at position 14 of SEQ ID NO:2;

(h) substituting a asparagine for the amino acid spatially corresponding to the amino acid at position 15 of SEQ ID NO:2;

(i) substituting a aspartic acid for the amino acid spatially corresponding to the amino acid at position 16 of SEQ ID NO:2;

5 (j) substituting a lysine for the amino acid spatially corresponding to the amino acid at position 18 of SEQ ID NO:2;

(k) substituting a lysine for the amino acid spatially corresponding to the amino acid at position 19 of SEQ ID NO:2;

10 (l) substituting a leucine for the amino acid spatially corresponding to the amino acid at position 20 of SEQ ID NO:2;

(m) substituting a serine for the amino acid spatially corresponding to the amino acid at position 22 of SEQ ID NO:2; and

(n) substituting a asparagine for the amino acid spatially corresponding to the amino acid at position 23 of SEQ ID NO:2.

15 One embodiment is a scaffold protein comprising all of the afore-mentioned substitutions.

Another preferred embodiment of the present invention is a scaffold protein comprising at least one substitution selected from the group consisting of:

20 (a) substituting a lysine for the amino acid spatially corresponding to the amino acid at position 1 of SEQ ID NO:4;

(b) substituting a arginine for the amino acid spatially corresponding to the amino acid at position 3 of SEQ ID NO:4;

(c) substituting a isoleucine for the amino acid spatially corresponding to the amino acid at position 5 of SEQ ID NO:4;

25 (d) substituting a isoleucine for the amino acid spatially corresponding to the amino acid at position 6 of SEQ ID NO:4;

(e) substituting a lysine for the amino acid spatially corresponding to the amino acid at position 7 of SEQ ID NO:4;

30 (f) substituting a threonine for the amino acid spatially corresponding to the amino acid at position 8 of SEQ ID NO:4;

(g) substituting a phenylalanine for the amino acid spatially corresponding to the amino acid at position 9 of SEQ ID NO:4; and,

(h) substituting a serine for the amino acid spatially corresponding to the amino acid at position 10 of SEQ ID NO:4.

One embodiment is a scaffold protein comprising all of the afore-mentioned substitutions.

5 In one embodiment, an immunogen comprises amino acids 2, 5, 6, 8, 9, 16, 18, 19, 20, 22 and 23 of SEQ ID NO:2 substituted at the spatially corresponding positions of a scaffold protein. In one embodiment, an immunogen comprises amino acids 2, 5, 6, 9, 16, 18, 19, 20, 22 and 23 of SEQ ID NO:2 substituted at the spatially corresponding positions of a scaffold protein.

10 One embodiment of the present invention is an immunogen comprising an amino acid sequence of a protein selected from the group consisting of 1lp1b_001 (SEQ ID NO:18), 1lp1b_002 (SEQ ID NO:21), 1lp1b_003 (SEQ ID NO:24), 1lp1b_004 (SEQ ID NO:149), 1s2xa_001 (SEQ ID NO:152), 1s2xa_002 (SEQ ID NO:155), 1s2xa_003 (SEQ ID NO:158), 1s2xa_004 (SEQ ID NO:164), 2eiaa_001 (SEQ ID NO:167), and 2eiaa_002
15 (SEQ ID NO:170), the amino acid sequences of which are disclosed in the Examples. One embodiment is an immunogen comprising 1lp1b_001 (SEQ ID NO:18). One embodiment is an immunogen comprising 1lp1b_002 (SEQ ID NO:21). One embodiment is an immunogen comprising 1lp1b_003 (SEQ ID NO:24). One embodiment is an immunogen comprising 1lp1b_004 (SEQ ID NO:149). One embodiment is an immunogen comprising
20 1s2xa_001 (SEQ ID NO:152). One embodiment is an immunogen comprising 1s2xa_002 (SEQ ID NO:155). One embodiment is an immunogen comprising 1s2xa_003 (SEQ ID NO:158). One embodiment is an immunogen comprising 1s2xa_004 (SEQ ID NO:164). One embodiment is an immunogen comprising 2eiaa_001 (SEQ ID NO:167). One embodiment is an immunogen comprising 2eiaa_002 (SEQ ID NO:170). One
25 embodiment is a variant of any of such immunogens, wherein the variant shares at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity with such immunogen. Preferably the variant retains contact residues of such immunogen.

One embodiment is an immunogen comprising an amino acid sequence of protein 1s2xa_003_PADRE (SEQ ID NO:161). One embodiment is a variant of such immunogen,
30 wherein the variant shares at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity with such immunogen. Preferably the variant retains contact residues of such immunogen.

One embodiment is an immunogen comprising an amino acid sequence of a protein selected from the group consisting of 1s2xa_001_N_His (SEQ ID NO:177), 1s2xa_002_N_His (SEQ ID NO:178), 1s2xa_003_N_His (SEQ ID NO:179), 1s2xa_004_N_His (SEQ ID NO:180), and 2eiaa_002_N_His (SEQ ID NO:181), the amino acid sequences of which are disclosed in the Examples. One embodiment is a variant of any of such immunogens, wherein the variant shares at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity with such immunogen. Preferably the variant retains contact residues of such immunogen.

In addition to substituting amino acids in the portion of the scaffold protein spatially corresponding to the antibody-binding domain of RSV F peptide, other changes can be made to the immunogen as well, as long as such changes do not reduce the affinity of motavizumab or 101F for the immunogen. For example, amino acid residues outside of the antibody-binding domain may be altered in order to reduce steric interference between the backbone atoms of the antibody and the backbone atoms of the scaffold protein portion of the immunogen. In addition, amino acids that are outside of the antibody-binding domain can be substituted with an amino acid that allows the formation of a new ionic bond, thereby strengthening the interaction between the immunogen and the antibody. Such alteration of the scaffold protein is herein referred to as epitope conformation stabilization.

One embodiment of the invention is an immunogen comprising a motavizumab-binding domain spatially corresponding to a motavizumab-binding domain from an RSV F peptide, wherein the scaffold protein sequence of such immunogen has been subject to epitope stabilization conformation to reduce steric hindrance and/or increase the affinity of the immunogen for motavizumab. Another embodiment of the invention is an immunogen comprising a 101F-binding domain spatially corresponding to a 101F-binding domain from an RSV F peptide, wherein the scaffold protein sequence of such immunogen has been subject to epitope stabilization conformation to reduce steric hindrance and/or increase the affinity of the immunogen for 101F.

In addition to alterations of the immunogen sequence that result in reduced steric hindrance and/or increase the affinity of the immunogen for motavizumab or 101F, the immunogen can be subject to a process referred to as resurfacing, addition of N-linked glycosylation sites, or PEGylation. As used herein, the term resurfacing refers to a process whereby amino acid substitutions are introduced into scaffold sequences that are outside

of the antibody-binding domain in order to eliminate or hide immunodominant epitopes. For example, amino acids within an immunodominant epitope can be substituted with neutral amino acids (i.e., having an uncharged R group) so that the epitope is no longer bound by an antibody. In some embodiments, N-linked glycosylation sites can be introduced into the protein, resulting in glycosylation of the immunogen such that immunodominant epitopes are hidden from the immune system and thus do not elicit a strong humoral or cell mediated immune response. In some embodiments, a scaffold can be PEGylated (i.e., treated with polyethylene glycol), or otherwise treated, to mask immunodominant epitopes. Such processes can also be referred to as cloaking. Methods of producing resurfaced proteins have been previously described in, for example, PCT International Publication No. WO/2009/100376 entitled, "Antigenic Cloaking and Its Use", published 13 August 2009, which is hereby incorporated by reference in its entirety. As used herein, the phrase immunodominant epitope refers to an epitope within a protein or peptide that is most easily recognized by the immune system and thus has the greatest influence on the specificity of an antibody elicited by a protein or peptide containing the immunodominant epitope.

One embodiment of the present invention is an immunogen comprising sequences from a scaffold protein, wherein such immunogen binds motavizumab or 101F, and wherein scaffold protein sequences outside of the antibody-binding domain of such immunogen have been subject to resurfacing. In one embodiment, amino acids in the scaffold protein sequences of the immunogen are substituted with neutral amino acids. In another embodiment, glycosylation sites are introduced into scaffold protein sequences of the immunogen, or the immunogen is submitted to PEGylation methodology such that immunodominant epitopes present in the immunogen are hidden from the immune system by glycosylation or PEGylation of the immunogen. It should be appreciated that immunogens of the present invention can comprise combinations of the amino acid alterations discussed above. Whether scaffold protein sequences will require the introduction of neutral amino acids, glycosylation or PEGylation or combinations of such types of alterations depends on the nature of the sequences present in the scaffold protein. It is within the ability of those skilled in the art to determine which alterations will best eliminate or hide immunodominant epitopes outside of the antibody-binding domain. Moreover, methods of substituting amino acids into a protein or peptide, introducing

glycosylation sites into a protein or peptide or PEGylating such protein or peptide are known to those skilled in the art.

A preferred embodiment of the present invention is an immunogen comprising an amino acid sequence of a protein selected from the group consisting of

5 mota_1lp1b.m1.c1.d1_glyc1 (SEQ ID NO:55), mota_1lp1b.m1.c1.d1_glyc2 (SEQ ID NO:56), mota_1lp1b.m1.c1.d1_des1_1 (SEQ ID NO:66), mota_1lp1b.m1.c1.d1_des1_2 (SEQ ID NO:67), mota_1lp1b.m1.c1.d1_des1_3 (SEQ ID NO:68),
mota_1lp1b.m1.c1.d1_des1_5 (SEQ ID NO:69), mota_1lp1b.m1.c1.d1_des1_6 (SEQ ID NO:70), mota_1lp1b.m1.c1.d1_des1_7 (SEQ ID NO:71), mota_1lp1b.m1.c1.d1_des1_8
10 (SEQ ID NO:72), mota_1lp1b.m1.c1.d1_des1_9 (SEQ ID NO:73),
mota_1lp1b.m1.c1.d1_des1_10 (SEQ ID NO:74), and mota_1lp1b.m1.c1.d1_des1_11 (SEQ ID NO:75), the amino acid sequences of which are disclosed in the Examples.

One embodiment is an immunogen that has one or more N-linked glycosylation sites, such as, but not limited to, mota_1lp1b.m1.c1.d1_glyc1 (SEQ ID NO:55),

15 mota_1lp1b.m1.c1.d1_glyc2 (SEQ ID NO:56), 1lp1b_003_Glyc1 (SEQ ID NO:39), 1lp1b_003_Glyc2 (SEQ ID NO:42), 1lp1b_003_Glyc3 (SEQ ID NO:45), 1lp1b_003_Glyc4 (SEQ ID NO:48), 1lp1b_003_Glyc5 (SEQ ID NO:51), or 1lp1b_003_Glyc6 (SEQ ID NO:54). One embodiment is an immunogen comprising mota_1lp1b.m1.c1.d1_glyc1 (SEQ ID NO:55). One embodiment is an immunogen comprising

20 mota_1lp1b.m1.c1.d1_glyc2 (SEQ ID NO:56). One embodiment is an immunogen comprising 1lp1b_003_Glyc1 (SEQ ID NO:39). One embodiment is an immunogen comprising 1lp1b_003_Glyc2 (SEQ ID NO:42). One embodiment is an immunogen comprising 1lp1b_003_Glyc3 (SEQ ID NO:45). One embodiment is an immunogen comprising 1lp1b_003_Glyc4 (SEQ ID NO:48). One embodiment is an immunogen
25 comprising 1lp1b_003_Glyc5 (SEQ ID NO:51). One embodiment is an immunogen comprising 1lp1b_003_Glyc6 (SEQ ID NO:54). One embodiment is a variant of any of such immunogens, wherein the variant shares at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity with such immunogen. Preferably the variant retains contact residues of such immunogen.

30 One embodiment is an immunogen that has been resurfaced, such as, but not limited to, mota_1lp1b.m1.c1.d1_des1_1 (SEQ ID NO:66), mota_1lp1b.m1.c1.d1_des1_2 (SEQ ID NO:67), mota_1lp1b.m1.c1.d1_des1_3 (SEQ ID NO:68),
mota_1lp1b.m1.c1.d1_des1_5 (SEQ ID NO:69), mota_1lp1b.m1.c1.d1_des1_6 (SEQ ID

NO:70), mota_1lp1b.m1.c1.d1_des1_7 (SEQ ID NO:71), mota_1lp1b.m1.c1.d1_des1_8 (SEQ ID NO:72), mota_1lp1b.m1.c1.d1_des1_9 (SEQ ID NO:73), mota_1lp1b.m1.c1.d1_des1_10 (SEQ ID NO:74), or mota_1lp1b.m1.c1.d1_des1_11 (SEQ ID NO:75). Additional examples are 1lp1b_003_Surf1 (SEQ ID NO:59), 1lp1b_003_Surf6 (SEQ ID NO:62), and 1lp1b_003_Surf8 (SEQ ID NO:65). One embodiment is an immunogen comprising mota_1lp1b.m1.c1.d1_des1_1 (SEQ ID NO:66). One embodiment is an immunogen comprising mota_1lp1b.m1.c1.d1_des1_2 (SEQ ID NO:67). One embodiment is an immunogen comprising mota_1lp1b.m1.c1.d1_des1_3 (SEQ ID NO:68). One embodiment is an immunogen comprising mota_1lp1b.m1.c1.d1_des1_5 (SEQ ID NO:69). One embodiment is an immunogen comprising mota_1lp1b.m1.c1.d1_des1_6 (SEQ ID NO:70). One embodiment is an immunogen comprising mota_1lp1b.m1.c1.d1_des1_7 (SEQ ID NO:71). One embodiment is an immunogen comprising mota_1lp1b.m1.c1.d1_des1_8 (SEQ ID NO:72). One embodiment is an immunogen comprising mota_1lp1b.m1.c1.d1_des1_9 (SEQ ID NO:73). One embodiment is an immunogen comprising mota_1lp1b.m1.c1.d1_des1_10 (SEQ ID NO:74). One embodiment is an immunogen comprising mota_1lp1b.m1.c1.d1_des1_11 (SEQ ID NO:75). One embodiment is an immunogen comprising 1lp1b_003_Surf1 or 1lp1b_003_Surf8. One embodiment is an immunogen comprising 1lp1b_003_Surf1 (SEQ ID NO:59). One embodiment is an immunogen comprising 1lp1b_003_Surf6 (SEQ ID NO:62). One embodiment is an immunogen comprising 1lp1b_003_Surf8 (SEQ ID NO:65). One embodiment is a variant of any of such immunogens, wherein the variant shares at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity with such immunogen. Preferably the variant retains contact residues of such immunogen.

The disclosure provides for immunogens of the embodiments that are multivalent. Without being bound by theory, it is believed that multivalent immunogens can elicit enhanced neutralizing antibody responses. A multivalent immunogen of the embodiments is an immunogen of the disclosure that includes a particle enabling attachment of one or more immunogens. Such a particle can be of a material known to those skilled in the art. Examples of particles include, but are not limited to, ferritin, viral capsid proteins, virus-like particles, and other proteins that assemble into high-copy, large particles. Such attachment is accomplished so as to not significantly reduce the ability of an immunogen of the embodiments to elicit a neutralizing humoral response against RSV. Such

attachment can be accomplished by covalently binding an immunogen to such a particle or can be accomplished by designing a nucleic acid molecule than encodes an immunogen of the embodiments and a particle, or subunit thereof. In one embodiment, a multivalent immunogen can be administered as a prime and/or boost. In one embodiment, a multivalent immunogen can be administered as a prime. In one embodiment, a multivalent immunogen can be administered as a boost.

One embodiment is an immunogen of the embodiments that is attached to ferritin. Ferritin, a globular protein complex consisting of 24 protein subunits, is a ubiquitous intracellular protein that stores iron and releases it in a controlled manner. The use of ferritin fusion proteins as vaccines has been described, for example, by Carter DC, et al., United States Patent No. 7,097,841 B2, issued August 29, 2006. One embodiment is a multivalent immunogen comprising an amino acid sequence of a protein selected from the group consisting of 1lp1b_003_ferritin (SEQ ID NO:138), 1lp1b_003_eumS (SEQ ID NO:140), 1lp1b_003_eumSP (SEQ ID NO:142), 1lp1b_003_eumL (SEQ ID NO:144), and 1lp1b_003_eumLP (SEQ ID NO:146). One embodiment is an immunogen comprising 1lp1b_003_ferritin (SEQ ID NO:138). One embodiment is an immunogen comprising 1lp1b_003_eumS (SEQ ID NO:140). One embodiment is an immunogen comprising 1lp1b_003_eumSP (SEQ ID NO:142). One embodiment is an immunogen comprising 1lp1b_003_eumL (SEQ ID NO:144). One embodiment is an immunogen comprising 1lp1b3_eumLP (SEQ ID NO:146). One embodiment is a variant of any of such immunogens, wherein the variant shares at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity with such immunogen. Preferably the variant retains contact residues of such immunogen.

The disclosure also provides an immunogen comprising an amino acid sequence of a protein selected from the group consisting of 1lp1b_003_K46A (SEQ ID NO:78), 1lp1b_003_Q52A (SEQ ID NO:81), 1lp1b_003_I13L_F27A (SEQ ID NO:87), 1lp1b_003_L41I_L42V (SEQ ID NO:90), 1lp1b-003_L41I_L42A (SEQ ID NO:93), 1lp1b_003_I13A (SEQ ID NO:105), 1lp1b_003_L16A (SEQ ID NO:108), 1lp1b_003_F27A (SEQ ID NO:111), 1lp1b_003_L41A (SEQ ID NO:114), 1lp1b_003_L42A (SEQ ID NO:117), and 1lp1b_003_Neg1 (SEQ ID NO:132). One embodiment is an immunogen comprising an amino acid sequence of a protein selected from the group consisting of 1lp1b_003_C43 (SEQ ID NO:33), 1lp1b_003_C47 (SEQ ID NO:36), 1lp1b_003_Glyc3 (SEQ ID NO:45), 1lp1b_003_Glyc4 (SEQ ID NO:48),

1lp1b_003_Glyc5 (SEQ ID NO:51), 1lp1b_003_Glyc6 (SEQ ID NO:54),
 1lp1b_003_K46A_Q52A (SEQ ID NO:84), 1lp1b_003_Glycine1 (SEQ ID NO:120),
 1lp1b_003_Glycine2 (SEQ ID NO:123), 1lp1b_003_Pos1 (SEQ ID NO:126),
 1lp1b_003_Pos2 (SEQ ID NO:129), and 1lp1b_003_Neg2 (SEQ ID NO:135). One
 5 embodiment is an immunogen comprising an amino acid sequence of a protein selected
 from the group consisting of 1lp1b_003_I13A_L42A (SEQ ID NO:96), 1lp1b_003_L19A
 (SEQ ID NO:99), and 1lp1b_003_L19A_L41I (SEQ ID NO:102). Such immunogens
 differ from immunogen 1lp1b_003 with respect to surface charge, glycosylation pattern
 and intrinsic flexibility. One embodiment is a variant of any of such immunogens,
 10 wherein the variant shares at least 95%, at least 96%, at least 97%, at least 98%, or at least
 99% amino acid sequence identity with such immunogen. Preferably the variant retains
 contact residues of such immunogen.

One embodiment is an immunogen comprising an amino acid sequence of protein
 1lp1b_003_K272E (SEQ ID NO:30). This immunogen lacks a key contact residue of the
 15 motavizumab-binding domain.

One embodiment is an immunogen comprising an amino acid sequence of protein
 1lp1b_003_F2Y_H15N (SEQ ID NO:27). This immunogen was expressed in HEK293
 cells and complexed with motavizumab, crystallized, and a three-dimensional model
 defined therefrom.

20 The disclosure provides an RSV immunogen that comprises an amino acid
 sequence of 1LP1_b (SEQ ID NO: 11) having from one to twenty amino acid
 substitutions, wherein at least one amino acid substitution is selected from the group
 consisting of:

- (a) substitution of a serine at amino acid position N-25 in SEQ ID NO:11;
- 25 (b) substitution of a leucine at amino acid position I-28 in SEQ ID NO:11;
- (c) substitution of a serine at amino acid position Q-29 in SEQ ID NO: 11;
- (d) substitution of an isoleucine at amino acid position L-31 in SEQ ID NO:11;
- (e) substitution of an asparagine at amino acid position K-32 in SEQ ID NO:11;
- (f) substitution of an aspartic acid at amino acid position K-4 in SEQ ID NO:11;
- 30 (g) substitution of a lysine at amino acid position Q-6 in SEQ ID NO:11;
- (h) substitution of a lysine at amino acid position Q-7 in SEQ ID NO:11;
- (i) substitution of a leucine at amino acid position N-8 in SEQ ID NO:11;
- (j) substitution of a serine at amino acid position F-10 in SEQ ID NO:11; and

(k) substitution of an asparagine at amino acid position Y-11 in SEQ ID NO:11. Such an RSV immunogen can comprise all of the substitutions (a) through (k). An RSV immunogen can elicit a humoral immune response against RSV. The phrase “from one to twenty amino acid substitutions” allows for substitutions beyond those that are specified in (a) through (k). In one embodiment, the three-dimension structure of the antibody-binding domain is maintained or improved when such substitution(s) are made. One embodiment is an RSV immunogen that comprises an amino acid sequence that has up to nine substitutions (i.e., any number ranging from 0 through 9 substitutions) in an amino acid sequence of a protein selected from the group consisting of 1lp1b_001 (SEQ ID NO:18), 1lp1b_002 (SEQ ID NO:21), 1lp1b_003 (SEQ ID NO:24), and 1lp1b_004 (SEQ ID NO:149). One embodiment is an RSV immunogen that comprises an amino acid sequence that has up to twelve substitutions in the amino acid sequence of protein 1lp1b_003 (SEQ ID NO:24). One embodiment is an RSV immunogen that comprises an amino acid sequence that is at least 95% identical to an amino acid sequence of a protein selected from the group consisting of 1lp1b_001 (SEQ ID NO:18), 1lp1b_002 (SEQ ID NO:21), 1lp1b_003 (SEQ ID NO:24), and 1lp1b_004 (SEQ ID NO:149). One embodiment is an RSV immunogen that comprises an amino acid sequence that is at least 95% identical to the amino acid sequence of 1lp1b_003 (SEQ ID NO:24). One embodiment is an RSV immunogen that comprises an amino acid sequence of a protein selected from the group consisting of 1lp1b_001 (SEQ ID NO:18), 1lp1b_002 (SEQ ID NO:21), 1lp1b_003 (SEQ ID NO:24), and 1lp1b_004 (SEQ ID NO:149). One embodiment is an RSV immunogen comprising 1lp1b_003; such an immunogen comprises amino acid sequence SEQ ID NO:24. One embodiment is an RSV immunogen that is N-linked glycosylated. One embodiment is an RSV immunogen that comprises an amino acid sequence of a protein selected from the group consisting of mota_1lp1b.m1.c1.d1_glyc1 (SEQ ID NO:55), mota_1lp1b.m1.c1.d1_glyc2 (SEQ ID NO:56), 1lp1b_003_Glyc1 (SEQ ID NO:39), 1lp1b_003_Glyc2 (SEQ ID NO:42), 1lp1b_003_Glyc3 (SEQ ID NO:45), 1lp1b_003_Glyc4 (SEQ ID NO:48), 1lp1b_003_Glyc5 (SEQ ID NO:51), and 1lp1b_003_Glyc6 (SEQ ID NO:54). One embodiment is an RSV immunogen that is resurfaced. One embodiment is an RSV immunogen that comprises an amino acid sequence of a protein selected from the group consisting of mota_1lp1b.m1.c1.d1_des1_1 (SEQ ID NO:66), mota_1lp1b.m1.c1.d1_des1_2 (SEQ ID NO:67), mota_1lp1b.m1.c1.d1_des1_3 (SEQ ID NO:68), mota_1lp1b.m1.c1.d1_des1_5 (SEQ ID NO:69), mota_1lp1b.m1.c1.d1_des1_6

(SEQ ID NO:70), mota_1lp1b.m1.c1.d1_des1_7 (SEQ ID NO:71),
mota_1lp1b.m1.c1.d1_des1_8 (SEQ ID NO:72), mota_1lp1b.m1.c1.d1_des1_9 (SEQ ID
NO:73), mota_1lp1b.m1.c1.d1_des1_10 (SEQ ID NO:74), mota_1lp1b.m1.c1.d1_des1_11
(SEQ ID NO:75), 1lp1b_003_Surf1 (SEQ ID NO:59), 1lp1b_003_Surf6 (SEQ ID NO:62),
5 and 1lp1b_003_Surf8 (SEQ ID NO:65). One embodiment is an RSV immunogen that is a
multivalent immunogen. One embodiment is an RSV immunogen that comprises an
amino acid sequence of a protein selected from the group consisting of 1lp1b_003 ferritin
(SEQ ID NO:138), 1lp1b_003_eumS (SEQ ID NO:140), 1lp1b_003_eumSP (SEQ ID
NO:142), 1lp1b_003_eumL (SEQ ID NO:144), and 1lp1b_003_eumLP (SEQ ID
10 NO:146). One embodiment is an RSV immunogen that comprises an amino acid sequence
of a protein selected from the group consisting of 1lp1b_003_K46A (SEQ ID NO:78),
1lp1b_003_Q52A (SEQ ID NO:81), 1lp1b_003_I13L_F27A (SEQ ID NO:87),
1lp1b_003_L41I_L42V (SEQ ID NO:90), 1lp1b_003_L41I_L42A (SEQ ID NO:93),
1lp1b_003_I13A (SEQ ID NO:105), 1lp1b_003_L16A (SEQ ID NO:108),
15 1lp1b_003_F27A (SEQ ID NO:111), 1lp1b_003_L41A (SEQ ID NO:114),
1lp1b_003_L42A (SEQ ID NO:117), and 1lp1b_003_Neg1 (SEQ ID NO:132). One
embodiment is an immunogen comprising an amino acid sequence of a protein selected
from the group consisting of 1lp1b_003_C43 (SEQ ID NO:33), 1lp1b_003_C47 (SEQ ID
NO:36), 1lp1b_003_Glyc3 (SEQ ID NO:45), 1lp1b_003_Glyc4 (SEQ ID NO:48),
20 1lp1b_003_Glyc5 (SEQ ID NO:51), 1lp1b_003_Glyc6 (SEQ ID NO:54),
1lp1b_003_K46A_Q52A (SEQ ID NO:84), 1lp1b_003_Glycine1 (SEQ ID NO:120),
1lp1b_003_Glycine2 (SEQ ID NO:123), 1lp1b_003_Pos1 (SEQ ID NO:126),
1lp1b_003_Pos2 (SEQ ID NO:129), and 1lp1b_003_Neg2 (SEQ ID NO:135). One
embodiment is an immunogen comprising an amino acid sequence of a protein selected
25 from the group consisting of 1lp1b_003_I13A_L42A (SEQ ID NO:96), 1lp1b_003_L19A
(SEQ ID NO:99), and 1lp1b_003_L19A_L41I (SEQ ID NO:102). An RSV immunogen
disclosed herein can elicit a humoral immune response against RSV. In one embodiment,
such an RSV immunogen can elicit a humoral immune response against RSV, but does not
elicit a cellular immune response. An RSV immunogen as disclosed herein can have a
30 motavizumab antibody binding domain comprising less than 12 consecutive amino acids
from a motavizumab antibody binding domain of RSV fusion protein. In one
embodiment, an RSV immunogen has a motavizumab antibody-binding domain

comprising less than 9 consecutive amino acids from a motavizumab antibody-binding domain of RSV fusion protein.

The disclosure provides an RSV immunogen comprising an amino acid sequence of truncated 1S2X_a (SEQ ID NO:13) having from one to twenty-five amino acid

5 substitutions, wherein at least one amino acid substitution is selected from the group consisting of:

- (a) substitution of a serine at amino acid position 92 in SEQ ID NO:13;
 - (b) substitution of a leucine at amino acid position 95 in SEQ ID NO:13;
 - (c) substitution of a serine at amino acid position 96 in SEQ ID NO:13;
 - 10 (d) substitution of an isoleucine at amino acid position 98 in SEQ ID NO:13;
 - (e) substitution of an asparagine at amino acid position 99 in SEQ ID NO:13;
 - (f) substitution of an aspartic acid at amino acid position 100 in SEQ ID NO:13;
 - (g) substitution of an asparagine at amino acid position 105 in SEQ ID NO:13;
 - (h) substitution of an aspartic acid at amino acid position 106 in SEQ ID NO:13;
 - 15 (i) substitution of a lysine at amino acid position 108 in SEQ ID NO:13;
 - (j) substitution of a lysine at amino acid position 109 in SEQ ID NO:13;
 - (k) substitution of a leucine at amino acid position 110 in SEQ ID NO:13;
 - (l) substitution of a serine at amino acid position 112 in SEQ ID NO:13; and
 - (m) substitution of an asparagine at amino acid position 113 in SEQ ID NO:13.
- 20 Such an RSV immunogen can comprise all of the substitutions (a) through (m). An RSV immunogen can elicit a humoral immune response against RSV. The phrase “from one to twenty-five amino acid substitutions” allows for substitutions beyond those that are specified in (a) through (m). In one embodiment, the three-dimension structure of the antibody-binding domain is maintained or improved when such substitution(s) are made.
- 25 One embodiment is an RSV immunogen that comprises an amino acid sequence that has up to twelve substitutions (i.e., any number ranging from 0 through 12 substitutions) in an amino acid sequence of a protein selected from the group consisting of 1s2xa_001 (SEQ ID NO:152), 1s2xa_002 (SEQ ID NO:155), 1s2xa_003 (SEQ ID NO:158), and 1s2xa_004 (SEQ ID NO:164). One embodiment is an RSV immunogen that comprises an amino acid
- 30 sequence that has up to twelve substitutions in the amino acid sequence of protein 1s2xa_003 (SEQ ID NO:158). One embodiment is an RSV immunogen that comprises an amino acid sequence that is at least 95% identical to an amino acid sequence of a protein selected from the group consisting of 1s2xa_001 (SEQ ID NO:152), 1s2xa_002 (SEQ ID

NO:155), 1s2xa_003 (SEQ ID NO:158), and 1s2xa_004 (SEQ ID NO:164). One embodiment is an RSV immunogen that comprises an amino acid sequence that is at least 95% identical to an amino acid sequence of protein 1s2xa_003 (SEQ ID NO:158). One embodiment is an RSV immunogen that comprises an amino acid sequence of a protein selected from the group consisting of 1s2xa_001 (SEQ ID NO:152), 1s2xa_002 (SEQ ID NO:155), 1s2xa_003 (SEQ ID NO:158), and 1s2xa_004 (SEQ ID NO:164). One embodiment is an RSV immunogen comprising 1s2xa_003; such an immunogen comprises amino acid sequence SEQ ID NO:158. One embodiment is an RSV immunogen that is N-linked glycosylated. One embodiment is an RSV immunogen that is resurfaced. One embodiment is an RSV immunogen that is a multivalent immunogen. An RSV immunogen disclosed herein can elicit a humoral immune response against RSV. In one embodiment, such an RSV immunogen can elicit a humoral immune response against RSV, but does not elicit a cellular immune response. An RSV immunogen as disclosed herein can have a motavizumab antibody binding domain comprising less than 12 consecutive amino acids from a motavizumab antibody binding domain of RSV fusion protein. In one embodiment, an RSV immunogen has a motavizumab antibody-binding domain comprising less than 9 consecutive amino acids from a motavizumab antibody-binding domain of RSV fusion protein.

An immunogen of the embodiments does not have an amino acid sequence that consists of SEQ ID NO:1. An immunogen of the embodiments does not have an amino acid sequence that consists of SEQ ID NO:2. An immunogen of the embodiments does not have an amino acid sequence that consists of SEQ ID NO:3. An immunogen of the embodiments does not have an amino acid sequence that consists of SEQ ID NO:9. An immunogen of the embodiments does not have an amino acid sequence that consists of SEQ ID NO:10.

The present invention also discloses an immunogen comprising an RSV F protein that is stabilized in its pre-fusion, trimeric state. Such an immunogen comprises an RSV F protein in which the furin cleavage sites can (but need not be) mutated to reduce or prevent cleavage and a trimerization motif (such as a fibrin T4 trimerization motif) preferably appended to a truncated C terminus lacking the F protein transmembrane and cellular domain so that the resultant RSV F protein remains in a trimeric, pre-fusion conformation.

One embodiment of the present invention is an immunogen comprising an amino acid sequence of a protein selected from RSV F₀ Fd (also referred to as RSV F0 Fd) (SEQ ID NO:174), RSV F Fd (SEQ ID NO:175), and RSV F0 Fd GAG (SEQ ID NO:176), the amino acid sequences of which are disclosed in the Examples. One embodiment is an immunogen comprising RSV F₀ Fd (SEQ ID NO:174). One embodiment is an immunogen comprising RSV F Fd (SEQ ID NO:175). One embodiment is an immunogen comprising RSV F0 Fd GAG (SEQ ID NO:176). One embodiment is a variant of any of such immunogens, wherein the variant shares at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% amino acid sequence identity with such immunogen. Preferably the variant retains contact residues of such immunogen.

Immunogens of the instant invention can be produced recombinantly or they can be produced synthetically. Also encompassed are immunogens that are combinations of recombinant and synthetic molecules. General methods for producing and isolating recombinant or synthetic proteins or peptides are known to those skilled in the art. It should be noted that, as used herein, an isolated, or biologically pure, molecule, is one that has been removed from its natural milieu. As such the terms isolated, biologically pure, and the like, do not necessarily reflect the extent to which the immunogen has been purified.

An immunogen of the embodiments can also comprise one or more motifs that can aid in purification of the immunogen, processing of the immunogen, and/or the immunogenicity of the immunogen. Examples include, but are not limited to, an HRV3C site, a caspase 3 site, a His tag, a Strep tag, MBP (maltose binding protein) or a functional fragment thereof, a factor Xa site, a TEV site, and a PADRE motif. A PADRE (Pan HLA DR-binding epitope peptide) motif has been shown to elicit T-cell help to stimulate a good antibody response; see, e.g., Alexander J, et al., 1994, Immunity 1, 751-761.

One embodiment is a protein comprising an amino acid sequence of an immunogen of the embodiments. Such a protein can be produced recombinantly or synthetically.

One embodiment of the present invention is a nucleic acid molecule that encodes an immunogen of the present disclosure. Such a nucleic acid molecule comprises a nucleic acid sequence that encodes an amino acid sequence of an immunogen of the embodiments. A nucleic acid molecule of the embodiments can include DNA, RNA, or derivatives of either DNA or RNA. A nucleic acid molecule can encode one or more immunogens of the

embodiments. Nucleic acid molecules of the disclosure have been subjected to human manipulation. Such a nucleic acid molecule can be produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification, cloning), chemical synthesis, or a combination of recombinant DNA technology and chemical synthesis. In one embodiment, a nucleic acid molecule, such as a nucleic acid molecule encoding a scaffold protein, can be modified by inserting, deleting, substituting, and/or inverting one or more nucleotides to yield a nucleic acid molecule that encodes an immunogen of the present invention. A nucleic acid molecule can also be modified to introduce codons that are better recognized by the system used to produce protein from a nucleic acid molecule of the disclosure.

One embodiment is a nucleic acid molecule encoding an immunogen comprising a scaffold protein with one or more contact residues, as described herein, embedded in it. Such embedding can be accomplished using techniques described herein as well as techniques known by one skilled in the art.

Nucleic acid molecules of the present invention can be produced using a number of methods known to those skilled in the art; see, for example, Sambrook J et al., 2001, *Molecular Cloning: a Laboratory Manual*, 3rd edition, Cold Spring Harbor Laboratory Press, and Ausubel F et al., 1994, *Current Protocols in Molecular Biology*, John Wiley & Sons. For example, nucleic acid molecules can be modified using a variety of techniques including, but not limited to, classic mutagenesis techniques and recombinant DNA techniques, such as site-directed mutagenesis, chemical treatment of a nucleic acid molecule to induce mutations, restriction enzyme cleavage of a nucleic acid fragment, ligation of nucleic acid fragments, polymerase chain reaction (PCR) amplification and/or mutagenesis of selected regions of a nucleic acid sequence, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules and combinations thereof. Nucleic acid molecules of the embodiments can be selected from a mixture of modified nucleic acids by screening for the function of the protein encoded by the nucleic acid (e.g., the ability of such a nucleic acid molecule to encode an immunogen that binds to motavizumab or 101F).

The disclosure provides a recombinant molecule that comprises a nucleic acid encoding an immunogen of the embodiments operatively linked to at least one transcription control sequence capable of effecting expression of the nucleic acid molecule in a recombinant cell. A recombinant cell is a host cell that is transformed with a

recombinant molecule of the embodiments; i.e., a recombinant cell comprises a recombinant molecule. A recombinant molecule can comprise one or more nucleic acid molecules encoding an immunogen of the embodiments operatively linked to one or more transcription control sequences. A recombinant cell can comprise one or more
5 recombinant molecules. In one embodiment, a nucleic acid molecule is operatively linked to a recombinant vector that includes a transcription control sequence to produce a recombinant molecule. Such a vector can be a plasmid vector, a viral vector, or other vector. Such a vector can be DNA, RNA, or a derivative of DNA or RNA. Host cells to transform can be selected based on their ability to effect expression of a nucleic acid
10 molecule of the embodiments. Host cells can also be selected that effect post-translational modifications. Methods to select, produce and use recombinant vectors, recombinant molecules, and recombinant cells of the embodiments are known to those skilled in the art. Proteins and immunogens of the embodiments can be produced by culturing recombinant cells of the embodiments. Methods to effect such production and recovery of such
15 proteins and immunogens are known to those skilled in the art, see for example Sambrook J et al., *ibid*, and Ausubel, F et al., *ibid*.

The disclosure also provides a recombinant molecule that is a nucleic acid immunogen or vaccine. That is, such a recombinant molecule can be administered to a subject to elicit a humoral immune response against RSV. Such a response can be a
20 neutralizing humoral immune response. Such a response can be protective. Such a vaccine comprises a recombinant molecule comprising a nucleic acid molecule that encodes an immunogen of the embodiments. In one embodiment, the recombinant molecule is a nucleic acid molecule of the embodiments operatively linked to a recombinant vector. Suitable vectors can be selected by one skilled in the art. Examples include, but are not
25 limited, to adenovirus, adeno-associated virus, cytomegalovirus (CMV), herpes virus, poliovirus, retrovirus, sindbis virus, vaccinia virus, or any other DNA or RNA virus vector.

The present invention also discloses methods of making an immunogen of the present invention. One embodiment is a method that involves using the three-dimensional
30 structure of the antibody-binding domain of a RSV F peptide, when such peptide is bound to an RSV neutralizing antibody, to identify a protein comprising a similar three-dimensional structure, and then substituting the contact residues from the RSV F peptide into the spatially corresponding positions in the native scaffold protein to create an

immunogen. In one embodiment, the RSV-neutralizing antibody is palivizumab (SYNAGIS[®]). In another embodiment, the RSV-neutralizing antibody is motavizumab. In yet another embodiment, the RSV-neutralizing antibody is 101F. In one embodiment the three-dimensional structure of complex between the RSV F peptide and the antibody is represented by atomic coordinates defining backbone atoms that have a root mean square deviation of less than 10 angstroms from the backbone atoms of the complex defined by the coordinates recited in PDB acc code 3IXT or in PDB acc code 3O41. A preferred embodiment of the present invention is a method to produce an immunogen that elicits a potent neutralizing humoral response against RSV, the method comprising:

(a) obtaining a three-dimensional model substantially corresponding to the three-dimensional model represented by the coordinates set forth in PDB acc code 3IXT or in PDB acc code 3O41;

(b) using the model obtained in (a) model to identify a native protein scaffold having a three-dimensional structure represented by atomic coordinates that have a root mean square deviation of protein backbone atoms of less than 10 angstroms when superimposed on the three-dimensional model of the RSV F peptide defined by atomic coordinates in PDB acc code 3IXT or in PDB acc code 3O41;

(c) substituting amino acids in the native protein scaffold that spatially correspond to contact residues in the RSV F protein in such three-dimensional model, with the spatially corresponding contact residues in the F protein in such three-dimensional model to create an immunogen containing a transplanted epitope; and

(d) producing said immunogen comprising such transplanted epitope.

As has previously been discussed, immunogens produced using the disclosed methods can also be modified to remove sequences related to the RSV F protein, reduce steric

hindrance and/or to increase the affinity of the immunogen for motavizumab or 101F.

Thus, in one embodiment, the method further comprises modifying the immunogen created in step (c) by substituting amino acids outside of the antibody-binding domain to (a) reduce steric hindrance, (b) introduce new ionic bonds between the immunogen and the antibody, (c) stabilize the protein in a conformation that maintains the transplanted epitope in the spatial conformation found in the three-dimensional model represented by the coordinates in PDB acc code 3IXT or in PDB acc code 3O41. Such a method can also include, but not be limited to, introducing flexibility, N-linked glycosylation sites,

positively or negatively charged amino acids, shielding against immunodominant epitopes, or other beneficial features.

The disclosure provides a method to produce an immunogen that elicits a potent neutralizing humoral immune response against RSV. The method comprises:

- 5 (a) obtaining a three-dimensional model substantially corresponding to the three-dimensional model of a complex between an RSV F peptide consisting of amino acid sequence SEQ ID NO:2 and motavizumab, wherein the model substantially represents the atomic coordinates specified in Protein Data Bank accession code 3IXT;
- 10 (b) using the model to identify a candidate protein scaffold having a three-dimensional structure represented by atomic coordinates that have a root mean square deviation of protein backbone atoms of less than 10 angstroms when superimposed on the three-dimensional model of the RSV F peptide defined by atomic coordinates in Protein Data Bank accession code 3IXT;
- 15 (c) substituting amino acids in the candidate protein scaffold that spatially correspond to contact residues in the RSV F protein in the three-dimensional model, with the spatially corresponding contact residues in the F protein in the three-dimensional model to create a protein containing a transplanted epitope; and
- (d) producing the protein comprising the transplanted epitope.

20 The method can further include the step of modifying the protein of step (c) to stabilize the protein in a conformation that maintains the transplanted epitope in the antibody-bound conformation of the three-dimensional model of a complex between an RSV F peptide consisting of amino acid sequence SEQ ID NO:2 and motavizumab, wherein the model substantially represents the atomic coordinates specified in Protein Data Bank accession code 3IXT.

25 The disclosure also provides a method to produce an immunogen that elicits a potent neutralizing humoral immune response against RSV. The method comprises:

- (a) obtaining a three-dimensional model substantially corresponding to the three-dimensional model of a complex between an RSV F peptide consisting of amino acid sequence SEQ ID NO:4 and 101F antibody, wherein the model substantially
- 30 represents the atomic coordinates specified in Protein Data Bank accession code 3O41;
- (b) using the model to identify a candidate protein scaffold having a three-dimensional structure represented by atomic coordinates that have a root mean square deviation of protein backbone atoms of less than 10 angstroms when superimposed on the

three-dimensional model of the RSV F peptide defined by atomic coordinates in Protein Data Bank accession code 3O41;

(c) substituting amino acids in the candidate protein scaffold that spatially correspond to contact residues in the RSV F protein in the three-dimensional model, with the spatially corresponding contact residues in the F protein in the three-dimensional model to create a protein containing transplanted epitope; and

(d) producing the protein comprising the transplanted epitope.

The method can further include the step of modifying the protein of step (c) to stabilize the protein in a conformation that maintains the transplanted epitope in the antibody-bound conformation of the three-dimensional model of a complex between an RSV F peptide consisting of amino acid sequence SEQ ID NO:4 and 101F antibody, wherein the model substantially represents the atomic coordinates specified in Protein Data Bank accession code 3O41.

The three-dimensional model of a complex between the RSV F peptide and motavizumab, or 101F, disclosed herein provides an understanding of how residues in each molecule interact to form a complex. As disclosed herein, such information is useful in producing immunogens that stimulate a humoral immune response against the RSV F protein. Such information can also be used to produce an antibody (also referred to herein as an antibody protein) that has a higher, or lower, affinity for the RSV F peptide. More specifically, by knowing how the peptide and the antibody align in three-dimensional space, the sequence of the antibody can be altered to introduce new amino acids capable of forming bonds with amino acids in the peptide. Thus, one embodiment of the present invention is a modified RSV neutralizing antibody that is more potent than motavizumab, or 101F; such modified antibody comprises a peptide-binding site for the RSV F peptide, wherein such modified antibody contains amino acid substitutions when compared to the amino acid sequence of motavizumab or 101F, wherein such substitutions result in the formation of new ionic bonds between the modified antibody and the RSV F peptide, and wherein such new ionic bonds result in the modified antibody having a higher affinity for the RSV F protein. In one embodiment, the modified antibody is created by introducing sequence alterations into the amino acid sequence of motavizumab. In another embodiment, the modified antibody is created by introducing sequence alterations into the amino acid sequence of 101F. An antibody protein of the embodiments can be of any size that exhibits more potent neutralization of RSV than does motavizumab or 101F antibody.

For example, an antibody protein can comprise an entire heavy chain and an entire light chain or can comprise a portion thereof that retains more potent neutralization activity. In one embodiment an antibody protein is an antigen-binding fragment. In one embodiment, an antibody protein is a single polypeptide chain.

5 A preferred embodiment of the present invention is a modified neutralizing antibody that exhibits more potent neutralization of RSV than does motavizumab, wherein such modified antibody is produced by:

 (a) obtaining a three-dimensional model of a complex between an RSV F peptide consisting of amino acid sequence SEQ ID NO:2 and motavizumab, such complex
10 being set forth in the three-dimensional model defined by the atomic coordinates in PDB acc code 3IXT;

 (b) identifying at least one amino acid change in the interface between motavizumab and the RSV F protein, wherein such at least one change, if incorporated into motavizumab, would yield an antibody protein with a higher affinity for RSV F
15 protein; and

 (c) producing such antibody protein comprising such at least one change.

Another embodiment is a modified neutralizing antibody that exhibits more potent neutralization of RSV than does 101F antibody, wherein such modified antibody is produced by:

20 (a) obtaining a three-dimensional model of a complex between an RSV F peptide consisting of amino acid sequence SEQ ID NO:4 and 101F antibody, the complex being set forth in the three-dimensional model defined by the atomic coordinates in PDB acc code 3O41;

 (b) identifying at least one amino acid change in the interface between 101F
25 antibody and the RSV F protein, wherein such at least one change, if incorporated into 101F antibody, would yield an antibody protein with a higher affinity for RSV F protein; and

 (c) producing such antibody protein comprising such at least one change.

As used herein, an antibody protein that exhibits more potent neutralization of
30 RSV than does motavizumab means that a lower titer of such antibody protein is required to neutralize a given amount of RSV, as compared to the titer of motavizumab required to neutralize the same amount of RSV. As used herein, an antibody that exhibits more potent neutralization of RSV than does 101F antibody means that a lower titer of such antibody is

required to neutralize a given amount of RSV, as compared to the titer of 101F required to neutralize the same amount of RSV. Suitable amino acid changes to the sequence of motavizumab that result in an antibody protein having a higher affinity for the RSV F protein are disclosed herein. This technique can also be used to modify other antibodies
5 that bind to the motavizumab-binding site or the 101F antibody-binding site of RSV F protein.

One embodiment of the present invention is a modified antibody, wherein the heavy chain of such antibody comprises SEQ ID NO:5, except that such heavy chain comprises at least one amino acid substitution selected from the group consisting of:

- 10 (a) the amino acid at position 32 of SEQ ID NO:5 is substituted with a histidine or a glutamic acid;
- (b) the amino acid at position 35 of SEQ ID NO:5 is substituted with an alanine;
- (c) the amino acid at position 52 of SEQ ID NO:5 is substituted with an amino acid selected from the group consisting of lysine, histidine, threonine, serine and arginine;
- 15 (d) the amino acid at position 53 of SEQ ID NO:5 is substituted with a histidine or a serine;
- (e) the amino acid at position 54 of SEQ ID NO:5 is substituted with a phenylalanine or an arginine;
- (f) the amino acid at position 56 of SEQ ID NO:5 is substituted with an amino
20 acid selected from the group consisting of isoleucine, serine, glutamic acid, and aspartic acid;
- (g) the amino acid at position 58 of SEQ ID NO:5 is substituted with a tyrosine or a tryptophan;
- (h) the amino acid at position 97 of SEQ ID NO:5 is substituted with an aspartic
25 acid, a histidine or an arginine;
- (i) the amino acid at position 99 of SEQ ID NO:5 is substituted with aspartic acid;
- (j) the amino acid at position 100 of SEQ ID NO:5 is substituted with a tyrosine, a tryptophan or a histidine; and
- (k) the amino acid at position 100A of SEQ ID NO:5 is substituted with a serine or
30 a threonine.

Another embodiment of the present invention is a modified antibody, wherein the light chain of such antibody comprises SEQ ID NO:6, except that such light chain comprises at least one amino acid substitution selected from the group consisting of:

(a) the amino acid at position 32 of SEQ ID NO:6 is substituted with a phenylalanine;

(b) the amino acid at position 49 of SEQ ID NO:6 is substituted with an histidine or an arginine;

5 (c) the amino acid at position 92 of SEQ ID NO:6 is substituted with lysine;

(d) the amino acid at position 94 of SEQ ID NO:6 is substituted with a histidine; and,

(e) the amino acid at position 96 of SEQ ID NO:6 is substituted with a histidine.

In addition to the substitutions described above, analysis of the three-dimensional
10 model of the complex between the RSV F peptide and motavizumab, or 101F, disclosed herein, indicates that additional contacts between the antibody and the peptide can be made by increasing the length of the CDRH2 loop in the antibody (which spans amino acids 50 through 58 of the heavy chain) by 2 residues. One embodiment of the present invention is a modified antibody, wherein the heavy chain of such antibody comprises
15 SEQ ID NO:5, except that amino acids 50 through 58 of SEQ ID NO:5 have been replaced with an 11 amino acid sequence defined as follows:

(a) position one of said 11 amino acid sequence is a glutamic acid, a serine, or a methionine;

(b) position two of said 11 amino acid sequence is an isoleucine;

20 (c) position three of said 11 amino acid sequence is a histidine, an arginine, or a phenylalanine;

(d) position four of said 11 amino acid sequence is a serine;

(e) position five of said 11 amino acid sequence is a glycine;

(f) position six of said 11 amino acid sequence is an amino acid selected from the
25 group consisting of glycine, histidine, lysine, leucine, asparagine, glutamine, serine, aspartic acid, threonine, and arginine;

(g) position seven of said 11 amino acid sequence is an amino acid selected from the group consisting of phenylalanine, lysine, serine, threonine, aspartic acid, and arginine;

(h) position eight of said 11 amino acid sequence is a glutamic acid, an asparagine,
30 or an aspartic acid;

(i) position nine of said 11 amino acid sequence is an amino acid selected from the group consisting of aspartic acid, histidine, leucine, serine, arginine, and threonine;

(j) position ten of said 11 amino acid sequence is a tyrosine; and

(k) position eleven of said 11 amino acid sequence is a tyrosine, a phenylalanine or a histidine.

It should be understood that any combination of the above-described substitutions can be made. That is, in addition to substituting the eleven amino acid sequence described above, other substitutions can be made outside of amino acids 50-58 of SEQ ID NO:5, (e.g., substitutions into positions 32, 35, 97, 99, 100 or 100A of SEQ ID NO:5, and/or substitutions in to the light chain), so long as the resultant antibody exhibits more potent neutralization of RSV than does motavizumab or 101F.

The disclosure provides a protein comprising an amino acid sequence of any of the antibody proteins of the embodiments. The disclosure also provides a nucleic acid molecule encoding any of the antibody proteins of the embodiments. Such a nucleic acid molecule can encode one or more antibody proteins. The disclosure further provides a recombinant molecule that comprises a nucleic acid molecule encoding an antibody protein of the embodiments operatively linked to at least one transcription control sequence capable of effecting expression of the nucleic acid molecule in a recombinant cell. A recombinant molecule can comprise one or more nucleic acid molecules encoding an antibody protein of the embodiments operatively linked to one or more transcription control sequences. The disclosure also provides a recombinant cell transformed with a recombinant molecule of the embodiments; i.e., a recombinant cell comprises a recombinant molecule. A recombinant cell can comprise one or more recombinant molecules.

The disclosure provides methods to produce antibody proteins of the embodiments. An antibody protein can be produced synthetically, recombinantly, or by a combination of synthetic and recombinant methods. Methods such as those taught herein for production of immunogens can be used. In addition, methods are known to those skilled in the art.

The disclosure provides a method to produce a composition comprising a neutralizing antibody protein that exhibits more potent neutralization of RSV than does motavizumab. The method comprises:

(a) obtaining a three-dimensional model of a complex between an RSV F peptide consisting of amino acid sequence SEQ ID NO:2 and motavizumab, the complex being set forth in the 3-dimensional model defined by the atomic coordinates in Protein Data Bank accession code 3IXT;

(b) identifying at least one amino acid change in the interface between motavizumab and the RSV F protein, wherein the at least one change, if incorporated into motavizumab, would yield an antibody protein with a higher affinity for RSV F protein; and

5 (c) producing the antibody protein comprising the at least one change.

The disclosure also provides a method to produce a composition comprising a neutralizing antibody protein that exhibits more potent neutralization of RSV than does 101F antibody. The method comprises:

(a) obtaining a three-dimensional model of a complex between an RSV F peptide consisting of amino acid sequence SEQ ID NO:4 and 101F antibody, the complex being set forth in the 3-dimensional model defined by the atomic coordinates in Protein Data Bank accession code 3O41;

(b) identifying at least one amino acid change in the interface between the 101F antibody and the RSV F protein, wherein the at least one change, if incorporated into the 101F antibody, would yield an antibody protein with a higher affinity for RSV F protein; and

(c) producing the antibody protein comprising the at least one change.

One embodiment of the present invention is an isolated crystal of a complex between an RSV F peptide and motavizumab. Another embodiment of the present invention is an isolated crystal of a complex between an RSV F peptide and 101F. As used herein, an isolated crystal is a crystal of a protein, or complex of proteins, that has been produced in a laboratory; that is, an isolated crystal is produced by an individual and is not an object found in situ in nature. It is appreciated by those skilled in the art that there are a variety of techniques to produce crystals including, but not limited to, vapor diffusion using a hanging or sitting drop methodology, vapor diffusion under oil, and batch methods; see, for example, Ducruix et al., eds., 1991, Crystallization of nucleic acids and proteins; A practical approach, Oxford University Press, and Wyckoff et al., eds., 1985, Methods in Enzymology 11, 49-185; each reference is incorporated by reference herein in its entirety. It is also to be appreciated that crystallization conditions can be adjusted depending on a protein's inherent characteristics as well as on a protein's concentration in a solution and that a variety of precipitants can be added to a protein solution in order to effect crystallization; such precipitants are known to those skilled in the art. In a preferred embodiment, a crystal of a complex between an RSV F peptide and

motavizumab or 101F is produced in a solution by adding a precipitant such as polyethylene glycol (PEG) or PEG monomethylether.

One embodiment of the present invention is an isolated crystal of a complex between an RSV F peptide and motavizumab, or 101F, such crystal being produced by the vapor diffusion method using a reservoir solution comprising about 17.5% (w/v) PEG 8000, 0.2 M zinc acetate, and 0.1 M cacodylate pH 6.5. Another embodiment of the present invention is an isolated crystal of a complex between an RSV F peptide and motavizumab, or 101F, wherein obtained by a method comprising :

- (a) producing an initial crystal using the vapor diffusion method at a temperature of about 20°C, with a reservoir solution comprising about 17.5% (w/v) PEG 8000, 0.2 M zinc acetate, and 0.1 M cacodylate pH 6.5; and
- (b) streak-seeding the initial crystal obtained in (a) into hanging drops consisting of 1 μ l of protein complex and 1 μ l of 30% (w/v) PEG 1500.

Isolated crystals of the present invention can include heavy atom derivatives, such as, but not limited to, gold, platinum, mercury, selenium, copper, and lead. Such heavy atoms can be introduced randomly or introduced in a manner based on knowledge of three-dimensional models of the present invention. Additional crystals of the present invention are not derivatized.

A preferred crystal of the present invention diffracts X-rays to a resolution of about 4.5 angstroms or higher (i.e., lower number meaning higher resolution), with resolutions of about 4.0 angstroms or higher, about 3.5 angstroms or higher, about 3.25 angstroms or higher, about 3 angstroms or higher, about 2.5 angstroms or higher, about 2.3 angstroms or higher, about 2 angstroms or higher, about 1.5 angstroms or higher, and about 1 angstrom or higher being increasingly more preferred. It is appreciated, however, that additional crystals of lower resolutions can have utility in discerning overall topology of the structures, e.g., location of a contact residues between an F peptide and its respective antibody. Preferred are crystals are those described in Table 3 and Table 4.

Table 3: Data collection and refinement statistics (molecular replacement)

		<u>Motavizumab/peptide</u>
Data collection		
	Space group	<i>P</i> 4 ₃ 2 ₁ 2
5	Cell dimensions	
	<i>a</i> = <i>b</i> , <i>c</i> (Å)	90.75, 232.06
	Resolution (Å)	50-2.75
	<i>R</i> _{merge}	11.3 (52.9)
	<i>I</i> / σ <i>I</i>	12.2 (1.8)
10	Completeness (%)	93.9 (88.3)
	Redundancy	4.6 (3.5)
Refinement		
	Resolution (Å)	2.75
	No. reflections	23,502
15	<i>R</i> work / <i>R</i> free (%)	21.3/27.4
	No. atoms	
	Protein	6464
	Ligand/ion	396
	Water	150
20	<i>B</i> -factors	
	Protein	57.1
	Ligand/ion	89.2
	Water	47.0
	R.m.s. deviations	
25	Bond lengths (Å)	0.004
	Bond angles (°)	0.742

Values in parentheses are for highest-resolution shell.

Table 4: Data collection and refinement statistics (molecular replacement)

		<u>101F/peptide</u>
	Data collection	
5	Space group	<i>P</i> 2 ₁ 2 ₁ 2 ₁
	Cell dimensions	
	<i>a</i> , <i>b</i> , <i>c</i> (Å)	79.90, 92.98, 141.22
	Resolution (Å)	50-1.95
	<i>R</i> _{merge}	10.7 (47.9)
10	<i>I</i> / σ <i>I</i>	15.5 (2.0)
	Completeness (%)	95.7 (77.4)
	Redundancy	5.9 (4.3)
	Refinement	
	Resolution (Å)	1.95
15	No. reflections	69,877
	<i>R</i> work / <i>R</i> free (%)	17.7/22.0
	No. atoms	
	Protein	6695
	Ligand/ion	223
20	Water	727
	<i>B</i> -factors	
	Protein	43.1
	Ligand/ion	61.2
	Water	50.7
25	R.m.s. deviations	
	Bond lengths (Å)	0.006
	Bond angles (°)	1.042

Values in parentheses are for highest-resolution shell.

30 One embodiment of the present invention is a three-dimensional model of a complex between an RSV F peptide consisting of SEQ ID NO:2 and motavizumab, wherein said model is substantially represented by the atomic coordinates specified in PDB acc code 3IXT. Another embodiment of the present invention is a three-dimensional

35 model of a complex between an RSV F peptide consisting of SEQ ID NO:4 and 101F, wherein said model is substantially represented by the atomic coordinates specified in PDB acc code 3O41. Another embodiment of the present invention is a three-dimensional model of a complex between an RSV F peptide consisting of SEQ ID NO:9 and 101F, wherein the model is substantially represented by the atomic coordinates specified in PDB

40 acc code 3O45. As used herein, a model that is substantially represented by atomic

coordinates listed herein includes not only those models literally represented by the coordinates but also models representing a coordinate transformation of atomic coordinates disclosed herein, for example, by changing the relative spatial orientation of the coordinates. A three-dimensional model of a complex between an RSV F peptide and motavizumab, or 101F, is a representation, a mathematical model, or image that predicts the actual structure of the corresponding complex. As such, a three-dimensional model is a tool that can be used to probe the relationship between the region's structure and function at the atomic level and to design immunogens and modified. It is well known to those skilled in the art, however, that a three-dimensional model of a protein derived by analysis of protein crystals is not identical to the inherent structure of the protein. See, for example, Branden et al., *Introduction to Protein Structure*, Garland Publishing Inc., New York and London, 1991, especially on page 277, which states "not surprisingly the model never corresponds precisely to the actual crystal." Furthermore, the model can be subjected to further refinements to more closely correspond to the actual structure of a complex between an RSV F peptide and motavizumab or 101F. Such a refined model, which is an example of a modification of the present invention, is a better predictor of the actual structure and mechanism of action of the protein that the model represents. Refinements can include models determined to more preferred degrees of resolution, preferably to about 4.5 angstroms, more preferably to about 4 angstroms, more preferably to about 3.5 angstroms, more preferably to about 3.25 angstroms, more preferably to about 3 angstroms, more preferably to about 2.5 angstroms, more preferably to about 2.3 angstroms, more preferably to about 2 angstroms, more preferably to about 1.5 angstroms, and even more preferably to about 1 angstrom. Preferred refinements are obtained using the three-dimensional model as a basis for such improvements.

One embodiment of the present invention is a composition comprising an immunogen or an antibody protein of the present invention. Another embodiment is a composition comprising a nucleic acid molecule, protein, recombinant molecule or recombinant cell of the embodiments. One type of composition is a vaccine. A composition of the present invention can be formulated in an excipient that a patient to be treated can tolerate. Examples of such excipients include water, saline, Ringer's solution, dextrose solution, Hank's solution, and other aqueous physiologically balanced salt solutions. Excipients can also contain minor amounts of additives, such as substances that enhance isotonicity and chemical or biological stability. Examples of buffers include

phosphate buffer, bicarbonate buffer, and Tris buffer. Standard formulations can either be liquids or solids that can be taken up in a suitable liquid as a suspension or solution for administration to a patient. In one embodiment, a non-liquid formulation may comprise the excipient salts, buffers, stabilizers, etc., to which sterile water or saline can be added

5 prior to administration.

A composition of the present invention may also include one or more adjuvants or carriers. Adjuvants are typically substances that enhance the immune response of a patient to a specific antigen, and carriers include those compounds that increase the half-life of a composition in the treated patient.

10 Immunogens and antibodies of the present invention are intended for use in protection against infection by RSV. The immunogens disclosed herein protect against RSV infection by eliciting a humoral immune response against the F protein of RSV. This humoral response results in neutralization of the virus. Antibodies of the present invention protect against infection with RSV by binding and neutralizing the virus. Thus one

15 embodiment of the present invention is a method to protect a patient from RSV infection, the method comprising administering to the patient an immunogen or an antibody produced using the methods disclosed herein. One embodiment is a method to elicit a neutralizing humoral immune response against RSV, the method comprising administering an immunogen of the embodiments, wherein such administration elicits a neutralizing

20 humoral immune response against RSV. In one embodiment, the immunogen is administered to a patient. One embodiment is a method to protect a patient from RSV infection comprising administering to the patient an immunogen, wherein such administration protects the patient from RSV infection. One embodiment is a method to elicit a neutralizing humoral immune response against RSV, the method comprising

25 administering a nucleic acid vaccine of the embodiments, wherein such administration elicits a neutralizing humoral immune response against RSV. In one embodiment, the nucleic acid vaccine is administered to a patient. One embodiment is a method to protect a patient from RSV infection comprising administering to the patient a nucleic acid vaccine, wherein such administration protects the patient from RSV infection. One

30 embodiment is a method to protect a patient from RSV infection comprising administering to the patient an antibody protein, wherein such administration protects the patient from RSV infection.

As used herein the phrase protect a patient from RSV infection includes preventing a patient from being infected by RSV, as well as treating a patient already infected with RSV. As used herein the term patient refers to any animal in need of such prevention or treatment. The animal can be a human or a non-human animal. A preferred animal to
5 treat is a mammal. A patient can be of any age. In one embodiment, an immunogen or antibody can be administered to an infant. In one embodiment, an immunogen or antibody can be administered to a patient that is older than an infant. An immunogen or antibody can be administered or applied per se, or as a composition. An immunogen or antibody of the present invention, or a composition thereof, can be administered to a patient by a
10 variety of routes, including, but limited to, by injection (e.g., intravenous, intramuscular, subcutaneous, intrathecal, intraperitoneal), by inhalation, by oral (e.g., in a pill, tablet, capsule, powder, syrup, solution, suspension, thin film, dispersion or emulsion.), transdermal, transmucosal, pulmonary, buccal, intranasal, sublingual, intracerebral, intravaginal rectal or topical administration or by any other convenient method known to
15 those of skill in the art.

The amount of an immunogen or antibody of the present invention, and/or a composition thereof that will be effective can be determined by standard clinical techniques known in the art. Such an amount is dependent on, among other factors, the patient being treated, including, but not limited to the weight, age, and condition of the
20 patient, the intended effect of the composition, the manner of administration and the judgment of the prescribing physician.

An immunogen or antibody of the present invention, or a composition thereof, can be administered alone or in combination with one or more other pharmaceutical agents, including other immunogens or antibodies of the present invention. The specific
25 composition depends on the desired mode of administration, as is well known to the skilled artisan. One composition can include an immunogen of the present invention comprising motavizumab-binding contact residues. Another composition can include an immunogen of the present invention comprising 101F-binding contact residues. One composition comprises a combination of both immunogens. Another composition is an
30 antibody of the present invention. Yet another composition comprises a nucleic acid vaccine comprising at least one nucleic acid molecule encoding an immunogen of the present invention. The disclosure also provides for a combination comprising one or more immunogens and/or antibodies (i.e., antibody proteins) of the embodiments with one or

more other RSV immunogens and/or antibodies. The disclosure also provides for a combination comprising one or more immunogens and/or antibodies (i.e., antibody proteins) of the embodiments with one or more protective agents, such as, but not limited to, an agent that protects from infection by a virus, bacterium, parasite, or other infectious agents.

In one embodiment, administration can comprise a prime followed by one or more boosts. A prime can comprise a composition comprising at least one of the immunogens disclosed herein, or a nucleic acid encoding such an immunogen. A boost can comprise at least one of the immunogen disclosed herein, or a nucleic acid encoding such an immunogen. In one embodiment the boost comprises an immunogen that has been resurfaced (compared to the first immunogen) to further boost the humoral immune response against RSV contact residues in the motavizumab or 101F binding domains. In one embodiment the boost comprises a multivalent immunogen.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the embodiments, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, and temperature is in degrees Celsius. Standard abbreviations may be used. For example, amino acids can be denoted by either the standard 3-letter or 1-letter code.

Example 1: Three-dimensional structure of RSV F protein and motavizumab

This Example describes the crystallization and determination of the 3-dimensional structure of a complex between motavizumab and the 24-residue RSV fusion (F) peptide spanning amino acids 254-277 of the F protein (i.e., NSELLSLIND MPITNDQKKL MSNN, also denoted herein as SEQ ID NO:2) that includes the binding domain of motavizumab. The amino acid sequence of the F protein used in these studies is as follows: MELLILKANA ITTILTAVTF CFASGQNITE EFYQSTCSAV SKGYLSALRT GWYTSVITIE LSNIKENKCN GTDAKVLIK QELDKYKNAV TELQLMQST

PATNNRARRE LPRFMNYTLN NAKKTNVTLS KKRKRRFLGF LLGVGSAIAS
 GVAVSKVLHL EGEVNIKISA LLSTNKAVVS LSNGVSVLTS KVLDLKNYID
 KQLLPVINKQ SCSISNIETV IEFQQKNRL LEITREFSVN AGVTTPVSTY
 MLTNSSELLSL INDMPITNDQ KKLMSNNVQI VRQQSYSIMS IIEEVLAYV
 5 VQLPLYGVID TPCWKLHTSP LCTTNTKEGS NICLTRTDRG WYCDNAGSVS
 FFPQAETCKV QSNRVFCDTM NSLTLPSEVN LCNVDIFNPK YDCKIMTSKT
 DVSSSVITSL GAIVSCYGKT KCTASNKNRG IKTFSNGCD YVSNKGVDTV
 SVGNTLYYVN KQEGKSLYVK GEPIINFYDP LVFPSDEFDA SISQVNEKIN
 QSLAFIRKSD ELL, also denoted herein as SEQ ID NO:1.

10 To enhance the potency of palivizumab, each residue in the six complementarity-
 determining regions (CDRs) was individually substituted with the other 19 amino acids (a
 total number of 1,121 unique single variants were assayed), and combinations of
 beneficial substitutions assessed (Wu H et al., 2007, J. Mol. Biol. 368, 652-665; Wu H et
 al., 2005, J. Mol. Biol. 350, 126-144). This led to the development of a second-generation
 15 antibody, motavizumab, which is ~10 times more potent than palivizumab (Wu H et al.,
 2007, *ibid.*). Only 13 amino acids differ between motavizumab and palivizumab. Of these,
 seven individually increase the affinity of the antibody to the F glycoprotein, resulting in a
 0.035 nM K_d (versus 1.4 nM for palivizumab) (Johnson S et al., 1997, J. Infect. Dis. 176,
 1215-1224; Wu H et al., 2007, *ibid.*; Wu H et al., 2005, *ibid.*). This disclosure includes a
 20 characterization of the structural basis of motavizumab affinity, model mutations with
 enhanced affinity, and structural implications for motavizumab binding in the trimeric F
 glycoprotein context.

Motavizumab is ~10-fold more potent than its predecessor, palivizumab
 (SYNAGIS[®]), the FDA-approved monoclonal antibody used to prevent respiratory
 25 syncytial virus (RSV) infection. The structure of motavizumab in complex with a 24-
 residue peptide corresponding to its epitope on the RSV-fusion (F) glycoprotein reveals
 the structural basis for its increased potency. Modeling suggests that motavizumab
 recognizes a different quaternary configuration of the F glycoprotein than observed in a
 homologous structure.

30 Recombinant motavizumab IgG molecules that were shown to neutralize RSV
 potently (Figure 1a) were used to create antigen-binding fragments (Fabs) for
 crystallographic analysis. Crystals of the Fab were obtained in complex with a 24-residue
 peptide, which corresponds to residues 254-277 of the RSV F glycoprotein A2 strain

(NSELLSLIND MPITNDQKKL MSNN) and represents the known epitope for palivizumab/motavizumab (Arbiza J et al., 1992, J. Gen. Virol. 74, 2225-2234). The crystals diffracted X-rays to 2.75 Å, and a molecular replacement solution was obtained containing two molecules of the previously-determined unliganded palivizumab structure (Johnson LS et al., US Patent No. 7,229,618, issued June 12, 2007) per asymmetric unit. Initial maps showed two regions of well-defined helical density near the CDRs of each Fab. These regions were modeled as the peptide, and the structure refined to an $R_{\text{crys}}/R_{\text{free}}$ = 21.3/27.4%; see Table 3.

The peptide forms a helix-loop-helix (Figure 1b), in agreement with secondary structure predictions of the RSV F glycoprotein (Smith BJ et al., 2002, Protein Eng. 15, 365-371). The main-chain electron density for the peptide was good for all residues and the side chain density was good for residues 262-276, but weak or non-existent for residues N- and C-terminal to this region (Figure 3). The variable domains of the peptide-bound motavizumab structure and the unbound palivizumab structure are similar (rmsd 1.8 Å for variable domain Ca), with the largest differences occurring in the three heavy chain CDRs.

To understand the structural basis for the high affinity interaction between motavizumab and the RSV F protein, the structure of the peptide/Fab complex was analyzed. The interface between the peptide and Fab buries a total of 1,304 Å² of surface area (680 Å² on the peptide and 624 Å² on the Fab, as calculated by PISA, Krissinel E et al., 2007, J. Mol. Biol. 372, 774-797) and has a shape complementarity (S_c) value of 0.76, which is substantially higher than the typical range of 0.64-0.68 for antibody/antigen complexes (Lawrence MC et al., 1993, J. Mol. Biol. 234, 946-950). The electrostatic potentials on the surface of the peptide and Fab are also complementary, with several acidic patches on the Fab interacting with positively charged regions on the peptide (Figure 4). Approximately 73% of the surface area buried on the Fab is located on the heavy chain, which possesses a large hydrophobic region consisting of residues from the second and third CDRs (Figure 1c). This region contacts peptide residues located along the length of both helices. The four peptide residues between the two helices do not contribute significantly to motavizumab binding to RSV F, having only 8 Å² buried at the interface. Interactions between the peptide and heavy chain include hydrogen bonds formed between the peptide side chain of Asn262 and the Fab side chains of Asp54 and Lys56, as well as a hydrogen bond between the peptide side chain of Ser275 and the

carbonyl oxygen of Fab residue Ile97. There are also several interactions between the peptide and light chain. These include a hydrogen bond between the side chain of peptide residue Asn268 and the carbonyl oxygen of Gly90, as well as a salt-bridge between the peptide side chain of Lys272 and the side chain of Asp49 in the second CDR (Figure 1c).

5 The interactions between the peptide and motavizumab Fab are consistent with RSV F glycoprotein mutations known to disrupt antibody binding to this epitope. It has been demonstrated that mutations N262Y, N268I and K272E decrease the binding of several antibodies that recognize this region of the F glycoprotein (Arbiza J et al., *ibid.*). The mutations K272M and K272Q have also been found in RSV F glycoprotein escape
10 mutants that are resistant to palivizumab (Zhao X et al., 2004, J. Infect. Dis. 190, 1941-1946). The side chains of these three peptide residues all form hydrogen bonds or salt bridges with residues in the Fab (Figure 1c), interactions that would be lost by the mutations listed above.

To investigate the structural basis for motavizumab's enhanced potency over
15 palivizumab, the positions of the 7 altered residues that increase the affinity to the F glycoprotein were analyzed in the peptide-bound crystal structure (Figure 1d). Three of the seven altered residues (S32A, T98F and W100F in the heavy chain) directly contact the peptide and are located in the large hydrophobic patch described earlier. Both the S32A and T98F substitutions increase the hydrophobicity of this patch, favoring interactions
20 with the peptide. As for the W100F mutation, the smaller Phe side chain is able to pack tightly against peptide residues N268 and K272. The larger Trp side chain found in palivizumab would likely alter the conformation of these residues, which make hydrogen bond and salt bridge interactions with the Fab, respectively. When the three palivizumab residues were modeled into the complex, the S_c value decreased from 0.76 to 0.70,
25 reflecting the poorer fit between the peptide and Fab.

The other four substitutions that increase the potency of motavizumab do not contact the peptide directly. Two of the mutations (D58H and S95D in the heavy chain) are located near the interface with the peptide, and their side chains interact with other residues in the CDRs. Thus, they likely exert their effects indirectly by altering the
30 position of other amino acids that do contact the peptide. The side chains of the two remaining substitutions, S65D in the heavy chain and S29R in the light chain, have weak electron density and do not contact any residues in the peptide or Fab. However, both substitutions increase the on-rate of motavizumab for the F glycoprotein, and the S29R

mutation alone results in a 4.4-fold increase in RSV neutralization *in vitro*⁹. Collectively, these data suggest that the S65D and S29R side chains either bind to residues in the F glycoprotein located outside the primary epitope or increase favorable long-range electrostatic interactions. Relevant to this, motavizumab binds to the peptide ~6,000-fold weaker than the full-length F protein (230 nM vs 0.035 nM) (Wu H et al., 2007, *ibid.*;
5 Tous GI et al., 2006, US Patent Application 11/230,593), though some fraction of the decrease in peptide affinity is likely due to the peptide not adopting the helix-loop-helix conformation in solution (Lopez JA et al., 1993, J. Gen. Virol. 74, 2567-2577).

An earlier version of motavizumab contained residues Phe52, Phe53, and Asp55 in the light chain CDR2, which increased *in vitro* RSV neutralization ~2-fold (Wu H et al.,
10 2007, *ibid.*). However, these residues also increased non-specific tissue binding and decreased the *in vivo* potency (Wu H et al., 2007, *ibid.*), perhaps due to the two solvent-exposed Phe residues (Figure 1d). Thus, they were ultimately returned to the amino acids found in palivizumab (Ser52, Lys53, and Ala55).

To visualize the binding of motavizumab to the full-length F glycoprotein, a model was generated based on the pre-fusion parainfluenza virus 5 (PIV5) structure (Yin HS et al., 2006, Nature 439, 38-44) (12.4% sequence identity to RSV F (Smith BJ et al., *ibid.*)). A sequence alignment (Figure 5a) identified a similar helix-loop-helix, and structural analysis provided a precise alignment (Figure 5b), shifting the PIV5 sequence by three
15 amino acids to provide a motavizumab epitope/PIV5 superposition of 2.1 Å rmsd for all 24 peptide Ca atoms (Figure 2a). A model was generated by orienting the Fab via superposition of the bound peptide onto the corresponding epitope in the PIV5 F glycoprotein structure. The resulting model shows no clashes between the Fab and F glycoprotein monomer to which it is bound (Figure 2b).

In the pre-fusion trimeric context, however, both the heavy and light chains of the Fab clash with an adjacent RSV F monomer that packs against the same face of the helix-loop-helix that motavizumab binds (Figure 2c,d). The location of the epitope at a subunit interface may explain why this neutralizing epitope is so highly conserved in RSV strains. Since motavizumab neutralizes RSV by preventing the fusion of the viral and cellular
20 membranes (Johnson S et al., 1997, *ibid.*), motavizumab must bind to the F glycoprotein before or during the transition to the post-fusion state. The extensive clashes in the trimer model, however, suggest that motavizumab would be unable to bind the pre-fusion trimeric F glycoprotein as it exists in the PIV5 structure. To address this issue

experimentally, a soluble RSV F glycoprotein was expressed and purified in a form similar to the PIV5 F glycoprotein used in the modeling. Specifically, the known furin cleavage sites were mutated and a fibrin trimerization motif (Tao Y et al., 1997, Structure 5, 789-798) was appended to the truncated C terminus to keep the protein in a trimeric, pre-fusion conformation. This stabilized RSV F glycoprotein, referred to as RSV F₀ Fd, eluted from a gel filtration column with a retention volume consistent with that of a glycosylated trimer (Figure 2e). To determine whether motavizumab or palivizumab is able to bind the pre-fusion trimeric RSV F glycoprotein, palivizumab Fab was added in excess to a solution of RSV F₀ Fd, and the mixture was passed over a gel filtration column. The elution profile contained two peaks, corresponding to excess Fab and a complex of the Fab and F glycoprotein (Figure 2f). The elution volume of the complex peak was consistent with a trimeric F glycoprotein bound by three Fabs, in agreement with the ratio (1:2.97) of F glycoprotein and Fab bands observed on a Coomassie stained SDS-PAGE gel containing fractions from the complex peak (Figure 2f).

Collectively, these data suggest that motavizumab binds to or induces a conformation of the trimeric F glycoprotein that is different from that observed in the PIV5 F pre-fusion structure. One possibility is that the structure of the RSV F glycoprotein differs significantly from that of PIV5, although the predicted RSV F glycoprotein secondary structure appears similar to that observed in the PIV5 F pre-fusion crystal structure (Figure 5). Another possibility is that motavizumab traps an intermediate between pre- and post-fusion forms. It has been suggested that during this transition, which is one of the largest structural rearrangements known, the F glycoprotein monomers transiently dissociate prior to forming the trimeric post-fusion conformation (Yin HS et al., *ibid*). It is to be noted in this regard that glutaraldehyde crosslinking of the soluble F glycoprotein trimer does not inhibit motavizumab binding (Figure 6). Alternatively, the RSV F glycoprotein in its pre-fusion conformation may contain sufficient flexibility to bind three motavizumab Fabs. Modeling studies indicate that a ~30° rotation of domain III parallel to the 3-fold axis would allow clash-free binding of three Fabs. A similar degree of rotation has been observed in cryo-EM tomograms after the binding of neutralizing antibodies to Dengue virus (Lok S-M, et al., 2008, Nat Struct Mol Biol 15, 312-317) and HIV-1 (Liu J et al., 2008, Nature 455, 109-113). Such flexibility may be a more common feature of viral fusion proteins than previously thought.

Materials and Methods

a. Cloning, expression and purification of motavizumab IgG. Two DNA fragments encoding the variable heavy and light chains of motavizumab (Wu H et al., 2007, J. Mol. Biol. 368, 652-665) with appropriate signal sequences were synthesized by GeneArt (Regensburg, Germany) and cloned in-frame into mammalian expression vectors containing human IgG1 heavy and light constant domains, respectively. The amino acid sequence of the variable heavy chain of motavizumab is as follows: QVTLRESGPA LVKPTQTLTL TCTFSGFSLs TAGMSVGWIR QPPGKALEWL ADIWWDDKKH YNPSLKDRLT ISKDTSKNQV VLKVTNMDPA DTATYYCARD MIFNFYFDVW GQGTTVTVSS, also denoted herein as SEQ ID NO:5. The amino acid sequence of the variable light chain of motavizumab is as follows: DIQMTQSPST LSASVGDRVIT ITCSASSRVG YMHWYQQKPG KAPKLLIYDT SKLASGVPSR FSGSGSGTEF TLTISSLQPD DFATYYCFQG SGYPFTFGGG TKVEIK, also denoted herein as SEQ ID NO:6.

Both vectors were co-transfected at a 1:1 ratio into HEK293F cells (Invitrogen, Life Technologies, Carlsbad, CA) in serum-free 293Freestyle medium (Invitrogen). After 3 hours, valproic acid (Sigma, St. Louis, MO) was added to 4 mM final concentration. Expression lasted for four days at 37°C with 10% CO₂ and shaking at 125 rpm in disposable flasks. The supernatant was collected, filtered, and passed over 5 ml of Protein A agarose resin (Pierce). After washing with several column volumes of phosphate-buffered saline (PBS), the resin was eluted with 25 ml of IgG Elution Buffer (Pierce, Thermo Fisher Scientific, Rockford, IL) and immediately neutralized with 1 M Tris pH 8.0. The eluted protein was dialyzed against PBS and stored at 4°C.

b. Measurement of antibody-mediated neutralization. RSV expressing green fluorescent protein (GFP) was provided by Mark Peeples and Peter Collins and constructed as previously reported (Hallak LK, et al., 2000, Virology 271, 264-275). Antibody-mediated neutralization was measured using HEp-2 cells. GFP-RSV was added to serial four-fold dilutions of serum and/or antibody in 96-well plates and incubated at 37°C for one hour. Serum concentrations ranged from 1:10 to 1:40,960. After one hour, 100 µl of virus/serum mixture was added to 5x10⁴ cells/100 µl per well in 96-well plates. Infection was monitored as a function of GFP expression (encoded by the viral genome) at 18 hours post-infection by flow cytometry (LSR II, BD Bioscience, CA, USA). Prior to assessment by flow cytometry, cells were treated with trypsin to ensure a single-cell

suspension optimal for analysis and fixed with 0.5% paraformaldehyde, Data were analyzed by curve fitting and non-linear regression (GraphPad Prism, GraphPad Software Inc., San Diego CA) in order to demonstrate the percent neutralization at a given antibody concentration, and the neutralization activity was compared based on the EC50.

5 c. Digestion and purification of motavizumab Fab fragments. The purified motavizumab IgG protein was reduced with 100 mM dithiothreitol at 37°C for 1 hour and then alkylated with 2 mM iodoacetamide for 48 hours at 4 °C. 10 ml of reduced and alkylated IgG in PBS at 3.5 mg/ml was combined with 15 µg of endoproteinase Lys-C (Roche) and incubated at 37 °C for 6 hours. The reaction was quenched by the addition of
10 TLCK and leupeptin to 50 µg/ml and 2 µg/ml, respectively. To remove the Fc fragments from the Fab fragments, the quenched reaction was passed over 5 ml of Protein A agarose. The Fab-containing flow through was further purified over an S200 gel filtration column and concentrated aliquots were stored frozen at -80 °C.

 d. Protein crystallization and data collection. A peptide with the sequence
15 NSELLSLIND MPITNDQKKL MSNN, corresponding to residues 254-277 of the RSV F protein and also denoted herein as SEQ ID NO:2) was synthesized by American Peptide (Sunnyvale, CA) with an acetylated N-terminus and an amidated C-terminus. A five-fold molar excess of peptide was incubated with motavizumab Fab at 22 °C for 1.5 hours and then concentrated to give a 13.1 mg/ml solution of Fab/peptide complex in 2 mM Tris pH
20 7.5, 150 mM NaCl. Initial crystals were grown by the vapor diffusion method in sitting drops at 20 °C by mixing 0.1 µl of protein complex with 0.1 µl of reservoir solution (17.5% (w/v) PEG 8000, 0.2 M zinc acetate, 0.1 M cacodylate pH 6.5) using a Cartesian Honeybee crystallization robot (Genomic Solutions). These initial crystals were streak-seeded into hanging drops consisting of 1 µl protein complex and 1 µl 30% (w/v) PEG
25 1500. After several days rectangular crystals appeared in a single drop with dimensions 40 x 40 x 10 µm. These crystals were flash frozen in liquid nitrogen in 40% (w/v) PEG 1500, 30% (v/v) ethylene glycol and loaded into a cryopuck. Data were collected at a wavelength of 0.82656 Å at the SER-CAT beamline ID-22 using the robot automounter (Advanced Photon Source, Argonne National Laboratory).

30 e. Structure determination, model building and refinement. Diffraction data were processed with the HKL2000 suite (Otwinowski Z et al., 1997, Methods Enzymol. 276, 307-326, Academic Press) and a molecular replacement solution was found by PHASER (McCoy AJ et al, 2007, J. Appl. Crystallog. 40, 658-674) using the palivizumab Fab

structure (PDB ID: 2hwz) as a search model. Two Fab molecules were placed in the asymmetric unit. After rigid body and TLS refinement using PHENIX (Adams, PD et al., 2002, Acta Crystallogr., Section D 58, 1948-1954), helical peptide density was evident near the CDRs of each Fab. Model building was carried out using COOT (Emsley, P et al., 2004, Acta Crystallogr., Section D 60, 2126-2132) and refinement was performed with PHENIX using NCS restraints. Final data collection and refinement statistics are presented in Table 3. The atomic coordinates for the motavizumab/peptide complex have been deposited in the Protein Data Bank under PDB accession code 3IXT. The atomic coordinates for the peptide portion of the complex are indicated below in Table 6.

Table 6. Atomic coordinates for the motavizumab binding peptide

	ATOM	3243	N	ASN P 254	7.154	8.372	-9.660	1.00124.11	N
	ANISOU	3243	N	ASN P 254	13767	16195	17192	-3482 2530 2430	N
15	ATOM	3244	CA	ASN P 254	7.612	9.736	-9.412	1.00121.53	C
	ANISOU	3244	CA	ASN P 254	13313	15765	17097	-3858 2355 2424	C
	ATOM	3245	C	ASN P 254	6.768	10.583	-8.462	1.00111.67	C
	ANISOU	3245	C	ASN P 254	12385	14386	15658	-4047 1969 2168	C
	ATOM	3246	O	ASN P 254	5.706	11.098	-8.827	1.00107.07	O
20	ANISOU	3246	O	ASN P 254	12235	13626	14819	-4027 2035 2025	O
	ATOM	3247	CB	ASN P 254	7.853	10.480	-10.730	1.00123.56	C
	ANISOU	3247	CB	ASN P 254	13592	15864	17490	-3953 2745 2570	C
	ATOM	3248	CG	ASN P 254	9.265	10.333	-11.225	1.00130.78	C
	ANISOU	3248	CG	ASN P 254	13976	16914	18799	-4017 2965 2837	C
25	ATOM	3249	OD1	ASN P 254	10.213	10.485	-10.461	1.00132.18	O
	ANISOU	3249	OD1	ASN P 254	13727	17233	19260	-4212 2717 2883	O
	ATOM	3250	ND2	ASN P 254	9.416	10.009	-12.511	1.00136.19	N
	ANISOU	3250	ND2	ASN P 254	14673	17575	19498	-3837 3434 3010	N
	ATOM	3251	N	SER P 255	7.270	10.702	-7.237	1.00104.70	N
30	ANISOU	3251	N	SER P 255	11278	13608	14896	-4206 1562 2109	N
	ATOM	3252	CA	SER P 255	6.624	11.477	-6.192	1.00 96.74	C
	ANISOU	3252	CA	SER P 255	10531	12499	13728	-4386 1160 1872	C
	ATOM	3253	C	SER P 255	6.465	12.942	-6.584	1.00 85.08	C
	ANISOU	3253	C	SER P 255	9239	10769	12318	-4677 1173 1822	C
35	ATOM	3254	O	SER P 255	5.469	13.589	-6.245	1.00 75.10	O
	ANISOU	3254	O	SER P 255	8390	9349	10797	-4716 1012 1614	O
	ATOM	3255	CB	SER P 255	7.430	11.374	-4.900	1.00100.06	C
	ANISOU	3255	CB	SER P 255	10618	13086	14314	-4514 733 1850	C
	ATOM	3256	OG	SER P 255	8.819	11.450	-5.170	1.00101.92	O
40	ANISOU	3256	OG	SER P 255	10318	13442	14965	-4658 796 2057	O
	ATOM	3257	N	GLU P 256	7.456	13.465	-7.294	1.00 90.87	N
	ANISOU	3257	N	GLU P 256	9673	11460	13395	-4878 1375 2016	N
	ATOM	3258	CA	GLU P 256	7.446	14.869	-7.683	1.00100.04	C
	ANISOU	3258	CA	GLU P 256	11006	12355	14651	-5184 1410 1998	C
45	ATOM	3259	C	GLU P 256	6.561	15.110	-8.898	1.00 95.96	C

	ANISOU	3259	C	GLU	P	256	10919	11641	13901	-5014	1792	2007	C
	ATOM	3260	O	GLU	P	256	5.860	16.117	-8.970	1.00	93.69		O
	ANISOU	3260	O	GLU	P	256	11032	11111	13454	-5117	1734	1875	O
	ATOM	3261	CB	GLU	P	256	8.867	15.360	-7.952	1.00	117.93		C
5	ANISOU	3261	CB	GLU	P	256	12779	14646	17381	-5502	1494	2201	C
	ATOM	3262	CG	GLU	P	256	9.623	14.514	-8.951	1.00	137.85		C
	ANISOU	3262	CG	GLU	P	256	14946	17336	20096	-5326	1918	2463	C
	ATOM	3263	CD	GLU	P	256	10.877	15.200	-9.446	1.00	155.42		C
	ANISOU	3263	CD	GLU	P	256	16748	19544	22759	-5665	2092	2662	C
10	ATOM	3264	OE1	GLU	P	256	11.414	16.058	-8.713	1.00	159.74		O
	ANISOU	3264	OE1	GLU	P	256	17121	20045	23527	-6047	1784	2594	O
	ATOM	3265	OE2	GLU	P	256	11.323	14.883	-10.568	1.00	161.28		O
	ANISOU	3265	OE2	GLU	P	256	17337	20320	23624	-5559	2543	2879	O
	ATOM	3266	N	LEU	P	257	6.597	14.186	-9.853	1.00	101.08		N
15	ANISOU	3266	N	LEU	P	257	11497	12391	14515	-4733	2173	2157	N
	ATOM	3267	CA	LEU	P	257	5.751	14.295	-11.035	1.00	105.52		C
	ANISOU	3267	CA	LEU	P	257	12464	12797	14833	-4526	2529	2159	C
	ATOM	3268	C	LEU	P	257	4.279	14.282	-10.639	1.00	92.20		C
	ANISOU	3268	C	LEU	P	257	11263	11055	12715	-4343	2347	1883	C
20	ATOM	3269	O	LEU	P	257	3.455	14.971	-11.242	1.00	80.48		O
	ANISOU	3269	O	LEU	P	257	10189	9377	11014	-4288	2459	1793	O
	ATOM	3270	CB	LEU	P	257	6.036	13.160	-12.018	1.00	115.65		C
	ANISOU	3270	CB	LEU	P	257	13585	14225	16131	-4230	2932	2345	C
	ATOM	3271	CG	LEU	P	257	7.431	13.112	-12.643	1.00	125.63		C
25	ANISOU	3271	CG	LEU	P	257	14378	15562	17795	-4348	3209	2635	C
	ATOM	3272	CD1	LEU	P	257	7.455	12.109	-13.788	1.00	125.83		C
	ANISOU	3272	CD1	LEU	P	257	14393	15674	17744	-4003	3651	2792	C
	ATOM	3273	CD2	LEU	P	257	7.861	14.487	-13.125	1.00	131.86		C
	ANISOU	3273	CD2	LEU	P	257	15206	16112	18783	-4685	3323	2722	C
30	ATOM	3274	N	LEU	P	258	3.961	13.490	-9.619	1.00	87.87		N
	ANISOU	3274	N	LEU	P	258	10664	10685	12036	-4239	2072	1749	N
	ATOM	3275	CA	LEU	P	258	2.600	13.395	-9.107	1.00	83.75		C
	ANISOU	3275	CA	LEU	P	258	10548	10153	11119	-4083	1890	1479	C
	ATOM	3276	C	LEU	P	258	2.187	14.657	-8.352	1.00	89.10		C
35	ANISOU	3276	C	LEU	P	258	11470	10660	11724	-4309	1559	1292	C
	ATOM	3277	O	LEU	P	258	1.104	15.199	-8.579	1.00	87.97		O
	ANISOU	3277	O	LEU	P	258	11742	10389	11292	-4214	1564	1118	O
	ATOM	3278	CB	LEU	P	258	2.460	12.175	-8.196	1.00	74.89		C
	ANISOU	3278	CB	LEU	P	258	9305	9260	9891	-3929	1714	1410	C
40	ATOM	3279	CG	LEU	P	258	2.444	10.811	-8.889	1.00	75.87		C
	ANISOU	3279	CG	LEU	P	258	9350	9528	9951	-3633	2030	1520	C
	ATOM	3280	CD1	LEU	P	258	2.583	9.674	-7.882	1.00	52.50		C
	ANISOU	3280	CD1	LEU	P	258	6237	6763	6946	-3528	1837	1495	C
	ATOM	3281	CD2	LEU	P	258	1.177	10.648	-9.714	1.00	71.13		C
45	ANISOU	3281	CD2	LEU	P	258	9153	8864	9008	-3407	2256	1381	C
	ATOM	3282	N	SER	P	259	3.050	15.118	-7.452	1.00	84.33		N
	ANISOU	3282	N	SER	P	259	10608	10060	11373	-4593	1263	1317	N
	ATOM	3283	CA	SER	P	259	2.753	16.304	-6.662	1.00	78.21		C
	ANISOU	3283	CA	SER	P	259	10063	9112	10542	-4822	925	1137	C
50	ATOM	3284	C	SER	P	259	2.572	17.520	-7.562	1.00	81.91		C

	ANISOU	3284	C	SER	P	259	10820	9288	11015	-4936	1112	1161	C
	ATOM	3285	O	SER	P	259	1.932	18.496	-7.175	1.00	92.12		O
	ANISOU	3285	O	SER	P	259	12470	10393	12137	-5016	910	979	O
	ATOM	3286	CB	SER	P	259	3.845	16.550	-5.623	1.00	86.96		C
5	ANISOU	3286	CB	SER	P	259	10806	10283	11954	-5123	583	1172	C
	ATOM	3287	OG	SER	P	259	5.128	16.495	-6.216	1.00	111.78		O
	ANISOU	3287	OG	SER	P	259	13511	13467	15494	-5284	782	1427	O
	ATOM	3288	N	LEU	P	260	3.135	17.452	-8.765	1.00	80.16		N
	ANISOU	3288	N	LEU	P	260	10467	9019	10971	-4923	1507	1389	N
10	ATOM	3289	CA	LEU	P	260	2.966	18.509	-9.754	1.00	81.64		C
	ANISOU	3289	CA	LEU	P	260	10963	8919	11138	-4989	1747	1442	C
	ATOM	3290	C	LEU	P	260	1.590	18.417	-10.392	1.00	82.16		C
	ANISOU	3290	C	LEU	P	260	11501	8937	10780	-4636	1897	1295	C
	ATOM	3291	O	LEU	P	260	0.872	19.411	-10.499	1.00	82.48		O
15	ANISOU	3291	O	LEU	P	260	11967	8754	10617	-4633	1836	1160	O
	ATOM	3292	CB	LEU	P	260	4.035	18.407	-10.840	1.00	88.21		C
	ANISOU	3292	CB	LEU	P	260	11497	9732	12286	-5076	2148	1745	C
	ATOM	3293	CG	LEU	P	260	5.418	18.965	-10.516	1.00	96.65		C
	ANISOU	3293	CG	LEU	P	260	12153	10763	13805	-5501	2072	1901	C
20	ATOM	3294	CD1	LEU	P	260	6.324	18.821	-11.725	1.00	101.88		C
	ANISOU	3294	CD1	LEU	P	260	12556	11421	14733	-5537	2540	2197	C
	ATOM	3295	CD2	LEU	P	260	5.316	20.420	-10.084	1.00	95.37		C
	ANISOU	3295	CD2	LEU	P	260	12284	10293	13658	-5824	1844	1782	C
	ATOM	3296	N	ILE	P	261	1.234	17.212	-10.823	1.00	88.03		N
25	ANISOU	3296	N	ILE	P	261	12167	9892	11387	-4332	2090	1316	N
	ATOM	3297	CA	ILE	P	261	-0.060	16.970	-11.445	1.00	90.12		C
	ANISOU	3297	CA	ILE	P	261	12819	10164	11258	-3990	2230	1161	C
	ATOM	3298	C	ILE	P	261	-1.191	17.335	-10.489	1.00	88.77		C
	ANISOU	3298	C	ILE	P	261	12956	9997	10776	-3935	1882	852	C
30	ATOM	3299	O	ILE	P	261	-2.217	17.877	-10.901	1.00	91.98		O
	ANISOU	3299	O	ILE	P	261	13765	10301	10884	-3760	1914	694	O
	ATOM	3300	CB	ILE	P	261	-0.206	15.502	-11.893	1.00	84.69		C
	ANISOU	3300	CB	ILE	P	261	11965	9720	10492	-3712	2451	1212	C
	ATOM	3301	CG1	ILE	P	261	0.855	15.159	-12.941	1.00	83.55		C
35	ANISOU	3301	CG1	ILE	P	261	11551	9571	10622	-3717	2830	1515	C
	ATOM	3302	CG2	ILE	P	261	-1.598	15.246	-12.447	1.00	77.49		C
	ANISOU	3302	CG2	ILE	P	261	11434	8840	9169	-3386	2552	1011	C
	ATOM	3303	CD1	ILE	P	261	0.798	13.729	-13.426	1.00	52.78		C
	ANISOU	3303	CD1	ILE	P	261	7511	5886	6658	-3439	3060	1577	C
40	ATOM	3304	N	ASN	P	262	-0.990	17.044	-9.209	1.00	83.99		N
	ANISOU	3304	N	ASN	P	262	12161	9519	10233	-4069	1551	766	N
	ATOM	3305	CA	ASN	P	262	-1.986	17.356	-8.193	1.00	82.83		C
	ANISOU	3305	CA	ASN	P	262	12280	9392	9801	-4027	1218	479	C
	ATOM	3306	C	ASN	P	262	-2.127	18.861	-7.981	1.00	87.67		C
45	ANISOU	3306	C	ASN	P	262	13199	9728	10385	-4198	1038	385	C
	ATOM	3307	O	ASN	P	262	-3.234	19.372	-7.812	1.00	83.35		O
	ANISOU	3307	O	ASN	P	262	13029	9126	9514	-4047	926	157	O
	ATOM	3308	CB	ASN	P	262	-1.644	16.661	-6.874	1.00	78.11		C
	ANISOU	3308	CB	ASN	P	262	11416	8988	9275	-4124	919	432	C
50	ATOM	3309	CG	ASN	P	262	-2.826	16.596	-5.930	1.00	77.84		C

	ANISOU	3309	CG	ASN	P	262	11645	9043	8886	-3998	662	140	C
	ATOM	3310	OD1	ASN	P	262	-3.953	16.917	-6.307	1.00	76.30		O
	ANISOU	3310	OD1	ASN	P	262	11788	8814	8388	-3808	726	-36	O
	ATOM	3311	ND2	ASN	P	262	-2.576	16.177	-4.698	1.00	81.46		N
5	ANISOU	3311	ND2	ASN	P	262	11948	9632	9373	-4089	373	85	N
	ATOM	3312	N	ASP	P	263	-0.999	19.566	-8.004	1.00	97.04		N
	ANISOU	3312	N	ASP	P	263	14221	10740	11909	-4514	1020	558	N
	ATOM	3313	CA	ASP	P	263	-0.983	21.013	-7.804	1.00	100.49		C
	ANISOU	3313	CA	ASP	P	263	14953	10871	12357	-4727	858	490	C
10	ATOM	3314	C	ASP	P	263	-1.686	21.770	-8.934	1.00	102.79		C
	ANISOU	3314	C	ASP	P	263	15690	10934	12433	-4545	1113	477	C
	ATOM	3315	O	ASP	P	263	-2.186	22.876	-8.727	1.00	106.47		O
	ANISOU	3315	O	ASP	P	263	16552	11164	12737	-4576	960	335	O
	ATOM	3316	CB	ASP	P	263	0.455	21.520	-7.650	1.00	102.30		C
15	ANISOU	3316	CB	ASP	P	263	14866	10975	13029	-5142	815	689	C
	ATOM	3317	CG	ASP	P	263	1.092	21.093	-6.337	1.00	108.70		C
	ANISOU	3317	CG	ASP	P	263	15314	11971	14017	-5337	452	646	C
	ATOM	3318	OD1	ASP	P	263	0.402	20.464	-5.506	1.00	114.86		O
	ANISOU	3318	OD1	ASP	P	263	16137	12945	14560	-5155	235	467	O
20	ATOM	3319	OD2	ASP	P	263	2.291	21.390	-6.141	1.00	105.41		O
	ANISOU	3319	OD2	ASP	P	263	14566	11512	13972	-5673	387	789	O
	ATOM	3320	N	MET	P	264	-1.719	21.172	-10.122	1.00	99.31		N
	ANISOU	3320	N	MET	P	264	15204	10558	11973	-4337	1494	624	N
	ATOM	3321	CA	MET	P	264	-2.353	21.793	-11.286	1.00	101.70		C
25	ANISOU	3321	CA	MET	P	264	15924	10663	12055	-4121	1756	628	C
	ATOM	3322	C	MET	P	264	-3.853	21.998	-11.096	1.00	101.82		C
	ANISOU	3322	C	MET	P	264	16354	10719	11614	-3798	1606	325	C
	ATOM	3323	O	MET	P	264	-4.487	21.285	-10.318	1.00	107.68		O
	ANISOU	3323	O	MET	P	264	17006	11714	12195	-3678	1411	137	O
30	ATOM	3324	CB	MET	P	264	-2.113	20.952	-12.540	1.00	102.27		C
	ANISOU	3324	CB	MET	P	264	15845	10841	12173	-3929	2178	829	C
	ATOM	3325	CG	MET	P	264	-0.678	20.946	-13.026	1.00	108.04		C
	ANISOU	3325	CG	MET	P	264	16229	11494	13326	-4205	2416	1147	C
	ATOM	3326	SD	MET	P	264	-0.528	20.158	-14.640	1.00	100.61		S
35	ANISOU	3326	SD	MET	P	264	15237	10623	12367	-3926	2946	1371	S
	ATOM	3327	CE	MET	P	264	-1.176	18.532	-14.285	1.00	169.29		C
	ANISOU	3327	CE	MET	P	264	23718	19718	20885	-3627	2880	1227	C
	ATOM	3328	N	PRO	P	265	-4.429	22.971	-11.821	1.00	98.01		N
	ANISOU	3328	N	PRO	P	265	16332	9993	10916	-3647	1708	279	N
40	ATOM	3329	CA	PRO	P	265	-5.855	23.274	-11.736	1.00	96.48		C
	ANISOU	3329	CA	PRO	P	265	16538	9840	10281	-3308	1576	-11	C
	ATOM	3330	C	PRO	P	265	-6.600	22.538	-12.832	1.00	99.88		C
	ANISOU	3330	C	PRO	P	265	17031	10449	10470	-2920	1854	-35	C
	ATOM	3331	O	PRO	P	265	-6.903	23.135	-13.862	1.00	107.99		O
45	ANISOU	3331	O	PRO	P	265	18396	11298	11338	-2724	2054	4	O
	ATOM	3332	CB	PRO	P	265	-5.906	24.778	-12.034	1.00	96.43		C
	ANISOU	3332	CB	PRO	P	265	17008	9434	10197	-3353	1558	-9	C
	ATOM	3333	CG	PRO	P	265	-4.471	25.182	-12.423	1.00	101.02		C
	ANISOU	3333	CG	PRO	P	265	17421	9763	11198	-3739	1743	308	C
50	ATOM	3334	CD	PRO	P	265	-3.756	23.909	-12.728	1.00	99.21		C

	ANISOU	3334	CD	PRO	P	265	16674	9802	11219	-3787	1950	497	C
	ATOM	3335	N	ILE	P	266	-6.871	21.257	-12.630	1.00	92.24		N
	ANISOU	3335	N	ILE	P	266	15761	9817	9470	-2812	1869	-98	N
	ATOM	3336	CA	ILE	P	266	-7.583	20.481	-13.634	1.00	85.03		C
5	ANISOU	3336	CA	ILE	P	266	14890	9088	8331	-2466	2115	-145	C
	ATOM	3337	C	ILE	P	266	-8.590	19.538	-12.993	1.00	86.91		C
	ANISOU	3337	C	ILE	P	266	15019	9666	8336	-2297	1962	-412	C
	ATOM	3338	O	ILE	P	266	-8.549	19.293	-11.785	1.00	82.19		O
	ANISOU	3338	O	ILE	P	266	14252	9177	7798	-2465	1709	-505	O
10	ATOM	3339	CB	ILE	P	266	-6.617	19.675	-14.518	1.00	78.06		C
	ANISOU	3339	CB	ILE	P	266	13719	8232	7709	-2518	2455	147	C
	ATOM	3340	CG1	ILE	P	266	-5.546	19.003	-13.661	1.00	80.88		C
	ANISOU	3340	CG1	ILE	P	266	13614	8689	8428	-2834	2367	295	C
	ATOM	3341	CG2	ILE	P	266	-5.970	20.572	-15.555	1.00	79.39		C
15	ANISOU	3341	CG2	ILE	P	266	14092	8085	7986	-2562	2711	381	C
	ATOM	3342	CD1	ILE	P	266	-4.627	18.100	-14.447	1.00	81.89		C
	ANISOU	3342	CD1	ILE	P	266	13428	8887	8799	-2850	2692	565	C
	ATOM	3343	N	THR	P	267	-9.499	19.019	-13.812	1.00	80.38		N
	ANISOU	3343	N	THR	P	267	14300	9006	7236	-1969	2120	-540	N
20	ATOM	3344	CA	THR	P	267	-10.486	18.064	-13.339	1.00	74.40		C
	ANISOU	3344	CA	THR	P	267	13432	8579	6258	-1823	2026	-796	C
	ATOM	3345	C	THR	P	267	-9.782	16.825	-12.802	1.00	74.01		C
	ANISOU	3345	C	THR	P	267	12977	8689	6456	-2022	2059	-671	C
	ATOM	3346	O	THR	P	267	-8.683	16.485	-13.251	1.00	67.48		O
25	ANISOU	3346	O	THR	P	267	11949	7775	5917	-2152	2249	-391	O
	ATOM	3347	CB	THR	P	267	-11.443	17.644	-14.465	1.00	73.99		C
	ANISOU	3347	CB	THR	P	267	13523	8679	5910	-1462	2221	-933	C
	ATOM	3348	OG1	THR	P	267	-10.701	17.004	-15.514	1.00	60.56		O
	ANISOU	3348	OG1	THR	P	267	11695	6938	4375	-1444	2539	-686	O
30	ATOM	3349	CG2	THR	P	267	-12.174	18.855	-15.024	1.00	77.11		C
	ANISOU	3349	CG2	THR	P	267	14340	8931	6028	-1209	2179	-1063	C
	ATOM	3350	N	ASN	P	268	-10.417	16.159	-11.840	1.00	59.47		N
	ANISOU	3350	N	ASN	P	268	11025	7080	4491	-2033	1884	-876	N
	ATOM	3351	CA	ASN	P	268	-9.888	14.920	-11.286	1.00	48.08		C
35	ANISOU	3351	CA	ASN	P	268	9252	5793	3225	-2179	1909	-783	C
	ATOM	3352	C	ASN	P	268	-9.596	13.891	-12.375	1.00	55.47		C
	ANISOU	3352	C	ASN	P	268	10056	6797	4225	-2062	2237	-637	C
	ATOM	3353	O	ASN	P	268	-8.630	13.133	-12.273	1.00	54.86		O
	ANISOU	3353	O	ASN	P	268	9713	6728	4405	-2194	2331	-421	O
40	ATOM	3354	CB	ASN	P	268	-10.855	14.328	-10.257	1.00	53.90		C
	ANISOU	3354	CB	ASN	P	268	9967	6772	3740	-2156	1726	-1056	C
	ATOM	3355	CG	ASN	P	268	-10.927	15.144	-8.978	1.00	58.38		C
	ANISOU	3355	CG	ASN	P	268	10612	7284	4285	-2303	1394	-1166	C
	ATOM	3356	OD1	ASN	P	268	-10.158	16.083	-8.779	1.00	75.62		O
45	ANISOU	3356	OD1	ASN	P	268	12839	9243	6652	-2460	1280	-1032	O
	ATOM	3357	ND2	ASN	P	268	-11.852	14.783	-8.101	1.00	47.61		N
	ANISOU	3357	ND2	ASN	P	268	9272	6124	2693	-2264	1246	-1417	N
	ATOM	3358	N	ASP	P	269	-10.431	13.863	-13.413	1.00	50.59		N
	ANISOU	3358	N	ASP	P	269	9628	6233	3359	-1795	2401	-762	N
50	ATOM	3359	CA	ASP	P	269	-10.226	12.943	-14.528	1.00	56.37		C

	ANISOU	3359	CA	ASP	P	269	10286	7018	4115	-1657	2711	-646	C
	ATOM	3360	C	ASP	P	269	-8.905	13.221	-15.234	1.00	59.80		C
	ANISOU	3360	C	ASP	P	269	10635	7235	4851	-1743	2918	-294	C
	ATOM	3361	O	ASP	P	269	-8.203	12.299	-15.639	1.00	62.89		O
5	ANISOU	3361	O	ASP	P	269	10824	7662	5411	-1754	3128	-108	O
	ATOM	3362	CB	ASP	P	269	-11.370	13.039	-15.538	1.00	71.42		C
	ANISOU	3362	CB	ASP	P	269	12391	8940	5804	-1348	2747	-844	C
	ATOM	3363	CG	ASP	P	269	-12.648	12.395	-15.041	1.00	84.67		C
	ANISOU	3363	CG	ASP	P	269	13937	10746	7487	-1286	2495	-1096	C
10	ATOM	3364	OD1	ASP	P	269	-12.604	11.690	-14.010	1.00	92.63		O
	ANISOU	3364	OD1	ASP	P	269	14755	11867	8574	-1464	2388	-1136	O
	ATOM	3365	OD2	ASP	P	269	-13.698	12.591	-15.688	1.00	83.85		O
	ANISOU	3365	OD2	ASP	P	269	13907	10648	7302	-1064	2423	-1224	O
	ATOM	3366	N	GLN	P	270	-8.573	14.497	-15.390	1.00	64.57		N
15	ANISOU	3366	N	GLN	P	270	11405	7611	5517	-1803	2873	-206	N
	ATOM	3367	CA	GLN	P	270	-7.335	14.872	-16.063	1.00	71.87		C
	ANISOU	3367	CA	GLN	P	270	12255	8322	6732	-1916	3088	124	C
	ATOM	3368	C	GLN	P	270	-6.111	14.487	-15.232	1.00	66.25		C
	ANISOU	3368	C	GLN	P	270	11165	7611	6395	-2220	3022	333	C
20	ATOM	3369	O	GLN	P	270	-5.112	14.014	-15.771	1.00	63.28		O
	ANISOU	3369	O	GLN	P	270	10569	7210	6264	-2269	3258	593	O
	ATOM	3370	CB	GLN	P	270	-7.334	16.364	-16.420	1.00	77.84		C
	ANISOU	3370	CB	GLN	P	270	13324	8807	7444	-1924	3063	156	C
	ATOM	3371	CG	GLN	P	270	-8.148	16.691	-17.673	1.00	80.33		C
25	ANISOU	3371	CG	GLN	P	270	13996	9079	7446	-1579	3248	70	C
	ATOM	3372	CD	GLN	P	270	-8.616	18.136	-17.723	1.00	83.13		C
	ANISOU	3372	CD	GLN	P	270	14748	9212	7627	-1516	3120	-21	C
	ATOM	3373	OE1	GLN	P	270	-8.413	18.901	-16.780	1.00	84.81		O
	ANISOU	3373	OE1	GLN	P	270	14984	9302	7938	-1737	2880	-48	O
30	ATOM	3374	NE2	GLN	P	270	-9.256	18.512	-18.824	1.00	79.56		N
	ANISOU	3374	NE2	GLN	P	270	14633	8701	6894	-1195	3272	-78	N
	ATOM	3375	N	LYS	P	271	-6.196	14.678	-13.918	1.00	60.48		N
	ANISOU	3375	N	LYS	P	271	10357	6924	5697	-2403	2697	213	N
	ATOM	3376	CA	LYS	P	271	-5.116	14.283	-13.018	1.00	55.55		C
35	ANISOU	3376	CA	LYS	P	271	9378	6334	5394	-2666	2580	374	C
	ATOM	3377	C	LYS	P	271	-4.934	12.773	-13.079	1.00	57.38		C
	ANISOU	3377	C	LYS	P	271	9365	6774	5663	-2571	2725	433	C
	ATOM	3378	O	LYS	P	271	-3.825	12.271	-13.244	1.00	62.74		O
	ANISOU	3378	O	LYS	P	271	9755	7458	6624	-2654	2863	681	O
40	ATOM	3379	CB	LYS	P	271	-5.428	14.700	-11.578	1.00	63.38		C
	ANISOU	3379	CB	LYS	P	271	10389	7354	6338	-2828	2186	190	C
	ATOM	3380	CG	LYS	P	271	-5.798	16.165	-11.399	1.00	71.40		C
	ANISOU	3380	CG	LYS	P	271	11709	8164	7255	-2891	2007	77	C
	ATOM	3381	CD	LYS	P	271	-5.976	16.508	-9.923	1.00	73.41		C
45	ANISOU	3381	CD	LYS	P	271	11962	8448	7481	-3058	1618	-89	C
	ATOM	3382	CE	LYS	P	271	-6.348	17.972	-9.729	1.00	79.73		C
	ANISOU	3382	CE	LYS	P	271	13100	9024	8170	-3106	1436	-210	C
	ATOM	3383	NZ	LYS	P	271	-6.484	18.328	-8.289	1.00	81.22		N
	ANISOU	3383	NZ	LYS	P	271	13306	9231	8324	-3263	1055	-373	N
50	ATOM	3384	N	LYS	P	272	-6.045	12.061	-12.936	1.00	59.97		N

	ANISOU	3384	N	LYS	P	272	9815	7272	5698	-2396	2694	196	N
	ATOM	3385	CA	LYS	P	272	-6.067	10.609	-13.005	1.00	64.48		C
	ANISOU	3385	CA	LYS	P	272	10236	8018	6247	-2295	2835	211	C
	ATOM	3386	C	LYS	P	272	-5.540	10.125	-14.347	1.00	65.65		C
5	ANISOU	3386	C	LYS	P	272	10337	8126	6481	-2144	3203	414	C
	ATOM	3387	O	LYS	P	272	-4.759	9.179	-14.420	1.00	73.32		O
	ANISOU	3387	O	LYS	P	272	11076	9153	7629	-2143	3344	594	O
	ATOM	3388	CB	LYS	P	272	-7.498	10.111	-12.791	1.00	71.71		C
	ANISOU	3388	CB	LYS	P	272	11343	9099	6804	-2148	2769	-110	C
10	ATOM	3389	CG	LYS	P	272	-7.654	8.607	-12.842	1.00	69.02		C
	ANISOU	3389	CG	LYS	P	272	10904	8914	6405	-2062	2916	-129	C
	ATOM	3390	CD	LYS	P	272	-9.070	8.192	-12.486	1.00	63.72		C
	ANISOU	3390	CD	LYS	P	272	10380	8374	5458	-1978	2784	-475	C
	ATOM	3391	CE	LYS	P	272	-9.260	6.706	-12.712	1.00	72.38		C
15	ANISOU	3391	CE	LYS	P	272	11382	9479	6638	-1889	2842	-522	C
	ATOM	3392	NZ	LYS	P	272	-10.652	6.276	-12.439	1.00	78.98		N
	ANISOU	3392	NZ	LYS	P	272	12280	10361	7368	-1861	2666	-820	N
	ATOM	3393	N	LEU	P	273	-5.982	10.782	-15.412	1.00	72.34		N
	ANISOU	3393	N	LEU	P	273	11427	8876	7183	-1992	3357	382	N
20	ATOM	3394	CA	LEU	P	273	-5.528	10.465	-16.757	1.00	79.75		C
	ANISOU	3394	CA	LEU	P	273	12374	9757	8168	-1828	3715	570	C
	ATOM	3395	C	LEU	P	273	-4.010	10.595	-16.852	1.00	79.24		C
	ANISOU	3395	C	LEU	P	273	12026	9589	8493	-1996	3846	913	C
	ATOM	3396	O	LEU	P	273	-3.330	9.710	-17.371	1.00	75.30		O
25	ANISOU	3396	O	LEU	P	273	11345	9139	8126	-1917	4088	1097	O
	ATOM	3397	CB	LEU	P	273	-6.200	11.395	-17.767	1.00	94.04		C
	ANISOU	3397	CB	LEU	P	273	14528	11450	9753	-1649	3815	487	C
	ATOM	3398	CG	LEU	P	273	-5.770	11.277	-19.227	1.00	113.16		C
	ANISOU	3398	CG	LEU	P	273	17028	13782	12187	-1462	4190	682	C
30	ATOM	3399	CD1	LEU	P	273	-6.239	9.956	-19.811	1.00	112.29		C
	ANISOU	3399	CD1	LEU	P	273	16900	13764	12001	-1203	4252	540	C
	ATOM	3400	CD2	LEU	P	273	-6.307	12.451	-20.037	1.00	120.74		C
	ANISOU	3400	CD2	LEU	P	273	18353	14581	12941	-1316	4239	626	C
	ATOM	3401	N	MET	P	274	-3.485	11.700	-16.334	1.00	79.04		N
35	ANISOU	3401	N	MET	P	274	11957	9425	8650	-2231	3683	989	N
	ATOM	3402	CA	MET	P	274	-2.059	11.987	-16.418	1.00	74.78		C
	ANISOU	3402	CA	MET	P	274	11127	8792	8495	-2431	3795	1296	C
	ATOM	3403	C	MET	P	274	-1.203	11.155	-15.457	1.00	65.60		C
	ANISOU	3403	C	MET	P	274	9560	7778	7586	-2574	3665	1398	C
40	ATOM	3404	O	MET	P	274	-0.024	10.928	-15.710	1.00	74.49		O
	ANISOU	3404	O	MET	P	274	10378	8910	9013	-2651	3830	1656	O
	ATOM	3405	CB	MET	P	274	-1.801	13.479	-16.198	1.00	74.94		C
	ANISOU	3405	CB	MET	P	274	11256	8595	8623	-2663	3660	1326	C
	ATOM	3406	CG	MET	P	274	-2.416	14.374	-17.260	1.00	74.28		C
45	ANISOU	3406	CG	MET	P	274	11579	8324	8320	-2509	3832	1289	C
	ATOM	3407	SD	MET	P	274	-2.167	16.114	-16.892	1.00	88.02		S
	ANISOU	3407	SD	MET	P	274	13510	9770	10163	-2789	3656	1307	S
	ATOM	3408	CE	MET	P	274	-0.385	16.157	-16.707	1.00	90.50		C
	ANISOU	3408	CE	MET	P	274	13341	10039	11004	-3149	3763	1647	C
50	ATOM	3409	N	SER	P	275	-1.786	10.698	-14.357	1.00	58.19		N

	ANISOU	3409	N	SER	P	275	8622	6967	6520	-2596	3377	1198	N
	ATOM	3410	CA	SER	P	275	-1.010	9.947	-13.374	1.00	64.47		C
	ANISOU	3410	CA	SER	P	275	9078	7895	7523	-2708	3223	1286	C
	ATOM	3411	C	SER	P	275	-0.910	8.458	-13.721	1.00	74.91		C
5	ANISOU	3411	C	SER	P	275	10295	9363	8806	-2489	3436	1353	C
	ATOM	3412	O	SER	P	275	0.101	7.817	-13.440	1.00	72.80		O
	ANISOU	3412	O	SER	P	275	9708	9176	8775	-2514	3463	1541	O
	ATOM	3413	CB	SER	P	275	-1.561	10.161	-11.957	1.00	60.08		C
	ANISOU	3413	CB	SER	P	275	8580	7392	6856	-2841	2817	1068	C
10	ATOM	3414	OG	SER	P	275	-2.883	9.673	-11.829	1.00	70.03		O
	ANISOU	3414	OG	SER	P	275	10112	8736	7759	-2675	2778	807	O
	ATOM	3415	N	ASN	P	276	-1.963	7.919	-14.334	1.00	83.21		N
	ANISOU	3415	N	ASN	P	276	11620	10445	9551	-2269	3580	1189	N
	ATOM	3416	CA	ASN	P	276	-1.973	6.536	-14.813	1.00	85.57		C
15	ANISOU	3416	CA	ASN	P	276	11892	10842	9777	-2054	3812	1230	C
	ATOM	3417	C	ASN	P	276	-1.096	6.359	-16.042	1.00	96.38		C
	ANISOU	3417	C	ASN	P	276	13146	12159	11314	-1929	4178	1491	C
	ATOM	3418	O	ASN	P	276	-0.144	5.567	-16.049	1.00	92.15		O
	ANISOU	3418	O	ASN	P	276	12348	11689	10978	-1880	4301	1694	O
20	ATOM	3419	CB	ASN	P	276	-3.381	6.118	-15.237	1.00	75.58		C
	ANISOU	3419	CB	ASN	P	276	10961	9605	8152	-1863	3840	940	C
	ATOM	3420	CG	ASN	P	276	-4.332	5.986	-14.084	1.00	72.43		C
	ANISOU	3420	CG	ASN	P	276	10672	9306	7544	-1958	3565	685	C
	ATOM	3421	OD1	ASN	P	276	-3.988	5.453	-13.032	1.00	74.52		O
25	ANISOU	3421	OD1	ASN	P	276	10786	9642	7885	-2060	3406	716	O
	ATOM	3422	ND2	ASN	P	276	-5.554	6.460	-14.280	1.00	76.67		N
	ANISOU	3422	ND2	ASN	P	276	11472	9834	7825	-1890	3468	404	N
	ATOM	3423	N	ASN	P	277	-1.460	7.108	-17.082	1.00	109.71		N
	ANISOU	3423	N	ASN	P	277	15053	13728	12903	-1847	4339	1470	N
30	ATOM	3424	CA	ASN	P	277	-0.906	6.972	-18.430	1.00	126.53		C
	ANISOU	3424	CA	ASN	P	277	17182	15771	15122	-1646	4669	1637	C
	ATOM	3425	C	ASN	P	277	0.332	6.120	-18.713	1.00	157.12		C
	ANISOU	3425	C	ASN	P	277	20737	19702	19260	-1569	4888	1908	C
	ATOM	3426	O	ASN	P	277	0.450	5.458	-19.705	1.00	165.30		O
35	ANISOU	3426	O	ASN	P	277	21836	20704	20266	-1305	5122	1951	O
	ATOM	3427	CB	ASN	P	277	-0.925	8.285	-19.231	1.00	126.96		C
	ANISOU	3427	CB	ASN	P	277	17414	15664	15160	-1697	4809	1710	C
	ATOM	3428	CG	ASN	P	277	0.019	9.368	-18.832	1.00	135.53		C
	ANISOU	3428	CG	ASN	P	277	18295	16661	16538	-2011	4771	1919	C
40	ATOM	3429	OD1	ASN	P	277	-0.273	10.174	-17.959	1.00	144.85		O
	ANISOU	3429	OD1	ASN	P	277	19523	17793	17721	-2213	4463	1791	O
	ATOM	3430	ND2	ASN	P	277	0.991	9.583	-19.696	1.00	136.64		N
	ANISOU	3430	ND2	ASN	P	277	18299	16728	16891	-2014	5086	2191	N
	END												

45

The Ramachandran plot shows 95.6% of all residues in favored regions and 99.3% of all residues in allowed regions. All structural images were created using PyMol (Delano Scientific, <http://www.pymol.org>).

f. Cloning, expression and purification of RSV F₀ Fd, also referred to as RSV F₀ Fd. A codon-optimized DNA fragment encoding amino acid residues 1-513 of the RSV F protein strain A2 with mutations R106Q, R109S, R135S and R136S was synthesized by GeneArt with a 3' fragment encoding the residues SAIGGYIPEA PRDGQAYVRK
 5 DGEWVLLSTF LGGIEGRHHH HHH, also denoted herein as SEQ ID NO:15). This gene was cloned into a variant of the pHLSec mammalian expression vector (Aricescu AR, et. al., 2006, Acta Crystallogr. D Biol. Crystallogr. 62, 1243-1250) and protein was expressed using the 293Freestyle expression system as described above for the motavizumab IgG expression. Protein was purified from the supernatant using Ni-NTA
 10 resin (Qiagen, Venlo, the Netherlands) followed by gel filtration on a SUPEROSE™6 column with a running buffer of 2 mM Tris-HCl pH 7.5, 150 mM NaCl. The peak corresponding to a trimer was pooled, concentrated and stored at 4 °C.

g. RSV F₀ Fd cross-linking and immunoprecipitation. RSV F₀ Fd (5 µg, 0.2 µM) in PBS was incubated with glutaraldehyde at concentrations of 0, 1, and 10 mM for 5 min
 15 at room temperature. Glycine was added to a final concentration of 100 mM to quench the reaction. The cross-linked and control proteins were incubated with 5 µg of motavizumab IgG for 30 min at room temperature. 20 µl of a Protein A agarose slurry (Pierce) was added and incubated for 90 min at room temperature. The resin was centrifuged, washed with PBS containing Tween 20, and then boiled in reducing SDS-PAGE loading buffer.

Example 2: Three-dimensional structure of RSV F protein and 101F antibody

This Example describes the crystallization and determination of the 3-dimensional structure of a complex between the 101F antibody and the 15-residue RSV fusion (F) peptide corresponding to amino acids 422-436 of the F protein (i.e., STASNKNRGI
 25 IKTFS, also denoted herein as SEQ ID NO:3) that includes the binding domain of 101F.

Recombinant 101F IgG and a peptide comprising the 101F binding domain were combined and the resultant complex submitted to crystallization and analysis as follows.

a. Cloning, expression and purification of 101F IgG: Two DNA fragments encoding the variable heavy and light chains of 101F with signal sequences were
 30 synthesized by GeneArt and cloned in-frame into mammalian expression vectors containing mouse IgG1 heavy and light constant domains, respectively. The amino acid sequence of the variable heavy chain of 101F is as follows: QVTLKESGPG
 ILQPSQTLSTL TCSFSGFSLT TSGMGVSWIR QPSGKGLEWL AHYWDGDDKR

YNPSLKSRILT ISKDTSRNQV FLKITSVDTA DTATYYCARL YGFTYGFAYW
GQGTLLTVSA, also denoted herein as SEQ ID NO:7. The amino acid sequence of the
variable light chain of 101F is as follows: DIVLTQSPAS LAVSLGQRAT

IFCRASQSVD YNGISYMHWF QQKPGQPPKL LIYAASNPE GIPARFTGSG

5 SGTDFTLNIH PVEEEDAATY YCQQIIEDPW TFGGGTKLEI K, also denoted herein as
SEQ ID NO:8.

Both vectors were co-transfected at a 1:1 ratio into HEK293F cells (Invitrogen) in
serum-free 293Freestyle medium (Invitrogen). After 3 hours, valproic acid (Sigma) was
added to 4 mM final concentration. Expression lasted for five days at 37°C with 10% CO₂
10 and shaking at 125 rpm in disposable flasks. The supernatant was collected, filtered, and
passed over 5 ml of Protein G agarose resin (Pierce). After washing with several column
volumes of phosphate-buffered saline (PBS), the resin was eluted with 15 ml of IgG
Elution Buffer (Pierce) and immediately neutralized with 1 M Tris pH 8.0. The eluted
protein was dialyzed against PBS and stored at 4°C.

15 b. Digestion and purification of 101F Fab fragments. The purified 101F IgG
protein was reduced with 100 mM dithiothreitol at 37°C for 1 hour and then alkylated with
2 mM iodoacetamide for 48 hours at 4 °C. Ten ml of reduced and alkylated IgG in PBS at
1.5 mg/ml was combined with .275 ODs of Ficin (Sigma), 20 mM L-cysteine, 1 mM
EDTA and incubated at 37 °C for 1 hour. The reaction was quenched by the addition of
20 iodoacetamide to 40 mM final concentration. To remove the Fc fragments from the Fab
fragments, the quenched reaction was passed over 1 ml of Protein A agarose. The Fab-
containing flow through was further purified over an S200 gel filtration column and
concentrated aliquots were stored frozen at -80 °C.

c. Protein crystallization and data collection. A peptide with the sequence
25 STASNKNRGI IKTFS (SEQ ID NO:3), corresponding to the originally identified 101F
epitope of CTASNKNRGI IKTFS (residues 422-436 of RSV F protein), also denoted
herein as SEQ ID NO:10, was synthesized by American Peptide with an acetylated N-
terminus and an amidated C-terminus. A five-fold molar excess of peptide was incubated
with 101F Fab at 22 °C for 1.5 hours and then concentrated to give an 8.3 mg/ml solution
30 of Fab/peptide complex. Crystals were grown by the vapor diffusion method in sitting
drops at 20 °C by mixing 1 µl of protein complex with 1 µl of reservoir solution (15%
(w/v) PEG 4000, 0.2 M lithium sulfate, 0.1 M Tris pH 8.5). These crystals were flash
frozen in liquid nitrogen in 20% (w/v) PEG 4000, 0.2M lithium sulfate, 0.1M Tris pH 8.5

and 15% (v/v) 2R,3R-butanediol. Data were collected at a wavelength of 0.82656 Å at the SER-CAT beamline ID-22 (Advanced Photon Source, Argonne National Laboratory).

The structure of the 101F/peptide complex was determined and a model built and refined using a method similar to that described in Example 1. Final data collection and

5 refinement statistics are presented in Table 4.

The atomic coordinates for the complex between the 15-residue F peptide and 101F antibody are indicated in PDB acc code 3O41. The atomic coordinates for the peptide portion of the complex are indicated below in Table 7.

10 Table 7. Atomic coordinates of the 101F binding peptide

15	ATOM	6696	O	LYS	P 427	-17.126	3.607	14.869	1.00	82.36	O	
	ANISOU	6696	O	LYS	P 427	11025	9697	10570	618	188	376	O
	ATOM	6697	N	LYS	P 427	-17.615	3.567	18.200	1.00	90.82	N	
	ANISOU	6697	N	LYS	P 427	12312	10602	11594	727	198	440	N
	ATOM	6698	CA	LYS	P 427	-18.312	3.323	16.942	1.00	89.25	C	
	ANISOU	6698	CA	LYS	P 427	12014	10493	11404	684	241	396	C
20	ATOM	6699	C	LYS	P 427	-17.352	2.880	15.838	1.00	83.29	C	
	ANISOU	6699	C	LYS	P 427	11189	9761	10696	633	229	378	C
	ATOM	6700	CB	LYS	P 427	-19.092	4.566	16.507	1.00	92.06	C	
	ANISOU	6700	CB	LYS	P 427	12357	10914	11709	704	213	400	C
	ATOM	6701	CG	LYS	P 427	-18.301	5.861	16.568	1.00	92.91	C	
	ANISOU	6701	CG	LYS	P 427	12507	11004	11790	733	117	442	C
25	ATOM	6702	CD	LYS	P 427	-19.182	7.057	16.249	1.00	93.83	C	
	ANISOU	6702	CD	LYS	P 427	12615	11181	11854	756	94	446	C
	ATOM	6703	CE	LYS	P 427	-18.427	8.362	16.431	1.00	95.87	C	
	ANISOU	6703	CE	LYS	P 427	12924	11417	12084	790	-2	490	C
	ATOM	6704	NZ	LYS	P 427	-19.183	9.529	15.890	1.00	97.27	N	
	ANISOU	6704	NZ	LYS	P 427	13079	11662	12216	804	-30	491	N
30	ATOM	6705	O	ASN	P 428	-17.432	0.121	13.533	1.00	63.07	O	
	ANISOU	6705	O	ASN	P 428	8415	7298	8250	494	373	266	O
	ATOM	6706	N	ASN	P 428	-16.796	1.681	15.999	1.00	77.41	N	
	ANISOU	6706	N	ASN	P 428	10437	8975	10000	607	264	364	N
	ATOM	6707	CA	ASN	P 428	-15.846	1.113	15.044	1.00	72.01	C	
	ANISOU	6707	CA	ASN	P 428	9689	8305	9366	558	258	347	C
35	ATOM	6708	C	ASN	P 428	-16.459	0.867	13.665	1.00	62.88	C	
	ANISOU	6708	C	ASN	P 428	8429	7243	8221	511	299	301	C
	ATOM	6709	CB	ASN	P 428	-15.262	-0.194	15.597	1.00	75.94	C	
	ANISOU	6709	CB	ASN	P 428	10204	8738	9913	541	296	340	C
	ATOM	6710	CG	ASN	P 428	-14.397	-0.927	14.583	1.00	78.67	C	
	ANISOU	6710	CG	ASN	P 428	10478	9101	10312	488	302	315	C
40	ATOM	6711	OD1	ASN	P 428	-13.646	-0.310	13.824	1.00	78.53	O	
	ANISOU	6711	OD1	ASN	P 428	10433	9107	10298	475	246	324	O
	ATOM	6712	ND2	ASN	P 428	-14.493	-2.255	14.574	1.00	79.70	N	
	ANISOU	6712	ND2	ASN	P 428	10577	9219	10484	456	368	283	N

	ATOM	6713	O	ARG	P	429	-16.017	-0.008	9.350	1.00	42.31		O
	ANISOU	6713	O	ARG	P	429	5527	4851	5699	350	340	193	O
	ATOM	6714	N	ARG	P	429	-15.878	1.492	12.642	1.00	51.34		N
	ANISOU	6714	N	ARG	P	429	6920	5825	6761	490	250	301	N
5	ATOM	6715	CA	ARG	P	429	-16.374	1.375	11.272	1.00	46.50		C
	ANISOU	6715	CA	ARG	P	429	6208	5304	6157	445	280	260	C
	ATOM	6716	C	ARG	P	429	-15.659	0.274	10.490	1.00	43.56		C
	ANISOU	6716	C	ARG	P	429	5770	4938	5843	392	309	230	C
	ATOM	6717	CB	ARG	P	429	-16.163	2.690	10.515	1.00	44.81		C
10	ANISOU	6717	CB	ARG	P	429	5973	5139	5912	450	211	277	C
	ATOM	6718	CG	ARG	P	429	-17.099	3.817	10.872	1.00	45.89		C
	ANISOU	6718	CG	ARG	P	429	6145	5300	5990	492	190	294	C
	ATOM	6719	CD	ARG	P	429	-18.397	3.691	10.094	1.00	45.47		C
	ANISOU	6719	CD	ARG	P	429	6021	5331	5924	469	249	254	C
15	ATOM	6720	NE	ARG	P	429	-19.454	3.112	10.897	1.00	43.13		N
	ANISOU	6720	NE	ARG	P	429	5752	5019	5617	488	315	242	N
	ATOM	6721	CZ	ARG	P	429	-20.614	2.677	10.413	1.00	45.12		C
	ANISOU	6721	CZ	ARG	P	429	5947	5330	5866	467	382	204	C
	ATOM	6722	NH1	ARG	P	429	-20.876	2.723	9.114	1.00	37.74		N
20	ANISOU	6722	NH1	ARG	P	429	4925	4475	4939	426	393	173	N
	ATOM	6723	NH2	ARG	P	429	-21.514	2.173	11.239	1.00	49.71		N
	ANISOU	6723	NH2	ARG	P	429	6560	5890	6437	487	439	197	N
	ATOM	6724	O	GLY	P	430	-12.500	0.641	9.683	1.00	33.44		O
	ANISOU	6724	O	GLY	P	430	4482	3601	4623	360	174	272	O
25	ATOM	6725	N	GLY	P	430	-14.632	-0.322	11.084	1.00	41.59		N
	ANISOU	6725	N	GLY	P	430	5560	4614	5628	392	296	246	N
	ATOM	6726	CA	GLY	P	430	-13.790	-1.264	10.361	1.00	40.69		C
	ANISOU	6726	CA	GLY	P	430	5390	4501	5571	344	312	222	C
	ATOM	6727	C	GLY	P	430	-12.815	-0.528	9.449	1.00	37.79		C
30	ANISOU	6727	C	GLY	P	430	4989	4161	5207	327	244	234	C
	ATOM	6728	O	ILE	P	431	-13.217	0.553	6.316	1.00	33.85		O
	ANISOU	6728	O	ILE	P	431	4303	3864	4696	252	220	183	O
	ATOM	6729	N	ILE	P	431	-12.341	-1.205	8.408	1.00	36.65		N
	ANISOU	6729	N	ILE	P	431	4768	4051	5106	277	263	203	N
35	ATOM	6730	CA	ILE	P	431	-11.431	-0.596	7.441	1.00	31.75		C
	ANISOU	6730	CA	ILE	P	431	4108	3463	4494	256	204	210	C
	ATOM	6731	C	ILE	P	431	-12.075	0.617	6.767	1.00	31.38		C
	ANISOU	6731	C	ILE	P	431	4033	3489	4399	264	172	213	C
	ATOM	6732	CB	ILE	P	431	-10.982	-1.638	6.397	1.00	32.82		C
40	ANISOU	6732	CB	ILE	P	431	4158	3629	4682	197	243	170	C
	ATOM	6733	CG1	ILE	P	431	-10.183	-2.745	7.083	1.00	34.89		C
	ANISOU	6733	CG1	ILE	P	431	4452	3813	4992	191	263	172	C
	ATOM	6734	CG2	ILE	P	431	-10.131	-1.010	5.305	1.00	30.23		C
	ANISOU	6734	CG2	ILE	P	431	3781	3343	4360	173	186	174	C
45	ATOM	6735	CD1	ILE	P	431	-8.873	-2.256	7.701	1.00	35.00		C
	ANISOU	6735	CD1	ILE	P	431	4532	3755	5013	216	188	220	C
	ATOM	6736	O	ILE	P	432	-11.819	4.214	4.076	1.00	32.03		O
	ANISOU	6736	O	ILE	P	432	4015	3759	4396	253	-4	243	O
	ATOM	6737	N	ILE	P	432	-11.352	1.736	6.711	1.00	32.07		N
50	ANISOU	6737	N	ILE	P	432	4149	3570	4466	283	90	251	N

5	ATOM	6738	CA	ILE	P	432	-11.905	2.955	6.116	1.00	34.42	C	
	ANISOU	6738	CA	ILE	P	432	4426	3934	4719	293	53	257	C
	ATOM	6739	C	ILE	P	432	-11.284	3.355	4.773	1.00	30.32	C	
	ANISOU	6739	C	ILE	P	432	3834	3476	4211	255	16	247	C
	ATOM	6740	CB	ILE	P	432	-11.919	4.150	7.094	1.00	37.38	C	
	ANISOU	6740	CB	ILE	P	432	4889	4268	5045	352	-10	306	C
10	ATOM	6741	CG1	ILE	P	432	-10.559	4.332	7.760	1.00	40.47	C	
	ANISOU	6741	CG1	ILE	P	432	5345	4580	5453	372	-74	349	C
	ATOM	6742	CG2	ILE	P	432	-13.019	3.969	8.144	1.00	37.48	C	
	ANISOU	6742	CG2	ILE	P	432	4955	4256	5031	387	36	306	C
	ATOM	6743	CD1	ILE	P	432	-10.449	5.644	8.535	1.00	44.51	C	
	ANISOU	6743	CD1	ILE	P	432	5936	5058	5916	426	-148	399	C
15	ATOM	6744	O	LYS	P	433	-8.171	0.912	3.440	1.00	32.29	O	
	ANISOU	6744	O	LYS	P	433	3981	3672	4616	147	29	212	O
	ATOM	6745	N	LYS	P	433	-10.167	2.730	4.420	1.00	30.10	N	
	ANISOU	6745	N	LYS	P	433	3782	3424	4229	226	7	244	N
	ATOM	6746	CA	LYS	P	433	-9.620	2.800	3.062	1.00	33.41	C	
	ANISOU	6746	CA	LYS	P	433	4120	3905	4670	181	-10	224	C
20	ATOM	6747	C	LYS	P	433	-8.993	1.454	2.691	1.00	33.94	C	
	ANISOU	6747	C	LYS	P	433	4143	3957	4797	137	36	194	C
	ATOM	6748	CB	LYS	P	433	-8.572	3.917	2.925	1.00	32.29	C	
	ANISOU	6748	CB	LYS	P	433	4003	3751	4514	196	-105	267	C
	ATOM	6749	CG	LYS	P	433	-9.060	5.302	3.329	1.00	29.89	C	
	ANISOU	6749	CG	LYS	P	433	3748	3457	4153	241	-159	301	C
25	ATOM	6750	CD	LYS	P	433	-7.969	6.352	3.086	1.00	33.80	C	
	ANISOU	6750	CD	LYS	P	433	4260	3944	4639	251	-253	342	C
	ATOM	6751	CE	LYS	P	433	-8.434	7.761	3.476	1.00	31.89	C	
	ANISOU	6751	CE	LYS	P	433	4067	3711	4339	296	-310	376	C
	ATOM	6752	NZ	LYS	P	433	-7.389	8.761	3.068	1.00	34.77	N	
	ANISOU	6752	NZ	LYS	P	433	4436	4078	4697	300	-400	412	N
30	ATOM	6753	O	THR	P	434	-7.088	0.909	0.074	1.00	30.52	O	
	ANISOU	6753	O	THR	P	434	3548	3608	4441	34	6	154	O
	ATOM	6754	N	THR	P	434	-9.396	0.919	1.541	1.00	35.59	N	
	ANISOU	6754	N	THR	P	434	4261	4237	5023	90	83	148	N
	ATOM	6755	CA	THR	P	434	-8.888	-0.360	1.047	1.00	35.52	C	
	ANISOU	6755	CA	THR	P	434	4201	4225	5071	45	130	114	C
35	ATOM	6756	C	THR	P	434	-7.729	-0.147	0.049	1.00	31.98	C	
	ANISOU	6756	C	THR	P	434	3704	3800	4647	13	82	116	C
	ATOM	6757	CB	THR	P	434	-10.029	-1.234	0.462	1.00	32.20	C	
	ANISOU	6757	CB	THR	P	434	3712	3862	4659	12	217	60	C
	ATOM	6758	OG1	THR	P	434	-10.738	-0.492	-0.539	1.00	32.65	O	
	ANISOU	6758	OG1	THR	P	434	3712	4008	4686	-3	211	44	O
40	ATOM	6759	CG2	THR	P	434	-11.021	-1.639	1.567	1.00	32.06	C	
	ANISOU	6759	CG2	THR	P	434	3748	3808	4627	43	267	59	C
	ATOM	6760	N	PHE	P	435	-7.440	-1.137	-0.802	1.00	31.73	N	
	ANISOU	6760	N	PHE	P	435	3600	3795	4659	-36	125	77	N
	ATOM	6761	CA	PHE	P	435	-6.234	-1.085	-1.658	1.00	31.87	C	
	ANISOU	6761	CA	PHE	P	435	3576	3826	4707	-66	84	79	C
50	ATOM	6762	C	PHE	P	435	-6.339	-0.069	-2.801	1.00	29.79	C	
	ANISOU	6762	C	PHE	P	435	3258	3646	4417	-82	44	77	C

	ATOM	6763	O	PHE P 435	-7.419	0.179	-3.308	1.00	30.98		O
	ANISOU	6763	O	PHE P 435	3369	3862	4540	-91	73	54	O
	ATOM	6764	CB	PHE P 435	-5.931	-2.446	-2.304	1.00	30.00		C
	ANISOU	6764	CB	PHE P 435	3274	3599	4525	-116	144	35	C
5	ATOM	6765	CG	PHE P 435	-5.662	-3.565	-1.320	1.00	31.76		C
	ANISOU	6765	CG	PHE P 435	3540	3741	4784	-108	183	33	C
	ATOM	6766	CD1	PHE P 435	-6.441	-4.717	-1.337	1.00	32.77		C
	ANISOU	6766	CD1	PHE P 435	3637	3880	4934	-130	267	-9	C
	ATOM	6767	CD2	PHE P 435	-4.621	-3.473	-0.406	1.00	29.15		C
10	ANISOU	6767	CD2	PHE P 435	3282	3327	4468	-81	136	75	C
	ATOM	6768	CE1	PHE P 435	-6.193	-5.770	-0.448	1.00	35.62		C
	ANISOU	6768	CE1	PHE P 435	4037	4167	5329	-124	303	-11	C
	ATOM	6769	CE2	PHE P 435	-4.360	-4.515	0.490	1.00	29.66		C
	ANISOU	6769	CE2	PHE P 435	3386	3317	4566	-75	172	73	C
15	ATOM	6770	CZ	PHE P 435	-5.153	-5.665	0.468	1.00	32.59		C
	ANISOU	6770	CZ	PHE P 435	3725	3700	4958	-97	256	30	C
	ATOM	6771	O	SER P 436	-2.853	0.265	-4.625	1.00	35.91		O
	ANISOU	6771	O	SER P 436	3954	4425	5266	-146	-87	105	O
	ATOM	6772	N	SER P 436	-5.195	0.475	-3.216	1.00	28.46		N
20	ANISOU	6772	N	SER P 436	3084	3474	4257	-89	-22	102	N
	ATOM	6773	CA	SER P 436	-5.063	1.195	-4.487	1.00	33.17		C
	ANISOU	6773	CA	SER P 436	3613	4149	4841	-116	-56	94	C
	ATOM	6774	C	SER P 436	-3.845	0.674	-5.250	1.00	35.26		C
	ANISOU	6774	C	SER P 436	3829	4414	5152	-155	-68	84	C
25	ATOM	6775	CB	SER P 436	-4.908	2.702	-4.256	1.00	31.56		C
	ANISOU	6775	CB	SER P 436	3455	3947	4591	-79	-139	141	C
	ATOM	6776	OG	SER P 436	-6.064	3.244	-3.639	1.00	36.23		O
	ANISOU	6776	OG	SER P 436	4084	4544	5136	-44	-129	148	O
	END										
30											

In the complex between the 15-residue F peptide and 101F, only the amino acids KNRGIIKTFS (SEQ ID NO:4) are modeled in the crystal structure as the remaining residues are disordered. As such, the coordinates disclosed in PDB acc code 3O41 only include those 10 amino acids of the 15-residue peptide.

Example 3: Production and characterization of scaffold-based immunogens

This Example describes the production and testing of scaffold-based immunogens designed using the atomic coordinates in PDB acc code 3IXT, i.e., the atomic coordinates of the complex between the 24-residue F peptide comprising the motavizumab binding domain and motavizumab.

Several scaffolds were designed to present the motavizumab epitope, or binding domain, using the superposition method (also referred to as side chain grafting) and the multi-segment side chain grafting method (also referred to as double superpositioning).

Multi-segment side chain grafting is an extension of the superposition method described in WO 2008/025015 A2. Multi-segment side chain grafting is intended for transplantation of certain complex epitopes to scaffold proteins, in which the epitope contains two or more backbone segments in a fixed orientation relative to each other; e.g., the motavizumab epitope is composed of two helices. Although the description herein focuses on two backbone segments, it is to be appreciated that the algorithm is generalizable to any number of segments. The method works very similarly to the original superposition method, except that scaffolds are scanned for structural similarity to each of the two epitope segments individually, and whenever a match is found to one of the segments, that scaffold is searched a second time for structural similarity to the other epitope segment, with the rigid body position of the second epitope segment relative to the scaffold pre-determined by the superposition of the first segment to the scaffold. "Double" matches are identified if (a) one epitope segment matches a scaffold with backbone rmsd/nsup < threshold 1 and the other segment matches with backbone rmsd/nsup < threshold 2, where threshold 1 is typically 0.15 and threshold 2 is typically 0.2, or (b) if both segments are superimposed simultaneously onto the scaffold and the backbone rmsd/nsup is < threshold 3, where threshold 3 is typically 0.2. As used herein, backbone rmsd refers to the root-mean square deviation of a structural alignment computed over the backbone atoms N, CA, C, O; and backbone rmsd/nsup refers to backbone rmsd divided by the number of aligned residues.

An automated search of monomeric and non-monomeric proteins without co-factors within the PDB (RCSB Protein Data Bank, Brookhaven, NY) was conducted in March 2009 to identify candidate scaffolds having similar three-dimensional structures to the three-dimensional structure defined by the atomic coordinates specified in PDB accession code 3IXT. Nearly all of the structural matches contained significant backbone clashes with the motavizumab antibody (multiple atomic overlaps). Several hundred structural matches were screened by eye to identify structures that could be trimmed to eliminate backbone clash. Ten variants of 3 different scaffold proteins (1lp1b (*Staphylococcus aureus* Protein A), 1s2xa (*Helicobacter pylori* CagZ protein) and 2eiaa (equine infectious anemia virus) were chosen either because no trimming was necessary (1lp1b, 2eiaa) or the necessary trimming was restricted to a terminus and could be done easily (1s2xa). It is to be appreciated that this method can also identify scaffolds requiring more advanced trimming by flexible backbone protein design.

Residues required for elicitation of a humoral immune response against RSV were then implanted into the scaffolds and epitope conformation was stabilized by genetic engineering using a method similar to that described in WO 2008/025015 A2. For example, Figure 7 shows a final scaffold comparison for immunogen 1lp1b_003. Amino acids in bold are motavizumab contact residues. Amino acids that are underlined were substituted to stabilize the structure.

The amino acid sequences of ten scaffold-based immunogens are:

1lp1b_001 (SEQ ID NO:18)

VDNSFNDEKKLASNEIAHLPNLNNEEQRSAFLSSINDDPSQSANLLAEAKKLNDAAQ
APK

1lp1b_002 (SEQ ID NO:21)

VDNSFNDEKKLASNEIQHLPNLNNEEQRSAFISSLNDDPSQSANLLAEAKKLNDAAQ
APK

1lp1b_003 (SEQ ID NO:24)

SFNDEKKLASNEIAHLPNLNNEEQRSAFLSSINDDPSQSANLLAEAKKLNDAAQAPK

1lp1b_004 (SEQ ID NO:149)

SFNDEKKLASNEIQHLPNLNNEEQRSAFISSLNDDPSQSANLLAEAKKLNDAAQAPK

1s2xa_001 (SEQ ID NO:152)

GSPNSRVDELGFNEAERQKILDSNSSLMRNANEVRDKFIQNYATSLKDSNDPQDF
LRRVQELRINMQKNFISFDAYYNYLNNLVLASANRCSQELSFANDTIKNNDTKKL
FSNEIADNFNNFTADEVARISDLVASYLPREYLPPFIDGNMMGVAFQILGIDDFGK
KLNEIVQDIGTKY

1s2xa_002 (SEQ ID NO:155)

GSPNSRVDELGFNEAERQKILDSNSSLMRNANEVRDKFIQNYATSLKDSNDPQDF
LRRVQELRINMQKNFISFDAYYNYLNNLVLASANRASQELSFINDTIKNNDTKKLF
SNEAADNFNNFTADEVARISDLVASYLPREYLPPFIDGNMMGVAFQILGIDDFGKK
LNEIVQDIGTKY

1s2xa_003 (SEQ ID NO:158)

GSPNSRVDELGFNEAERQKILDSNSSLMRNANEVRDKFIQNYATSLKDSNDPQDF
LRRVQELRINMQKNFISFDAYYNYLNNLVLASANRASQELSFANDTIKNNDTKKL
FSNEIADNFNNFTADEVARISDLVASYLPREYLPPFIDGNMMGVAFQILGIDDFGK
KLNEIVQDIGTK

1s2xa_004 (SEQ ID NO:164)

GSPNSRVDELGFNEAERQKILDSNSSLMRNANEVRDKFIQNYATSLKDSNDPQDF
 LRRVQELRINMQKNFISFDAYYNYLNNLVLASANRASQELSFINDTIKNNDTKKLF
 SNEAADNFNNFTADEVARISDLVASYLPREYLPPFIDGNMMGVAFQILGIDDFGKK
 LNEIVQDIGTK

5 2ciaa_001 (SEQ ID NO:167)

APRGYTTWVNTIQTNGLLNEASQNLFGILSVDATSEEMNAFLDVVPGQAGQKQIL
 LDAIDKIADDWDNRHPLPNAPLVAPPQGPIPMTARFIRGLGVPRERQMEPAFDQFR
 QTYRQWIIIEAMSEGIKVMIGKPKAQNIRQGAKPEPYPEFVSRLLSQINDEGHPNDIK
 KLRSTLTIQNANEECRNAMRHLRPSDTGAEKMYACRDIG

10 2ciaa_002 (SEQ ID NO:170)

GKPKAQNIRQGAKPEPYPEFVSRLLSQINDEGHPNDIKKLRSTLTIQNANEECRNA
 MRHLRPSDTGAEKMYACRDIG

These immunogens have been further tailored to include amino (N) or carboxyl
 (C) tags or motifs to aid in purification of immunogens of the embodiments. Examples
 15 include of production of the following immunogens:

1sx2a_001_N_His (SEQ ID NO:177)

HHHHHHGSPNSRVDELGFNEAERQKILDSNSSLMRNANEVRDKFIQNYATSLKDS
 NDPQDFLRRVQELRINMQKNFISFDAYYNYLNNLVLASANRCSQELSFANDTIKN
 NDTKKLFSNEIADNFNNFTADEVARISDLVASYLPREYLPPFIDGNMMGVAFQILG
 20 IDDFGKKLNEIVQDIGTKY*

1sx2a_002_N_His (SEQ ID NO:178)

HHHHHHGSPNSRVDELGFNEAERQKILDSNSSLMRNANEVRDKFIQNYATSLKDS
 NDPQDFLRRVQELRINMQKNFISFDAYYNYLNNLVLASANRASQELSFINDTIKN
 DTKKLFSNEAADNFNNFTADEVARISDLVASYLPREYLPPFIDGNMMGVAFQILGI
 25 DDFGKKLNEIVQDIGTKY*

1sx2a_003_N_His (SEQ ID NO:179)

HHHHHHGSPNSRVDELGFNEAERQKILDSNSSLMRNANEVRDKFIQNYATSLKDS
 NDPQDFLRRVQELRINMQKNFISFDAYYNYLNNLVLASANRASQELSFANDTIKN
 NDTKKLFSNEIADNFNNFTADEVARISDLVASYLPREYLPPFIDGNMMGVAFQILG
 30 IDDFGKKLNEIVQDIGTKY*

1sx2a_004_N_His (SEQ ID NO:180)

HHHHHHGSPNSRVDELGFNEAERQKILDSNSSLMRNANEVRDKFIQNYATSLKDS
 NDPQDFLRRVQELRINMQKNFISFDAYYNYLNNLVLASANRASQELSFINDTIKN

DTKKLFSNEAADNFNNFTADEVARISDLVASYLPREYLPPFIDGNMMGVAFQILGI
DDFGKKLNEIVQDIGTK*

2ciaa_002_N_His (SEQ ID NO:181)

HHHHHHGKPKAQNIRQGAKEPYPEFVSRLLSQINDEGHPNDIKKLSNTLTIQNAN

5 EECRNAMRHLRPSDTGAEKMYACRDIG*

Each of the four 1lp1b-based immunogens, namely 1lp1b_001, 1lp1b_002, 1lp1b_003 and 1lp1b_004 was expressed with a HRV3C site, PADRE, Caspase3 site, 6XHis tag and StrepTagII in 293F mammalian cells (Invitrogen) transformed with paH (also known as p(alpha)H) vector comprising a nucleic acid sequence encoding the
10 respective immunogen. The paH vector is a modified version of the pHLSec vector (Aricescu AR et al, *ibid*) that includes changes to the multi-cloning site (MCS) and removal of certain restriction enzyme sites. The resultant immunogens were purified by nickel IMAC and STREP-TACTIN® chromatography followed by gel filtration. His and Strep tags were cleaved by pro-caspase. As an example, the following sequence is

15 1lp1b_001 with a HRV3C site (LEVLFQGP (SEQ ID NO:182)), PADRE (AKFVAAWTLKAAA (SEQ ID NO:183)), caspase3 site (DEV (SEQ ID NO:184)), 6xHis tag (HHHHHH (SEQ ID NO:185)) and StrepTagII (WSHPQFEK (SEQ ID NO:186)) (each underlined) at its carboxyl terminus:

VDNSFNDEKKLASNEIAHLPLNNEEQRSAFLSSINDDPSQSANLLAEAKKLNDQAQ

20 APKLEVLFQGPGAKFVAAWTLKAAAGDEVDGSHHHHHHSAWSHPQFEK, also denoted herein as SEQ ID NO:187.

Each of three of the four 1s2xa-based immunogens, namely 1s2xa_001, 1s2xa_002, and 1s2xa_003, was expressed as a maltose binding protein fusion in BL21(DE3) bacteria transformed with MBP-HTSHP vector comprising a nucleic acid
25 sequence encoding the respective immunogen. The MBP-HTSHP vector is a modified version of the pMal-c2x vector (New England Biolabs, Ipswich, MA) that includes a linker region with all the various tags and restriction sites. Fusion protein was recovered by nickel chromatography. The fusion protein was cleaved with pro-caspase 3 and subjected to nickel chromatography and S75 gel filtration. An anion exchange column can
30 also be used as part of the procedure. As an example, the following sequence in bold is 1s2xa_001 with maltose-binding protein, a factor Xa site, his-tag, TEV site, strep-tag, his-tag, HRV3C site and caspase 3 site (each underlined) at its amino terminus:

MKIEEGKLVIWINGDKGYNGLAEVGGKFEKDTGIKVTVEHPDKLEEKFPQVAAT
GDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPI
AVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAA
DGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIK NKHMNADTDYSIAEAA
5 FNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASP
NKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAO
KGEIMPNI PQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSSSNNNNNNNNNN
NLGIEGRISGHHHHHHHHHDYDIPSSENLYFQGSASWSHPQFEKSGHHHHHHHHHDY
DIPSSLEVLFGQPGSDEV D GSPNSRVDELGFNEAERQKILDSNSSLMRNANEVR
10 **DKFIQNYATSLKDSNDPQDFLRRVQELRINMQKNFISFDAYNYLNNLVLASA**
NRCSQELSFANDTIKNNDTKKLFSNEIADNFNNFTADEVARISDLVASYLPREY
LPPFIDGNMMGVAFQILGIDDFGKKLNEIVQDIGTKY, also denoted herein as
 SEQ ID NO:151.

Each immunogen of the present invention can be produced in the manner described
 15 herein.

Scaffold-based immunogens 1lp1b_001, 1lp1b_002, 1lp1b_003, 1lp1b_004,
 1s2xa_001, 1s2xa_002 and 1s2xa_003 were submitted to surface plasmon resonance
 binding analysis. Experiments were carried out on a Biacore 3000 instrument (GE
 Healthcare). Motavizumab fragment of antigen binding (Fab) was covalently coupled to a
 20 CM5 chip, and a blank surface with no antigen was created under identical coupling
 conditions for use as a reference. Scaffolds were serially diluted 2-fold, starting at 10 mM,
 into 10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA and 0.005% polysorbate 20 and
 injected over the immobilized motavizumab Fab and reference cell at 40 ml/min. The
 surface was regenerated with 10 mM glycine pH 3 at a flow rate of 40 ml/min. The data
 25 were processed with SCRUBBER-2 and double referenced by subtraction of the blank
 surface and a blank injection (no analyte).

Results are shown in Table 5, which indicates the K_d in nanomolar (nM)
 concentrations for each of the tested scaffold-based immunogens.

Table 5. Affinity of scaffold-based immunogens for motavizumab

Scaffold	Motavizumab affinity (K_d in nM)
1lp1b_003	66
1lp1b_001	181
1lp1b_004	296
1lp1b_002	700
1s2xa_003	3005
1s2xa_002	3638
1s2xa_001	4755

Immunogen 1lp1b_003 exhibited the best affinity for motavizumab in this assay.

- 5 This immunogen was also tested for binding to an irrelevant IgG and irrelevant Fab. No significant binding above baseline was detected.

Immunogen 1lp1b_003 proteins, produced in both mammalian cells and bacteria as described above, were also tested for their three-dimensional structure. The data in Figure 8 demonstrate that both mammalian- and bacterial-produced 1lp1b_003 proteins are alpha
10 helical in solution and have a melting temperature of about 60°C, which confirms that the proteins are folded in solution.

Example 4: Production of resurfaced scaffold-based immunogens

- This example describes the production of scaffold-based immunogens that have
15 been resurfaced to neutralize deleterious immunodominant epitopes.

Resurfaced variants of the 1lp1b_003 scaffold were designed using protocols similar to those described in WO 2009/100376 A2. This scaffold has the highest binding affinity of the initial designs. In these resurfaced variants, a significant fraction of the non-epitope surface area on the scaffold was mutated to generate antigenic surfaces
20 distinct from the original design. In a few cases the mutations were designed to introduce N-linked glycosylation sites, but in most cases the mutations did not. These variants are useful in heterologous prime-boost immunizations using a non-resurfaced immunogen as the prime and one or more of the resurfaced variants as a boost, with the aim of focusing the antibody response onto the antibody binding domain, or epitope, which is intended to

be the only antigenic surface conserved between the non-resurfaced and resurfaced immunogens.

The sequences for several resurfaced 11p1b immunogens are below. The first two immunogens include substitutions resulting in glycan-masked immunogens; the remaining

5 are non-glycan resurfaced immunogens.

mota_11p1b.m1.c1.d1_glyc1 (SEQ ID NO:55)

SFNDEKKLASNEIAHLPNLNETQRS AFLSSINDDPSQSANLLANATKLNDAQAP

mota_11p1b.m1.c1.d1_glyc2 (SEQ ID NO:56)

SFNDEKKLASNEIAHLPNLNETQRS AFLSSINDDPNQSANLLANATKLNDAQAP

10 mota_11p1b.m1.c1.d1_des1_1 (SEQ ID NO:66)

SFNDEKKLASNR IANLPNLNEEQRS AFLSKINDDPSQSANLLEEAL KLND AQAQK

mota_11p1b.m1.c1.d1_des1_2 (SEQ ID NO:67)

SFNDDKKLASNR IANLPNLNEEQRS AFLSKINDDPSQSRNLLEEAL KLND AQAQK

mota_11p1b.m1.c1.d1_des1_3 (SEQ ID NO:68)

15 SFNDKKK LASNR IANLPNLNEEQRS AFLSKINDDPSKSEELLEKAL KLND AQAQK

mota_11p1b.m1.c1.d1_des1_5 (SEQ ID NO:69)

SFNDKKK LASNEI ANLPNLNEEQRS AFLSKINDDPSKSEELLEEAL KLND AQAQK

mota_11p1b.m1.c1.d1_des1_6 (SEQ ID NO:70)

SFNDKKK LASNR IAKLPNLNEKQRS AFLSKINDDPSKSEELLKKAL KLNKAQAQK

20 mota_11p1b.m1.c1.d1_des1_7 (SEQ ID NO:71)

SFNDEKK LASNR IANLPNLNQEQRS AFLSKINDDPSQSANLLEEAL KLND NQAQK

mota_11p1b.m1.c1.d1_des1_8 (SEQ ID NO:72)

SFNDDKK LASNR IANLPNLNQEQRS AFLSKINDDPSQSRNLLEEAL KLND NQAQK

mota_11p1b.m1.c1.d1_des1_9 (SEQ ID NO:73)

25 SFNDKKK LASNR IANLPNLNQEQRS AFLSKINDDPSKSEELLEKAL KLND NQAQK

mota_11p1b.m1.c1.d1_des1_10 (SEQ ID NO:74)

SFNDKKK LASNEI ANLPNLNQEQRS AFLSKINDDPSKSEELLEEAL KLND NQAQK

mota_11p1b.m1.c1.d1_des1_11 (SEQ ID NO:75)

SFNDKKK LASNR IAKLPNLNEKQRS AFLSNINDDPSKSEELLEKAL KLNQAQAQK

30 Production of these resurfaced immunogens can occur using methods similar to those described in Example 3.

Example 5: Production of trimeric F immunogens

The production of RSV F₀ Fd immunogen was described in Example 1. Additional soluble trimeric F immunogens, stabilized in the pre-fusion conformation, namely RSV F Fd and RSV F₀ Fd GAG, have been produced in a similar manner. The amino acid sequence of each of these immunogens, with the T4 fibrin trimerization domain (also referred to as Foldon, or Fd) underlined, is as follows:

RSV F₀ Fd (SEQ ID NO:174)

MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVI
 TIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTPATNNQARSELP
 10 RFMNYTLNNAKKTNTVLSKKRKSSFLGFLGVSASIASGVAVSKVLHLEGEVNKI
 KSALLSTNKAVVSLSNGVSVLTSTKVLDLKNYIDKQLLPVINKQSCSISNIETVIEFQ
 QKNNRLLLEITREFSVNAGVTTPVSTYMLTNSSELLSLINDMPITNDQKKLMSNNVQI
 VRQQSYSIMSIIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRT
 DRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNVDIFNPKY
 15 DCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIKTFSNGCDYVSNKGV
 DTVSVGNTLYYVVKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSL
 AFIRKSDELLSAIGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGIEGRHHHHHH—

RSV F Fd (SEQ ID NO:175)

MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVI
 20 TIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELP
 RFMNYTLNNAKKTNTVLSKKRKRRLGFLGVSASIASGVAVSKVLHLEGEVNKI
 KSALLSTNKAVVSLSNGVSVLTSTKVLDLKNYIDKQLLPVINKQSCSISNIETVIEFQ
 QKNNRLLLEITREFSVNAGVTTPVSTYMLTNSSELLSLINDMPITNDQKKLMSNNVQI
 VRQQSYSIMSIIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRT
 25 DRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNVDIFNPKY
 DCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIKTFSNGCDYVSNKGV
 DTVSVGNTLYYVVKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSL
 AFIRKSDELLSAIGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGLVPRGSHHHHHH
 SAWSHQPFEK--

RSV F₀ Fd GAG (SEQ ID NO:176)

GAGMELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWY
 TSVITIELSNIKENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTPATNNRAR
 GAGKRRFLGFLGVSASIASGVAVSKVLHLEGEVNKIKSALLSTNKAVVSLSNGV

SVLTSKVLDLKNYIDKQLLPIVNKQSCSISNIETVIEFQQKNNRLLLEITREFSVNAGV
 TTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIIKEEVLAYV
 VQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQA
 ETCKVQSNRVFCDTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITSLG
 5 AIVSCYGKTKCTASNKNRGIKTFSNGCDYVSNKGVDTVSVGNTLYYVNBKQEGKS
 LYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLSAIGGYIPEAPR
DGQAYVRKDGEWVLLSTFLGGLVPRGSHHHHHHSAWSHPQFEK--

Gel filtration analysis of RSV F₀ Fd immunogen indicates that this immunogen has
 a calculated molecular weight of about 240 kilodaltons (kD), indicative of a trimer (Figure
 10 2 e). Binding studies indicate that RSV F₀ Fd immunogen binds motavizumab,
 SYNAGIS[®] and 101F with affinities similar to RSV F protein. RSV F₀ Fd immunogen can
 bind motavizumab and 101F simultaneously.

Example 6: Design of antibodies having higher affinity binding for the motavizumab-
 15 binding domain

This Example describes a method to design antibodies having a higher binding
 affinity for the motavizumab-binding domain. Such antibodies can have utility as passive
 immunotherapy compositions.

Additional interactions were identified between the motavizumab antibody and its
 20 epitope using a modified Rosetta interface design protocol. The crystal structure of the
 complex between motavizumab and its binding domain was prepacked to remove clashes
 as calculated by Rosetta. The iterations of local refine docking between epitope and
 antibody were carried out to generate an ensemble of slightly different rigid body
 orientations, then the backrub algorithm was applied to both epitope and antibody to
 25 generate backbone conformational variation, and finally iterative design and minimization
 of sidechains in the interface was carried out. This protocol was carried out hundreds of
 thousands of times; 20,000 low-energy structures were generated, and the top 1% were
 selected based on calculated ddG. From those 200 structures, approximately 50 were
 selected based on the total calculated free energy. The top 50 structures were aligned and
 30 the mutations listed below were identified as ones that could enhance binding between the
 antibody and peptide. Several candidate mutations are listed at many of the positions. A
 DNA library encoding all of these mutations within a single-chain fragment variable

(ScFv) construct for motavizumab will be built and screened using yeast surface display to identify the tightest binding clones.

The mutations identified that may increase binding affinity are as follows:

	<u>Motavizumab heavy (H) chain residue</u>	<u>Encoded mutation</u>
5	32	H or E
	35	A
	52	K, H, T, S or R
	53	H or S
	54	F or R
10	56	I, S, E or D
	58	Y or W
	97	D, H or R
	99	D
	100	Y, W or H
15	100A	S or T
	<u>Motavizumab light (L) chain residue</u>	<u>Encoded mutation</u>
	32	F
	49	H or R
20	92	K
	94	H
	96	H

It was also recognized that additional contacts between the antibody and epitope could be made by increasing the length of the CDRH2 loop by 2 residues. The CDRH2 loop in the heavy chain of motavizumab spans amino acids H50 through H58. The above protocol was used for these simulations as well but it included an additional step in which the loop was explicitly rebuilt to increase the length prior to the iterations of docking, backrub and design. Following is the library of mutations for that lengthened loop. This library is merged with the library above and screened on yeast as described above.

Specifically, the 9-residue stretch between H50-H58 was removed and replaced with a variation of the 11-residue sequence below, where 1 is the first amino acid in that sequence:

	<u>Position</u>	<u>Encoded mutation</u>
	1	E, S or M
	2	I
	3	H, R or F
5	4	S
	5	G
	6	G, H, K, L, N, Q, S, D, T or R
	7	F, K, S, T, D or R
	8	E, N or D
10	9	D, H, L, S, R or T
	10	Y
	11	Y, F or H.

Example 7. Binding of immunogens to motavizumab

15 This Example demonstrates the ability of immunogens of the present disclosure to bind to motavizumab.

 a. Expression protocol for 1lp1b-based immunogens: Mammalian codon-optimized genes encoding 1lp1b-based immunogens were synthesized by GeneArt with an N-terminal secretion signal (MGS LQPLATLYLLGMLVASVLA (SEQ ID NO:188)) and
20 a C-terminal HRV3C cleavage site, PADRE epitope (AKFVAAWTLKAAA (SEQ ID NO:183)), Caspase-3 cleavage site, 6x His-tag and Strep-tag II. The genes were cloned into the mammalian expression vector pαH. Proteins were expressed from the plasmids by transient transfection using the FREESTYLE™ 293 expression system (Invitrogen). 1lp1b proteins were purified from the media using Ni²⁺-NTA resin (Qiagen) and then STREP-
25 TACTIN® resin (IBA, Goettingen, Germany) as per manufacturer's instructions, followed by passage over a 16/60 Superdex 75 column (GE Healthcare). For SPR, ITC and CD measurements, all tags were retained. For immunization experiments, Pro-caspase 3 was added to remove the 6x His-tag and Strep-tag II. The tags and protease were removed from cleaved 1lp1b by passage over Ni²⁺-NTA resin.

30 b. Expression protocol for 1s2xa-based immunogens: *E. coli* codon-optimized genes encoding 1s2xa-based immunogens were synthesized by GeneArt and cloned into a custom vector based on pMAL-c2X (New England Biolabs). The expression vectors were transformed into BL21(DE3) cells, and the cells were grown in Terrific

Broth (Difco, Becton Dickinson, Franklin Lakes, NJ) at 37°C until OD₆₀₀ = 2.0. The temperature was then reduced to 22°C, and isopropyl β-D-thiogalactoside (IPTG) was added to 1 mM. After overnight incubation at 22°C, the cells were harvested and lysed with BUGBUSTER™ Protein Extraction Reagent (Novagen, EMD Chemicals,

5 Gibbstown, NJ), and 1s2xa proteins were purified using Ni²⁺-NTA resin (Qiagen). Fusion tags were removed by incubation with Pro-caspase 3 and passage over Ni²⁺-NTA resin. 1s2xa proteins were further purified by passage over a 16/60 SUPERDEX™ 75 column (GE Healthcare), and anion exchange chromatography using a MonoQ column (GE Healthcare).

10 c. Production of ferritin-containing immunogens. The gene encoding 1lp1b_003 fused to the N-terminus of the coding region of human ferritin was subcloned into a mammalian expression vector, such as pVRC8405 (Barouch D et al., 2005, J. Virol. 79, 8828-8834). Proteins were expressed from this plasmid by transient transfection in HEK293 GnTI^{-/-} cells. 1lp1b_003_ferritin was initially purified from the media by anion
15 exchange chromatography using a MonoQ HR 10/10 column (GE Healthcare). The eluted protein was then passed over a column consisting of SYNAGIS® IgG covalently coupled to Protein A agarose resin (Pierce). The column was washed with phosphate-buffered saline (PBS) and eluted with Actisep Elution Medium (Sterogene, Carlsbad, CA). The eluted 1lp1b_003_ferritin was dialyzed against PBS, concentrated, and further purified by
20 passage over a 16/70 SUPEROSE™6 column (GE Healthcare). Additional ferritin-containing immunogens, such as 1lp1b_003 eumS, 1lp1b_003 eumSP, 1lp1b_003eumL, and 1lp1b_003 eumLP, were produced in a similar manner.

Immunogens were produced as described herein and submitted to surface plasmon resonance binding analysis as described in Example 3. Some of the immunogens were
25 also submitted to isothermal titration calorimetry (ITC). Table 8 indicates which immunogens were tested and the results obtained. K_d refers to the K_d of motavizumab for a tested immunogen by either Biacore or ITC measurement, respectively. dH and -TdS relate to measurements of enthalpy and entropy, respectively. Table 8 also indicates the cell type (i.e., HEK293 mammalian cells or *E. coli* bacteria) used to produce the respective
30 immunogen. Table 8 also provides the name of the immunogen used in Table 9. A blank square indicates that the respective immunogen was not tested in that assay. “nd” means not detectable; i.e., below the limit of detection of the assay.

Table 8. Affinity of immunogens for motavizumab and ITC measurements

Name	Name used in Table 9	Immunogen production system	Biacore	ITC		
			K _d (nM)	dH	-TdS	K _d (nM)
1lp1b_001		HEK293	181			
1lp1b_002		HEK293	700			
1lp1b_003	1lp1b	HEK293	66	1.1	-10.5	126
1lp1b_003	1lp1b(Ecoli)	E. coli				
1lp1b_004		HEK293	296			
1lp1b_003_K272E		HEK293	nd			
1lp1b_003_Glyc1	1lp1b_Glyc1	HEK293	400			
1lp1b_003_Glyc2	1lp1b_Glyc2	HEK293	812			
1lp1b_003_Surf1	1lp1b_Surf1	HEK293	120			
1lp1b_003_Surf8	1lp1b_Surf8	HEK293	117			
1s2xa_001		E. coli	4755			
1s2xa_002		E. coli	3638			
1s2xa_003	1s2xa or Cag-Z	E. coli	3005	-19.2	9.9	147
1s2xa_004		E. coli	3950	-19.6	10.7	275
2e1aa_001		HEK293	nd			
1lp1b_003_K46A	K46A	HEK293	148	-5.3	-5.1	21
1lp1b_003_Q52A	Q52A	HEK293	233	-1.8	-8.8	16
1lp1b_003_I13L_F27A	I13L_F27A	HEK293	541	-19.4	10.1	159
1lp1b_003_L41I_L42A	L41I_L42A	HEK293	247	-1.3	-9.0	24
1lp1b_003_L41I_L42V	L41I_L42V	HEK293	77	-1.7	-7.9	90
1lp1b_003_I13A_L42A	I13A_L42A	HEK293	>10,000	-25.6	18.8	10,700
1lp1b_003_L19A	L19A	HEK293	>10,000	-27.3	20.9	21,000
1lp1b_003_L19A_L41I	L19A_L41I	HEK293	>10,000			
1lp1b_003_I13A	I13A	HEK293	2300	-16.2	7.4	380
1lp1b_003_L16A	L16A	HEK293	2300	-12.3	3.4	341
1lp1b_003_F27A	F27A	HEK293	490	-12.4	2.7	83
1lp1b_003_L41A	L41A	HEK293	310	-4.0	-5.7	79
1lp1b_003_L42A	L42A	HEK293	218	-4.4	-5.1	93
1lp1b_003_ferritin	1lp1b_ferritin	HEK293				
1lp1b_003_eumS	eumS	HEK293				
1lp1b_003_eumSP	eumSP	HEK293				
1lp1b_003_eumL	eumL	HEK293				
1lp1b_003_eumLP	eumLP	HEK293				
1lp1b_003_Neg1	Neg1	HEK293				

The results indicate that a number of the immunogens bind motavizumab with a K_d within approximately an order of magnitude of the K_d of RSV F peptide for motavizumab (K_d of ~250 nM). Immunogen 1lp1b_003_K272E has a mutation resulting in removal of a key contact residue within the motavizumab-binding domain; as such, that immunogen would not be expected to bind to motavizumab.

Example 8. Immunogenicity data: Binding and Neutralization

This Example demonstrates the ability of immunogens of the embodiments to elicit a humoral immune response that yields immune sera capable of binding to F protein, F

peptide (having SEQ ID NO:2), and scaffold immunogens. The Example further demonstrates the ability of immunogens of the embodiments to elicit a neutralizing humoral immune response against RSV.

Mice were immunized as follows: Six- to eight-week old female C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME) were used for all experiments. Mice were immunized with immunogen and 25 μ g CpG/mouse intramuscularly. Table 9 indicates which immunogens were tested, as well as the immunogen doses, administration regimen (including identification of immunogen administered as a prime (dose 1), identification of immunogen(s) administered as a boost(s), and number of boosts (i.e., dose 2, dose 3, etc.), interval of time between doses, and number of mice used. Please note that the immunogen names in Table 9 are an abbreviated form of immunogens listed in Table 8; Table 8 provides both names. Table 9 also indicates if the immunogens did not include a PADRE motif (-PADRE). Sera were collected on day 10 or day 14 and tested as described herein.

The abilities of the murine immune sera to bind 11p1b_003 immunogen (labeled as 11p1b in Table 9), 11p1b(K272E) protein (the mutation causing loss of motavizumab binding, and labeled as 11p1b(K272E) in Table 9), RSV F peptide, and RSV F protein were tested using a kinetic ELISA as follows: The RSV F protein and scaffold proteins were diluted in PBS and coated onto 96-well flat bottom ELISA plates at a concentration of 1 μ g/ml and incubated overnight at 4°C. For RSV F peptide binding, a biotinylated RSV F peptide (biotin-peptide) was used; the biotin-peptide was coated onto a Neutravidin plate (Thermo Scientific, Rockford, IL) and incubated for 2 hours at room temperature. For all samples, nonspecific adsorption was prevented with 200 μ L/well of blocking buffer (2% BSA in PBS) for 1 hour at 37°C. Plates were then washed four times on an automated plate washer (Bio-Tek Instruments, Winooski, VT) with wash buffer (0.02% Tween-20 in PBS). One hundred μ L of diluted test sera (1:100 in blocking buffer) or positive serum control were added to each well. Plates were incubated for one hour at room temperature, washed four times, and incubated for 1 hour at room temperature with HRP-conjugated goat anti-mouse IgG antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). Plates were washed with wash buffer four times followed by distilled water. One hundred μ L of Super AquaBlue ELISA substrate (eBioscience, San Diego, CA) was added to each well, and plates were read immediately using a Dynex Technologies microplate reader (Chantilly, VA). The rate of color change in mOD/min was read at a

wavelength of 405 nm every 9 seconds for 5 minutes with the plates shaken before each measurement. The mean mOD/min reading of duplicate wells was calculated, and the background mOD/min was subtracted from the corresponding control well.

Neutralization activity was measured using a flow cytometric neutralization assay as described in the Examples herein.

The abilities of immune sera elicited by immunogens of the embodiments to bind to RSV F protein and RSV F peptide are indicated in Table 9. Table 9 also provides data from the neutralization assay, expressed as number of mice (between 0 and 5) showing a specified result (Frequency) and reciprocal dilution of immune sera at which EC₅₀ was achieved (Magnitude). For certain immunogens, the immune sera, while not achieving 50% neutralization under the stipulated conditions, did show lower amounts of neutralization. Those results are shown in the last column (Comments). These data are expressed as % of neutralization at a specified dilution of sera. For example, “40% neutralization in 1:10” means that all 5 mice showed 40% neutralization when the respective immune sera were diluted by a factor of 10. “3/5 20% neutralization in 1:10” means that 3 of the 5 mice showed 20% neutralization when the respective immune sera were diluted by a factor of 10. A blank square indicates that the respective immunogen was not tested in that assay. “nd” means not detectable; i.e., below the limit of detection of the assay.

Table 9. Immunogenicity of immune sera elicited by immunogens

Trial	Immunogen Dose (immunogen+25µg CpG)	dose 1	dose 2	dose 3	Interval	N	1lp1b binding (kinetic ELISA)	1lp1b(K272E) binding (kinetic ELISA)	RSV F Peptide binding (kinetic ELISA)	RSV F protein binding (kinetic ELISA)	Neutralization assay		Comments
							mean	mean	mean	mean	(flow cytometry)		
											Frequency	Magnitude	
2	10 µg	1lp1b	1lp1b		3 weeks	5	75.8	67.4		nd	nd	nd	
	10 µg	1lp1b(-) PADRE	1lp1b(-) PADRE			5	2.8	2.3		0.2	nd	nd	
	10 µg	Is2xa (E.coli)	Is2xa (E.coli)			5	0.2	0.8		1.2	nd	nd	
	10 µg	1lp1b (E.coli)	1lp1b (E.coli)			5	0.4	0.2		0.4	nd	nd	
	10 µg	1lp1b	1lp1b	1lp1b	4 weeks	5	113	88		nd	nd	nd	
	10 µg	1lp1b	1lp1b	1lp1b		5	3.8	4.2		nd	nd	nd	
	10 µg	Is2xa (E.coli)	Is2xa (E.coli)	Is2xa (E.coli)		5	0.2	0.4		nd	nd	nd	
	10 µg	1lp1b (E.coli)	1lp1b (E.coli)	1lp1b (E.coli)		5	1	nd		2.21	nd	nd	
3	20 µg	1lp1b_ Ferritin	1lp1b_ Ferritin	1lp1b_ Ferritin	2 weeks	5	12	15.33			nd	nd	
	20 µg	1lp1b_ Glyc 1	1lp1b_ Glyc 1	1lp1b_ Glyc 1		5	3.6	2			nd	nd	
	20 µg	1lp1b_ surf 1	1lp1b_ Glyc 1	1lp1b_ surf 1		5	0.6	1			nd	nd	
	20 µg	1lp1b_ surf 8	1lp1b_ Glyc 1	1lp1b_ surf 8		5	0.8	0.4			nd	nd	
4	20 µg	1lp1b	1lp1b		2 weeks	5			1.8	2.2	nd	nd	
	20 µg	L41A	L41A			5			0.4	1.6	nd	nd	
	20 µg	L42A	L42A			5			0	2.6	nd	nd	
	20 µg	Glyc 2	Glyc 2			5			2.6	0.8	nd	nd	
	20 µg	113L F27A	113L F27A			5			0.4	1.4	nd	nd	
	20 µg	113L F27A	L42A			5			0.4	1	nd	nd	Additional Neutralization Results:
	20 µg	1lp1b	1lp1b	1lp1b- Ferritin	2 weeks	5	18.8	19	0.2	1.8	2	15-20	40% neutralization in 1:10
	20 µg	L41A	L41A	1lp1b- Ferritin		5	15.2	15.6	0.2	1.2	2	10-17	40% neutralization in 1:10
	20 µg	L42A	L42A	1lp1b- Ferritin		5	12.8	10.2	nd	2.2	2	15	40% neutralization in 1:10
	20 µg	Glyc 2	Glyc 2	1lp1b- Ferritin		5	1.8	1.8	0.6	0.4	2	10-13	40% neutralization in 1:10
	20 µg	113L F27A	113L F27A	1lp1b- Ferritin		5	6.2	5.4	nd	0.6	2	10	40% neutralization in 1:10
	20 µg	113L F27A	L42A	Glyc 2		5	11.4	11.4	0.4	0.8	3	9-15	40% neutralization in 1:10
5	20 µg	k46A	k46A	1lp1b_ Ferritin	2 weeks	5	14.8	6.6	nd	1.75	4	10-20	
	20 µg	q52A	q52A	1lp1b_ Ferritin		5	7.8	7.2	nd	3.6	4	11-20	
	20 µg	L141L/L42V	L141L/L42V	1lp1b_ Ferritin		5	19	12.8	nd	2.75	3	10-16	
	20 µg	eumS	eumS	1lp1b_ Ferritin		5	12	11.6	nd	2.2	3	10-24	
	20 µg	eumSP	eumSP	1lp1b_ Ferritin		5	25.4	22.8	nd	3	3	10-20	
	20 µg	eumL	eumL	1lp1b_ Ferritin		5	15.4	14.8	0.4	3.4	nd	~10	50% neutralization in 1:10
	20 µg	eumLP	eumLP	1lp1b_ Ferritin		5	13.5	15	0.2	2.8	4	13-27	
6	20 µg	L16A	L16A	L16A	2 weeks	5	13	15.4	0.6	1.4	nd	nd	3/5 20% neutralization in 1:10
	20 µg	Neg 1	Neg 1	Neg 1		5	10.6	14.2	0.2	3	nd	nd	3/4 50% neutralization in 1:10
	20 µg	113A	113A	113A		5	1.8	2	0.4	1.2	nd	nd	3/5 35% neutralization in 1:10
	20 µg	F27A	F27A	F27A		5	3	2.2	0.2	2.8	nd	nd	3/5 25% neutralization in 1:10
PROSCI							1lp1b binding	1lp1b(K272E) binding	RSV F Peptide binding	RSV F protein binding	Neutralization assay		
Trial	Vaccination	dose 1,2	dose 3,4	dose 5,6	Interval	N	(kinetic ELISA)	(kinetic ELISA)	(kinetic ELISA)	(kinetic ELISA)	(flow cytometry)		
1139	20µg scaffold + 50µg CpG – 25µl in 2% alum	2X 1lp1b	2X 1lp1b	2X 1lp1b_ Ferritin	2 weeks	5			nd	4.6	nd	nd	
1140		2X 1lp1b	2X 1s2xa (CagZ)	2X 1lp1b_ Ferritin		5			4.25	4.75	nd	nd	
1141		2X 1lp1b	2X 1lp1b_surf1			5			nd	59	nd	nd	
1142		2X 1s2xa (CagZ)	2X 1s2xa (CagZ)			5			0.4	1.4	nd	nd	
1143		2X 1s2xa (CagZ)	2X 1lp1b			5			0.2	3.8	nd	nd	
1144		2X peptide	2X 1lp1b			5			49.8	9.4	nd	nd	

The results in Table 9 indicate that immune sera elicited by immunogens of the embodiments demonstrated binding to 1lp1b_003 (referred to in Table 9 as 1lp1b) under the conditions tested. Some binding was also observed to 1lp1b_003_K272E under the conditions tested. A number of the immune sera also bound to RSV F peptide or RSV F protein under the conditions tested. Interestingly, immune sera from all 5 mice immunized twice with 1lp1b_003 followed by two boosts with 1lp1b_003_Surf1 (trial 1141) showed high binding to RSV F protein. Some of the immunogens also elicited a neutralizing humoral immune response. Interestingly, boosting with a multivalent immunogen comprising ferritin stimulated a neutralizing humoral immune response. It is to be appreciated that while a number of immunogen immunizations led to undetectable neutralization, those results do not preclude other prime and/or boost conditions being found that could enable neutralization with such immunogens.

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims.

What is claimed:

1. An RSV immunogen comprising an amino acid sequence of 1LP1_b (SEQ ID NO: 11) having from one to twenty amino acid substitutions, wherein at least one amino acid substitution is selected from the group consisting of:

- 5 (a) substitution of a serine at amino acid position N-25 in SEQ ID NO:11;
- (b) substitution of a leucine at amino acid position I-28 in SEQ ID NO:11;
- (c) substitution of a serine at amino acid position Q-29 in SEQ ID NO: 11;
- (d) substitution of an isoleucine at amino acid position L-31 in SEQ ID NO:11;
- (e) substitution of an asparagine at amino acid position K-32 in SEQ ID NO:11;
- 10 (f) substitution of an aspartic acid at amino acid position K-4 in SEQ ID NO:11;
- (g) substitution of a lysine at amino acid position Q-6 in SEQ ID NO:11;
- (h) substitution of a lysine at amino acid position Q-7 in SEQ ID NO:11;
- (i) substitution of a leucine at amino acid position N-8 in SEQ ID NO:11;
- (j) substitution of a serine at amino acid position F-10 in SEQ ID NO:11; and
- 15 (k) substitution of an asparagine at amino acid position Y-11 in SEQ ID NO:11.

2. The RSV immunogen of Claim 1, wherein said RSV immunogen comprises all of said substitutions (a) through (k).

3. The RSV immunogen of Claim 1, wherein said RSV immunogen comprises an amino acid sequence that is at least 95% identical to an amino acid sequence of a protein
20 selected from the group consisting of 1lp1b_001 (SEQ ID NO:18), 1lp1b_002 (SEQ ID NO:21), 1lp1b_003 (SEQ ID NO:24), and 1lp1b_004 (SEQ ID NO:149).

4. The RSV immunogen of Claim 1, wherein said RSV immunogen comprises an amino acid sequence of a protein selected from the group consisting of 1lp1b_001 (SEQ ID NO:18), 1lp1b_002 (SEQ ID NO:21), 1lp1b_003 (SEQ ID NO:24), and 1lp1b_004
25 (SEQ ID NO:149).

5. An RSV immunogen comprising amino acid sequence SEQ ID NO:24.
6. The RSV immunogen of Claim 1, wherein said RSV immunogen comprises an amino acid sequence of a protein selected from the group consisting of mota_1lp1b.m1.c1.d1_glyc1 (SEQ ID NO:55), mota_1lp1b.m1.c1.d1_glyc2 (SEQ ID NO:56), 1lp1b_003_Glyc1 (SEQ ID NO:39), 1lp1b_003_Glyc2 (SEQ ID NO:42), 1lp1b_003_Glyc3 (SEQ ID NO:45), 1lp1b_003_Glyc4 (SEQ ID NO:48), 1lp1b_003_Glyc5 (SEQ ID NO:51), and 1lp1b_003_Glyc6 (SEQ ID NO:54).
7. The RSV immunogen of Claim 1, wherein said RSV immunogen comprises an amino acid sequence of a protein selected from the group consisting of mota_1lp1b.m1.c1.d1_des1_1 (SEQ ID NO:66), mota_1lp1b.m1.c1.d1_des1_2 (SEQ ID NO:67), mota_1lp1b.m1.c1.d1_des1_3 (SEQ ID NO:68), mota_1lp1b.m1.c1.d1_des1_5 (SEQ ID NO:69), mota_1lp1b.m1.c1.d1_des1_6 (SEQ ID NO:70), mota_1lp1b.m1.c1.d1_des1_7 (SEQ ID NO:71), mota_1lp1b.m1.c1.d1_des1_8 (SEQ ID NO:72), mota_1lp1b.m1.c1.d1_des1_9 (SEQ ID NO:73), mota_1lp1b.m1.c1.d1_des1_10 (SEQ ID NO:74), mota_1lp1b.m1.c1.d1_des1_11 (SEQ ID NO:75), 1lp1b_003_Surf1 (SEQ ID NO:59), 1lp1b_003_Surf6 (SEQ ID NO:62), and 1lp1b_003_Surf8 (SEQ ID NO:65).
8. The RSV immunogen of Claim 1, wherein said RSV immunogen is a multivalent immunogen.
9. The RSV immunogen of Claim 1, wherein said RSV immunogen comprises an amino acid sequence of a protein selected from the group consisting of 1lp1b_003_ferritin (SEQ ID NO:138), 1lp1b_003_eumS (SEQ ID NO:140), 1lp1b_003_eumSP (SEQ ID NO:142), 1lp1b_003_eumL (SEQ ID NO:144), and 1lp1b_003_eumLP (SEQ ID NO:146).
10. The RSV immunogen of Claim 1, wherein said RSV immunogen comprises an amino acid sequence of a protein selected from the group consisting of 1lp1b_003_K46A (SEQ ID NO:78), 1lp1b_003_Q52A (SEQ ID NO:81), 1lp1b_003_I13L_F27A (SEQ ID NO:87), 1lp1b_003_L41I_L42V (SEQ ID NO:90), 1lp1b_003_L41I_L42A (SEQ ID NO:93), 1lp1b_003_I13A (SEQ ID NO:105), 1lp1b_003_L16A (SEQ ID NO:108),

1lp1b_003_F27A (SEQ ID NO:111), 1lp1b_003_L41A (SEQ ID NO:114),
1lp1b_003_L42A (SEQ ID NO:117), and 1lp1b_003_Neg1 (SEQ ID NO:132).

11. An RSV immunogen comprising an amino acid sequence of truncated 1S2X_a
(SEQ ID NO:13) having from one to twenty-five amino acid substitutions, wherein at least
5 one amino acid substitution is selected from the group consisting of:

- (a) substitution of a serine at amino acid position 92 in SEQ ID NO:13;
- (b) substitution of a leucine at amino acid position 95 in SEQ ID NO:13;
- (c) substitution of a serine at amino acid position 96 in SEQ ID NO:13;
- (d) substitution of an isoleucine at amino acid position 98 in SEQ ID NO:13;
- 10 (e) substitution of an asparagine at amino acid position 99 in SEQ ID NO:13;
- (f) substitution of an aspartic acid at amino acid position 100 in SEQ ID NO:13;
- (g) substitution of an asparagine at amino acid position 105 in SEQ ID NO:13;
- (h) substitution of an aspartic acid at amino acid position 106 in SEQ ID NO:13;
- (i) substitution of a lysine at amino acid position 108 in SEQ ID NO:13;
- 15 (j) substitution of a lysine at amino acid position 109 in SEQ ID NO:13;
- (k) substitution of a leucine at amino acid position 110 in SEQ ID NO:13;
- (l) substitution of a serine at amino acid position 112 in SEQ ID NO:13; and
- (m) substitution of an asparagine at amino acid position 113 in SEQ ID NO:13.

12. The RSV immunogen of Claim 11, wherein said RSV immunogen comprises all of
20 said substitutions (a) through (m).

13. The RSV immunogen of Claim 11, wherein said RSV immunogen comprises an
amino acid sequence that is at least 95% identical to an amino acid sequence of a protein
selected from the group consisting of 1s2xa_001 (SEQ ID NO:152), 1s2xa_002 (SEQ ID
NO:155), 1s2xa_003 (SEQ ID NO:158), and 1s2xa_004 (SEQ ID NO:164).

14. The RSV immunogen of Claim 11, wherein said RSV immunogen comprises an amino acid sequence of a protein selected from the group consisting of 1s2xa_001 (SEQ ID NO:152), 1s2xa_002 (SEQ ID NO:155), 1s2xa_003 (SEQ ID NO:158), and 1s2xa_004 (SEQ ID NO:164).
- 5 15. The RSV immunogen of any of Claims 1-14, wherein said RSV immunogen binds motavizumab antibody.
16. The RSV immunogen of any of Claims 1-14, wherein said RSV immunogen elicits a humoral immune response against RSV.
- 10 17. The RSV immunogen of Claim 16, wherein said RSV immunogen does not elicit a cellular immune response.
18. The RSV immunogen of any of Claims 1-14, wherein said RSV immunogen has a motavizumab antibody-binding domain comprising less than 12 consecutive amino acids from a motavizumab antibody-binding domain of RSV fusion protein.
- 15 19. The RSV immunogen of any of Claims 1-14, wherein said RSV immunogen has a motavizumab antibody-binding domain comprising less than 9 consecutive amino acids from a motavizumab antibody-binding domain of RSV fusion protein.
20. A nucleic acid molecule comprising a nucleic acid sequence that encodes an immunogen of Claims 1-19.
- 20 21. A recombinant molecule comprising a nucleic acid molecule of Claim 20 operatively linked to a transcription control sequence.
22. The recombinant molecule of Claim 21 wherein said recombinant molecule is a nucleic acid vaccine.
23. A recombinant cell comprising a recombinant molecule of Claim 21.
24. A method to produce an RSV immunogen of any of Claims 1-19 comprising
25 culturing a recombinant cell of Claim 23.
25. A composition comprising an immunogen, nucleic acid molecule, recombinant molecule, or recombinant cell of any of Claims 1-23.

26. A vaccine comprising an RSV immunogen of any of Claims 1-19.

27. A method to elicit a neutralizing humoral immune response against RSV, said method comprising administering an RSV immunogen of any of Claims 1-19, a vaccine of Claim 26, or a vaccine of Claim 22, wherein said administration elicits a neutralizing
5 humoral immune response against RSV.

28. A method to protect a patient from RSV infection comprising administering to said patient an RSV immunogen of any of Claims 1-19, a vaccine of Claim 26, or a vaccine of Claim 22 to a patient, wherein said administration protects said patient from RSV infection.

10 29. An immunogen comprising an antibody-binding domain that binds an antibody selected from the group consisting of motavizumab and 101F antibody,

wherein the three-dimensional structure of said antibody-binding domain of said immunogen spatially corresponds to a three-dimensional structure of an antibody-binding domain of a fusion (F) peptide derived from respiratory syncytial virus (RSV) fusion (F)
15 protein in a complex selected from the group consisting of:

(a) a complex between a F peptide consisting of amino acid sequence SEQ ID NO:2 and motavizumab, said complex being set forth in the three-dimensional model defined by the atomic coordinates specified in Protein Data Bank accession code 3IXT; and

20 (b) a complex between a F peptide consisting of amino acid sequence SEQ ID NO:4 and 101F antibody, said complex being set forth in the three-dimensional model defined by the atomic coordinates specified in Protein Data Bank accession code 3O41;

wherein said antibody-binding domain of said immunogen comprises less than 12
25 consecutive amino acids from a motavizumab-binding domain or a 101F antibody-binding domain of RSV F protein, and wherein said immunogen elicits a humoral immune response against RSV.

30. The immunogen of Claim 29, wherein said antibody-binding domain of said immunogen comprises contact residues that have a spatial orientation represented by

atomic coordinates that have a root mean square deviation of protein backbone atoms of less than 10 angstroms from the corresponding backbone atoms of contact residues in an RSV F peptide that contacts motavizumab or 101F antibody in a complex set forth in Claim 29.

5 31. The immunogen of Claim 29, wherein said immunogen comprises contact residues of the motavizumab-binding domain embedded in a protein scaffold comprising a three-dimensional structure having two alpha helices defined by atomic coordinates that have a root mean square deviation of protein backbone atoms of less than 10 angstroms when superimposed on the two alpha helices of the peptide consisting of amino acid sequence
10 SEQ ID NO:2 when complexed with motavizumab, the three-dimensional model of said complex being defined by the coordinates specified in Protein Data Bank accession code 3IXT.

32. The immunogen of Claim 29, wherein said immunogen comprises contact residues of the 101F antibody-binding domain embedded in a protein scaffold comprising a three-
15 dimensional structure with atomic coordinates that have a root mean square deviation of protein backbone atoms of less than 10 angstroms when superimposed on the peptide consisting of amino acid sequence SEQ ID NO:4 when complexed with 101F antibody, the 3-dimensional model of said complex being defined by the coordinates specified in Protein Data Bank accession code 3O41.

20 33. The immunogen of Claim 31, wherein said scaffold comprises *Staphylococcus aureus* protein A, *Helicobacter pylori* CagZ protein, or equine infectious anemia virus p26 protein.

34. An RSV immunogen of Claim 29, wherein said antibody-binding domain of said immunogen comprises less than 9 consecutive amino acids from a motavizumab-binding
25 domain or a 101F antibody-binding domain of RSV F protein.

35. The immunogen of Claim 29, wherein the motavizumab-binding domain of said immunogen comprises less than 15 amino acids of the motavizumab-binding domain from said RSV F peptide, wherein said amino acids are in clusters of no more than 8 consecutive amino acids per cluster.

36. The immunogen of Claim 29, wherein the motavizumab-binding domain of said immunogen comprises less than 15 amino acids of the motavizumab-binding domain from said RSV F peptide, wherein said amino acids are in clusters of no more than 3 consecutive amino acids per cluster.

5 37. The immunogen of Claim 29, wherein the 101F antibody-binding domain of said immunogen comprises no more than 10 amino acids of the 101F antibody-binding domain of said RSV F peptide, wherein said amino acids are in clusters of no more than 8 consecutive amino acids per cluster.

10 38. The immunogen of Claim 29, wherein said immunogen comprises an amino acid sequence of a Protein Data Bank accession code selected from the group consisting of 1LP1, 1S2X and 2EIA.

15 39. The immunogen of Claim 29, wherein said immunogen comprises an amino acid sequence of a protein selected from the group consisting of 1LP1_b (SEQ ID NO:11), 1S2X_a (SEQ ID NO:12), truncated 1S2X_a (SEQ ID NO:13), and 2EIA_a (SEQ ID NO:14).

20 40. The immunogen of Claim 29, wherein said immunogen comprises an amino acid sequence selected from the group consisting of 1LP1_b (SEQ ID NO:11), 1S2X_a (SEQ ID NO:12), truncated 1S2X_a (SEQ ID NO:13), and 2EIA_a (SEQ ID NO:14), wherein said amino acid sequence comprises at least one substitution selected from the group consisting of:

(a) substitution of a serine for the amino acid spatially corresponding to the amino acid at position 2 of SEQ ID NO:2;

(b) substitution of a leucine for the amino acid spatially corresponding to the amino acid at position 5 of SEQ ID NO:2;

25 (c) substitution of a serine for the amino acid spatially corresponding to the amino acid at position 6 of SEQ ID NO:2;

(d) substitution of an isoleucine for the amino acid spatially corresponding to the amino acid at position 8 of SEQ ID NO:2;

(e) substitution of an asparagine for the amino acid spatially corresponding to the amino acid at position 9 of SEQ ID NO:2;

(f) substitution of an aspartic acid for the amino acid spatially corresponding to the amino acid at position 10 of SEQ ID NO:2;

5 (g) substitution of a threonine for the amino acid spatially corresponding to the amino acid at position 14 of SEQ ID NO:2;

(h) substitution of an asparagine for the amino acid spatially corresponding to the amino acid at position 15 of SEQ ID NO:2;

10 (i) substitution of an aspartic acid for the amino acid spatially corresponding to the amino acid at position 16 of SEQ ID NO:2;

(j) substitution of a lysine for the amino acid spatially corresponding to the amino acid at position 18 of SEQ ID NO:2;

(k) substitution of a lysine for the amino acid spatially corresponding to the amino acid at position 19 of SEQ ID NO:2;

15 (l) substitution of a leucine for the amino acid spatially corresponding to the amino acid at position 20 of SEQ ID NO:2;

(m) substitution of a serine for the amino acid spatially corresponding to the amino acid at position 22 of SEQ ID NO:2; and

20 (n) substitution of an asparagine for the amino acid spatially corresponding to the amino acid at position 23 of SEQ ID NO:2.

41. The immunogen of Claim 40, wherein said immunogen comprises all of said substitutions.

42. The immunogen of Claim 32, wherein said protein scaffold comprises an amino acid sequence comprising at least one substitution selected from the group consisting of:

25 (a) substitution of a lysine for the amino acid spatially corresponding to the amino acid at position 1 of SEQ ID NO:4;

(b) substitution of an arginine for the amino acid spatially corresponding to the amino acid at position 3 of SEQ ID NO:4;

(c) substitution of a isoleucine for the amino acid spatially corresponding to the amino acid at position 5 of SEQ ID NO:4;

5 (d) substitution of a isoleucine for the amino acid spatially corresponding to the amino acid at position 6 of SEQ ID NO:4;

(e) substitution of a lysine for the amino acid spatially corresponding to the amino acid at position 7 of SEQ ID NO:4;

10 (f) substitution of a threonine for the amino acid spatially corresponding to the amino acid at position 8 of SEQ ID NO:4;

(g) substitution of a phenylalanine for the amino acid spatially corresponding to the amino acid at position 9 of SEQ ID NO:4; and,

(h) substitution of a serine for the amino acid spatially corresponding to the amino acid at position 10 of SEQ ID NO: 4.

15 43. The immunogen of Claim 42, wherein said immunogen comprises all of said substitutions.

44. The immunogen of Claim 29, wherein said immunogen comprises an amino acid sequence of a protein selected from the group consisting of 1lp1b_001 (SEQ ID NO:18), 1lp1b_002 (SEQ ID NO:21), 1lp1b_003 (SEQ ID NO:24), 1lp1b_004 (SEQ ID NO:149),
20 1s2xa_001 (SEQ ID NO:152), 1s2xa_002 (SEQ ID NO:155), 1s2xa_003 (SEQ ID NO:158), 1s2xa_004 (SEQ ID NO:164), 2eiaa_001 (SEQ ID NO:167), and 2eiaa_002 (SEQ ID NO:170).

45. The RSV immunogen of Claim 29, wherein said RSV immunogen comprises an amino acid sequence of a protein selected from the group consisting of
25 mota_1lp1b.m1.c1.d1_glyc1 (SEQ ID NO:55), mota_1lp1b.m1.c1.d1_glyc2 (SEQ ID NO:56), 1lp1b_003_Glyc1 (SEQ ID NO:39), 1lp1b_003_Glyc2 (SEQ ID NO:42), 1lp1b_003_Glyc3 (SEQ ID NO:45), 1lp1b_003_Glyc4 (SEQ ID NO:48), 1lp1b_003_Glyc5 (SEQ ID NO:51), and 1lp1b_003_Glyc6 (SEQ ID NO:54).

46. The RSV immunogen of Claim 29, wherein said RSV immunogen comprises an amino acid sequence of a protein selected from the group consisting of mota_1lp1b.m1.c1.d1_des1_1 (SEQ ID NO:66), mota_1lp1b.m1.c1.d1_des1_2 (SEQ ID NO:67), mota_1lp1b.m1.c1.d1_des1_3 (SEQ ID NO:68), mota_1lp1b.m1.c1.d1_des1_5 (SEQ ID NO:69), mota_1lp1b.m1.c1.d1_des1_6 (SEQ ID NO:70), mota_1lp1b.m1.c1.d1_des1_7 (SEQ ID NO:71), mota_1lp1b.m1.c1.d1_des1_8 (SEQ ID NO:72), mota_1lp1b.m1.c1.d1_des1_9 (SEQ ID NO:73), mota_1lp1b.m1.c1.d1_des1_10 (SEQ ID NO:74), mota_1lp1b.m1.c1.d1_des1_11 (SEQ ID NO:75), 1lp1b_003_Surf1 (SEQ ID NO:59), 1lp1b_003_Surf6 (SEQ ID NO:62), and 1lp1b_003_Surf8 (SEQ ID NO:65).

47. The RSV immunogen of Claim 29, wherein said RSV immunogen is a multivalent immunogen.

48. The RSV immunogen of Claim 29, wherein said RSV immunogen comprises an amino acid sequence of a protein selected from the group consisting of 1lp1b_003_ferritin (SEQ ID NO:138), 1lp1b_003_eumS (SEQ ID NO:140), 1lp1b_003_eumSP (SEQ ID NO:142), 1lp1b_003eum_L (SEQ ID NO:144), and 1lp1b_003eum_LP (SEQ ID NO:146).

49. The RSV immunogen of Claim 29, wherein said RSV immunogen comprises an amino acid sequence of a protein selected from the group consisting of 1lp1b_003_K46A (SEQ ID NO:78), 1lp1b_003_Q52A (SEQ ID NO:81), 1lp1b_003_I13L_F27A (SEQ ID NO:87), 1lp1b_003_L41I_L42V (SEQ ID NO:90), 1lp1b-003_L41I_L42A (SEQ ID NO:93), 1lp1b_003_I13A (SEQ ID NO:105), 1lp1b_003_L16A (SEQ ID NO:108), 1lp1b_003_F27A (SEQ ID NO:111), 1lp1b_003_L41A (SEQ ID NO:114), 1lp1b_003_L42A (SEQ ID NO:117), and 1lp1b_003_Neg1 (SEQ ID NO:132).

50. The RSV immunogen of Claim 29, wherein said immunogen elicits a humoral immune response against RSV, but not a cellular immune response.

51. An immunogen comprising a protein comprising an amino acid sequence of a protein selected from the group consisting of RSV F₀ Fd (SEQ ID NO:174), RSV F Fd (SEQ ID NO:175), and RSV F₀ Fd GAG (SEQ ID NO:176).

52. A nucleic acid molecule comprising a nucleic acid sequence that encodes an immunogen of any of Claims 29-51.

53. A recombinant molecule comprising a nucleic acid molecule of Claim 52.

54. The recombinant molecule of Claim 53 wherein said recombinant molecule is a nucleic acid vaccine.

55. A recombinant cell comprising a recombinant molecule of Claim 53.

56. A method to produce an immunogen of any of Claims 29-51 comprising culturing a recombinant cell of Claim 55.

57. A composition comprising an immunogen, nucleic acid molecule, or recombinant molecule, or recombinant cell of any of Claims 29-55.

58. A vaccine comprising an immunogen of any of Claims 29-51.

59. A vaccine comprising an immunogen comprising a motavizumab-binding domain and an immunogen comprising a 101F antibody-binding domain, wherein said immunogens are set forth in any of Claims 29-51.

60. A method to elicit a neutralizing humoral immune response against RSV, said method comprising administering an RSV immunogen of any of Claims 29-51, a vaccine of Claim 58, or a vaccine of Claim 54, wherein said administration elicits a neutralizing humoral immune response against RSV.

61. A method to protect a patient from RSV infection comprising administering to said patient an RSV immunogen of any of Claims 29-51, a vaccine of Claim 58, or a vaccine of Claim 54, wherein said administration protects said patient from RSV infection.

62. An antibody protein comprising a heavy chain comprising SEQ ID NO:5, except that said antibody protein comprises at least one amino acid substitution selected from the group consisting of:

(a) substitution of the amino acid corresponding to position 32 of SEQ ID NO:5, is a histidine or a glutamic acid;

(b) substitution of the amino acid at position 35 of SEQ ID NO:5 is substituted with an alanine;

(c) substitution of the amino acid at position 52 of SEQ ID NO:5 is substituted with an amino acid selected from the group consisting of lysine, histidine, threonine,
5 serine and arginine;

(d) substitution of the amino acid at position 53 of SEQ ID NO:5 is substituted with a histidine or a serine;

(e) substitution of the amino acid at position 54 of SEQ ID NO:5 is substituted with a phenylalanine or an arginine;

10 (f) substitution of the amino acid at position 56 of SEQ ID NO:5 is substituted with an amino acid selected from the group consisting of isoleucine, serine, glutamic acid, and aspartic acid;

(g) substitution of the amino acid at position 58 of SEQ ID NO:5 is substituted with a tyrosine or a tryptophan;

15 (h) substitution of the amino acid at position 97 of SEQ ID NO:5 is substituted with an aspartic acid, a histidine or an arginine;

(i) substitution of the amino acid at position 99 of SEQ ID NO:5 is substituted with aspartic acid;

(j) substitution of the amino acid at position 100 of SEQ ID NO:5 is
20 substituted with a tyrosine, a tryptophan or a histidine; and

(k) substitution of the amino acid at position 100A of SEQ ID NO:5 is substituted with a serine or a threonine.

63. An antibody protein comprising a heavy chain comprising SEQ ID NO:6, except that said antibody protein comprises at least one amino acid substitution selected from the
25 group consisting of:

(a) substitution of the amino acid at position 32 of SEQ ID NO:6 is substituted with a phenylalanine;

(b) substitution of the amino acid at position 49 of SEQ ID NO:6 is substituted with a histidine or an arginine;

(c) substitution of the amino acid at position 92 of SEQ ID NO:6 is substituted with a lysine;

5 (d) substitution of the amino acid at position 94 of SEQ ID NO:6 is substituted with a histidine; and

(e) substitution of the amino acid at position 96 of SEQ ID NO:6 is substituted with a histidine.

64. A nucleic acid molecule comprising a nucleic acid sequence that encodes an
10 antibody protein of any of Claims 62-63.

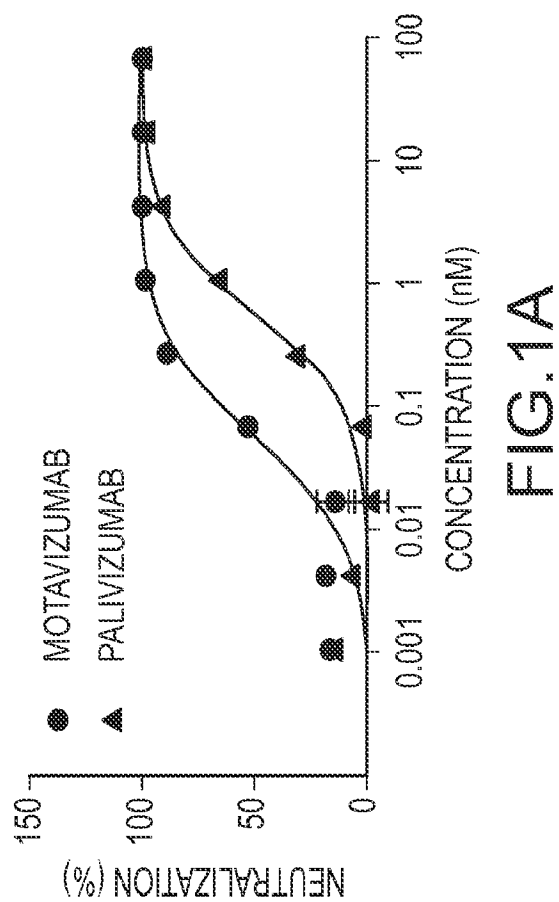
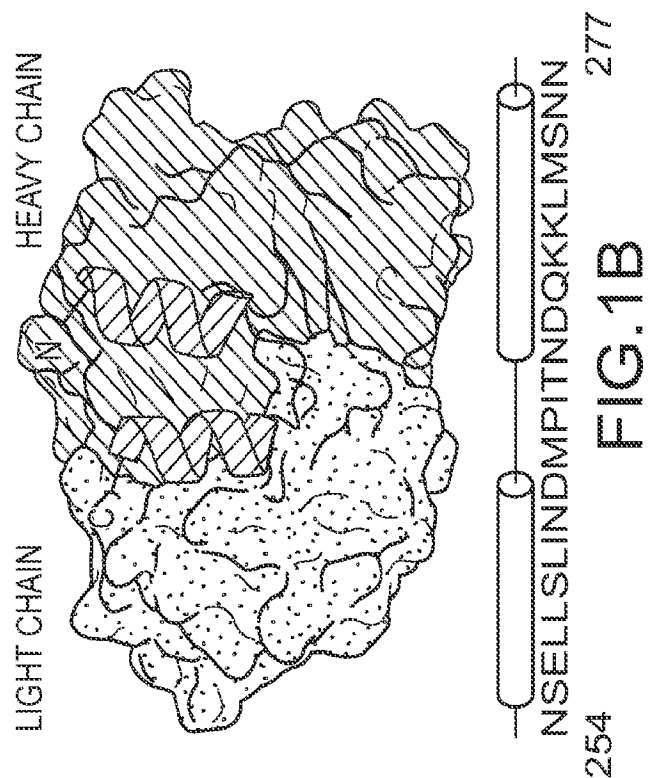
65. A recombinant molecule comprising a nucleic acid molecule of Claim 64.

66. A recombinant cell comprising a recombinant molecule of Claim 65.

67. A method to produce an antibody protein of any of Claims 62-63, comprising culturing a recombinant cell of Claim 66.

15 68. A composition comprising an antibody protein of any of Claims 62-63.

69. A method to protect a patient from RSV infection comprising administering to said patient an antibody protein of any of Claims 62-63, wherein said administration protects said patient from RSV infection.



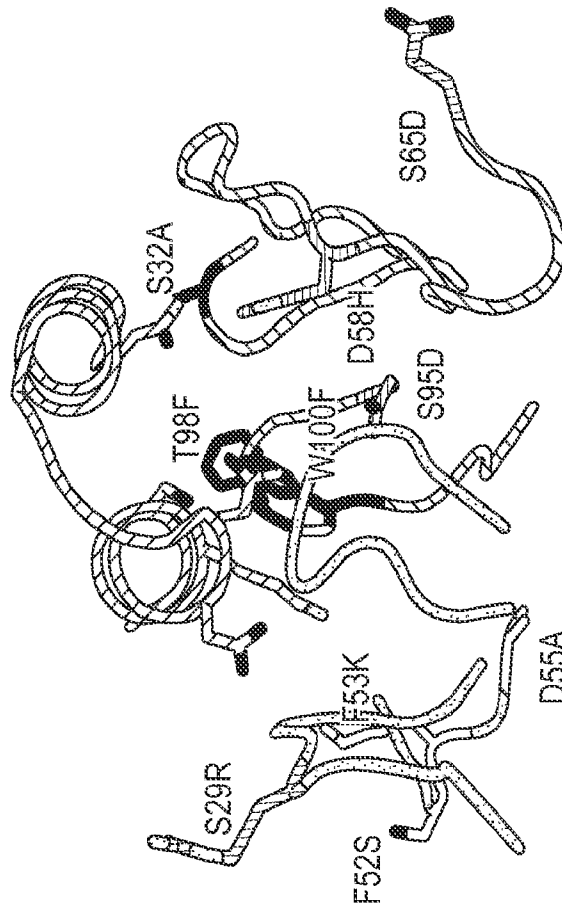


FIG.1D

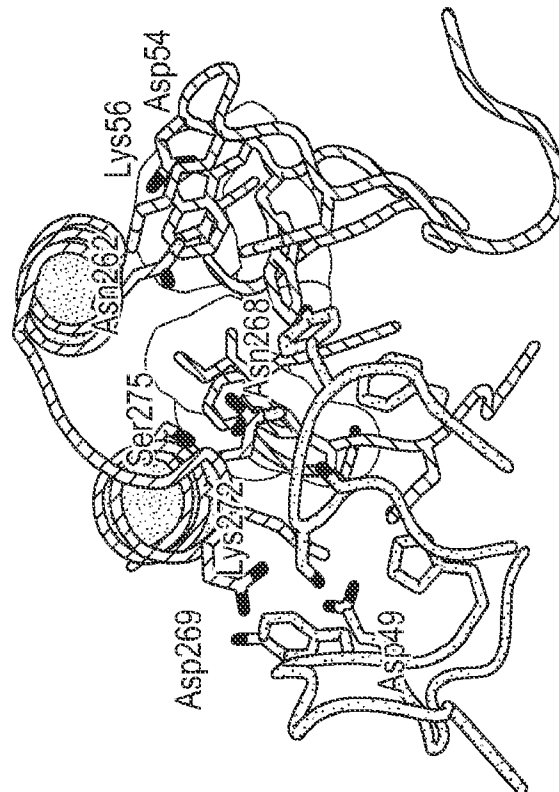


FIG.1C

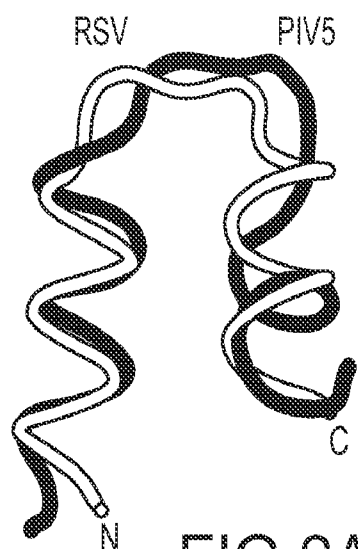


FIG.2A

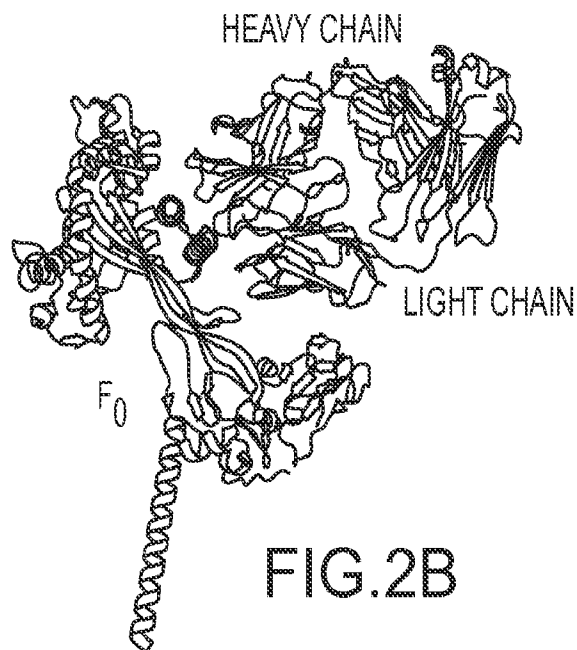


FIG.2B

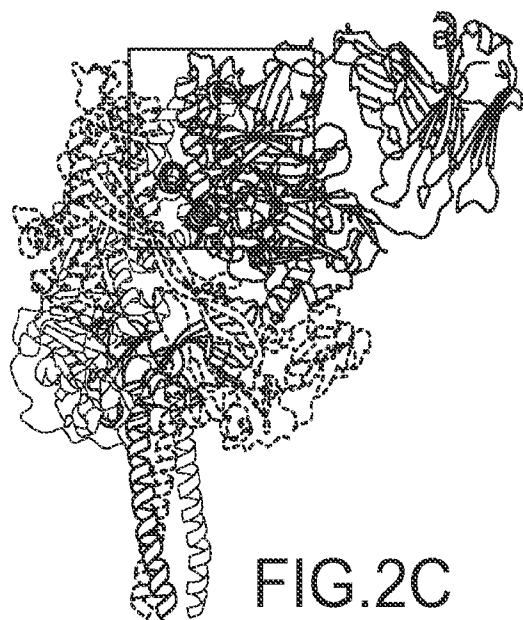


FIG.2C



FIG.2D

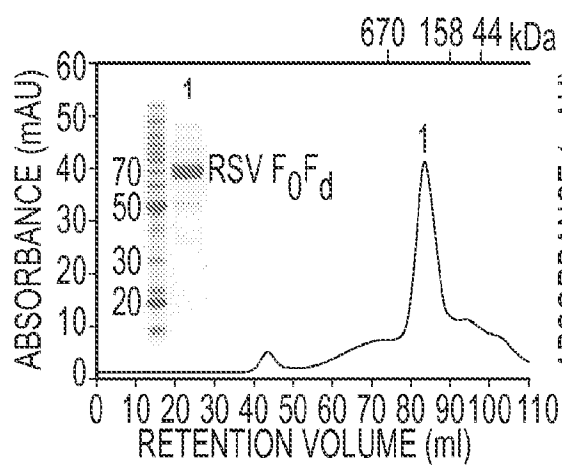


FIG.2E

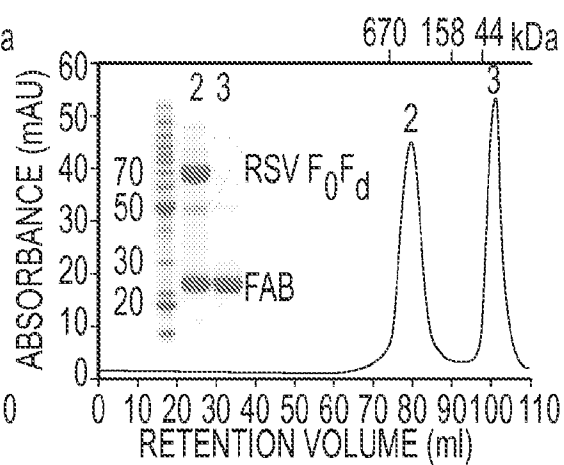


FIG.2F

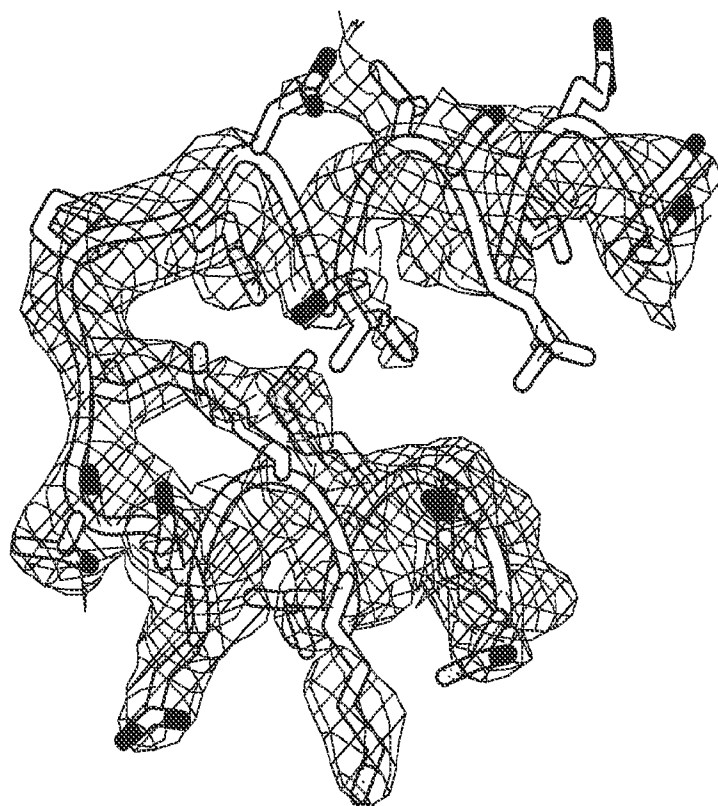


FIG.3B

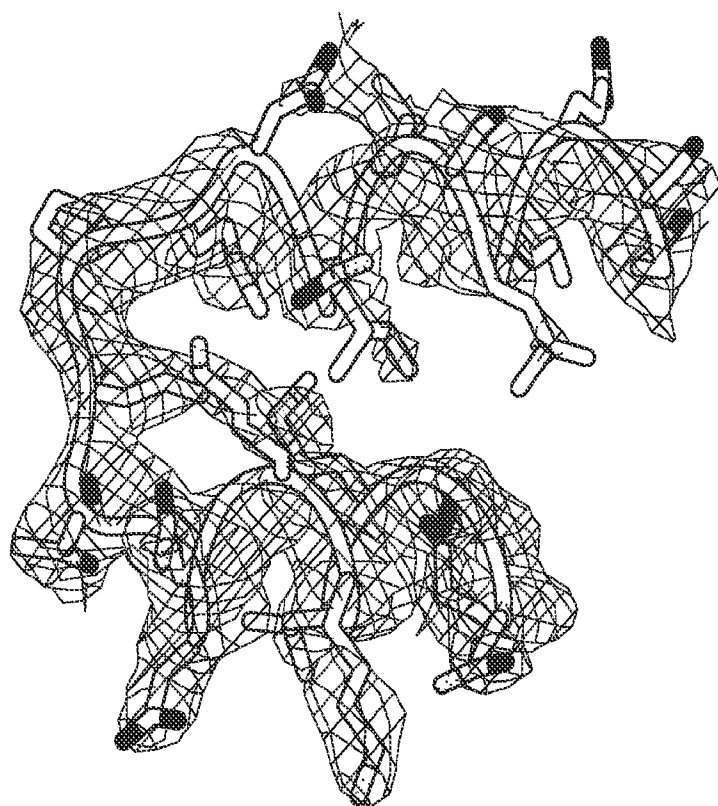


FIG.3A



FIG.4A

180°
↺

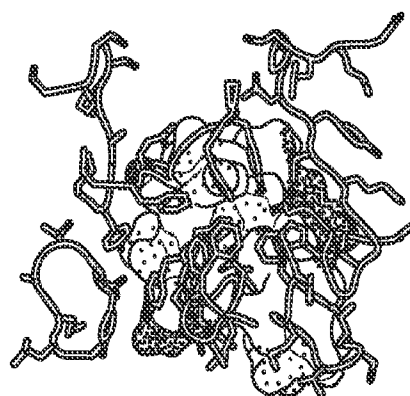
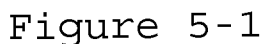


FIG.4B



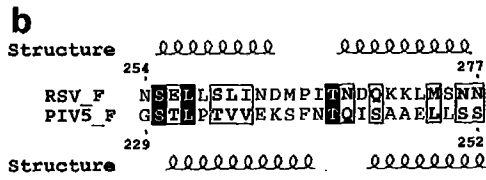


Figure 5-2

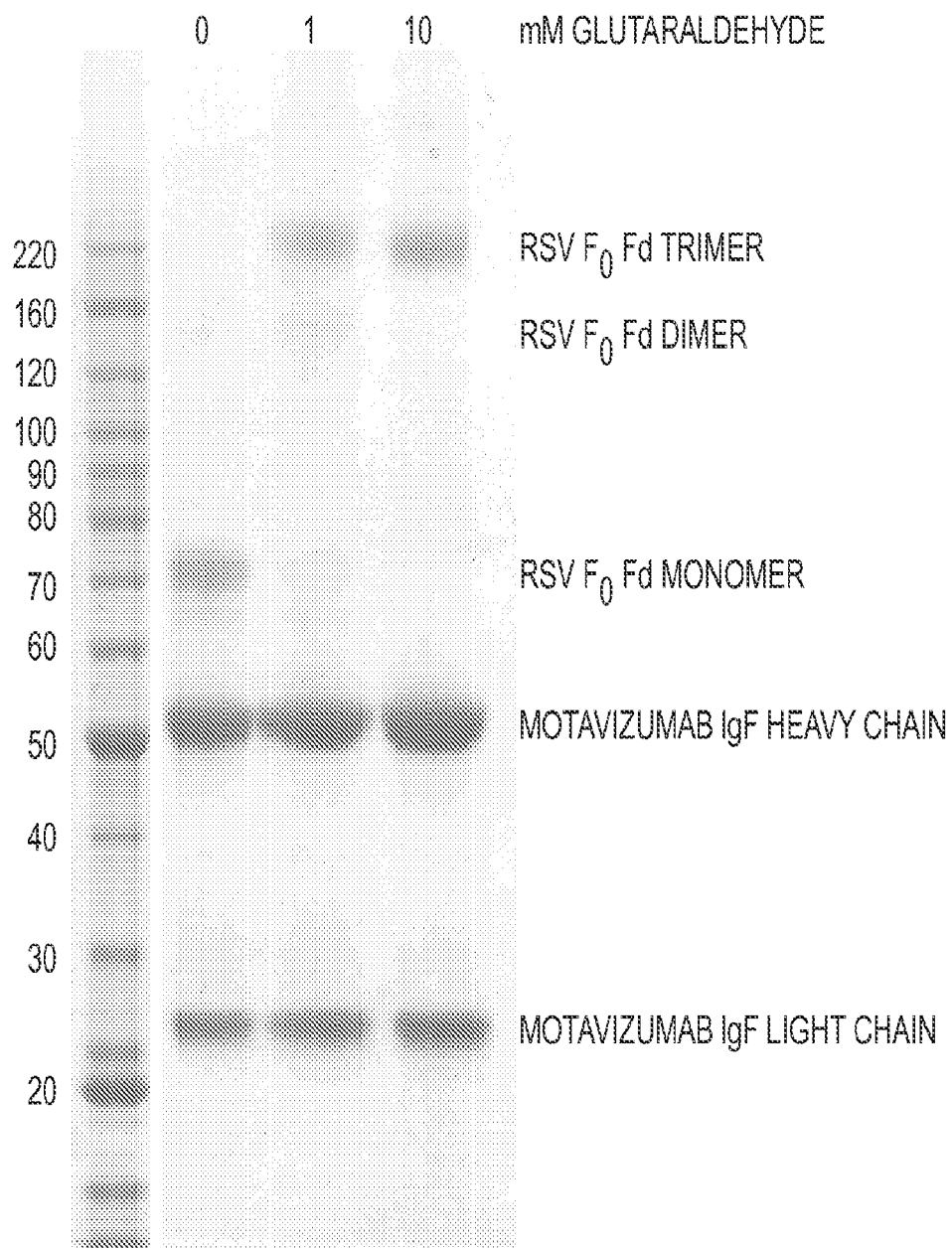
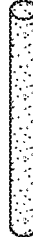


FIG.6

			
	NDQKKLMSNN	NSLLSLINDMPIT	NDQKKLMSNN
FINAL	<u>SFDEK</u> KLASNEI <u>A</u> HLPNLNEEQRS <u>A</u> FLSSINDDDPSQSANLLAEAKKLNDAAQAPK		
ORIGINAL	KFNKEQQNAFYEIILHLPNLNEEQRNAFIQSLKDDPSQSANLLAEAKKLNDAAQAPK		

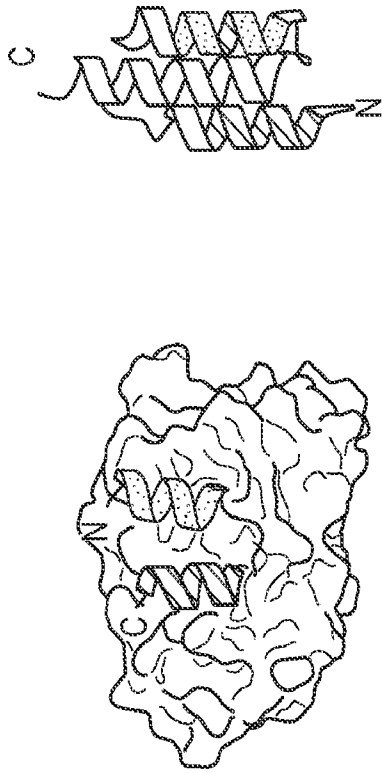


FIG.7

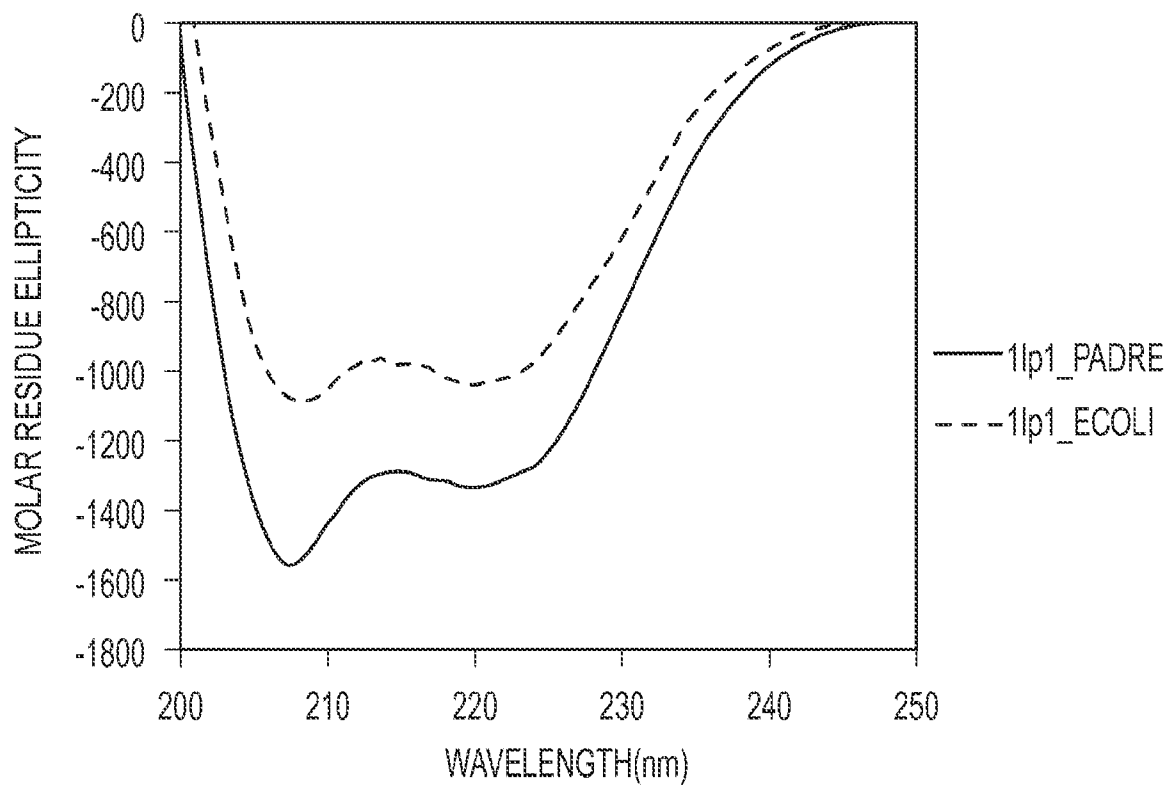


FIG.8A

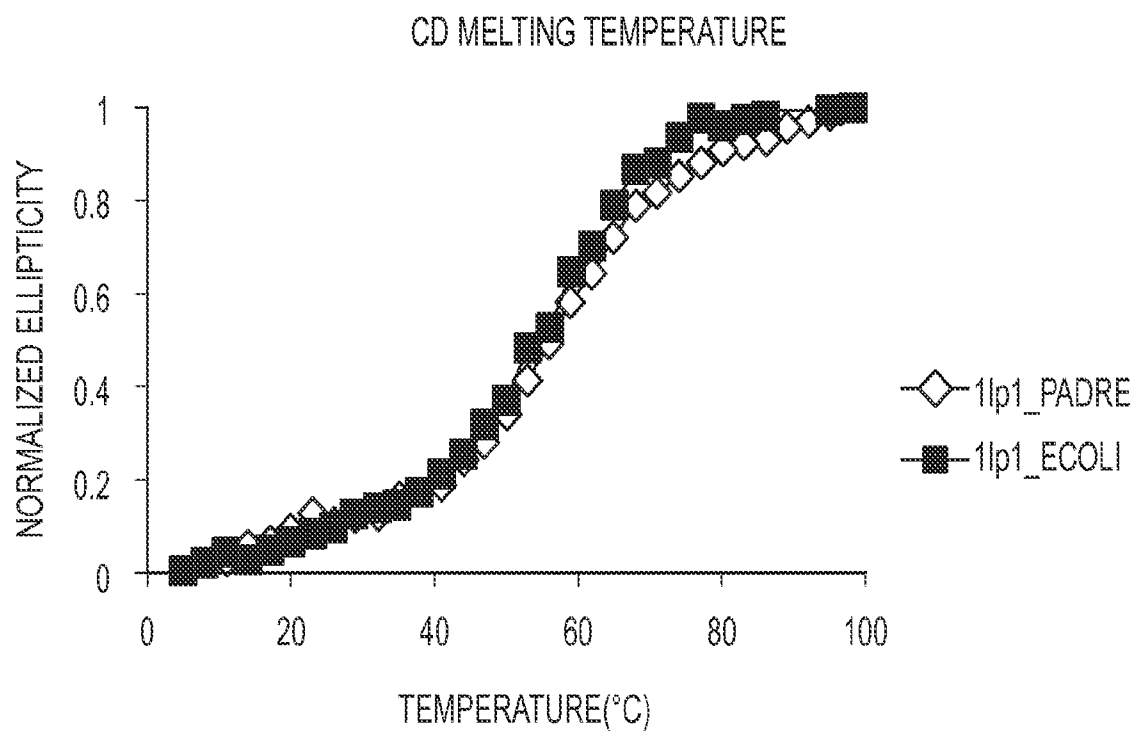


FIG.8B