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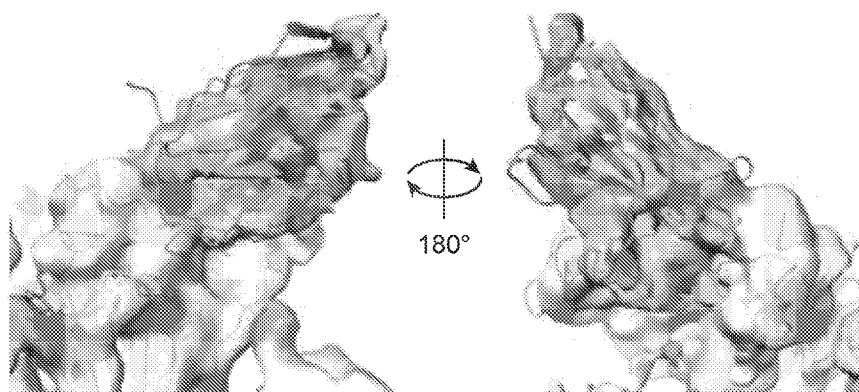


FIG. 25B

(57) Abstract: The present disclosure relates to single domain antibodies ("VHHs") against SARS-CoV-2, as well as to polypeptides comprising one or more of such VHHs. The disclosure also relates to nucleic acids encoding such VHHs and polypeptides; to methods of preparing such VHHs and polypeptides; to host cells expressing or capable of expressing such VHHs or polypeptides; to compositions comprising such VHHs, polypeptides, nucleic acids or host cells; and to uses of such VHHs, such polypeptides, such nucleic acids, such host cells or such compositions, in particular for prophylactic, therapeutic or diagnostic purposes.



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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
PATENT COOPERATION TREATY PATENT APPLICATION**

**CONSTRUCTS COMPRISING SINGLE DOMAIN VHH ANTIBODIES AGAINST
SARS-CoV-2**

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This Application claims priority to US Provisional Patent Application No. 63/129,877 filed December 23, 2020, the entire contents of which are incorporated by reference herein.

INCORPORATION BY REFERENCE OF THE SEQUENCE LISTING

[0002] The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: LUBI-033-01WO_SeqList_ST25.txt, date recorded: December 21, 2021, file size $\approx$ 238 kilobytes).

GOVERNMENT FUNDING

[0003] This disclosure was made with Government support under grant number MTEC-20-09-COVID19-029. The U.S. Government has certain rights to this disclosure.

FIELD

[0004] The present disclosure relates to single domain antibodies (“VHHs”) against SARS-CoV-2, as well as to polypeptides comprising one or more of such VHHs. The disclosure also relates to nucleic acids encoding such VHHs and polypeptides; to methods of preparing such VHHs and polypeptides; to host cells expressing or capable of expressing such VHHs or polypeptides; to compositions comprising such VHHs, polypeptides, nucleic acids or host cells; and to uses of such VHHs, such polypeptides, such nucleic acids, such host cells or such compositions, in particular for prophylactic, therapeutic or diagnostic purposes.

BACKGROUND OF THE DISCLOSURE

[0005] Coronaviruses (CoV) are known to cause relatively mild upper respiratory tract infections, and account for approximately 30% of the cases of the common cold in humans. However, a recently identified CoV, severe acute respiratory syndrome coronavirus (SARS-CoV-2), causes severe respiratory distress in humans leading to significant mortality in individuals infected. In 2020, SARS-

CoV-2 established efficient human to human transmission resulting in a worldwide pandemic. By December 20, 2021, SARS-CoV-2 was responsible for more than 5,331,019 confirmed deaths and 271,963,258 confirmed cases worldwide (see World Health Organization website, Coronavirus disease (COVID-19) pandemic).

[0006] The majority of non-hospitalized subjects infected with SARS-CoV-2 experience both GI and respiratory symptoms, and 25% experience only GI symptoms¹. Historically, 25% of MERS cases² and 16-73% of SARS cases in 2002-2003³ presented with significant gastrointestinal distress. SARS-CoV-1 is able to replicate in the GI tract and live virus can be isolated from stool samples⁴. SARS-CoV-2 shares these characteristics. Both use the ACE2 receptor, which is expressed on intestinal epithelial cells at levels nearly 100 times greater than on respiratory epithelial cells⁵; capsid proteins from SARS-CoV-2 have been identified in GI epithelial cells from subjects infected with SARS-CoV-2⁶; viral RNA is present in rectal and stool samples in 53% of hospitalized subjects and detected in stool samples in 23% of subjects after respiratory symptoms have resolved and nasopharyngeal swabs test negative^{6,7}. SARS-CoV-2 also clearly infects GI tissues of the best available animal model, shedding live virus in the stool⁸. In populations with weakened immune systems (e.g., the elderly) GI colonization may result in self-infection of the airways. The systemic inflammatory response associated with GI infection may also exacerbate pulmonary disease. Blunting the cytokine storm emanating from the gastrointestinal tract could reduce the frequency or severity of acute respiratory distress syndrome in infected subjects.

[0007] The Amoy Gardens SARS super-cluster was traced to faulty engineering that enhanced fecal-oral transmission⁹. Very high amounts of SARS-CoV-2 viral RNA are present in stool samples⁶, even in subjects who never suffer any respiratory symptoms¹. Like norovirus, SARS-CoV-2 outbreaks occur on cruise ships and in nursing homes¹⁰, where the risk of fecal-oral transmission is especially high. Similarly, a US aircraft carrier was recently forced to return to port after a SARS-CoV-2 outbreak, demonstrating the important implications for operational readiness¹⁰. Therapeutics and preventatives to treat this documented route of infection, this reservoir of ongoing viral disease, and this potential means of transmission should not be overlooked. However, no such therapeutic specifically targeting the GI viral reservoir has been developed.

[0008] The present disclosure relates to therapeutics derived from ultra-simple antibodies unique to the camelid family that possess antigen binding affinities and specificities that are comparable to the more complex heavy/light chain human antibodies. These therapeutics require only the isolated, small, single polypeptide antigen-binding protein domains, referred to as "VHHs." VHHs require no complex modifications for full bioactivity (i.e., no supramolecular assembly, no essential disulfide bonds for correct folding, and no glycosylation). VHHs are superior to human antibodies in their ability to bind to

epitopes inaccessible to the larger heavy/light chain antibodies. VHHs also tend to be more stable on the shelf and in the proteolytic environment of the gut lumen.

[0009] Antibody therapies, including VHHs, are proven therapies for enteric diseases. First successfully demonstrated in human clinical trials in 1988, the efficacy of anti-pathogen antibodies has since been replicated many times using a variety of antibody formats, disease targets, and various *in vivo* efficacy trial designs with both humans and a wide range of animal models. VHHs given orally have been shown to be effective in treating rotavirus infection in human infants, and in preventing pathogen infections in a variety of animal models including ETEC and rotavirus infection. Oral VHHs targeting TNF-alpha are an effective therapy in inflammatory bowel disease. For example, oral hyperimmune bovine colostrum is effective for preventing and treating *C. difficile* infection in piglets, and for preventing ETEC infection in humans.

[0010] Antibodies are powerful therapeutic tools; broadly reactive as a class, yet individually possessing exquisite specificity. But the enormous risk and complexity associated with their clinical development, and their slow and cumbersome manufacturing processes, have made them a poor choice for rapidly responding to threats like SARS-CoV-2. Accordingly, there remains an urgent need for a therapeutic to eliminate SARS-CoV-2 viral reservoirs to reduce overall viral burden, inhibit disease progression, accelerate viral clearance, and block the fecal-oral transmission route. The present disclosure addresses this urgent need by providing neutralizing VHHs against SARS-CoV-2.

BRIEF SUMMARY

[0011] Provided herein is a VHH antibody that binds SARS-CoV-2, wherein the VHH antibody comprises three complementarity determining regions (CDR1, CDR2, and CDR3) each of which is selected from an amino acid sequence comprising at least about 85% identity to a sequence in Table 1. In embodiments, the CDR1, CDR2, and CDR3 each comprise at least about 90%, 95%, 97%, or 99% identity to a sequence in Table 1. In embodiments, the CDR1, CDR2, and CDR3 correspond to SEQ ID NO: 610, 611, and 612. In embodiments, the VHH antibody comprises at least 85% identity with SEQ ID NO: 365. In embodiments, CDR1, CDR2, and CDR3 correspond to SEQ ID NO: 421, 422, and 423. In embodiments, the VHH antibody comprises at least 85% identity with SEQ ID NO: 302.

[0012] In embodiments, provided is a polypeptide comprising a VHH antibody. In embodiments, provided is a polypeptide comprising at least two VHH antibodies. In embodiments, the at least two VHH antibodies comprise SEQ ID NO: 365 or SEQ ID NO: 302. In embodiments, the at least two VHH antibodies are connected to each other via a linker. In embodiments, the linker comprises at least about 85% identity to SEQ ID NO: 390. In embodiments, provided is a polypeptide comprising at least three

VHH antibodies. In embodiments, provided is a polypeptide comprising at least four VHH antibodies. In embodiments, the VHH antibodies are different. In embodiments, the VHH antibodies are the same.

[0013] Provided is a pharmaceutical composition comprising a VHH antibody and a pharmaceutically acceptable carrier.

[0014] Provided is a method of treating a disease or disorder related to SARS-CoV-2, the method comprising: administering a pharmaceutical composition to a subject in need thereof, thereby treating the disease or disorder related to SARS-CoV-2. In embodiments, the subject in need thereof is infected with SARS-CoV-2 as determined by PCR assay. In embodiments, the subject in need thereof has been diagnosed as being infected with SARS-CoV-2. In embodiments, the subject in need thereof is at risk of developing Coronavirus Disease (COVID). In embodiments, the subject in need thereof is at risk of developing severe COVID. In embodiments, the subject in need thereof has one or more comorbidities. In embodiments, the subject in need thereof is treated for the one or more comorbidities. In embodiments, the pharmaceutical composition is delivered encapsulated in a spirulina cell. In embodiments, the spirulina cell is dried. In embodiments, the spirulina cell is of strain *A. platensis*. In embodiments, the pharmaceutical composition is delivered orally. In embodiments, the pharmaceutical composition is delivered via the airway. In embodiments, the pharmaceutical composition is delivered via inhalation. In embodiments, the pharmaceutical composition is delivered nasally. In embodiments, the SARS-CoV-2 is a variant of concern. In embodiments, the variant of concern is selected from the group consisting of: Alpha, Beta, Gamma, Delta, and Omicron. In embodiments, the SARS-CoV-2 evades an immune response associated with vaccine therapy.

[0015] Provided is a method of treating a disease or disorder related to SARS-CoV-2, the method comprising: administering a pharmaceutical composition to a subject in need thereof thereby treating the disease or disorder related to SARS-CoV-2, wherein the disease or disorder is recalcitrant to anti-SARS-CoV-2 vaccine therapy. In embodiments, the subject in need thereof has previously been administered an anti-SARS-CoV-2 monoclonal therapy. In embodiments, the subject in need thereof has received a booster of the anti-SARS-CoV-2 vaccine therapy. In embodiments, the subject in need thereof has not received a booster of the anti-SARS-CoV-2 vaccine therapy. In embodiments, the administering is effective in reducing or eliminating a symptom of the disease or disorder related to the SARS-CoV-2. In embodiments, the symptom is a gastrointestinal symptom. In embodiments, the symptom is a respiratory symptom. In embodiments, the administering is effective in reducing a period of illness associated with the disease or disorder related to the SARS-CoV-2 by at least about 1-3 days.

[0016] It is another object of the disclosure to provide compositions comprising a recombinant spirulina, wherein the recombinant spirulina comprises at least one VHH. In embodiments, the VHH is delivered orally to the gastrointestinal tract. In embodiments, the VHH is delivered by other non-parenteral routes such as the airway, inhalation, and/or intranasally. In embodiments, the VHH is delivered parenterally.

[0017] In embodiments, the disclosure relates to host or host cell that expresses or can express a VHH of the disclosure and/or a polypeptide of the disclosure; and/or that contains a nucleic acid encoding a VHH of the disclosure and/or a polypeptide of the disclosure.

[0018] The disclosure further relates to a product or composition containing or comprising a VHH of the disclosure, a polypeptide of the disclosure; and/or a nucleic acid of the disclosure. Such a product or composition may for example be a pharmaceutical composition or a product or composition for diagnostic use.

[0019] The disclosure further relates to methods for preparing or generating the VHHs, polypeptides, nucleic acids, host cells, products and compositions as described herein, which methods are as further described below.

[0020] The disclosure further relates to applications and uses of the above VHHs, polypeptides, nucleic acids, host cells, products and compositions described herein, which applications and uses include, but are not limited to, the applications and uses described herein below.

[0021] Other embodiments, embodiments, advantages, and applications of the disclosure will become clear from the further description herein below.

BRIEF DESCRIPTION OF DRAWINGS

[0022] **Figure 1** shows antigen binding kinetics of anti-SARS-CoV-2 VHHs of the disclosure. In brief, VHHs were purified from *E. coli*. Overall protein expression and quality were assessed using SDS-PAGE gel and protein concentrations were measured by absorbance. The antigen, recombinant RBD with the C-terminal biotin acceptor AviTag was expressed and purified from mammalian systems and the resultant protein was biotinylated using *E. coli* biotin ligase enzyme BirA (Avidity Bio). Biotinylated RBD was captured on streptavidin biosensors (ForteBio) and antigen loading was quenched using 50 μ M biocytin. Binding kinetics for anti-SARS-CoV-2 VHHs and SARS-CoV-2 RBD assayed using the BLI system. Binding curves were generated using global fitting with 1:1 interaction model and the k_D is indicated in red.

[0023] **Figure 2** shows characterization of the VHHs of the disclosure through neutralization, binding, and competition assays.

[0024] **Figure 3** shows microneutralization assay results of 141 VHHs screened at 10 µg/mL.

[0025] **Figure 4** shows representative results of microneutralization assay with serial dilution of VHHs.

[0026] **Figure 5** shows BLI-based epitope binning of SARS VHH-72 and VHHs of the disclosure. BLI-based epitope binning of SARS VHH-72 and anti-SARS-CoV-2 VHHs. Biotinylated RBD was captured on streptavidin biosensors (ForteBio) and antigen loading was quenched by incubating with 50 µM Biocytin. RBD-loaded biosensors were initially incubated with 10 µg/mL of VHH1 until saturation to assess epitope binning. This initial association step assays binding of VHH1. VHH1-saturated biosensors were transitioned to microwells containing 10 µg/mL of VHH2. Association of VHH2 can be measured during the second association step only if VHH1 and VHH2 bind different epitopes.

[0027] **Figure 6A -Figure 6C** show competition of selected VHHs of the disclosure against human ACE2-FC dimer, SARS VHH-72 dimer, and VHH R2B-D5 dimer for SARS-CoV-2-RBD binding. High-binding ELISA plates are coated with 1 µg/mL of the specified competing protein. Separately, SARS-CoV-2 RBD with C-terminal AviTag at 0.5 µg/mL was incubated for 1 hour at room temperature with a 5-fold VHH dilution series, starting at 20 µg/mL. After incubation, 100 µL of the VHH-RBD mix was added on to antigen-coated plates. RBD binding to ACE2-FC was detected using mouse anti-AviTag primary antibody (Avidity Bio) at 1:5,000 dilution and HRP conjugated goat anti-mouse secondary antibody at 1:5,000 dilution (BioRad). **Figure 6A** shows ELISA-based ACE2 competition for RBD binding where ELISA plates are coated with human ACE2-FC at 1 µg/mL. **Figure 6B** shows ELISA based SARS VHH-72-Ank1C2 dimer competition for RBD binding where ELISA plates are coated with SARS VHH-72-Ank1C2 at 1 µg/mL. **Figure 6C** shows ELISA based VHH R2B-D5-5HVZ-MBP dimer competition for RBD binding where ELISA plates are coated with R2B-D5-5HVZ-MBP at 1 µg/mL.

[0028] **Figure 7** shows protease resistance results.

[0029] **Figure 8A** shows an image of an expression gel. **Figure 8B** shows an image of a gel showing soluble protein. **Figure 8C** shows an image of a gel showing the final analysis.

[0030] **Figure 9A** shows an image of a gel showing protein expression. **Figure 9B** shows absorbance. Of note, is the presence of the two free Cys amino acids result in aggregate formation.

[0031] **Figure 10A** shows an image of a gel showing protein expression. **Figure 10B** shows absorbance. Of note, is that mutating the Cys residues to Ser resulted in monodispersed monomer protein.

[0032] **Figure 11A** shows an image of a gel showing protein expression. **Figure 11B** shows absorbance. Docking peptide fused VHH R2B-D5 expressed as MBP-fusion.

[0033] **Figure 12** shows Size-Exclusion Chromatography based comparison of the PP758 (2-R2B-D5), PP1625 (1-S3b-C8), and PP1896 (2-R2B-D5 and 1-S3b-C8) constructs. The Cerberbody PP1896 forms

a complex when mixed 1:1.5 molar ratio between the dimeric PP758 and the monomeric PP1625, thereby increasing binding avidity.

[0034] **Figure 13** shows kinetics binding responses for the Cerberbody forming components, PP758, PP917, and the Cerberbody PP1896.

[0035] **Figure 14** shows Size-Exclusion Chromatography based comparison of Cerberbody, PP1897 and complex forming components, PP917, and PP1625.

[0036] **Figure 15** shows kinetics binding responses for the Cerberbody forming components, PP758, PP917, and the Cerberbody complex PP1897.

[0037] **Figure 16** shows Size-Exclusion Chromatography based comparison of Cerberbody complex PP1898, and complex forming components, PP917, and PP1823.

[0038] **Figure 17** shows kinetics binding responses for the Cerberbody forming components, PP758, PP917, and the Cerberbody complex PP1898.

[0039] **Figure 18A** shows a graphic of an exemplary Hydrabody complex formation. **Figure 18B** shows a graphic of an exemplary Cerberbody complex formation.

[0040] **Figure 19** shows a gel of protein expression of exemplary Hydrabody forming monomeric dimer constructs PP1892, PP1893, PP1894, and PP1895.

[0041] **Figure 20A** shows an image of a gel of Ni-NTA purification results of construct PP1892. **Figure 20B** shows sizing analysis of Hydrabody forming dimeric construct PP1892.

[0042] **Figure 21A** shows an image of a gel of Hydrabody 1894. **Figure 21B** shows Size-Exclusion Chromatography analysis result for the same.

[0043] **Figure 22A** shows an image of a gel of Hydrabody 1895. **Figure 22B** shows Size-Exclusion Chromatography based analysis result for the same construct.

[0044] **Figure 23** shows a comparison of binding between referenced monomer, dimer, and trimer constructs.

[0045] **Figure 24** shows results of a binding assay showing a comparison of binding among Hydrabody forming components.

[0046] **Figure 25A** and **Figure 25B** show Cryo-EM structures of PP758 in complex with SARS-CoV-2 Spike. **Figure 25A** shows a cryo-EM reconstruction of PP758 in complex with prefusion stabilized SARS-CoV-2 S trimer at 3.9 Å. Two receptor binding domains (RBDs) are visible, colored in shades of green, with the VHH in magenta, and the rest of the spike colored in light purple with glycans in darker purple. **Figure 25B** is a close-up view of the VHH bound to the RBD. The VHH model was generated by homology modeling and rigid body docked into the electron density map.

[0047] **Figure 26** shows a graphic of epitope binning.

[0048] **Figure 27** shows results of an ELISA assay completed evaluating binding of exemplary VHHs against SARS-CoV-2 RBD variants Alpha, Beta, Gamma, and Delta.

[0049] **Figure 28** shows IC₅₀ measurement of purified VHHs PP917, PP758, PP1146 and PP1148.

[0050] **Figure 29A-Figure 29C** is a comparison of an in-house pseudovirus assay and IITRI live virus assay. **Figure 29A** shows % reduction of infectivity of an initial in-house pseudovirus assay. **Figure 29B** shows % reduction of infectivity of a pre-live in-house pseudovirus assay. **Figure 29C** shows % reduction of infectivity of an IITR pseudovirus assay.

[0051] **Figure 30A-Figure 30AK** show percent reduction of viral growth per the IITRI live virus assay. P: purified VHH, P+M: Purified VHH +matrix, C: cell extract, and REM: Remdesivir (positive control).

[0052] **Figure 31** shows TRII live virus assay results, position effect corrected and rescaled. Observed (points) rescaled TRII assay data (y-axis) are shown by dilution (x-axis) with model fit (lines) superimposed. Fits with quality parameter estimates (defined as a Dil80 estimate with $p < 0.1$ significance) are shown with solid lines; fits lacking quality parameter estimates are shown as dashed lines. Top and bottom of the assay values are given as dotted, horizontal line.

[0053] **Figure 32** shows estimates of dilution yielding 80% inhibition of viral infection (Dil80Dil80) parameter estimates are shown as points with 95% confidence intervals (range, if available) shown. An exact match between live virus assay and pseudovirus assay assessment of potency would be when Dil80Dil80 is 1.0. At values > 1.0 , the live virus assessment of potency is lower than the pseudovirus assessment. At values < 1.0 , the live virus assessment of potency is higher than the pseudovirus assessment.

[0054] **Figure 33A** shows LabChip Results of Non-Reduced (left) and Reduced (right) Proj113R3 Airway Powder: Size and Estimated Purity shown on expected peak. **Figure 33B** shows airway powder from Proj113: Absorbance 450nm vs ln (protein ug/ml) with 4-Par Logistic Curve Fit to Estimate EC₅₀ ug/ml and ELISA results for PP917 Standard and the Proj113R3 Airway Powder applying Reduced Model. **Figure 33C** shows Airway Powder from Proj137: Absorbance 450nm vs log (protein ug/ml) with 4-Par Logistic Curve Fit to Estimate EC₅₀ ug/ml and ELISA results for the PP917 Standard and the Proj137R1 Airway Powder applying Reduced Mode.

OVERVIEW

Definitions

[0055] While the following terms are believed to be well understood by one of ordinary skill in the art, the following definitions are set forth to facilitate explanation of the presently disclosed subject matter.

[0056] All technical and scientific terms used herein, unless otherwise defined below, are intended to have the same meaning as commonly understood by one of ordinary skill in the art. References to

techniques employed herein are intended to refer to the techniques as commonly understood in the art, including variations on those techniques and/or substitutions of equivalent techniques that would be apparent to one of skill in the art.

[0057] Following long-standing patent law convention, the terms “a”, “an”, and “the” refer to “one or more” when used in this application, including the claims, unless clearly indicated otherwise.

[0058] Any ranges listed herein are intended to be inclusive of endpoints. For example, a range of 2-4 includes 2 and 4 and values between.

[0059] The term “about” or “approximately” when immediately preceding a numerical value means a range plus or minus 10% of that value. For example, “about 50” can mean 45 to 55, “about 25,000” can mean 22,500 to 27,500, etc., unless the context of the disclosure indicates otherwise, or is inconsistent with such an interpretation. In the context of a list of numerical values such as “about 49, about 50, about 55, ...”, “about 50” means a range extending to less than half the interval(s) between the preceding and subsequent values, e.g., more than 49.5 to less than 52.5. Furthermore, the phrases “less than about” a value or “greater than about” a value should be understood in view of the definition of the term “about” provided herein.

[0060] When referring to a nucleic acid sequence or protein sequence, the term “identity” is used to denote similarity between two sequences. Unless otherwise indicated, percent identities described herein are determined using the BLAST algorithm available at the world wide web address: blast.ncbi.nlm.nih.gov/Blast.cgi using default parameters.

[0061] The term “subject” as used herein refers to a vertebrate or an invertebrate, and includes mammals, birds, fish, reptiles, and amphibians. Subjects include humans and other primates, including non-human primates such as chimpanzees and other apes and monkey species. Subjects include farm animals such as cattle, sheep, pigs, goats, and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats, and guinea pigs; birds, including domestic, wild, and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like; and aquatic animals such as fish, shrimp, and crustaceans.

Anti-SARS-CoV-2 Single variable domain on a heavy chain (VHH) antibodies

[0062] In embodiments, provided herein are single variable domain on a heavy chain (VHH) antibodies that target SARS-CoV-2 as well as to polypeptides comprising or essentially consisting of one or more of such VHHs. The present disclosure also relates to nucleic acids encoding such VHHs and polypeptides; to methods for preparing such VHHs and polypeptides; to host cells expressing or capable

of expressing such VHHs or polypeptides; to compositions comprising such VHHs, polypeptides, nucleic acids or host cells; and to uses of such VHHs, such polypeptides, such nucleic acids, such host cells or such compositions, in particular for prophylactic, therapeutic or diagnostic purposes mentioned below. Advantages and applications of the disclosure will become clear from the further description herein below.

[0063] It is a general object of the present disclosure is to provide VHHs that specifically bind to SARS-CoV-2. In particular, it is an object of the present disclosure to provide VHHs that specifically bind to SARS-CoV-2, and to provide proteins or polypeptides comprising the same, that are suitable for therapeutic and/or diagnostic use for the prevention, treatment and/or diagnosis of infection caused by SARS-CoV-2, and/or that can be used in the preparation of a pharmaceutical composition for the prevention and/or treatment of infection caused by SARS-CoV-2.

[0064] These objects are achieved by the VHHs, proteins and polypeptides described herein. These VHHs are also referred to herein as "VHHs of the disclosure"; and these proteins and polypeptides are also collectively referred to herein "polypeptides of the disclosure".

[0065] The present disclosure is not particularly limited to or defined by a specific antigenic determinant, epitope, part, domain, subunit or confirmation (where applicable) of SARS-CoV-2 against which the VHHs and polypeptides of the disclosure are directed. In embodiments, the VHHs of the disclosure can bind any region of SARS-CoV-2.

[0066] In embodiments, a VHH of the disclosure targets any one of the four structural proteins of SARS-CoV-2 including but not limited to: spike protein (S), envelope protein (E), membrane protein (M) and/or nucleocapsid protein (N). The spike protein (S protein) is responsible for receptor-recognition, attachment to the cell, infection via the endosomal pathway, and the genomic release driven by fusion of viral and endosomal membranes. Though sequences between the different family members vary, there are conserved regions and motifs within the S protein making it possible to divide the S protein into two subdomains: S1 and S2. While the S2, with its transmembrane domain, is responsible for membrane fusion, the S1 domain recognizes the virus-specific receptor and binds to the target host cell. In embodiments, the VHHs of the disclosure recognize the receptor binding domain (RBD) or spike protein of SARS-CoV-2.

[0067] In embodiments, VHHs comprising one or more of the CDR's explicitly listed in **Table 1** below are particularly preferred. In embodiments, VHHs comprising two or more of the CDR's explicitly listed in **Table 1** to **Table 3** are more particularly preferred. In embodiments, VHHs comprising three of the CDR's explicitly listed in any one of **Table 1** to **Table 3** are most particularly preferred.

[0068] Some particularly preferred, but non-limiting combinations of CDR sequences can be seen in **Table 1** to **Table 3** below, which lists the CDR's and framework sequences that are present in a number of preferred (but non-limiting) VHHs of the disclosure. As will be clear to the skilled person, a combination of CDR1, CDR2 and CDR3 sequences that occur in the same clone (i.e., CDR1, CDR2 and CDR3 sequences which are mentioned on the same line in any one of **Table 1** to **Table 3**) will usually be preferred (although the disclosure in its broadest sense is not limited thereto, and also comprises other suitable combinations of the CDR sequences mentioned in any one of **Table 1** to **Table 3**).

[0069] In another embodiment, a VHH that specifically binds to a SARS-CoV-2 antigen or component comprises, or alternatively consists of, a polypeptide having an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical, to any one of the amino acid sequences recited in **Table 2** or **Table 3**. In another embodiment, a VHH that specifically binds to a SARS-CoV-2 antigen or component comprises, or alternatively consists of, a polypeptide having an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical, to any one of the CDRs recited in **Table 1**. Nucleic acid molecules encoding these antibodies are also encompassed by the disclosure.

[0070] In embodiments, the VHHs of the disclosure comprise combinations of CDRs recited in **Table 1** or **Table 3**, where each CDR is replaced by a CDR having at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity to one or more of the CDRs listed in **Table 1**.

[0071] However, as will be clear to the skilled person, the combinations of CDR sequences recited in **Table 1** are generally preferred.

[0072] Thus, in the VHHs of the disclosure, at least one of the CDR1, CDR2 and/or CDR3 sequences present is chosen from the group of the CDR1, CDR2 and CDR3 sequences, respectively, listed in **Table 1** or **Table 3**. In embodiments, the VHHs of the disclosure comprise at least one of the CDR1, CDR2, and CDR3 sequences; or from the group of CDR1, CDR2 and CDR3 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in **Table 1**.

[0073] Preferably, in the VHHs of the disclosure, at least two of the CDR1, CDR2 and CDR3 sequences present are chosen from the group of CDR1, CDR2 and CDR3 sequences, respectively, listed in **Table**

1 or from the group of CDR1, CDR2 and CDR3 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in **Table 1**.

[0074] Most preferably, in the VHHs of the disclosure, all three CDR1, CDR2 and CDR3 sequences present are chosen from the group of the CDR1, CDR2 and CDR3 sequences, respectively, listed in **Table 1** or from the group of CDR1, CDR2 and CDR3 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in **Table 1**.

[0075] Even more preferably, in the VHHs of the disclosure, all three CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group of the CDR1, CDR2 and CDR3 sequences, respectively, listed in **Table 1**.

[0076] Also, generally, the combinations of CDR's listed in **Table 1** (i.e., those mentioned on the same line in **Table 1**) are preferred. Thus, it is generally preferred that, when a CDR in a VHH of the disclosure is a CDR sequence mentioned in **Table 1** or is chosen from the group of CDR sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with a CDR sequence listed in **Table 1**, that at least one and preferably both of the other CDR's are chosen from the CDR sequences that belong to the same combination in **Table 1** (i.e. mentioned on the same line in **Table 1**) or are chosen from the group of CDR sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the CDR sequence(s) belonging to the same combination.

[0077] Thus, by means of non-limiting examples, a VHH of the disclosure can for example comprise a CDR1 sequence that has more than 80% sequence identity with one of the CDR1 sequences mentioned in **Table 1**, a CDR2 sequence that has 3, 2 or 1 amino acid difference with one of the CDR2 sequences mentioned in **Table 1** (but belonging to a different combination), and a CDR3 sequence.

[0078] In the most preferred in the VHHs of the disclosure, the CDR1, CDR2 and CDR3 sequences present are suitably chosen from the one of the combinations of CDR1, CDR2 and CDR3 sequences, respectively, listed in **Table 1**.

Table 1: Exemplary CDR Sequences of single domain VHH antibodies

Construct	CDR1	SEQ ID NO	CDR2	SEQ ID NO	CDR3	SEQ ID NO
R1E-B2	GQITAENY	391	ITTGGST	392	NADRSYFPDY	393

R1E-D4	GSIREINA	394	IDSGSLD	395	AASRSGDYRY	396
R1I-A11	GSILQINA	397	ITSGGST	398	AATSTRGGLSDY	399
R1I-C8	GRQFGAWR	400	ITSGTSG	401	AATSHTTYHAEY	402
R1I-E7	GTEYDISA	403	VTSDNST	404	TATVKTSQYRY	405
R1I-G7	GSSININY	406	ITSGGGT	407	AASEAWRRFDS	408
R2A-C5	GTWGNIQY	409	ITPNNGT	410	TAVLWYTTKEGPYDY	411
R2A-F3	GSITSINW	412	ISQTGRG	413	AAASRTGTPYNY	414
R2B-C4	GSSSWNA	415	ITSGTST	416	NGYEHTTGYDY	417
R2B-D4	GSYASIAS	418	LTSGGSR	419	TALILHYAGMYDY	420
R2B-D5	GYADEINM	421	IASGGST	422	AAIPRPSLSYAHDYAY	423
R2B-E6	GGVTRGNA	424	IDNGGSH	425	AVFPDHDSEQASIDRVY	426
R2C-B3	GTYASYET	427	ITGAGSS	428	NADSIPPALRAANTGY	429
R2C-F6	GRILAANI	430	ITTGGSN	431	NALGLGSASGSYES	432
R2C-G6	GSYKYSD	433	LTGAGTT	434	AAGQGFKATRTFDS	435
R2D-A6	GSGASIGA	436	ITSAGNT	437	NALAGANYESQWEEYNY	438
R2D-D3	GHIFSINN	439	IHSGGGT	440	NAEVSTGAGSRGRVSDVY	441
R2D-G5	GSAMMINR	442	ITWQGST	443	NALSADGAYVRRYKDY	444
R2E-D2	GHQLSTNA	445	IGSGSSS	446	TALGGDELDGMTYDY	447
R2E-F4	GETFSSNA	448	ITSGGND	449	NAPATEDTSHYPRNY	450
R5A-A1	GAELSYTA	451	INSGGSS	452	TALLDSTYDY	453
R5A-A5	GSAFATNG	454	ITSNGSI	455	TALLMSYGGGEEYDY	456
R5A-B3	GQLDRSNA	457	ITSGAST	458	AADRYRAIQRTQYS	459
R5A-B5	GDDFIENA	460	VTLGGHS	461	NYPVGIPAVAIIDGRRSYGY	462
R5A-B7	GAFFSYGA	463	ITSGGST	464	NAGVVWLPSNAAAYNL	465
R5A-C5	GDIFSINA	466	ITSGGST	467	AAQQARGGLSDY	468
R5A-D4	GSAMMINR	469	ITWQGST	470	NAVAPVHENENY	471
R5A-D6	GNDWYINS	472	VSRGGSN	473	AALLETSSGDYTY	474

R5A-E5	GYDFDILR	475	ITSAESV	476	ATYDYTDDYGYAS	477
R5A-F2	GSISSIEA	478	MASGGDT	479	AAQTYRPDTWGRYTF	480
R5A-F5	GYHASILA	481	MTWSDMT	482	AATYVTRDRPWITH	483
R5B-C3	GGISVANV	484	LTSEGSR	485	AAEPTGNTHEFEY	486
R5B-C5	GSPFKTQT	487	ITSGSGT	488	AAANDGYGVD	489
R5B-G1	GSIGQIVL	490	ITSPSST	491	NTELEGKTQGFTGKTVWYDY	492
R5C-A1	GLDVSINF	493	ITWQGST	494	ATVWAGGTPNDY	495
R5C-A2	GDIFSKNA	496	IYSDGSS	497	AALDYSARYRT	498
R5C-B1	GSGFSNNA	499	ITSGSVS	500	AAVTGSSTGNAGQGYLT	501
R5C-B2	GSIFSLNW	502	INSGGHA	503	AAGPYGAYNFNS	504
R5C-E1	YIFSATV	505	INSTGSS	506	AADGADVVGNYRY	507
R5C-E3	SFFSINA	508	ISVGDSR	509	AAQIYITTYELYDY	510
R5C-F3	TIPSINY	511	LTSGDNR	512	NAKTVGSSYRDSYNY	513
R5C-F5	SYFKINS	514	IHSGDGT	515	AADQAWVRAGTYDY	516
R5C-F6	GQTNLSILA	517	ITSGSNS	518	AARNGKLSPEANQRLY	519
R5C-G2	GSATSINR	520	IDSGGYT	521	NAVPTTDDKTPMYQY	522
R5C-G6	GSIESINA	523	ITENDVD	524	ASLSSGYQYVGFRY	525
R5D-A1	GSISSYNA	526	LTSSGSD	527	NAFSYHTYSRYDF	528
R5D-B2	GSIWSIAF	529	ITTGNDE	530	AANTYERTPDGRTFSY	531
R5D-B4	GSSTSINI	532	ITGGSNG	533	AAVATSYTGVEYDY	534
R5D-B5	GSRDSIVA	535	ITSRWVT	536	NAPVYYDTEWYDI	537
R5D-B6	GRILAANI	538	ITSGGGS	539	NALGLGQSSLYRSADPYDS	540
R5D-D4	GSDFTINA	541	ITSTSST	542	AAFAVRDADTAGWTARDY	543
R5D-E1	GSSFSINW	544	INSTGSD	545	AAAITQPMPQGKGAPPGFYY	546
R5D-E3	GSIYVINT	547	IAKGGSG	548	AADRTYGYEYSTYQY	549
R5D-E4	GQISSNNG	550	ATTGGTT	551	TALLMSRAGEFDY	552
R5D-F3	GSQLSINY	553	ITITGSS	554	NTVDRTDNYGYDY	555

R5D-G1	GSIFGIND	556	ITNGLSA	557	AAQVYITTYELYDY	558
R5D-G6	GRILAANI	559	ITSGGST	560	AAKPLGSPDDFDY	561
R5E-C1	GSIPYVQA	562	IGWGGGT	563	AAKFRENFSFYDY	564
R5E-D1	GFEFSTSA	565	ITSGGSD	566	AAVVGTDNYGYDY	567
R5E-G2	GTIKSINA	568	IGSNGAK	569	TALITVAESDDYDY	570
S1a-C4	GSRDSINT	571	LDNSTTG	572	AAVTGTPGGLGFYDY	573
S1b-E11	GSYASIAS	574	VTSGGST	575	NALAGANYESQWEEYNY	576
S1b-E7	GGEFSDNG	577	ASSDGST	578	TALLMSSAGEYDV	579
S2a-C4	GFSESINA	580	LDGGGSD	581	AAQLTSSFRGYDFTPSNYDY	582
S2b-A7	GSSDSWNA	583	ISSAGSG	584	NGYEHTTGYDY	585
S2b-C10	GSAFSLNY	586	IGSGGSS	587	AAQPNGSPDDFDY	588
S2b-F7	GSTLSNNA	589	IGSSDST	590	TALLDSGPGEYNY	591
S2b-G8	GSIQSYEA	592	ITWGDST	593	AADVRQGYSS	594
S3a-C5	GSIKNTNA	595	ITSGGNS	596	AAFGYRYDQDYTYDY	597
S3a-H1	GGIYSPYS	598	IVGGGGT	599	NANYQEYDEYDY	600
S3a-H2	GAAFSKNA	601	ITSGSGT	602	AALAYSGGYDY	603
S3b-A7	GSIASINA	604	ITSGGST	605	AAQPGQSYAV	606
S3b-A8	GSIIIRVK	607	IDSGTAS	608	AAENRGYGVD	609
S3b-C8	GSNIENIER	610	IDSGGTT	611	AAVWLTNAESTQEY	612
S3b-E11	GDIYSQSA	613	ITTTGSR	614	AAFAVRSSSVSDAYDY	615
RN-01	GYIFEGGP	616	ITTSGET	617	NATDRDETGYDY	618
RN-02	GSYFSKNA	619	LTNSGSG	620	TANISESTGQGYDY	621
RN-03	GFEFTYRA	622	VVSGGST	623	NAGPQAEEDTYDY	624
RN-05	GSSFRILA	625	ITSNGNT	626	NADTLDDLDDGATYNY	627
RN-09	GLRFAMTE	628	ITEGGSG	629	AALHYTEGANTESATTFDY	630
RN-12	GSSFEGNA	631	ITTSTSY	632	AARLKDRGPQSEYDY	633
RN-14	GENFSGNL	634	VTSTGSG	635	AAKHQKGEIPYYD	636

RN-16	GADFSSNA	637	IDSDKSG	638	NAYSGTDNYGYDY	639
RN-17	GSMYSRAV	640	IGRTGDS	641	AADNLDVVYGNRY	642
RN-18	GYFQIVA	643	ITSTGST	644	NAVSTDAQGAAEY	645
RN-19	GIDDSIGA	646	LSGTGTT	647	AAAFPAGGDY	648
RN-20	GNYFEINK	649	IGSGGST	650	NALIYLADRRDGYYSYDY	651
RN-21	GLQTSDNA	652	LTSGTSR	653	AAFPDVGDAASSIGFVY	654
RN-22	GSISEINL	655	MTSGGST	656	AADEFTKYGHGRY	657
RN-23	GGEKSISA	658	ITAASTT	659	TAVLYDSYDY	660
RN-25	GEDYSYNA	661	INSGGGT	662	AYLGALDRDGYDA	663
RN-26	GSISSINS	664	VSSYGSD	665	AARTVTGFTYGDY	666
RN-27	GPQFSSNA	667	ITSGGST	668	TAPSVSDADRRTRDY	669
RN-28	GSQFSHNL	670	ITSFGST	671	AALSSSEDTSYPRNY	672
RN-29	GYILSEN	673	VTSGDYT	674	AAGTSDWEVKWSH	675
RN-30	GIPASYLN	676	AGGTNSN	677	AALDGAESYDYVYPV	678
RN-31	GSTGSDYA	679	ISSYGSD	680	AALQASAGAGYYYYDY	681

Table 2: Exemplary Framework Region 2 (FW2) Sequences of single domain VHH antibodies

ID	CDR1+FW2:	SEQ ID NO :	FW2+CDR2:	SEQ ID NO :	CDR3:	SEQ ID NO :
R1E-B2	GQITAENY MHWYR	1	KQREWVA AITTGGSTL YAD	98	CNADRSYFPDYW	195
R1E-D4	GSIREINAM HVWR	2	KGRELVA IDSGLDYY AD	99	CAASRSGDYRYW	196
R1I-A11	GSILQINAI HWFR	3	KQREEVA ITSGGSTGY AD	100	CAATSTRGGLDSDYW	197
R1I-C8	GRQFGAWRM HWYR	4	KQREWVAG ITSGTSGN YAD	101	CAATSHTTYHAEYW	198

R1I-E7	GTEYDISAAHWY R	5	KQREWWAGVTS ~~DN~~ STF YAD	102	CTATVKTSQYRYW	199
R1I-G7	GSSININYMHW R	6	KGREGVASITS ~~GG~~ GTY ED	103	CAASEAWRRFDSW	200
R2A-C5	GTWGNIQYLSW FR	7	KRREFVAAITP ~~NN~~ GTNY AD	104	CTAVLWYTTKEGPYDYW	201
R2A-F3	GSITSINWMHW R	8	KDREGVASIS ~~QT~~ GRGSY AD	105	CAAASRTGTPYNYW	202
R2B-C4	GSSSWNAIHW R	9	KQREFVARITS ~~GT~~ STYY VD	106	CNGYEHTTGYDYW	203
R2B-D4	GSYASIASMHW R	10	KQREFVAGLT ~~SG~~ GSRIY GD	107	CTALILHYAGMYDYW	204
R2B-D5	GYADEINMVHW YR	11	KGREFVAAIAS ~~GG~~ STFY AD	108	CAAIPRPSLSYAHDYAYW	205
R2B-E6	GGVTRGNAMAW YR	12	KQREIVAAID ~~NG~~ GS ~~SH~~ AY GD	109	CAVFPDHDSEQASIDRVYW	206
R2C-B3	GTYASYETIAWY R	13	KQREKVARIT ~~GAG~~ SSFY AD	110	CNADSIPPALRAANTGYW	207
R2C-F6	GRILAA~~NI~~VHWY R	14	KEREGVAGIT ~~TGG~~ SNYY SD	111	CNALGLGSASGSYESW	208
R2C-G6	GS~~IY~~KYSDVHW R	15	KAREAVALL ~~TGAG~~ TTIY GD	112	CAAGQGFKATR~~TF~~DSW	209
R2D-A6	GSGASIGAMHW R	16	KGREWWASITS ~~AG~~ NTYY GD	113	CNALAGANYESQWEEYNY W	210
R2D-D3	GHIFSINNAAWV R	17	KSREGVATI ~~HSG~~ G ~~GG~~ TYY SD	114	CNAEVSTGAGSRGRVSDVY W	211
R2D-G5	GSAMMINRTHW FR	18	KEREGVAGITW ~~QG~~ STY YAD	115	CNALSADGAYVRRYKDYW	212
R2E-D2	GHQLSTNATHW FR	19	KNREFVASI ~~GS~~ GSSSYA D	116	CTALGGDELDGMTYDYW	213
R2E-F4	GETFSSNAMHW R	20	KGREFVAAIT ~~SG~~ GNDNY SD	117	CNAPATEDTSHYPRNYW	214
R5A-A1	GAELSYTAIHW R	21	KQREFVATIN ~~SG~~ GSSYY AD	118	CTALLDSTYDYW	215
R5A-A5	GSAFATNGLHW R	22	KQREFVAGITS ~~NG~~ SINY KD	119	CTALLMSYGGGEEYDYW	216

R5A-B3	QQLDRSNAMAW FR	23	KGREWVASITSGASTQY AD	120	CAADRYRAIQRTQYSW	217
R5A-B5	GDDFIENAI SWY R	24	KGREWVAAVTLGGHSN YAD	121	CNYPVGIPAV AIDGRRSYGY W	218
R5A-B7	GAFFSYGAI HW R	25	KQREEVAAITSGGSTGY AD	122	CNAGVVWLPSNAAYNLW	219
R5A-C5	GDIFSINAL HWFR	26	KQREEVAAITSGGSTGY AD	123	CAAQQARGGLDSDYW	220
R5A-D4	GSAMMINR THW FR	27	KEREGVAGITWQGSTY YAD	124	CNAVAPVHENENYW	221
R5A-D6	GNDWYIN SAHW YR	28	KEREFVASVSRGGSNNY RD	125	CAALLETSSGDYTYW	222
R5A-E5	GYDFDILR MAWF R	29	KEREMVAAITSAESVNY ED	126	CATYDYTDDYGYASW	223
R5A-F2	GSISSIEAM SWFR	30	KQREGVATMASGGDTS YAD	127	CAAQTYRPDTWGRYTFW	224
R5A-F5	GYHASILAM HW VR	31	KEREWVASMTWSDMT QYSD	128	CAATYVTRDRPWTHW	225
R5B-C3	GGISVANVM HW FR	32	KEREGVAGLTSEGSRY YAD	129	CAAAPTGNTHFEYW	226
R5B-C5	GSPFKTQT VHWF R	33	KGREWVASITSGSGTQY MD	130	CAAANDGYGVDW	227
R5B-G1	GSIGQIVL VHWF R	34	KQRELVAGITSPSSTYY AD	131	CNTELEGKTQGFTGKT VWYDYW	228
R5C-A1	GLDVSINF MHWY R	35	KEREGVAGITWQGSTY YAD	132	CATVWAGGTPNDYW	229
R5C-A2	GDIFSKNAM HW VR	36	KQREFVAGIYSDGSSLY AD	133	CAALDYSARYRTW	230
R5C-B1	GSGFSNNAM HW YR	37	KQREGVAGITSGSVSFY AD	134	CAAVTGSSTGNAGQGYLTW	231
R5C-B2	GSIFSLNW VHWF R	38	KSREGVASINSGGHADY SD	135	CAAGPYGAYNFNSW	232
R5C-E1	GYIFSATVM HW R	39	KQREGVASINSTGSSQY AD	136	CAADGADVYGNRYW	233
R5C-E3	GSFFSINA VHWF R	40	KEREFVASISVGDSRSY D	137	CAAQIYITTYELYDYW	234

R5C-F3	GTIPSINYTAWYR	41	KQRELVASLTSGDNRYT TD	138	CNAKTVGSSYRDSYNYW	235
R5C-F5	GSYFKINSLAWF R	42	KAREVVAAIHSGDGTN YAD	139	CAADQAWVRAGTYDYW	236
R5C-F6	GQTNSILAMHW YR	43	KQREAVATITSGSNSTY AD	140	CAARNGKLSPKANQRLYW	237
R5C-G2	GSATSINRMAWV R	44	KEREWVAVIDSGGYTY YAD	141	CNAVPTTDDKTPMYQYW	238
R5C-G6	GSIESINAIHWFR	45	KAREEVAAITENDVDYY AD	142	CASLSSGYQYVGFRYW	239
R5D-A1	GSISSYNAMHWF R	46	KQREWWAGLTSSGSDG YVD	143	CNAFSYHTYSRYDFW	240
R5D-B2	GSIWIAFMWSY R	47	KGREVVAAITTGND EAYAD	144	CAANTYERTPDGRFTSYW	241
R5D-B4	GSSTSINIMAWY R	48	KQREGVAGITGGSNGY YAD	145	CAAVATSYTGVEYEW	242
R5D-B5	GSRDSIVAMHWY R	49	KGREGVAGITSRWVTY YAD	146	CNAPVYYDTEWYDIW	243
R5D-B6	GRILAANIVHWY R	50	KEREGVAGITSGGGSYY AD	147	CNALGLGQSSLYRSADPYDS W	244
R5D-D4	GSDF TINAMHWF R	51	KQREFVATITSTSSTAYR D	148	CAAFVRDADTAGWTARD YW	245
R5D-E1	GSSFSINWMHWF R	52	KQREGVASINSTGSDYY AD	149	CAAAITQMPQGKGAPPGF YYW	246
R5D-E3	GSIVINTMHWF R	53	KQRELVALIAKGGSGYY AD	150	CAADR TYGYEYSTYQYW	247
R5D-E4	GQISSNNGMHWF R	54	KRREFVATATTGGTTSY AD	151	CTALLMSRAGEFDYW	248
R5D-F3	GSQLSINYVHWF R	55	KQREFVAAITITGSSYYA D	152	CNTVDRTDNYGYDYW	249
R5D-G1	GSIFGINDVHWF R	56	KEREFVATITNGLSARY KD	153	CAAQVYITTYELYDYW	250
R5D-G6	GRILAANIVHWY R	57	KNREGVAGITSGGSTYY AD	154	CAAKPLGSPDDFDYW	251
R5E-C1	GSIPYVQAMSWF R	58	KGREGVASIGWGGGT YAD	155	CAAKFRENFSFYDYW	252

R5E-D1	GFEFSTSAMHWR	59	KQREWVARITSGGSDNYGD	156	CAAYVGTDNYGYDYW	253
R5E-G2	GTIKSINAMHWR	60	KAREFVASIGSNGAKSYSD	157	CTALITVAESDDYDYW	254
S1a-C4	GSRDSINTAHWR	61	KAREWVASLDNSTTGSYAD	158	CAAVTGTGGLGFYDYW	255
S1b-E11	GSYASIASMHWR	62	KEREFVAGVTSGGSTNYGD	159	CNALAGANYESQWEEYNYW	256
S1b-E7	GGEFSDNGLHWR	63	KQREFVATASSDGSTQYRD	160	CTALLMSSAGEYDVW	257
S2a-C4	GFSESINATAWFR	64	KQREGVAGLDGGGSDY YSD	161	CAAQLTSSFRGYDFTPSNYD YW	258
S2b-A7	GSSDSWNALHWR	65	KEREFVAGISSAGSGYYAD	162	CNGYEHTTGYDYW	259
S2b-C10	GSAFSLNYMAWFR	66	KQREGVAIIGSGGSSDYAD	163	CAAQPNGSPDDFDYW	260
S2b-F7	GSTLSNNAMHWR	67	KAREFVAAIGSSDSTDYKD	164	CTALLDSGPGEYNYW	261
S2b-G8	GSIQSYEAMHWR	68	KAREWVAVITWGDSTQYGD	165	CAADVRRQGYSSW	262
S3a-C5	GSIKNTNAMHWR	69	KDREWVAGITSGGNSNYGD	166	CAAFGYRYDQDYTYDYW	263
S3a-H1	GGIYSPYSMHWR	70	KAREWVAGIVGGGGTTYSD	167	CNANYQEYDEYDYW	264
S3a-H2	GAAFSKNALHWR	71	KGREWVASITSGSGTQYMD	168	CAALAYSGGYDYW	265
S3b-A7	GSIASINAIHWVR	72	KQREEVAAITSGGSTGYAD	169	CAAQPGQSYAVW	266
S3b-A8	GSISRVKVHWR	73	KSREWVAGIDSGTASYYS	170	CAAENRGYGVW	267
S3b-C8	GSIENIERMHWR	74	KQRELVAVIDSGGTTRYAD	171	CAAVWLTNAESTQEYW	268
S3b-E11	GDIYSQSAMAWYR	75	KEREFVAAITTTGSRSYKD	172	CAAFAVRSSSVSDAYDYW	269
RN-01	GYIFEGGPTAWFR	76	KGREFVAAITTSGETSYAY	173	CNATDRDETGYDYW	270

RN-02	GSYFSKNAVHW YR	77	KGREWVAAL ~~T~~ NSGSGN YKD	174	CTANISESTGQGYDYW	271
RN-03	GFEFTYRAVHWF R	78	KGREWVAGVVSGGSTY YAD	175	CNAGPQAEEDTYDYW	272
RN-05	GSSFRILAMHWY R	79	KNREWVAVITSNGNTY YAD	176	CNADTLDDLDDGATYNYW	273
RN-09	GLRFAMTEMAW FR	80	KQREFVALITEGGSGSY GD	177	CAALHYTEGANTESATTFD YW	274
RN-12	GSSFEGNAVHWF R	81	KNREFVATITTTSTSYYY AD	178	CAARLKDRGPQSEYDYW	275
RN-14	GENFSGNLVHWF R	82	KAREGVATVTSTGSGY YAD	179	CAAKHQKGEIPYYDW	276
RN-16	GADFSSNAMHW FR	83	KAREFVASIDSDKSGYY AD	180	CNAYSGTDNYGYDYW	277
RN-17	GSMYSRAVMHW FR	84	KEREGVASIGRTGDSYY AD	181	CAADNLDVVYGNRYW	278
RN-18	GYFFQIVAMHW YR	85	KEREGVAGITSTGSTYY GD	182	CNAVSTDAQGAAEYW	279
RN-19	GIDDSIGAMHWF R	86	KGREFVAALSGTGTTSY AD	183	CAAAPAGGDIW	280
RN-20	GNIFEINKMHW FR	87	KQREFVATIGSGGSTAY AD	184	CNALIYLADRRDGYYSYD W	281
RN-21	GLQTS DNAMHW FR	88	KNRELVAGLTSGTSRNY GD	185	CAAFPDVGDASSIGFVYW	282
RN-22	GSISEINLLHWFR	89	KQREWVATMTSGGSTD YAD	186	CAADEFTKYGHGRYW	283
RN-23	GGEKSISAAHWY R	90	KEREFVAAITAASTTSY AD	187	CTAVLYDSYDYW	284
RN-25	GEDYSYNAAHW FR	91	KEREWVAAINSGGGTY YAD	188	CAYLGALDRDGYDAW	285
RN-26	GSISSINSMHWYR	92	KEREWVAAVSSYGSDY YAD	189	CAARTVTGFTYGDYW	286
RN-27	GPQFSSNAVSWF R	93	KEREWVAGITSGGSTDY AD	190	CTAPSVSDADRRTRDYW	287
RN-28	GSQFSHNLMSWF R	94	KQREFVAAITSFGSTGY AD	191	CAALSS EDTSHYPRNYW	288

RN-29	GYILSENLTAWF R	95	KAREVVAT VTSGDYTN YDD	192	CAAGTSDWEVKWSHW	289
RN-30	GIPASYLNMHWF R	96	KQREWVAG AGGTNSNY YQD	193	CAALDGAESYDYVYPVW	290
RN-31	GSTGSDYAMHW FR	97	KDREWVAG ISSYGSDSY GD	194	CAALQASAGAGYYYDYW	291

The bolded sequences refer to a CDR sequence.

Table 3: Exemplary sequences of single domain VHH antibodies

ID	VHH Sequence:	SEQ ID NO:
R1E-B2	QVQLVESGGGLVQPGGSLRLSCAASGQITAENYMHWFY RQAPGKQREWVAAITTGGSTLYADSVKGRFTISRDN AKNTVYLMNSLKPEDTAVYYCNADRSYFPDYWGQGTQV TVSS	292
R1E-D4	QVQLVESGGGLVQPGGSLRLSCAASGSIREINAMHWVR QAPGKGRELVASIDSGSLDYYADSVKGRFTISRDN AKNTVYLMNSLKPEDTAVYYCAASRSGDYRYWGQGTQV TVSS	293
R1I-A11	QVQLVESGGGLVQPGGSLRLSCAASGSILQINAIHWFRQ APGKQREEVAITSGGSTGYADSVKGRFTISRDN AKNTVYLMNSLKPEDTAVYYCAATSTRGGLDS DYWGQGTQVTVSS	294
R1I-C8	QVQLVESGGGLVQPGGSLRLSCAASGRQFGAWRMHWY RQAPGKQREWVAGITSGTSGNYADSVKGRFTISR DNAKNTVYLMNSLKPEDTAVYYCAATSHTTYHAEY WGQGTQVTVSS	295
R1I-E7	QVQLVESGGGLVQPGGSLRLSCAASGTEYDISAAHWYR QAPGKQREWVAGVTSDNSTFYADSVKGRFTISR DNAKNTVYLMNSLKPEDTAVYYCTATVKTSQYRY WGQGTQVTVSS	296
R1I-G7	QVQLVESGGGLVQPGGSLRLSCAASGSSININYMHWFR QAPGKGREGVASITSGGGTYEDSVKGRFTISR DNAKNTVYLMNSLKPEDTAVYYCAASEAWRRFDS WGQGTQVTVSS	297
R2A-C5	QVQLVESGGGLVQPGGSLRLSCAASGTWGNIQYLSWFR QAPGKRREFVAAITPNNGTNYADSVKGRFTISR DNAKNTVYLMNSLKPEDTAVYYCTAVLWYTTKEG PYDYWGQGTQVTVSS	298

R2A-F3	QVQLVESGGGLVQPGGSLRLSCAASGSITSINWMHWFR QAPGKDREGVASISQTGRGSYADSVKGRFTISRDNANKNT VYLQMNSLKPEDTAVYYCAAASRTGTPYNYWGQGTQV TVSS	299
R2B-C4	QVQLVESGGGLVQPGGSLRLSCAASGSSESWNAIHWFR QAPGKQREFVARITSGTSTYYVDSVKGRFTISRDNANKNT VYLQMNSLKPEDTAVYYCNGYEHTTGYDYWGQGTQV TVSS	300
R2B-D4	QVQLVESGGGLVQPGGSLRLSCAASGSYASIASMHWFR QAPGKQREFVAGLTSGGSRIYGDSVKGRFTISRDNANKNT VYLQMNSLKPEDTAVYYCTALILHYAGMYDYWGQGT QVTVSS	301
R2B-D5	QVQLVESGGGLVQPGGSLRLSCAASGYADEINMVHWY RQAPGKGREFVAAIASGGSTFYADSVKGRFTISRDNANKN TVYLQMNSLKPEDTAVYYCAAIPRPSLSYAHDYAYWG QGTQVTVSS	302
R2B-E6	QVQLVESGGGLVQPGGSLRLSCAASGGVTRGNAMAWY RQAPGKQREIVAAIDNGGSHAYGDSVKGRFTISRDNANK NTVYLQMNSLKPEDTAVYYCAVFPDHDSEQASIDRVY WGQGTQVTVSS	303
R2C-B3	QVQLVESGGGLVQPGGSLRLSCAASGTYASYETIAWYR QAPGKQREKVARITGAGSSFYADSVKGRFTISRDNANKNT VYLQMNSLKPEDTAVYYCNADSIPPALRAANTGYWGQ GTQVTVSS	304
R2C-F6	QVQLVESGGGLVQPGGSLRLSCAASGRILANIVHWYR QAPGKEREVAGITTGGSNYYSDSVKGRFTISRDNANKNT VYLQMNSLKPEDTAVYYCNALGLGSASGSYESWGQGT QVTVSS	305
R2C-G6	QVQLVESGGGLVQPGGSLRLSCAASGSIYKYSDVHWFR QAPGKAREAVALLTGAGTTIYGDSVKGRFTISRDNANKN TVYLQMNSLKPEDTAVYYCAAGQGFKATRFTDSWGQG TQVTVSS	306
R2D-A6	QVQLVESGGGLVQPGGSLRLSCAASGSGASIGAMHWFR QAPGKGREWVASITSAGNTYYGDSVKGRFTISRDNANKN TVYLQMNSLKPEDTAVYYCNALAGANYESQWEEYNY WGQGTQVTVSS	307
R2D-D3	QVQLVESGGGLVQPGGSLRLSCAASGHIFSINNAAWVR QAPGKSREGVATIHSGGGTYYSDSVKGRFTISRDNANKNT VYLQMNSLKPEDTAVYYCNAEVSTGAGSRGRVSDVYW GQGTQVTVSS	308

R2D-G5	QVQLVESGGGLVQPGGSLRLSCAASGSAMMINRTHWFR QAPGKEREKVAGITWQGSTYYADSVKGRFTISRDNANKN TVYLMNSLKPEDTAVYYCNALSADGAYVRRYKDYW GQGTQVTVSS	309
R2E-D2	QVQLVESGGGLVQPGGSLRLSCAASGHQLSTNATHWFR QAPGKNREFVASIGSGSSSYADSVKGRFTISRDNANKNT VYLMNSLKPEDTAVYYCTALGGDELDGMTYDYWGQ GTQVTVSS	310
R2E-F4	QVQLVESGGGLVQPGGSLRLSCAASGETFSSNAMHWFR QAPGKGREFVAAITSGGNDNYSDSVKGRFTISRDNANKN TVYLMNSLKPEDTAVYYCNAPATEDTSHYPRNYWGQ GTQVTVSS	311
R5A-A1	QVQLVESGGGLVQPGGSLRLSCAASGAELSYTAIHWFR QAPGKQREFVATINSGGSSSYADSVKGRFTISRDNANKNT VYLMNSLKPEDTAVYYCTALLDSTYDYWGQGTQVTV SS	312
R5A-A5	QVQLVESGGGLVQPGGSLRLSCAASGSAFATNGLHWFR QAPGKQREFVAGITSNGSINYKDSVKGRFTISRDNANKNT VYLMNSLKPEDTAVYYCTALLMSYGGGEEYDYWGQ GTQVTVSS	313
R5A-B3	QVQLVESGGGLVQPGGSLRLSCAASGQLDRSNAMAWF RQAPGKGREWVASITSGASTQYADSVKGRFTISRDNANK NTVYLMNSLKPEDTAVYYCAADRYRAIQRTQYSWGQ GTQVTVSS	314
R5A-B5	QVQLVESGGGLVQPGGSLRLSCAASGDDFIENAIWYR QAPGKGREWVA AVTLGGHSNYADSVKGRFTISRDNANK NTVYLMNSLKPEDTAVYYCNYPVGIPAV AIDGRRSYG YWGQGTQVTVSS	315
R5A-B7	QVQLVESGGGLVQPGGSLRLSCAASGAFFSYGAIHWFR QAPGKQREEVAAITSGGSTGYADSVKGRFTISRDNANKNT VYLMNSLKPEDTAVYYCNAGV VWLPSNAAYNLWGQ GTQVTVSS	316
R5A-C5	QVQLVESGGGLVQPGGSLRLSCAASGDIFSINALHWFRQ APGKQREEVAAITSGGSTGYADSVKGRFTISRDNANKNTV YLMNSLKPEDTAVYYCAAQQARGGLDSYWGQGTQ VTVSS	317
R5A-D4	QVQLVESGGGLVQPGGSLRLSCAASGSAMMINRTHWFR QAPGKEREKVAGITWQGSTYYADSVKGRFTISRDNANKN TVYLMNSLKPEDTAVYYCNAVAPVHENENYWGQGT QVTVSS	318

R5A-D6	QVQLVESGGGLVQPGGSLRLSCAASGNDWYINSAHWY RQAPGKEREFVASVSRGGSNNYRDSVKGRFTISRDN NTVYLQMNSLKPEDTAVYYCAALLETSSGDYTYWGQG TQVTVSS	319
R5A-E5	QVQLVESGGGLVQPGGSLRLSCAASGYDFDILRMAWFR QAPGKEREMVAAITSAESVNYEDSVKGRFTISRDN TVYLQMNSLKPEDTAVYYCATYDYTDDYGYASWGQG TQVTVSS	320
R5A-F2	QVQLVESGGGLVQPGGSLRLSCAASGSISSIEAMSWFRQ APGKQREGVATMASGGDTSYADSVKGRFTISRDN NTVYLQMNSLKPEDTAVYYCAAQTYRPDTWGRYTFWQ GTQVTVSS	321
R5A-F5	QVQLVESGGGLVQPGGSLRLSCAASGYHASILAMHWV RQAPGKEREWVASMTWSDMTQYSDSVKGRFTISRDN KNTVYLQMNSLKPEDTAVYYCAATYVTRDRPWTHWG QGTQVTVSS	322
R5B-C3	QVQLVESGGGLVQPGGSLRLSCAASGGISVANVMHWFR QAPGKEREGLVAGLTSEGSRYADSVKGRFTISRDN NTVYLQMNSLKPEDTAVYYCAAEPGTGNTHEFEYWGQGT QVTVSS	323
R5B-C5	QVQLVESGGGLVQPGGSLRLSCAASGSPFKTQTVHWFR QAPGKGREWVASITSGSGTQYMDSVKGRFTISRDN NTVYLQMNSLKPEDTAVYYCAAANDGYGVDWGQGTQV TVSS	324
R5B-G1	QVQLVESGGGLVQPGGSLRLSCAASGSIGQIVLVHWFR QAPGKQRELVAGITSPSSTYYADSVKGRFTISRDN NTVYLQMNSLKPEDTAVYYCNTELEGKTQGFTGKT VWYDYWGQGTQVTVSS	325
R5C-A1	QVQLVESGGGLVQPGGSLRLSCAASGLDVSINFMHWYR QAPGKEREGLVAGITWQGSTYYADSVKGRFTISRDN NTVYLQMNSLKPEDTAVYYCATVWAGGTPNDYWGQGT QVTVSS	326
R5C-A2	QVQLVESGGGLVQPGGSLRLSCAASGDIFSKNAMHWVR QAPGKQREFVAGIYSDGSSLYADSVKGRFTISRDN NTVYLQMNSLKPEDTAVYYCAALDYSARYRTWGQGTQV TVSS	327
R5C-B1	QVQLVESGGGLVQPGGSLRLSCAASGSGFSNNAMHWY RQAPGKQREGVAGITSGSVSFYADSVKGRFTISRDN NTVYLQMNSLKPEDTAVYYCAAVTGSSTGNAGQGYLTW GQGTQVTVSS	328

R5C-B2	QVQLVESGGGLVQPGGSLRLSCAASGSIFSLNWWHWFR QAPGKSREGVASINSGGHADYSDSVKGRFTISRDNANT VYLQMNSLKPEDTAVYYCAAGPYGAYNFNSWGQGTQ VTVSS	329
R5C-E1	QVQLVESGGGLVQPGGSLRLSCAASGYIFSATVMHWFR QAPGKQREGVASINSTGSSQYADSVKGRFTISRDNANT VYLQMNSLKPEDTAVYYCAADGADVVGNYRWGQGT QVTVSS	330
R5C-E3	QVQLVESGGGLVQPGGSLRLSCAASGSFFSINAVHWFR QAPGKREFVASISVGDSRSYRDSVKGRFTISRDNANT VYLQMNSLKPEDTAVYYCAAQIYITTYELDYWGQGT QVTVSS	331
R5C-F3	QVQLVESGGGLVQPGGSLRLSCAASGTIPSINYTAWYRQ APGKQRELVASLTSGDNRYTDSVKGRFTISRDNANT VYLQMNSLKPEDTAVYYCNAKTVGSSYRDSYNYWGQ GTQVTVSS	332
R5C-F5	QVQLVESGGGLVQPGGSLRLSCAASGSYFKINSLAWFR QAPGKAREVVAAIHSGDGTNYADSVKGRFTISRDNANT TVYLQMNSLKPEDTAVYYCAADQAWVRAGTYDYWGQ GTQVTVSS	333
R5C-F6	QVQLVESGGGLVQPGGSLRLSCAASGQTNILAMHWYR QAPGKQREAVATITSGSNSTYADSVKGRFTISRDNANT VYLQMNSLKPEDTAVYYCAARNGKLSPKANQRLYWG QGTQVTVSS	334
R5C-G2	QVQLVESGGGLVQPGGSLRLSCAASGSATSINRMAWVR QAPGKEREWVAVIDSGGYTTYADSVKGRFTISRDNANT TVYLQMNSLKPEDTAVYYCNAVPTTDKTPMYQYWG QGTQVTVSS	335
R5C-G6	QVQLVESGGGLVQPGGSLRLSCAASGSIESINAIHWFRQ APGKAREEVAAITENDVDYYADSVKGRFTISRDNANT VYLQMNSLKPEDTAVYYCASLSSGYQYVGFRYWGQGT QVTVSS	336
R5D-A1	QVQLVESGGGLVQPGGSLRLSCAASGSISSYNAMHWFR QAPGKQREWVAGLTSSGSDGYVDSVKGRFTISRDNANT TVYLQMNSLKPEDTAVYYCNAFSYHTYSRYDFWGQGT QVTVSS	337
R5D-B2	QVQLVESGGGLVQPGGSLRLSCAASGSIWSIAFMSWYR QAPGKGREWVAAITGNDEAYADSVKGRFTISRDNANT TVYLQMNSLKPEDTAVYYCAANTYERTPDGRTFSYWG QGTQVTVSS	338

R5D-B4	QVQLVESGGGLVQPGGSLRLSCAASGSSTSINIMAWYR QAPGKQREGVAGITGGSNGYYADSVKGRFTISRDNANKN TVYLMNSLKPEDTAVYYCAAVATSYTGVEYEYWGQG TQVTVSS	339
R5D-B5	QVQLVESGGGLVQPGGSLRLSCAASGSRDSIVAMHWYR QAPGKGREGVAGITSRWVTYYADSVKGRFTISRDNANKN TVYLMNSLKPEDTAVYYCNAPVYYDTEWYDIWGQGT QVTVSS	340
R5D-B6	QVQLVESGGGLVQPGGSLRLSCAASGRILAAANIVHWYR QAPGKEREVAGITSGGGSYYADSVKGRFTISRDNANKN TVYLMNSLKPEDTAVYYCNALGLGQSSLYRSADPYDS WGQGTQVTVSS	341
R5D-D4	QVQLVESGGGLVQPGGSLRLSCAASGSDFTINAMHWFR QAPGKQREFVATITSTSTAYRDSVKGRFTISRDNANKNT VYLMNSLKPEDTAVYYCAAFVRDADTAGWTARDY WGQGTQVTVSS	342
R5D-E1	QVQLVESGGGLVQPGGSLRLSCAASGSSFSINWMHWFR QAPGKQREGVASINSTGSDYYADSVKGRFTISRDNANKN TVYLMNSLKPEDTAVYYCAAITQMPQGGKGAPPGFY YWGQGTQVTVSS	343
R5D-E3	QVQLVESGGGLVQPGGSLRLSCAASGSIYVINTMHWFR QAPGKQRELVALIAKGGSGYYADSVKGRFTISRDNANKN TVYLMNSLKPEDTAVYYCAADRTYGYEYSTYQYWG QGTQVTVSS	344
R5D-E4	QVQLVESGGGLVQPGGSLRLSCAASGQISSNNGMHWFR QAPGKRREFVATATTGGTTSYADSVKGRFTISRDNANKN TVYLMNSLKPEDTAVYYCTALLMSRAGEFDYWGQGT QVTVSS	345
R5D-F3	QVQLVESGGGLVQPGGSLRLSCAASGSQLSINYVHWFR QAPGKQREFVAAITITGSSYYADSVKGRFTISRDNANKNT VYLMNSLKPEDTAVYYCNTVDRTDNYGYDYWGQGT QVTVSS	346
R5D-G1	QVQLVESGGGLVQPGGSLRLSCAASGSIFGINDVHWFRQ APGKEREFVATITNGLSARYKDSVKGRFTISRDNANKNTV YLMNSLKPEDTAVYYCAAQVYITTYELYDYWGQGTQ VTVSS	347
R5D-G6	QVQLVESGGGLVQPGGSLRLSCAASGRILAAANIVHWYR QAPGKNREGVAGITSGGSTYYADSVKGRFTISRDNANKN TVYLMNSLKPEDTAVYYCAAKPLGSPDDFDYWGQGT QVTVSS	348

R5E-C1	QVQLVESGGGLVQPGGSLRLSCAASGSIPYVQAMSWFR QAPGKGREGVASIGWGGGTYADSVKGRFTISRDNANKN TVYLQMNSLKPEDTAVYYCAAKFRENFSFYDYWGQGT QVTVSS	349
R5E-D1	QVQLVESGGGLVQPGGSLRLSCAASGFEFSTSAMHWYR QAPGKQREWARITSGGSDNYGDSVKGRFTISRDNANKN TVYLQMNSLKPEDTAVYYCAAYVGTDNYG DYWGQG TQVTVSS	350
R5E-G2	QVQLVESGGGLVQPGGSLRLSCAASGTIKSINAMHWFR QAPGKAREFVASIGSNGAKSYSDSVKGRFTISRDNANKNT VYLQMNSLKPEDTAVYYCTALITVAESDDYDYWGQGT QVTVSS	351
S1a-C4	QVQLVESGGGLVQPGGSLRLSCAASGSRDSINTAHWYR QAPGKAREWVASLDNSTTGSYADSVKGRFTISRDNANKN TVYLQMNSLKPEDTAVYYCAAVTGTGPGGLGFYDYWGQ GTQVTVSS	352
S1b-E11	QVQLVESGGGLVQPGGSLRLSCAASGSYASIASMHWFR QAPGKREFVAGVTSGGSTNYGDSVKGRFTISRDNANKN TVYLQMNSLKPEDTAVYYCNALAGANYESQWEEYNY WGQGTQVTVSS	353
S1b-E7	QVQLVESGGGLVQPGGSLRLSCAASGGEFSDNGLHWFR QAPGKQREFVATASSDGSTQYRDSVKGRFTISRDNANKN TVYLQMNSLKPEDTAVYYCTALLMSSAGEYDVWGQGT QVTVSS	354
S2a-C4	QVQLVESGGGLVQPGGSLRLSCAASGFSESINATAWFRQ APGKQREGVAGLDGGGSDYYSDSVKGRFTISRDNANKNT VYLQMNSLKPEDTAVYYCAAQLTSSFRGYDFTPSNYDY WGQGTQVTVSS	355
S2b-A7	QVQLVESGGGLVQPGGSLRLSCAASGSSDSWNALHWFR QAPGKREFVAGISSAGSGYYADSVKGRFTISRDNANKNT VYLQMNSLKPEDTAVYYCNGYEHTTGYDYWGQGTQV TVSS	356
S2b-C10	QVQLVESGGGLVQPGGSLRLSCAASGSASFSLNYMAWFR QAPGKQREGVAIIGSGGSSDYADSVKGRFTISRDNANKNT VYLQMNSLKPEDTAVYYCAAQPNGSPDDFDYWGQGTQ VTVSS	357
S2b-F7	QVQLVESGGGLVQPGGSLRLSCAASGSTLSNNAMHWFR QAPGKAREFVAAIGSSDSTDYKDSVKGRFTISRDNANKNT VYLQMNSLKPEDTAVYYCTALLDSGPGEYNYWGQGTQ VTVSS	358

S2b-G8	QVQLVESGGGLVQPGGSLRLSCAASGSIQSYEAMHWFR QAPGKAREWVAVITWGDSTQYGDSVKGRFTISRDN NTVYLQMNSLKPEDTAVYYCAADVRQGYSSWGQGTQ VTVSS	359
S3a-C5	QVQLVESGGGLVQPGGSLRLSCAASGSIKNTNAMHWFR QAPGKDREWVAGITSGGNSNYGDSVKGRFTISRDN TVYLQMNSLKPEDTAVYYCAAFGYRYDQDYTYDYWG QGTQVTVSS	360
S3a-H1	QVQLVESGGGLVQPGGSLRLSCAASGGIYSPYSMHWR QAPGKAREWVAGIVGGGGTTYSDSVKGRFTISRDN TVYLQMNSLKPEDTAVYYCNANYQEYDEYDYWGQGT QVTVSS	361
S3a-H2	QVQLVESGGGLVQPGGSLRLSCAASGAAFSKNALHWFR QAPGKGREWVASITSGSGTQYMDSVKGRFTISRDN TVYLQMNSLKPEDTAVYYCAALAYSGGYDYWGQGTQ VTVSS	362
S3b-A7	QVQLVESGGGLVQPGGSLRLSCAASGSIASINAIHWVRQ APGKQREEVAAITSGGSTGYADSVKGRFTISRDN NTVYLQMNSLKPEDTAVYYCAAQPGQSYAVWGQGTQVTVS S	363
S3b-A8	QVQLVESGGGLVQPGGSLRLSCAASGSIISRVKVHWFRQ APGKSREWVAGIDSGTASYSDSVKGRFTISRDN NAKNTVYLQMNSLKPEDTAVYYCAAENRGYGV DWGQGTQVTVSS	364
S3b-C8	QVQLVESGGGLVQPGGSLRLSCAASGSIENIERMHWR YRQAPGKQRELVAVIDSGGTTRYADSVKGRFTISRDN NAKNTVYLQMNSLKPEDTAVYYCAAVWLTNAESTQEYWGQ GTQVTVSS	365
S3b-E11	QVQLVESGGGLVQPGGSLRLSCAASGDIYSQSAMAWYR QAPGKEREVAAITTTGSRSYKDSVKGRFTISRDN NAKNTVYLQMNSLKPEDTAVYYCAAFVRSSSVSDAYDYWGQ GTQVTVSS	366
RN-01	QVQLVESGGGLVQPGGSLRLSCAASGYIFEGGPTAWFR QAPGKGREFVAAITTSGETSYAYSVKGRFTISRDN NAKNTVYLQMNSLKPEDTAVYYCNATDRDETGYDYWGQGTQ VTVSS	367
RN-02	QVQLVESGGGLVQPGGSLRLSCAASGSYFSKNAVHWY RQAPGKGREWVAALTNSGSGNYKDSVKGRFTISRDN NAKNTVYLQMNSLKPEDTAVYYCTANISESTGQGYDYWG QGTQVTVSS	368

RN-03	QVQLVESGGGLVQPGGSLRLSCAASGFETYRAVHWFR QAPGKGREWVAGVVSGGSTYYADSVKGRFTISRDN NTVYLMNSLKPEDTAVYYCNAGPQAEEDTYDYWGQ GTQVTVSS	369
RN-05	QVQLVESGGGLVQPGGSLRLSCAASGSSFRILAMHWYR QAPGKNREWVAVITSNGNTYYADSVKGRFTISRDN TVYLMNSLKPEDTAVYYCNADTLDDGATYNYWG GGTQVTVSS	370
RN-09	QVQLVESGGGLVQPGGSLRLSCAASGLRFAMTEMAWF RQAPGKQREFVALITEGSGSYGDSVKGRFTISRDN TVYLMNSLKPEDTAVYYCAALHYTEGANTESATT FDYWGQGTQVTVSS	371
RN-12	QVQLVESGGGLVQPGGSLRLSCAASGSSFEGNAVHWFR QAPGKNREFVATITTTSTSYYYADSVKGRFTISR DNANTVYLMNSLKPEDTAVYYCAARLKDRGPQSE DYWGQGTQVTVSS	372
RN-14	QVQLVESGGGLVQPGGSLRLSCAASGENFSGNLVHWFR QAPGKAREGVATVTSTGSGYYADSVKGRFTISR DNANTVYLMNSLKPEDTAVYYCAAKHQKGEIPY YDWGQGTQVTVSS	373
RN-16	QVQLVESGGGLVQPGGSLRLSCAASGADFSSNAMHWF RQAPGKAREFVASIDSDKSGYYADSVKGRFTISR DNANTVYLMNSLKPEDTAVYYCNAYSGTDNYGY DYWGQGTQVTVSS	374
RN-17	QVQLVESGGGLVQPGGSLRLSCAASGSMYSRAVMHWF RQAPGKEREVASIGRTGDSYYADSVKGRFTISR DNANTVYLMNSLKPEDTAVYYCAADNLDVVYGN RYWGQGTQVTVSS	375
RN-18	QVQLVESGGGLVQPGGSLRLSCAASGYFQIVAMHWY RQAPGKEREVAGITSTGSTYYGDSVKGRFTISR DNANTVYLMNSLKPEDTAVYYCNAVSTDAQGA AEYWGQGTQVTVSS	376
RN-19	QVQLVESGGGLVQPGGSLRLSCAASGIDDSIGAMHWF RQAPGKREFVAALSGTGTTSYADSVKGRFTISR DNANTVYLMNSLKPEDTAVYYCAAAPAGGDYWG QGTQVTVSS	377
RN-20	QVQLVESGGGLVQPGGSLRLSCAASGNYFEINKMHWFR QAPGKQREFVATIGSGGSTAYADSVKGRFTISR DNANTVYLMNSLKPEDTAVYYCNALIYLADRRD GYYSYDYWGQGTQVTVSS	378

RN-21	QVQLVESGGGLVQPGGSLRLSCAASGLQTSNAMHWF RQAPGKNREL VAGLTSGTSRNYGDSVKGRFTISRDN NTVYLMNSLKPEDTAVYYCAAFPDVGDASSIGFVYW GQGTQVTVSS	379
RN-22	QVQLVESGGGLVQPGGSLRLSCAASGSISEINLLHWFRQ APGKQREWVATMTSGGSTDYADSVKGRFTISRDN NTVYLMNSLKPEDTAVYYCAADEF TKYGHGRYWGQG TQVTVSS	380
RN-23	QVQLVESGGGLVQPGGSLRLSCAASGGEKSISAAHWYR QAPGKEREVAAITAASTTSYADSVKGRFTISRDN NTVYLMNSLKPEDTAVYYCTAVLYDSYDYWGQGTQVT VSS	381
RN-25	QVQLVESGGGLVQPGGSLRLSCAASGEDYSYNAAHWF RQAPGKEREWVAAINSGGGTYADSVKGRFTISRDN NTVYLMNSLKPEDTAVYYCAYLGALDRDGYDAWGQ GTQVTVSS	382
RN-26	QVQLVESGGGLVQPGGSLRLSCAASGSISSINSMHWYRQ APGKEREWVAAVSSYGSDYYADSVKGRFTISRDN NTVYLMNSLKPEDTAVYYCAARTVTGFTYGDYWGQGT QVTVSS	383
RN-27	QVQLVESGGGLVQPGGSLRLSCAASGPQFSSNAVSWFR QAPGKEREWVAGITSGGSTDYADSVKGRFTISRDN NTVYLMNSLKPEDTAVYYCTAPSVSDADRRTRDYWGQ GTQVTVSS	384
RN-28	QVQLVESGGGLVQPGGSLRLSCAASGSQFSHNLSWFR QAPGKQREFVAAITSFGSTGYADSVKGRFTISRDN NTVYLMNSLKPEDTAVYYCAALSSDTSHYPRNYWGQ TQVTVSS	385
RN-29	QVQLVESGGGLVQPGGSLRLSCAASGYILSENLTAWFR QAPGKAREVVATVTSGDYTNYYDDSVKGRFTISRDN NTVYLMNSLKPEDTAVYYCAAGTSDWEVKWSHWGQ TQVTVSS	386
RN-30	QVQLVESGGGLVQPGGSLRLSCAASGIPASYLNMHWFR QAPGKQREWVAGAGGTNSNYQDSVKGRFTISRDN NTVYLMNSLKPEDTAVYYCAALDGAESYDYVYPVW GQGTQVTVSS	387
RN-31	QVQLVESGGGLVQPGGSLRLSCAASGSTGSDYAMHWF RQAPGKDREWVAGISSYGSYGSYGSVKGRFTISRDN NTVYLMNSLKPEDTAVYYCAALQASAGAGYDYW GQGTQVTVSS	388

[0079] In embodiments, the VHHs of the disclosure include one or more amino acid substitutions, deletions, or additions. As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the VHHs of the disclosure.

[0080] The present disclosure provides VHHs that comprise, or alternatively consist of, variants (including derivatives) of the VHH CDRs described herein that specifically bind to a SARS-CoV-2 antigen or component. In embodiments, the present disclosure provides VHHs that comprise, or alternatively consist of, variants (including derivatives) of the VHH CDRs described herein that specifically bind to the spike protein of SARS-CoV-2. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a VHH of the disclosure, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis which result in amino acid substitutions. Preferably, the variants (including derivatives) encode less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the reference VHH domain. In a preferred embodiment, the variants have conservative amino acid substitutions at one or more predicted non-essential amino acid residues. A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity (e.g., the ability to bind a SARS-CoV-2 antigen or component). Following mutagenesis, the encoded protein may routinely be expressed and the functional and/or biological activity of the encoded protein, (e.g., ability to specifically bind to a SARS-CoV-2 antigen or component) can be determined using techniques described herein or by routinely modifying techniques known in the art.

[0081] In embodiments, the disclosure relates to a polypeptide that comprises at least one VHH against SARS-CoV-2 as defined herein. In embodiments, the disclosure also relates to a polypeptide that comprises at least two or at least three anti-SARS-CoV-2 binding moieties such constructs enabling

multimeric interactions are herein referred to as a Cerberbody and/or a Hydrabody complex. Complexes provided herein can be formed using any means. In embodiments, any number of higher-order complexes are formed. Higher order complexes can be formed that comprise 5HVZ. A 5HVZ can be a smAKAP AKB domain bound RIa dimerization/docking (D/D) complex. In embodiments, a composition provided herein comprises one or more of a 5HVZ.

Cerberbody

[0082] In embodiments, a Cerberbody construct can be formed when a *Spirulina* strain (either two independent strains or a single strain from different ORFs) express a 5HVZ mediated homodimer molecule and smAKAP linked monomer VHH. These independent complexes form a further complex structure that will have three antigen binding sites. The presence of increased antigen binding reagents (VHHs) results in increased binding affinity, mainly due to avidity-based interaction. The three binding VHHs can be derived from the same VHH forming a homo-trimer. The design also enables mixing diverse VHHs with desirable binding and virus neutralization property. Such a molecule complex will be able to bind multiple epitopes and mutant variants of a virus such as novel SARS-Cov-2 variants that may not be captured by conventional immune surveillance or conventional therapies. We have demonstrated that the Cerberbody complexes show increased apparent binding when compared to dimeric or monomeric forms.

Hydrabody

[0083] In embodiments, provided are also Hydrabody complexes. In embodiments, a Hydrabody comprises at least four antigen binding VHHs. Hydrabody complexes can be formed when a *Spirulina* strain (either two independent strains or a single strain from different ORFs) express a 5HVZ mediated homodimer molecule and smAKAP linked dimeric VHHs. The smAKAP mediated dimers contain VHHs on both the N- and C- terminal of smAKAP. The VHHs fused to the smAKAP can form homo-dimeric (the same VHH) or hetero-dimeric (two different VHHs on either end). Such a configuration can enable inclusion of multiple, therapeutically effective VHHs in a single complex.

[0084] In embodiments, a Cerberbody or a Hydrabody can comprise a sequence from **Table 1**, **Table 2**, or **Table 3**. In embodiments, a Cerberbody or Hydrabody comprise at least 2, 3, 4, 5, 6, 7, or 8 sequences selected from **Tables 1-3**. A Cerberbody or Hydrabody can comprise multiples of the same sequence or different sequences, for example CDR sequences in **Table 1**. In embodiments, a Cerberbody or Hydrabody can comprise from 1-4 duplications or combinations of any one of SEQ ID NO: 391-681 or SEQ ID NO: 292-388.

[0085] In addition to increased avidity-based apparent binding, the higher order complexes of Cerberbody and Hydrabody enable the formation of super potent and cross-reactive therapeutic complex that can bind and neutralize diverse virus variants. For example, in embodiments, a Hydrabody is administered to a subject in need thereof to neutralize two or more SARS-COV-2 variants. A Hydrabody that contains a dimer of dimers of two VHHs that bind and neutralize single variants can bind better than each separately and neutralize both variants. Such a design combines multiple components easily where a diverse epitope can be engaged resulting in decreased virus escape.

[0086] In embodiments, a composition provided herein, for example a Cerberbody and/or a Hydrabody can bind from about 1, 2, 3, 4, 5, 6, 7, or up to about 8 different SARS-CoV-2 variants.

[0087] Also provided are nucleic acids that encode any of the VHH of the disclosure and/or a polypeptide of the disclosure such as a Cerberbody and/or a Hydrabody. Such a nucleic acid will also be referred to below as a "nucleic acid of the disclosure" and may for example be in the form of a genetic construct. In embodiments, a multimeric construct, such as a Cerberbody or a Hydrabody, is more effective at neutralizing SARS-CoV-2 as compared to a monomeric or dimeric construct. In embodiments, the effectiveness is at least about 1-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 1000-fold, 5000-fold, 10,000-fold, 15,000-fold, 100,000-fold, 200,000-fold, 300,000-fold, 500,000-fold, 800,000-fold, or 1,000,000-fold more effective as compared to a non-multimeric construct. In embodiments, a multimeric construct, such as a Cerberbody or a Hydrabody, is more effective at reducing a symptom or disease of SARS-CoV-2 in a subject in need thereof as compared to a monomeric or dimeric construct. In embodiments, the effectiveness at reducing the symptom or disease is at least about 1-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 1000-fold, 5000-fold, 10,000-fold, 15,000-fold, 100,000-fold, 200,000-fold, 300,000-fold, 500,000-fold, 800,000-fold, or 1,000,000-fold more effective as compared to a non-multimeric construct. In embodiments, a multimeric construct, such as a Cerberbody or a Hydrabody, is more effective at neutralizing a variant of concern of SARS-CoV-2 that has evaded a vaccine therapy as compared to a monomeric or dimeric construct. In embodiments, the effectiveness is at least about 1-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, 100-fold, 1000-fold, 5000-fold, 10,000-fold, 15,000-fold, 100,000-fold, 200,000-fold, 300,000-fold, 500,000-fold, 800,000-fold, or 1,000,000-fold more effective as compared to the vaccine therapy.

[0088] In embodiments, provided are also linkers that can be utilized in the compositions and methods provided herein. Any linker can be utilized in the compositions of the disclosure for example to join one or more VHHs of the disclosure. In embodiments, a linker can also be utilized to join a Cerberbody and/or Hydrabody. In embodiments, a linker comprises smAKAP peptide. In embodiments, the

smAKAP peptide can be modified. A modification can be done for example to increase soluble protein expression. In embodiments, one or more residues in a linker can be mutated. In embodiments, two Cys residues in an exemplary linker, e.g., smAKAP peptide, are mutated to serine. In embodiments, a cleavable linker can also be utilized in compositions provided herein.

[0089] In embodiments, a linker comprises at least about or at most about: 80%, 82%, 84%, 86%, 88%, 90%, 92%, 94%, 96%, 98%, 100% identity to SEQ ID NO: 389 or SEQ ID NO: 390.

[0090] In embodiments, a linker can also be of any length. In embodiments, a linker is from about: 1-3, 2-6, 1-8, 3-10, 5-20, 3-15, 10-30, 15-35, 20-35, or 25-35 residues in length. In embodiments, a linker is about: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acids in length.

SARS-CoV-2

[0091] In embodiments, compositions and methods provided herein are used to reduce or treat a SARS-CoV-2 related disease or condition. In embodiments the SARS-CoV-2 related disease or condition comprises COVID. In embodiments the SARS-CoV-2 related disease or condition comprises COVID-19.

[0092] In embodiments, a strain of SARS-CoV-2 is a variant of concern (VOI) or a variant of interest (VOI) as determined by the WHO. In embodiments, a strain of SARS-CoV-2 is not a variant of concern (VOI) as determined by the WHO. In embodiments, a strain of SARS-CoV-2 is not a variant of interest (VOI) as determined by the WHO. A SARS-CoV-2 variant that meets the definition of a VOI and, through a comparative assessment, has been demonstrated to be associated with one or more of the following changes at a degree of global public health significance: (a) increase in transmissibility or detrimental change in COVID-19 epidemiology; (b) increase in virulence or change in clinical disease presentation; and/or (c) decrease in effectiveness of public health and social measures or available diagnostics, vaccines, therapeutics. In embodiments, a VOI or a VOC is selected from the group consisting of Alpha, Beta, Gamma, Delta, and Omicron. In embodiments, a strain of SARS-CoV-2 is one or more of: alpha, beta, gamma, delta, epsilon, zeta, eta, theta, iota, kappa, lambda, mu, nu, xi, omicron, pi, rho, sigma, tau, upsilon, phi, chi, psi, or omega.

[0093] In embodiments, a SARS-CoV-2 is a variant as determined by the WHO. A variant may be identified as comprising genetic changes that are predicted or known to affect virus characteristics such as transmissibility, disease severity, immune escape, diagnostic or therapeutic escape; and/or identified to cause significant community transmission or multiple COVID-19 clusters, in multiple countries with increasing relative prevalence alongside increasing number of cases over time, or other apparent

epidemiological impacts to suggest an emerging risk to global public health. In embodiments, a SARS-CoV-2 variant is lambda or Mu.

[0094] It is now widely accepted that SARS-CoV-2 colonizes the GI tract, and very high amounts of viral RNA are commonly found in stool samples. This viral reservoir exacerbates the pandemic in several ways. First, GI infection directly causes significant disease symptoms, with a majority of cases presenting with both GI and respiratory symptoms, and 25% presenting with only GI symptoms¹. In addition to contributing directly to disease progression and possibly liver damage¹¹, GI colonization may result in subsequent self-infection of the airways, particularly in vulnerable populations with weakened immune systems. Further, a systemic inflammatory response (cytokine storm) emanating from a GI infection may exacerbate pulmonary inflammation and contribute to acute respiratory distress syndrome (ARDS). Second, subjects with ongoing GI infection may test negative in nasopharyngeal swabs, yet silently carry and potentially transmit the virus for weeks.

[0095] In addition to colonizing the GI tract, SARS-CoV-2 can also colonize the respiratory tract. In embodiments, SARS-CoV-2, infects the cells along the airways by binding the ACE-2 receptor. Respiratory infection can lead to inflammation resulting in any one of: cough, chest tightness, and/or pain while breathing. In embodiments, airway inflammation can result in pneumonia. In embodiments, respiratory tract infection of SARS-CoV-2 can result in the need for lung transplant.

[0096] Respiratory diseases like influenza spread on a “one-to-few” basis through respiratory droplets and fomites. By contrast, diseases like norovirus that spread through the fecal-oral route are much more difficult to control in confined settings (nursing homes, cruise ships, naval vessels) because a single individual can spread the virus to many others — “one-to-many” transmission. Norovirus, is so contagious in confined settings that when researchers conduct human challenge clinical trials, extreme precautions must be taken to avoid infection of clinical staff. Fecal-oral transmission of SARS-CoV-2 may therefore explain some of the most dramatic local infection clusters. This is supported by the well-documented fecal-oral transmission of the SARS virus in 2003 (the Amoy Gardens event)^{9,12}, and the fact that many outbreak events apparently share “one-to-many” similarities to norovirus. The first major rich-world outbreak was a cruise ship (Diamond Princess) and the first major cluster of Covid-19 deaths in the U.S. occurred in a nursing home in Kirkland, WA. The foregoing is supported by the following observations.

[0097] The human host cell receptor used by SARS-CoV-2 for cell entry (ACE2) is “highly expressed in the lung AT2 cells, but also in upper and stratified epithelial cells and absorptive enterocytes from ileum and colon...”¹³. Infective virus is found in stool from infected humans⁶ and animals⁸. Many subjects test positive in rectal swabs after symptoms resolve and oral swabs are negative⁷. Some

individuals who test positive in rectal swabs are completely asymptomatic for Covid-19⁷. Positive test results in rectal samples occur even when oral swabs show negative results¹⁴. Modeling of outbreak dynamics China indicate that, “due to their greater numbers, undocumented infections were the source for 79% of documented cases”¹⁵ leading to speculation that GI-driven transmission may be responsible. Covid-19’s epidemiology may in part explain why SARS-CoV-2 has spread more rapidly than prior coronavirus outbreaks.¹⁶

[0098] The compositions of the disclosure are safe to be used prophylactically and unlike a vaccine, work immediately upon administration and do not require a competent host immune system. The approach also side-steps the risks of vaccine development, which can sometimes exacerbate the disease through the phenomenon of antibody-dependent enhancement. In embodiments, a composition of the disclosure is effective at reducing or eliminating COVID when a SARS-CoV-2 infection evades vaccine therapy.

[0099] Compositions of the disclosure may be delivered through non-parenteral routes of administration, has no known toxicity risks, and is shelf stable for >1 year at 42°C. Therefore, neither cold-chain distribution nor trained medical personnel for administration are required.

[0100] In embodiments, an infection with SARS-CoV-2 is determined utilizing an in vitro assay. Suitable in vitro assays comprise RT-PCR, ELISA, RT-LAMP, transcription-mediated amplification (TMA), rolling cycle amplification (RCA), CRISPR, microarray, or any combination thereof. In embodiments, serological testing may also be utilized to monitor progress of disease. In embodiments, serological testing comprises an analysis of blood serum, plasma, saliva, sputum, and other biological fluids for the presence of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies. Serological testing can provide an assessment of both short-term (days to weeks) and long-term (years or permanence) trajectories of antibody response, as well as antibody abundance and diversity.

Spirulina

[0101] Compositions of the present disclosure include recombinant spirulina in a non-living form, wherein the spirulina is engineered to contain at least one VHH. Using spirulina as both a manufacturing system and delivery vehicle enables faster development without compromising safety. These non-living spirulina containing at least one VHH are then administered to a subject to elicit a response in the subject. In embodiments, non-living recombinant spirulina comprising at least one VHH is prepared by drying the live culture of the recombinant spirulina. Methods of drying include heat drying, e.g., drying in an oven; air-drying, spray drying, lyophilizing, freeze-drying, thin-film drying, spray granulating, or

spray-freeze drying. Accordingly, in embodiments, compositions of the present disclosure comprise a dried biomass of a recombinant spirulina comprising at least one VHH as described herein.

[0102] Neutralizing VHH genes are stably engineered into spirulina, which manufactures and stores the antibodies in its cytoplasm. For oral administration, no trained medical personnel are required: the entire biomass is simply dried, whereby the desiccated (inviable) spirulina cell becomes an edible organic mini-capsule containing the VHH drug 'payload'. VHHs are stable within the dried biomass for at least 12 months without refrigeration ($>42^{\circ}\text{C}$), facilitating stockpiling and eliminating cold-chain distribution requirements.

[0103] There are several advantages to delivering VHHs encapsulated in spirulina. One of these advantages is the increased resistance of the encapsulated VHH to proteolysis. Pharmacokinetic studies show that the dry spirulina biomass retains the bio-encapsulated VHH and protects it from gastric digestion, and that the VHHs are rapidly released from the biomass following exit from the stomach. Following release, the VHH antibodies are bioactive for hours in the GI tract. Ingestion of different capsule or tablet formulations can modulate the site of VHH release from the spirulina biomass, thus allowing for esophageal, small intestine or colon delivery depending on the specific pathogen target. After release, the mode of action is to bind directly to pathogen-specific proteins present in the gut lumen that are required for infectivity, thus blocking interaction with the host and allowing them to pass harmlessly out of the body. Development is easier and faster than vaccine development and carries the added advantages of working immediately and equally well in subjects who may be immunocompromised (e.g., due to stress or age).

[0104] For example, encapsulation in spirulina protects the VHH from the enzymes and conditions of the digestive tract, thereby allowing the therapeutic to be delivered to the portion of the digestive tract that digests the spirulina cells and releases the therapeutic. In embodiments, the compositions of the present disclosure survive (e.g., remain substantially intact) at a pH of about 1.3 to about 8.0. In embodiments, the compositions of the present disclosure survive in the oral cavity. In embodiments, the compositions of the present disclosure survive in the stomach. In embodiments, the compositions survive in the small intestine. In embodiments, the compositions survive in a simulated stomach environment. In embodiments, the simulated stomach environment has an acidic pH and contains pepsin. In embodiments, the simulated stomach environment has a pH of about 3.0 and about 2000 U/mL of pepsin. In embodiments, the compositions can survive in the gastrointestinal conditions or simulated stomach environment for about 5 minutes to about 1 day. In embodiments, the compositions survive in the gastrointestinal conditions or simulated stomach environment for about 5 minutes, about 10 minutes, about 20 minutes, about 30 minutes, about 1 hour, about 2 hours, about 3 hours, about 4

hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 12 hours, about 24 hours. In embodiments, the compositions survive in the gastrointestinal conditions or simulated stomach environment overnight.

[0105] To facilitate delivery to the airway, the VHH may be extracted from the spirulina by lysing the spirulina, partially purifying the soluble proteins including the VHH, and drying the partially purified protein by an appropriate drying method including any drying method provided herein. For example, the harvested spirulina slurry may be lysed using high pressure homogenization, and the soluble protein separated from insoluble materials by filtration, then diafiltered and concentrated by tangential flow ultrafiltration. The partially purified extract may be formulated with appropriate stabilizers and mucoadhesives, including carbohydrates such as trehalose, hyaluronic acid, or chitosan; amino acids such as lysine or glycine; and polymers such as polyvinyl alcohol or polyvinyl pyrrolidone. In embodiments, the VHH will comprise a purity from about: 4%, 6%, 8%, 10%, 12%, 14%, 16%, 18%, 20%, 22%, 24%, 26%, 28%, 30%, 32%, 34%, 36%, 38%, 40%, 42%, 44%, 46%, 48%, 50%, 52%, 54%, 56%, 58%, 60%, or up to about 70% of solids.

[0106] Another advantage of the compositions of the present disclosure is their stability in storage. In embodiments, the compositions of the present disclosure are stable at elevated temperatures (e.g., greater than room temperature). In embodiments, the compositions of the present disclosure are stable at 42°C. In embodiments, the compositions of the present disclosure are stable at 42°C for about one day to 5 years. In embodiments, the compositions of the present disclosure are stable at 42°C for about one day, two days, three days, four days, five days, six days, seven days, one week, two weeks, three weeks, four weeks, one month, two months, three months, four months, five months, six months, or one year. In embodiments, the compositions of the present disclosure are stable at 42°C for one month or three months. In embodiments, the compositions of the present disclosure are stable at room temperature (e.g., about 20° to about 29°C). In embodiments, the compositions of the present disclosure are stable at 27°C. In embodiments, the compositions of the present disclosure are stable at 27°C for about one day to 5 years. In embodiments, the compositions of the present disclosure are stable at 27°C for about one day, two days, three days, four days, five days, six days, seven days, one week, two weeks, three weeks, four weeks, one month, two months, three months, four months, five months, six months, or one year. In embodiments, the compositions of the present disclosure are stable at 27°C for one month or three months.

[0107] As used herein “spirulina” is synonymous with “*Arthrospira*.” Compositions of the present disclosure can comprise any one of the following species of spirulina: *A. amethystine*, *A. ardissoni*, *A. argentina*, *A. balkrishnanii*, *A. baryana*, *A. boryana*, *A. braunii*, *A. breviararticulata*, *A. brevis*, *A. curta*,

A. desikacharyiensis, *A. funiformis*, *A. fusiformis*, *A. ghannae*, *A. gigantean*, *A. gomontiana*, *A. gomontiana* var. *crassa*, *A. indica*, *A. jenneri* var. *platensis*, *A. jenneri* Stizenberger, *A. jenneri* f. *purpurea*, *A. joshii*, *A. khannae*, *A. laxa*, *A. laxissima*, *A. laxissima*, *A. leopoliensis*, *A. major*, *A. margaritae*, *A. massartii*, *A. massartii* var. *indica*, *A. maxima*, *A. meneghiniana*, *A. miniata* var. *constricta*, *A. miniata*, *A. miniata* f. *acutissima*, *A. neapolitana*, *A. nordstedtii*, *A. oceanica*, *A. okensis*, *A. pellucida*, *A. platensis*, *A. platensis* var. *non-constricta*, *A. platensis* f. *granulate*, *A. platensis* f. *minor*, *A. platensis* var. *tenuis*, *A. santannae*, *A. setchellii*, *A. skujae*, *A. spirulinoides* f. *tenuis*, *A. spirulinoides*, *A. subsalsa*, *A. subtilissima*, *A. tenuis*, *A. tenuissima*, and *A. versicolor*. In embodiments, spirulina is *A. platensis*.

Pharmaceutical Compositions and Dosing

[0108] As used herein, the terms “oral composition” or “orally delivered composition” comprise compositions administered or delivered to the gastrointestinal tract (e.g., orally, compositions administered to the stomach via a feeding tube, etc.). Any appropriate area of the gastrointestinal tract may be targeted by the compositions of the present disclosure.

[0109] In embodiments, the compositions of the present disclosure are administered via the airway. In embodiments, the compositions of the present disclosure are administered by inhalation. In embodiments, the compositions of the present disclosure are administered intranasally. In embodiments, the compositions of the present disclosure are administered by a nebulizer, an inhaler, or a mist. In embodiments, the compositions of the present disclosure are lyophilized and delivered as a powder or a powder resuspended in a liquid.

[0110] In embodiments, the compositions of the present disclosure are formulated for administration via the airway. In embodiments, the compositions of the present disclosure are formulated for administration by inhalation. In embodiments, the compositions of the present disclosure are formulated for intranasal administration. In embodiments, the compositions of the present disclosure are formulated for administration by a nebulizer, an inhaler, a dry powder inhalation device, or a mist.

[0111] In embodiments, compositions of the present disclosure can comprise one or more pharmaceutically acceptable excipients. Pharmaceutically acceptable carriers include but are not limited to saline, buffered saline, dextrose, water, glycerol, sterile isotonic aqueous buffer, and combinations thereof. In embodiments, a pharmaceutically acceptable excipient is sodium bicarbonate.

Uses of Compositions

[0112] In embodiments, compositions of the present disclosure can be used to reduce the severity of a disease or disorder in a subject in need thereof. In embodiments, compositions can be used to prevent

a disease or disorder in a subject. In embodiments, compositions can be used to prevent initiation of a disease or disorder in a subject. In embodiments, compositions can be used to reduce the severity of a disease or disorder in a subject caused by a coronavirus e.g., SARS-CoV-2. In embodiments, compositions can be used to prevent or delay recurrence of a disease in a subject such as COVID-19.

Methods of Treatment

[0113] In embodiments, the present disclosure provides a method of treating COVID-19, ARDS, inflammation, organ or tissue damage, or inflammatory lung damage or symptoms, and/or related conditions comprising administering compositions comprising a VHH of the present disclosure to a subject in need.

[0114] In embodiments, the present disclosure provides a method of treating COVID-19, ARDS, inflammation, organ or tissue damage, inflammatory lung damage symptoms, and/or a COVID-19 related GI symptom in a subject in need thereof comprising administering a composition comprising a VHH of the present disclosure. In embodiments, the present disclosure provides a method of preventing COVID-19, ARDS, inflammation, organ or tissue damage, inflammatory lung damage symptoms, and/or a COVID-19 related GI symptom in a subject in need thereof comprising administering a composition comprising a VHH of the present disclosure to the subject. In embodiments, the present disclosure provides a method of slowing the progression of COVID-19, ARDS, inflammation, organ or tissue damage, inflammatory lung damage symptoms, and/or a COVID-19 related GI symptom in a subject in need thereof comprising administering compositions comprising a VHH of the present disclosure to the subject. In embodiments, the present disclosure provides a method of ameliorating the symptoms of COVID-19, ARDS, inflammation, organ or tissue damage, inflammatory lung damage symptoms, and/or a COVID-19 related GI symptom and/or respiratory symptom in a subject in need thereof comprising administering a composition comprising a VHH of the present disclosure to the subject.

[0115] In embodiments, the present disclosure provides a method of decreasing inflammation in a subject in need thereof comprising administering compositions comprising a VHH of the disclosure to the subject. In embodiments, the present disclosure provides a method of decreasing fibrosis in a subject in need thereof comprising administering compositions comprising a VHH of the disclosure to the subject. In embodiments, the present disclosure provides a method of decreasing expression of markers of inflammation or fibrosis in the organ or tissue in a subject in need thereof comprising administering compositions comprising a VHH of the disclosure to the subject. In embodiments, the present disclosure provides a method of decreasing expression of one or more pro-inflammatory cytokines in a subject in need thereof comprising administering compositions comprising a VHH of the disclosure to the subject.

In embodiments, the one or more pro-inflammatory cytokines is implicated in the inflammatory response to lung injury. In embodiments, the one or more pro-inflammatory cytokines include, but are not limited to, IL-17A, TNF α , NF- κ B, NLRP3, IL-18, IL-1, IL-1R, TNF α R, and IL-18R.

[0116] In embodiments, the present disclosure provides a method of increasing the expression of one or more anti-inflammatory cytokines in a subject in need thereof comprising administering compositions comprising a VHH of the disclosure to the subject. In embodiments, the one or more anti-inflammatory cytokines include, but are not limited to the receptors that bind to one or more pro-inflammatory cytokines, interleukin-1 receptor antagonist, IL-4, IL-6, IL-10, IL-11, and IL-13. In embodiments, the present disclosure provides a method of inducing the proliferation of protective T-cells in a subject in need thereof comprising administering compositions comprising a VHH of the disclosure to the subject.

[0117] The treatment, prevention, delay, or amelioration of inflammatory lung damage and/or the symptoms thereof may be measured by any means known in the art. For example, evaluation may include measurement of oxygen levels in the subject, exhaled nitric oxide test, measurement of respiratory rate, spirometry, and/or inflammatory lung damage is treated, prevented, delayed, or ameliorated for about 1 week, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 8 months, about 1 year, about 2 years, about 5 years, and/or about 10 years compared with the blood pressure in an untreated SARS-CoV-2 infected subject. In embodiments, inflammatory lung damage is prevented, delayed, or ameliorated by about 1%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90% compared with blood pressure in an untreated SARS-CoV-2 infected subject. In embodiments, this prevention, delay, or amelioration of inflammatory lung damage is observed at the time points disclosed herein.

[0118] In embodiments, administration of the compositions disclosed herein improve inflammatory lung damage in a subject compared to an untreated inflammatory lung damage subject. In embodiments, inflammatory lung damage is improved for about 1 week, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 8 months, about 1 year, about 2 years, about 5 years, and/or about 10 years compared with cardiac function of an untreated SARS-CoV-2 infected subject. In embodiments, inflammatory lung damage is improved by about 1%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90% compared with cardiac function of an untreated SARS-CoV-2 infected subject. In embodiments, this improvement in inflammatory lung damage is observed at the time points disclosed herein.

[0119] The treatment, prevention, delay, or amelioration of COVID-19, ARDS, inflammation, organ, or tissue damage and/or the symptoms thereof may be measured by any means known in the art. In embodiments, COVID-19, ARDS, inflammation, organ, or tissue damage and/or symptoms thereof are treated, prevented, delayed, or ameliorated for about 1 week, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 8 months, about 1 year, about 2 years, about 5 years, and/or about 10 years compared with the blood pressure in an untreated SARS-CoV-2 infected subject. In embodiments, COVID-19, ARDS, inflammation, organ, or tissue damage and/or the symptoms thereof are prevented, delayed, or ameliorated by about 1%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90% compared with blood pressure in an untreated SARS-CoV-2 infected subject. In embodiments, this prevention, delay, or amelioration of COVID-19, ARDS, inflammation, organ, or tissue damage and/or the symptoms thereof is observed at the time points disclosed herein. In embodiments, administration of a composition of the disclosure is effective in reducing a period of illness associated with the disease or disorder related to SARS-CoV-2 by at least about 1-3 days, 3-5 days, 4-6 days, a week, two weeks, or up to about three weeks. In embodiments, administration of a composition of the disclosure is effective in reducing a period of illness associated with the disease or disorder related to SARS-CoV-2 by at least about 1 day, 2 days, 4 days, 6 days, 8 days, 10 days, 12 days, 14 days, 16 days, 18 days, 20 days, 22 days, 24 days, 26 days, 28 days, or 30 days.

[0120] In embodiments, administration of the compositions disclosed herein improve COVID-19, ARDS, inflammation, organ, or tissue damage and/or the symptoms thereof in a subject compared to an untreated SARS-CoV-2 infected subject. In embodiments, COVID-19, ARDS, inflammation, organ, or tissue damage and/or the symptoms thereof are improved for about 1 week, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 8 months, about 1 year, about 2 years, about 5 years, and/or about 10 years compared with cardiac function of an untreated SARS-CoV-2 infected subject. In embodiments, COVID-19, ARDS, inflammation, organ, or tissue damage and/or the symptoms thereof are improved by about 1%, about 5%, about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, or about 90% compared with cardiac function of an untreated SARS-CoV-2 infected subject. In embodiments, this improvement in COVID-19, ARDS, inflammation, organ, or tissue damage and/or the symptoms thereof is observed at the time points disclosed herein.

Subject Populations

[0121] Any appropriate subject may be administered the compositions of the present disclosure. In embodiments, the subject is at risk of developing COVID-19, ARDS, inflammation, organ, or tissue

damage and/or the symptoms thereof. In embodiments, the subject is experiencing COVID-19, ARDS, inflammation, organ, or tissue damage and/or the symptoms thereof. In embodiments, the subject is at risk of developing inflammatory lung damage. In embodiments, the subject is experiencing inflammatory lung damage. In embodiments, the subject is infected with, or presumed to be infected with, SARS-CoV-2. In embodiments, the subject has COVID-19 and/or is experiencing symptoms associated with COVID-19. In embodiments, the subject is hospitalized. In embodiments, the subject has a mild case of COVID-19, and/or is experiencing mild symptoms associated with COVID-19. In embodiments, the subject has a moderate case of COVID-19, and/or is experiencing moderate symptoms associated with COVID-19. In embodiments, the subject has a severe case of COVID-19, and/or is experiencing severe symptoms associated with COVID-19 (e.g., rapid clinical deterioration, ARDS, and/or death). In embodiments, the subject requires assistance breathing. In embodiments, the subject is receiving oxygen. In embodiments, the subject is receiving oxygen by face mask or nasal cannula with prongs. In embodiments, the subject requires a ventilator to breathe. In embodiments, the subject exhibits low oxygen saturation levels. In embodiments, the subject exhibits an increased respiratory rate (e.g., greater than 24 breaths/minute). In embodiments, the subject exhibits an accompanying fever (e.g., temperature greater than 101°F). In embodiments, the subject is at risk of progressing to more severe COVID-19 or symptoms, and the composition of the present disclosure is administered before symptoms worsen.

[0122] In embodiments, a subject has been vaccinated against SARS-CoV-2. In embodiments, a subject has not been vaccinated against SARS-CoV-2. Suitable vaccines comprise mRNA vaccines, vector vaccines, protein subunit vaccines, and combinations thereof. In embodiments, a subject has received at least about 1, 2, 3, or 4 administrations of the vaccine. In embodiments, a subject has received a booster of the vaccine, such as a third or fourth administration.

[0123] In embodiments, a subject has previously been treated with an anti-SARS-CoV-2 therapy or COVID-19 therapy. Suitable therapies comprise: monoclonal antibodies (e.g., bamlanivimab, evusheld, sotrovimab, Regen-Cov, and etesevimab), Remdesivir, mechanical ventilation, oxygen therapy, and combinations thereof. In embodiments, a subject in need thereof is recalcitrant to an anti-SARS-CoV-2 therapy or COVID-19 therapy for example those suitable therapies provided herein.

[0124] In embodiments the subject is between the ages of 18 and 100. In embodiments, the subject is between the ages of 18 and 85. In embodiments, the subject is between the ages of 50 and 100. In embodiments, the subject is older than 65 years old. In embodiments, the subject is 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 years old or older. In embodiments, the subject who is older than

50 years old is considered high risk of developing severe disease (e.g., COVID-19 or ARDS). In embodiments, the subject who is older than 65 years old is considered high risk of developing severe disease (e.g., COVID-19 or ARDS).

[0125] In embodiments, a subject who possess one or more comorbidities is considered high risk for developing severe disease (e.g., COVID-19 or ARDS). In embodiments, the comorbidity includes, but is not limited to, obesity, hypertension, diabetes, an autoimmune disorder (e.g., rheumatoid arthritis), heart disease, heart failure, atherosclerosis, cancer (e.g., lung cancer), a history of smoking or exposure to other lung-damaging agents), liver disease, alcoholism, other pulmonary infection, and chronic kidney disease. In embodiments, the subject at risk presents with elevated markers of cardiac injury or dysfunction (e.g., hsTnI, NT-proBNP). In embodiments, race is a factor in a subject being considered at high risk of developing severe disease (e.g., COVID-19 or ARDS). In embodiments, socioeconomic status is a factor in a subject being considered at high risk of developing severe disease (e.g., COVID-19 or ARDS).

[0126] In embodiments, while being administered with a composition of the present disclosure, the subject continues to receive standard of care for any comorbidities.

Combination Therapies

[0127] The compositions disclosed herein may be administered with various therapies used to treat, prevent, delay, or ameliorate inflammatory lung damage, COVID-19, and/or ARDS. In embodiments, the composition is administered concomitantly with standard of care medications. The one or more therapeutic agents may be any compound, molecule, or substance that exerts therapeutic effect to a subject in need thereof.

[0128] In embodiments, the compositions disclosed herein are administered with therapeutic agents including, but not limited to, antiviral agents, anti-malarial agents, agents that protect epithelial cells, defibrotide, convalescent plasma, chloroquine, hydroxychloroquine, remdesivir, desferal, favipiravir, corticosteroids, clevudine, anti-inflammatory agents, anti-oxidant agents, dapagliflozin, IFX-1, ruxolitinib, baricitinib, interferon beta 1a, azithromycin, tocilizumab, acalabrutinib, umifenovir, ciclesonide, sarilumab, anti-interleukin agents, and telmisartan.

[0129] The one or more therapeutic agents may be “co-administered”, i.e., administered together in a coordinated fashion to a subject, either as separate compositions or admixed in a single composition. By “co-administered”, the one or more therapeutic agents may also be administered simultaneously with the present compositions, or be administered separately, including at different times and with different frequencies. The one or more therapeutic agents may be administered by any known route, such as orally, intravenously, intramuscularly, nasally, via aerosol, subcutaneously, intra-vaginally, intra-

rectally, and the like; and the therapeutic agent may also be administered by any conventional route. In embodiments, the composition is administered subcutaneously.

[0130] When two or more therapeutic agents are used in combination, the dosage of each therapeutic agent is commonly identical to the dosage of the agent when used independently. However, when a therapeutic agent interferes with the metabolism of others, the dosage of each therapeutic agent is properly adjusted. Alternatively, where the two or more therapeutic agents show synergistic effects, the dose of one or more may be reduced. Each therapeutic agent may be administered simultaneously or separately in an appropriate time interval.

Methods of making compositions

[0131] Provided are methods of making compositions described herein. Methods of making compositions comprise introducing into a spirulina a nucleic acid sequence encoding the at least one VHH. In embodiments, the methods of making compositions comprise introducing into a spirulina a polypeptide. In embodiments, the spirulina is *A. platensis*.

[0132] Any appropriate means for transforming spirulina may be used in the present disclosure. Exemplary methods for transforming spirulina to express a heterologous protein are described in U.S. Patent No. 10,131,870, which is incorporated by reference herein in its entirety.

[0133] In embodiments, methods of making a composition include introducing an expression vector having a nucleic acid sequence encoding the at least one VHH into a spirulina cell. In embodiments, the vector is not integrated into the spirulina genome. In embodiments, the vector is a high copy or a high expression vector. In embodiments the nucleic acid sequence encoding the at least one VHH is under the control of a strong promoter. In embodiments the nucleic acid sequence encoding the at least one VHH is under the control of a constitutive promoter. In embodiments the nucleic acid sequence encoding the at least one VHH is under the control of an inducible promoter.

[0134] In embodiments, the vector or a portion thereof (e.g., the VHH coding portion) is integrated into the spirulina genome. In embodiments, methods of making a composition include introducing a vector (e.g., via homologous recombination) having homology arms and a nucleic acid sequence encoding the at least one VHH into a spirulina cell.

[0135] In embodiments, a vector having homology arms and a nucleic acid sequence encoding the at least one VHH can be introduced into spirulina using electroporation. The electroporation is preferably carried out in the presence of an appropriate osmotic stabilizer.

[0136] Prior to introduction of the vector into spirulina, the spirulina may be cultured in any suitable media for growth of cyanobacteria such as SOT medium. SOT medium includes NaHCO₃ 1.68 g,

K₂HPO₄ 50 mg, NaNO₃ 250 mg, K₂SO₄ 100 mg, NaCl 100 mg, MgSO₄·7H₂O, 20 mg, CaCl₂·2H₂O 4 mg, FeSO₄·7H₂O 1 mg, Na₂EDTA·2H₂O 8 mg, A₅ solution 0.1 mL, and distilled water 99.9 mL. A₅ solution includes H₃BO₃ 286 mg, MnSO₄·5H₂O 217 mg, ZnSO₄·7H₂O 22.2 mg, CuSO₄·5H₂O 7.9 mg, Na₂MoO₄·2H₂O 2.1 mg, and distilled water 100 mL. Cultivation may occur with shaking (e.g., 100-300 rpm) at a temperature higher than room temperature (e.g., 25-37°C.) and under continuous illumination (e.g., 20-2,000, 50-500, or 100-200 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$). The growing cells may be harvested when the optical density at 750 nm reaches a predetermined threshold (e.g., OD₇₅₀ of 0.3-2.0, 0.5-1.0, or 0.6-0.8). A volume of the harvested cells may be concentrated by centrifugation then resuspended in a solution of pH balancer and salt. The pH balancer may be any suitable buffer that maintains viability of spirulina while keeping pH of the media between 6 and 9 pH, between 6.5 and 8.5 pH, or between 7 and 8 pH. Suitable pH balancers include HEPES, HEPES-NaOH, sodium or potassium phosphate buffer, and TES. The salt solution may be NaCl at a concentration of between 50 mM and 500 mM, between 100 mM and 400 mM, or between 200 mM and 300 mM. In an embodiment between 1-50 mL of 1-100 mM pH balance may be used to neutralize the pH.

[0137] Cells collected by centrifugation may be washed with an osmotic stabilizer and optionally a salt solution (e.g., 1-50 mL of 0.1-100 mM NaCl). Any amount of the culture may be concentrated by centrifugation. In an embodiment between 5-500 mL of the culture may be centrifuged. The osmotic stabilizer may be any type of osmotic balancer that stabilizes cell integrity of spirulina during electroporation. In an embodiment, the osmotic stabilizer may be a sugar (e.g., w/v 0.1-25%) such as glucose or sucrose. In an embodiment the osmotic stabilizer may be a simple polyol (e.g., v/v 1-25%) including glycerine, glycerin, or glycerol. In an embodiment the osmotic stabilizer may be a polyether including (e.g., w/v 0.1-20%) polyethylene glycol (PEG), poly(oxyethylene), or poly(ethylene oxide) (PEO). The PEG or PEO may have any molecular weight from 200 to 10,000, from 1000 to 6000, or from 2000 to 4000. In an embodiment the pH balancer or buffer may be used instead of or in addition to the osmotic stabilizer.

[0138] A vector having homology arms and a nucleic acid sequence encoding the at least one exogenous polypeptide can be introduced into spirulina cells (e.g., *A. platensis*.) that are cultured and washed with an osmotic stabilizer as described above. Electroporation can be used to introduce the vector.

[0139] Electroporation may be performed in a 0.1-, 0.2- or 0.4-cm electroporation cuvette at between 0.6 and 10 kV/cm, between 2.5 and 6.5 kV/cm, or between 4.0 and 5.0 kV/cm; between 1 and 100 μF , between 30 and 70 μF , or between 45 and 55 μF ; and between 10 and 500 m Ω , between 50 and 250 m Ω , or between 90 and 110 m Ω . In embodiments, electroporation may be performed at 4.5 kV/cm, 50 μF , and 100 m Ω .

[0140] Following electroporation, the cells may be grown in the presence of one or more antibiotics selected based on resistance conferred through successful transformation with the plasmid. Post-electroporation culturing may be performed at reduced illumination levels (e.g., 5-500, 10-100, or 30-60 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$). The culturing may also be performed with shaking (e.g., 100-300 rpm). The level of antibiotics in the media may be between 5 and 100 $\mu\text{g/mL}$. Post-electroporation culturing may be continued for 1-5 days or longer. Successful transformants identified by antibiotic resistance may be selected over a time course of 1 week to 1 month on plates or in 5-100 mL of SOT medium supplemented with 0.1-2.0 μg of appropriate antibiotics.

[0141] A vector used in the methods may be a plasmid, bacteriophage, or a viral vector into which a nucleic acid sequence encoding the at least one VHH may be inserted or cloned. A vector may comprise one or more specific sequences that allow recombination into a particular, desired site of the spirulina's chromosome. These specific sequences may be homologous to sequences present in the wild-type spirulina. A vector system can comprise a single vector or plasmid, two or more vectors or plasmids, some of which increase the efficiency of targeted mutagenesis, or a transposition. The choice of the vector will typically depend on the compatibility of the vector with the spirulina cell into which the vector is to be introduced. The vector can include a reporter gene, such as a green fluorescent protein (GFP), which can be either fused in frame to one or more of the encoded VHHs, or expressed separately. The vector can also include a positive selection marker such as an antibiotic resistance gene that can be used for selection of suitable transformants. The vector can also include a negative selection marker such as the type II thioesterase (*tesA*) gene or the *Bacillus subtilis* structural gene (*sacB*). Use of a reporter or marker allows for identification of those cells that have been successfully transformed with the vector.

[0142] In embodiments, the vector includes one or two homology arms that are homologous to DNA sequences of the spirulina genome that are adjacent to the targeted locus. The sequence of the homology arms can be partially or fully complementary to the regions of spirulina genome adjacent to the targeted locus.

[0143] The homology arms can be of any length that allows for site-specific homologous recombination. A homology arm may be any length between about 2000 bp and 500 bp. For example, a homology arm may be about 2000 bp, about 1500 bp, about 1000 bp, or about 500 bp. In embodiments having two homology arms, the homology arms may be the same or different length. Thus, each of the two homology arms may be any length between about 2000 bp and 500 bp. For example, each of the two homology arms may be about 2000 bp, about 1500 bp, about 1000 bp, or about 500 bp.

[0144] A portion of the vector adjacent to one homology arm or flanked by two homology arms modifies the targeted locus in the spirulina genome by homologous recombination. The modification may change a length of the targeted locus including a deletion of nucleotides or addition of nucleotides. The addition or deletion may be of any length. The modification may also change a sequence of the nucleotides in the targeted locus without changing the length. The targeted locus may be any portion of the spirulina genome including coding regions, non-coding regions, and regulatory sequences.

NUMBERED EMBODIMENTS

[0145] Notwithstanding the appended claims, the following numbered embodiments also form part of the instant disclosure.

[0146] 1. A VHH antibody that binds SARS-CoV-2, wherein the VHH antibody comprises three complementarity determining regions (CDR1, CDR2, and CDR3) each of which is selected from an amino acid sequence comprising at least about 85% identity to a sequence in Table 1.

[0147] 2. The VHH antibody of embodiment 1, wherein the CDR1, CDR2, and CDR3 each comprise at least about 90%, 95%, 97%, or 99% identity to a sequence in Table 1.

[0148] 3. The VHH antibody of any one of embodiments 1-2, wherein CDR1, CDR2, and CDR3 correspond to SEQ ID NO: 610, 611, and 612.

[0149] 4. The VHH antibody of embodiment 3, wherein the VHH antibody comprises at least 85% identity with SEQ ID NO: 365.

[0150] 5. The VHH antibody of any one of embodiments 1-2, wherein CDR1, CDR2, and CDR3 correspond to SEQ ID NO: 421, 422, and 423.

[0151] 6. The VHH antibody of embodiment 5, wherein the VHH antibody comprises at least 85% identity with SEQ ID NO: 302.

[0152] 7. A polypeptide comprising the VHH antibody of any one of embodiments 1-6.

[0153] 8. A polypeptide comprising at least two VHH antibodies of any one of embodiments 1-6.

[0154] 9. The polypeptide of embodiment 8, wherein the at least two VHH antibodies comprise SEQ ID NO: 365 or SEQ ID NO: 302.

[0155] 10. The polypeptide of embodiments 8-9, wherein the at least two VHH antibodies are connected to each other via a linker.

[0156] 11. The polypeptide of embodiment 10, wherein the linker comprises at least about 85% identity to SEQ ID NO: 390.

[0157] 12. A polypeptide comprising at least three VHH antibodies of any one of embodiments 1-6.

- [0158] 13. A polypeptide comprising at least four VHH antibodies of any one of embodiments 1-6.
- [0159] 14. The polypeptide of embodiment 12 or 13, wherein the VHH antibodies are different.
- [0160] 15. The polypeptide of embodiment 12 or 13, wherein the VHH antibodies are the same.
- [0161] 16. A pharmaceutical composition comprising the VHH antibody of any one of embodiments 1-6 and a pharmaceutically acceptable carrier.
- [0162] 17. A pharmaceutical composition comprising the polypeptide of any one of embodiments 7-15 and a pharmaceutically acceptable carrier.
- [0163] 18. A method of treating a disease or disorder related to SARS-CoV-2, the method comprising: administering the pharmaceutical composition of embodiment 16 to a subject in need thereof, thereby treating the disease or disorder related to SARS-CoV-2.
- [0164] 19. A method of treating a disease or disorder related to SARS-CoV-2, the method comprising: administering the pharmaceutical composition of embodiment 17 to a subject in need thereof, thereby treating the disease or disorder related to SARS-CoV-2.
- [0165] 20. The method of embodiment 18 or 19, wherein the subject in need thereof is infected with SARS-CoV-2 as determined by PCR assay.
- [0166] 21. The method of any one of embodiments 18-20, wherein the subject in need thereof has been diagnosed as being infected with SARS-CoV-2.
- [0167] 22. The method of any one of embodiments 18-21, wherein the subject in need thereof is at risk of developing Coronavirus Disease (COVID).
- [0168] 23. The method of embodiment 22, wherein the subject in need thereof is at risk of developing severe COVID.
- [0169] 24. The method of any one of embodiments 18-23, wherein the subject in need thereof has one or more comorbidities.
- [0170] 25. The method of embodiment 24, wherein the subject in need thereof is treated for the one or more comorbidities.
- [0171] 26. The method of any one of embodiments 18-25, wherein the pharmaceutical composition is delivered encapsulated in a spirulina cell.
- [0172] 27. The method of embodiment 26, wherein the spirulina cell is dried.
- [0173] 28. The method of embodiment 26 or 27, wherein the spirulina cell is of strain *A. platensis*.
- [0174] 29. The method of any one of embodiments 18-28, wherein the pharmaceutical composition is delivered orally.
- [0175] 30. The method of any one of embodiments 18-28, wherein the pharmaceutical composition is delivered via the airway.

- [0176] 31. The method of embodiment 30, wherein, the pharmaceutical composition is delivered via inhalation.
- [0177] 32. The method of embodiment 30 or 31, wherein the pharmaceutical composition is delivered nasally.
- [0178] 33. The method of any one of embodiments 18-32, wherein the SARS-CoV-2 is a variant of concern.
- [0179] 34. The method of embodiment 33, wherein the variant of concern is selected from the group consisting of: alpha, beta, gamma, delta, and omicron.
- [0180] 35. The method of any one of embodiments 18-34, wherein the SARS-CoV-2 evades an immune response associated with vaccine therapy.
- [0181] 36. A method of treating a disease or disorder related to SARS-CoV-2, the method comprising: administering the pharmaceutical composition of embodiment 16 or 17 to a subject in need thereof thereby treating the disease or disorder related to SARS-CoV-2, wherein the disease or disorder is recalcitrant to anti-SARS-CoV-2 vaccine therapy.
- [0182] 37. The method of embodiment 36, wherein the subject in need thereof has previously been administered an anti-SARS-CoV-2 monoclonal therapy.
- [0183] 38. The method of embodiments 36-37, wherein the subject in need thereof has received a booster of the anti-SARS-CoV-2 vaccine therapy.
- [0184] 39. The method of embodiments 36-37, wherein the subject in need thereof has not received a booster of the anti-SARS-CoV-2 vaccine therapy.
- [0185] 40. The method of any one of embodiments 36-39, wherein the administering is effective in reducing or eliminating a symptom of the disease or disorder related to the SARS-CoV-2.
- [0186] 41. The method of embodiment 40, wherein the symptom is a gastrointestinal symptom.
- [0187] 42. The method of embodiment 40, wherein the symptom is a respiratory symptom.
- [0188] 43. The method of any one of embodiments 36-42, wherein the administering is effective in reducing a period of illness associated with the disease or disorder related to the SARS-CoV-2 by at least about 1-3 days.

EXAMPLES

Example 1 - Binding characterization of anti-SARS-CoV-2 VHHs

[0189] The VHHs of the disclosure were assessed for affinity for the RBD or intact spike protein as measured by biolayer interferometry (BLI). The VHHs of the disclosure were expressed with scaffolds to promote solubility and bioactivity. Specifically, the VHHs were designed as monomers (MBP-VHH-

6His) and dimers (MBP-5HVZ-VHH-6His) for expression and purification from *E. coli*, and subsequent *in vitro* characterization and expression in spirulina. An additional set of VHHs were built into alternative multimeric scaffolds using the proline-rich linker.

[0190] To measure VHH binding affinity to RBD, binding kinetics were assessed by BLI. VHH binding activity was determined by a quantitative label-free assay using a biolayer interferometry instrument, a ForteBio Octet, which can measure the kinetic parameters k_{on} and k_{off} to determine the dissociation constant K_d . Rates of association and dissociation were calculated by incubating antigen-loaded biosensors with various concentrations of VHHs (monomers at 363.6 nM, 36.4 nM, and 3.64 nM and dimers at 163.9 nM, 16.4 nM, and 1.64 nM) (**Figure 1**). The binding kinetics confirm increased binding affinity by dimeric VHHs, mainly due to decreased off-rate. To further assess the effect of multimerization on binding, selected VHHs were designed as dimers, trimers, heptamers, and higher order multimers using a viral particle scaffold. RBD binding to these multimerized VHHs was assessed using BLI (**Figure 2**).

Example 2 - Viral neutralization characterization of anti-SARS-CoV-2 VHHs

[0191] The VHHs of the disclosure were assessed for neutralizing activity in an infectivity assay using SARS-CoV-2 pseudotyped lentivirus. A key criterion is neutralizing activity, since it is expected that some VHHs will bind to the target antigen without interfering with its function. Pseudoviruses expressing SARS-CoV-2 spike protein are produced by co-transfection into 293T cells of plasmids encoding a lentivirus backbone containing a luciferase reporter and the spike protein. Tested VHHs were mixed with the harvested pseudovirions, added to ACE2 expressing cells, and luciferase quantified at 3 days.

[0192] VHHs were screened at a single concentration of 10 $\mu\text{g/mL}$, equivalent to 150-180 nM (**Figure 3**) VHHs exhibiting more than 50% inhibition of infectivity were selected for further analysis. As shown in **Figure 3**, among the 141 VHHs screened, none of the monomers (as MBP fusion proteins) exhibited more than 50% inhibition of the infectivity. Twenty-five dimers, six trimers, ten heptamers and one 30-mer showed more than 50% inhibition of the infectivity (**Figure 3**). Neutralization assays were then performed on this set of 42 VHHs over a serial dilution curve ranging from 50 $\mu\text{g/mL}$ to 0.016 $\mu\text{g/mL}$ (**Figure 4**). IC_{50} values were calculated and are shown in **Figure 2**.

Example 3 - Epitope binning of anti-SARS-CoV-2 VHHs

[0193] VHHs that exhibited measurable affinity binding and bioactivity in pseudovirus neutralization were examined for competitive binding. This epitope binning analysis determines compatible VHHs for a therapeutic cocktail. BLI methods are used in epitope binning. In this experiment, VHHs were

compared for successive binding in an N-by-N format (**Figure 5**). Initial epitope binning experiments were conducted using dimeric forms of selected VHHs and SARS VHH-72, a neutralizing antibody developed by the McLellan lab (University of Texas at Austin), to exploit the slow off-rate due to high avidity interactions. The preliminary data showed one VHH that directly competes with SARS VHH-72 (**Figure 4**). The dimeric anti-SARS-CoV-2 VHH, R2D-D3, competed with SARS VHH-72 dimer. Still, no competition was observed against other VHHs tested in this initial binning experiment, suggesting the presence of a shared epitope region between SARS VHH-72 and R2D-D3. On the other hand, dimeric VHHs (VHH-5HVZ-MBP) R2A-F3, R2B-D5, R2B-E6, R2C-F6, and R2C-G6 compete for RBD binding and appear to recognize an overlapping epitope (**Figure 4**). These data define at least two neutralizing epitopes in the RBD that can be independently targeted by two different classes of VHHs.

Example 4 - ELISA-based competition of anti-SARS-CoV-2 VHHs

[0194] Orthogonal to BLI-based binning experiment, ELISA-based competition experiments against SARS VHH-72 and VHH R2B-D5 were used to assess the epitope space on SARS-CoV-2 RBD recognized by VHHs. In agreement with epitope binning experiments, the data showed that dimeric R2D-D3 competed with SARS VHH-72 while the other VHHs tested showed minimal or no competition against SARS VHH-72. In contrast, the competition experiment with R2B-D5 showed three possible groups where SARS VHH-72 and R5D-D4 did not compete for RBD binding at all, R2D-D3 S2B-C10 exhibited minimal competition while R5C-A2, R5A-B7, R5A-C5, and R2B-D5 showed good competition (**Figure 6**).

Example 5 - ACE2-competition of anti-SARS-CoV-2 VHHs

[0195] To complement pseudovirus-based virus neutralization, the VHHs of the disclosure were assayed in human ACE2 competition assay. In this assay, high binding ELISA plates are coated with human ACE2-FC and SARS-CoV-2 RBD incubated with a 5-fold VHH dilution series before application to the ACE2-FC coated plates. RBD binding to ACE2-FC was detected using mouse anti-AviTag antibody (Avidity Bio). In general, almost all monomeric VHHs showed little or no ACE2 competition while higher-order forms of some VHHs (dimeric, trimeric, or heptameric forms) competed for ACE2 strongly (**Figure 6**).

Example 6 - anti-SARS-CoV-2 VHHs were assessed for resistance to cleavage by intestinal proteases

[0196] The VHHs of the disclosure were assessed for resistance to intestine proteases using an ELISA based assay developed at Lumen. Desirable VHH pharmacokinetics within the GI tract are strongly dependent on the degree of resistance to GI proteases, and monomer VHHs differ dramatically their ability to remain functionally active following incubations with GI proteases (chyme). To rapidly screen VHHs for susceptibility to GI proteases VHH binding activity was evaluated after treatment with an intestinal protease. Following protease incubation, protease was neutralized with inhibitors and VHH binding activity was measured by capture ELISA using the target antigen. VHHs that functionally survived the protease incubations were able to bind the target antigen, which was detected with an anti-VHH or anti-VHH conjugate antibody. An EC₅₀ binding curve was calculated using a 4-parameter logistic curve fit of the results and the value was compared to an undigested control to calculate the percent activity remaining.

[0197] The VHHs of the invention were incubated with trypsin, chymotrypsin, and elastase. Protease resistance results agreed well between the ELISA and neutralization assays. Protease resistance was observed with trypsin, chymotrypsin, and elastase (**Figure 7**).

[0198] *Example 7– Protein Design to Improve anti-RBD VHH binding*

[0199] Exploiting the hetero-trimeric interaction between the PKA-R dimerization motif and the region of smAKAP that binds the dimer, high order complexes were generated that enable multimeric interactions of diverse molecules. Receptor Binding Domain (RBD)-interacting VHHs were designed in either N- and/or C- terminus of the smAKAP peptide. The new higher order forming (Cerberbody or Hydrabody) substrates were designed with or without the solubility enhancer scaffold, MBP. To increase soluble protein expression, two Cys residues in the smAKAP peptide were mutated to serine.

Mutated positions - C16S and C24S

Wild type -- TVILEYAHRLSQDILCDALQQWAC (SEQ ID NO: 389)

Mutant ----- TVILEYAHRLSQDILSDALQQWAS (SEQ ID NO: 390)

[0200] Cerberbody constructs are formed when a spirulina strain (either two independent strains or a single strains from different ORFs) express a 5HVZ mediated homodimer molecule and smAKAP linked monomer VHH. These independent complexes form a further complex structure that will have a valence of three binding moieties. The increased valence will result in increased apparent binding affinity, mainly due to avidity-based interaction. The three binding moieties can be derived from the same VHH, forming a homo-trimer. The design also enables mixing diverse VHHs with desirable binding and virus neutralization property. Such a molecule complex will be able to bind mutant variants of a virus.

[0201] As shown here, the Cerberbody complexes show increased apparent binding when compared to dimeric or monomeric forms. Building on the Cerberbody complexes, components were designed that are used to form Hydrabody which consists of four binding moieties. Hydrabody complexes are formed when spirulina strain (either two independent strains or a single strain from different ORFs) express a 5HVZ mediated homodimer molecule and smAKAP linked dimeric VHHs. The smAKAP containing dimers contain VHHs on both the N- and C- terminal of smAKAP. The VHHs fused to the smAKAP can form homo-dimeric (the same VHH) or hetero-dimeric (two different VHHs on either end). Such a configuration enables inclusion of multiple, therapeutically effective VHHs in a single complex.

[0202] In addition to increased avidity-based apparent binding, the higher order complexes of Cerberbody and Hydrabody enable the formation of super potent and cross-reactive therapeutic complex that can bind and neutralize diverse virus variants. For example, PP758 neutralizes SARS-COV-2 variants alpha, beta, and gamma but not delta while PP917 neutralizes alpha and delta SARS-CoV-2 variants. A Hydrabody that contains a dimer of dimers of these two VHHs will be able to bind better than each separately and neutralize both variants. Such a design combines multiple components easily where diverse epitope can be engaged resulting in decreased virus escape mutants.

[0203] Dimerization of the VHH sequences was shown to increase binding avidity of the VHHs to the receptor binding domain (RBD) of the SARS-CoV-2 spike protein. The presence of a 5HVZ dimerization motif in a polypeptide acts as a docking platform which mediates protein-protein interactions between three α -helical chains, placing the monomers antiparallel to one another. Asymmetric disulfide bond formation between the promoters of the construct further strengthens the dimerization. The cAMP dependent protein kinase (PKA) regulatory subunit (R) PKA-R dimerizes through the 5HVZ motif. (See Burgers et al. (2016) FEBS J. 283(11):2132-2148, the contents of which are incorporated by reference herein). This dimerization motif mediates the cellular interaction between cAMP dependent kinase and the A-kinase anchoring protein. The sAKAP polypeptide is a small membrane anchored peptide that binds the PKA-R dimer and localizes the enzyme to the substrate. The smAKAP used herein can comprise two mutated Cys residues (SEQ ID NO: 390) to improve protein folding and expression.

Table 4 shows anti-RBD VHHs that exhibit acceptable antigen binding as dimers and virus neutralization for complex formation. VHH S3B-C8 is the VHH of the dimeric PP917 construct; R2B-D5 is the VHH in the dimeric PP758 construct. In addition, monomeric forms were designed as fusion proteins with the wild type docking peptide, smAKAP (SEQ ID NO: 389) and cysteine-to-Serine mutant forms of the smAKAP docking peptide (SEQ ID NO: 390). These constructs were also designed with and without maltose binding protein (MBP) as a solubility enhancer scaffold.

Lane	Sample	Description	MW
1	Ladder		
2	PP1625-001	S3b-C8_smAKAP_C16S_8H	17
3	PP1864-001	R2B-D5_smAKAP_C16S_H8 *	17
4	PP1823-001	H8_MBP_smAKAP-C16S_R2B-D5	61
5	PP1824-001	R2B-D5_smAKAP_H8 *	17
6	PP1624-001	S3b-C8_smAKAP_8H **	17

[0204] These constructs were produced for expression in *E. coli*. As shown in **Figure 8A-Figure C**, the VHH R2B-D5 (indicated with * in **Table 4**) containing constructs (VHH in PP758 construct) did not express in an *E. coli* inducible expression system. Further, the constructs containing the wild type (e.g., cysteine containing) docking peptide (indicated with ** in **Table 4**) show higher order oligomerization through the free cysteine residues (see Lane 6 in the “non-reduced portion of Figure 8C).

[0205] The constructs were purified on a nickel charged affinity resin (Ni-NTA) and the subsequent elutions were then subjected to SEC purification and run on an agarose gel. The samples were also subjected to high resolution gel filtration to study aggregate formation. **Figure 9A- Figure 9B** shows the expression of the S3b-C8_smAKAP_8H construct which comprises the wild type smAKAP protein. The presence of the two free cysteine residues results in aggregate formation. In contrast, mutating the cysteine residues in the smAKAP protein resulted in monodispersed monomer protein. See **Figure 10A-Figure 10B**. Finally, the H8_MBP_smAKAP-C16S_R2B-D5 construct which comprises the smAKAP peptide fused to the R2B-D5 VHH and expressed as a MBP-fusion also resulted in monodispersed monomer protein See **Figure 11A-Figure 11B**.

Example 8- Comparison of binding of VHH complexes to receptor binding domain of SARS-CoV-2 spike protein

[0206] VHH sequences can associate to form a “Cerberbody” through the association of a dimeric VHH construct to another through a smAKAP protein. Several different configurations of VHH sequences

(see **Table 5**) were compared to determine which showed increased binding avidity to the receptor binding domain of the SARS-CoV-2 spike protein.

Table 5: Configurations of VHH sequences in exemplary Cerberbodies

Sample ID	Description	VHH1	VHH2
PP758	2-R2B-D5	R2B-D5	
PP917	2-S3b-C8	S3b-C8	
PP1625	1-S3b-C8		S3b-C8
PP1823		R2B-D5	R2B-D5
PP1896	2-R2B-D5 and 1-S3b-C8	R2B-D5	S3b-C8
PP1897	3-S3b-C8	S3b-C8	S3b-C8
PP1898	1-R2B-D5 and 2-S3b-C8	S3b-C8	R2B-D5

[0207] Hypotheses:

- Compared to the dimeric PP758, the Cerberbody complex PP1896 will exhibit comparable binding and neutralization
- Compared to the monomeric PP917, the Cerberbody complex PP1896 will exhibit increased binding and virus neutralization potency
- Compared to the monomeric PP917, the Cerberbody complex PP1897 will exhibit increased binding due to avidity-based interactions
- Compared to the monomeric PP917, the Cerberbody complex PB1898 will exhibit increased binding due to avidity-based interactions

[0208] Study and Results:

[0209] The constructs were purified on a nickel charged affinity resin (Ni-NTA) and the subsequent elutions were then subjected to SEC purification and run on an agarose gel. The samples were also subjected to high resolution gel filtration to study aggregate formation.

[0210] Increased binding was observed when the VHH valency was increased from monomer to trimer. This increase in binding is mediated by avid interactions. Additionally, the effect of antigen density on binding kinetics was studied. The data shows that when antigen density is increased, a concomitant increase in binding was observed. This effect may be due to a decreased rate of dissociation.

[0211] **Figure 12** shows the monomeric v dimeric v aggregate complex formation of PP758, PP1625, and PP1896. The Cerberbody PP1896 form a complex when mixed 1:1.5 molar ratio between the dimeric P758 and the monomeric PP1625. **Figure 13** and **Table 6** show that the Cerberbody complex demonstrates about 100-fold increased binding avidity compared to PP758.

Table 6

Sample ID	Description	VHH1	VHH2	KD (nM)	ka (1/Ms)	kdis (1/s)	Fold improvement (KD)
PP758	2-R2B-D5	R2B-D5		0.76	8.202E04	6.226E-05	
PP917	2-S3b-C8	S3b-C8		10.80	1.012E05	1.404E-03	
PP1896	2-R2B-D5 and 1-S3b-C8	R2B-D5	S3b-C8	<0.001	2.482E05	<1.0E-07	~100 X compared to PP758

[0212] The Cerberbody PP1897 was compared to the dimeric PP917 and the monomeric PP1625. **Figure 14** shows the Cerberbody PP1897 form a complex when mixed 1:15 molar ratio between the dimeric pP917 and the monomeric PP1625. **Figure 15** and **Table 7** show that the Cerberbody complex demonstrates about 15-fold greater binding avidity compared to PP917.

Table 7

Sample ID	Description	VHH1	VHH2	KD (nM)	ka (1/Ms)	kdis (1/s)	Fold improvement (KD)
PP758	2-R2B-D5	R2B-D5		0.76	8.202E04	6.226E-05	
PP917	2-S3b-C8	S3b-C8		10.80	1.012E05	1.404E-03	
PP1897	3-S3b-C8	S3b-C8	S3b-C8	0.73	5.212E05	3.818E-04	~15 X compared to PP917

[0213] **Figure 16** shows that the Cerberbody PP1897 form a minor complex when mixed 1:1.5 molar ratio between the dimeric PP917 and the monomeric PP1823. Most of the dimer remained uncomplexed,

and SDS-PAGE analysis of the MBP fused monomer PP1898 show degradation. **Figure 17** and **Table 8** show that PP1898 demonstrates about 30-fold binding avidity compared to PP917.

Table 8

Sample ID	Description	VHH1	VHH2	KD (nM)	ka (1/Ms)	kdis (1/s)	Fold improvement (KD)
PP758	2-R2B-D5	R2B-D5		0.76	8.202E04	6.226E-05	
PP917	2-S3b-C8	S3b-C8		10.80	1.012E05	1.404E-03	
PP1898	1-R2B-D5 and 2-S3b-C8	S3b-C8	R2B-D5	0.40	5.405E05	2.181E-04	~30 X compared to PP917

Example 9 – Hydrabody design, protein purification, and binding

[0214] In contrast with the Cerberbody complexes described above, which comprise three VHH sequences, constructs comprising four VHH sequences (“Hydrabodies”) were constructed and characterized. An exemplary Hydrabody is shown in **Figure 18A**. **Table 9** describes various constructs used in this study.

Table 9

Protein product	Constructs	Dimeric VHH	VHH1	VHH2	VHH3	Complex
PP1892	R2B-D5_smAKAP-C16S_S3B-C8_H6	PP758	R2B-D5	R2B-D5	S3B-C8	3xR2B-D5 and 1xS3B-C8
		PP917	S3B-C8	R2B-D5	S3B-C8	3xS3B-C8 and 1xR2B-D5
PP1893	R2B-D5_smAKAP-C16S_R2B-D5_H6	PP758	R2B-D5	R2B-D5	R2B-D5	4xR2B-D5
		PP917	S3B-C8	R2B-D5	R2B-D5	2xR2B-D5 and 2xS3B-C8
PP1894	S3B-C8_smAKAP-C16S_R2B-D5_H6	PP758	R2B-D5	S3B-C8	R2B-D5	3xR2B-D5 and 1xS3B-C8
		PP917	S3B-C8	S3B-C8	R2B-D5	3xS3B-C8 and 1xR2B-D5

PP1895	S3B-C8_smAKAP-C16S_S3B-C8_H6	PP758	R2B-D5	S3B-C8	S3B-C8	2xR2B-D5 and 2xS3B-C8
		PP917	S3B-C8	S3B-C8	S3B-C8	4xS3B-C8

[0215] In **Table 9**, the protein product column describes the Hydrabody component that contains the smAKAP docking peptide. The construct column describes the VHHs included and their orientation in the polypeptide chain. The third column describes an 5HVZ-containing protein construct. Columns 4 through 6 describe the different VHHs in the reaction mixture: VHH1 is from the 5HVZ motif dimerized construct (e.g., column 3; **Figure 18A**), VHH2 is the N-terminal VHH in the smAKAP-containing dimer, and VHH3 is the C-terminal VHH in the smAKAP-containing dimer.

[0216] Hypotheses:

The Hydrabody complex formed between the 5HVZ-containing dimers and the smAKAP-containing dimers will be compared with the dimers themselves (**Figure 18A**).

- PP1892 will demonstrate increased binding avidity and higher neutralization potency
- PP1893 will demonstrate increased binding avidity and higher neutralization potency.
- PP1894 will demonstrate increased binding avidity and higher neutralization potency
- PP1895 will demonstrate increased binding avidity and higher neutralization potency.

[0217] Study Design and Results:

[0218] The constructs were purified on a nickel charged affinity resin (Ni-NTA) and the subsequent elutions were then subjected to SEC purification and run on an agarose gel. The samples were also subjected to high resolution gel filtration to study aggregate formation.

[0219] **Figure 19** shows that the dimeric R2B-D5-VHH did not express, likely due to the intrinsic toxicity of the protein. **Figure 20A- Figure 10B** show the Ni-NTA purification and dimeric nature of R2B-D5_smAKAP-C16S_S3B-C8_H8. Lanes 2-13 show the IMAC wash and elution fractions following imidazole-based elution. **Figure 21A- Figure 21B** show the Ni-NTA purification and dimeric nature of S3B-C8_smAKAP-C16S_R2B-D5_H6. Lanes 2-12 show the IMAC wash and elution fractions following imidazole-based elution. **Figure 22A- Figure 22B** show the Ni-NTA purification and dimeric nature of S3B-C8_smAKAP-C16S_S3B-C8_H6. Lanes 2-14 show the IMAC wash and elution fractions following imidazole-based elution.

Binding Assay

[0220] The Cerberbodies show increased apparent binding when compared to dimeric or monomeric forms, see **Figure 23**, and **Tables 10** and **11** below. When compared to 1xVHH, 2xVHH and 3xVHH constructs show increased binding. The increased apparent binding with increased valency of VHH indicate an avidity-based interaction.

Table 10: RBD at 1ug/mL

Sample ID	KD (nM)	ka (1/Ms)	kdis (1/s)
PP758 (Dimer)	0.78	1.40E05	1.102E-04
PP917 (Dimer)	10.8	1.095E06	1.185E-02
Cerberbody	0.061	2.840E05	1.728E-05

Table 11: RBD at 10 ug/mL

Sample ID	KD (nM)	ka (1/Ms)	kdis (1/s)
PP758	0.52	4.656E04	2.435E-05
PP917	13.87	1.012E05	1.404E-03
Cerberbody	<0.001	9.296E04	<1.0E-07

[0221] By increasing the valence of the VHHs, we hypothesize that the Hydrabodies will show better binding. The other advantage of the Cerberbody and Hydrabody complexes is the ability to form a complex using VHHs that can bind and neutralize diverse variants. For example, PP758 neutralizes SARS-COV-2 variants alpha, beta, and gamma but not delta while PP917 neutralizes alpha and delta variants. A Hydrabody that contains these two VHHs will be able to bind better than each and neutralize both variants. The design of these constructs combine multiple components easily where diverse epitope can be engaged resulting in decreased escape mutants.

[0222] To evaluate binding of Hydrabody complexes, a binding assay was completed and showed that the 5HVZ-containing two chain dimers and the smAKAP-containing single-chain dimer VHHs exhibit comparable binding (KD comparison between PP1895 and PP917, 5x better binding). The single-chain heterodimer (PP1892) exhibits comparable binding to the high-affinity homodimer PP758, 2x better

binding, and about 30X better binding when compared to the weak binding homodimer PP917, see **Table 12 and Figure 24**.

Table 12: Hydrabody forming components binding comparison

Sample ID	KD (nM)	ka (1/Ms)	kdis (1/s)
R2B-D5_smAKAP-C16S_S3B-C8_H6 (PP1892)	0.33	1.226E06	4.115E-04
S3B-C8_smAKAP-C16S_R2B-D5_H6 (PP1894)	0.44	1.343E06	5.995E-04
S3B-C8_smAKAP-C16S_S3B-C8_H6 (PP1895)	2.06	3.693E06	7.612E-03
MBP-5HVZ-R2B-D5 (PP758)	0.78	4.656E+04	2.435E-05
MBP-5HVZ-S3b-C8 (PP917)	10.8	1.012E+05	1.404E-03

Example 10- production of spirulina extract powder for intranasal delivery

[0223] Frozen SP1741 biomass was added to 1mM ammonium acetate pH 9.3 yield a final concentration of between 10 and 20 g/L ash free dry weight. The suspension was then hand homogenized with a hand immersion blender before high pressure homogenization through a Microfluidics LM-20 high pressure homogenizer set to 14,000 psi, collecting the lysate on ice. Phenylmethylsulfonyl fluoride was added to the lysate to a final concentration of 2 mM and mixed. The mixture was then heated to 30°C and 2mM magnesium chloride was added, followed by benzonase nuclease HC added to a concentration of 1 microgram per 10 grams of biomass. The mixture was allowed to react at 30 °C for 30 minutes, then chilled to 20°C, pH adjusted to pH 9.18 with ammonium hydroxide, and then filtered through a 1000 kDa tangential flow membrane. The permeate was then concentrated and diafiltered using a 30 kDa tangential flow membrane using 1 mM ammonium acetate pH 9. The concentrate was split into several samples and formulated with trehalose (ranging from 50:1 to 300:1 trehalose to protein molar ratios) and lysine (ranging from 75:1 to 200:1 lysine to protein molar ratio). The resulting aqueous formulations were then spray dried using an outlet temperature between about 60 °C. The resulting powder was analyzed by capillary electrophoresis under reducing and non-reducing conditions (**Figure 33A**), and by ELISA using purified PP917 protein (**Figure 33B**).

[0224] In another example, Frozen SP1741 biomass was added to 1mM ammonium acetate pH 8.3 yield a final concentration of 15 g/L ash free dry weight. The pH was adjusted with ammonium hydroxide to

9.0. The suspension was then hand homogenized with a hand immersion blender before high pressure homogenization through a Microfluidics LM-20 high pressure homogenizer set to 20,000 psi, collecting the lysate on ice. Phenyl methyl sulfonyl fluoride was added to the lysate to a final concentration of 2mM and mixed. The mixture was then heated to 37°C and 2mM magnesium chloride was added, the pH was adjusted to pH 8.22 with ammonium hydroxide, and benzonase nuclease added to a concentration of 1 microgram per 10 grams of biomass. The mixture was allowed to react at 37 °C for 30 minutes, then chilled to 20°C and filtered through a 1000 kDa tangential flow membrane. The permeate was then concentrated and diafiltered using a 30 kDa tangential flow membrane using 1 mM ammonium acetate pH 7.95. After concentration the pH was adjusted to 7.4 with monobasic sodium phosphate and NaCl added to a final concentration of 130mM. The resulting aqueous formulations were then spray dried using an outlet temperature between about 60 °C. The resulting powder was analyzed by ELISA using purified PP917 protein (**Figure 33C**).

Example 11- Structural Analysis of Complex Binding

[0225] To evaluate binding of an exemplary nanobody, the complex formed between PP758 and SARS-CoV-2 was evaluated using Cryo-EM. Structural analysis helps determine the binding epitope and mode of virus neutralization. The binding epitope of PP758 was elucidated using single-particle cryo-EM reconstructions in complex with SARS-CoV-2 S2P-prefusion-stabilized trimer by imaging with a Titan Krios. In the distribution of particles of the complex, two predominant populations were observed: one particle class with three PP758 VHHS bound per spike in a 1-RBD-up conformation and a second particle class with two partially occupied PP758 VHH bound per spike in a 3-RBD-down conformation with missing density for one of the RBDs (**Figure 25A**). Nonuniform refinement for this particle class revealed density for the VHH and majority of the binding interface near the receptor binding motif (RBM), however resolution was limited by molecular motion. The rigid body docked model (**Figure 25A** and **Figure 25B**) show that PP758 recognizes a region on the ACE2 binding site of RBD. The positioning of the VHH would cause clashes with ACE2 in the spike-ACE2 complex, suggesting that blockage of receptor binding likely accounts for neutralization.

Example 12- Epitope Binning

[0226] Antibody therapeutics that recognize multiple neutralizing epitopes are potent neutralizers when used as a cocktail. Notable advantages of delivering two or more antibodies against a target pathogen include the decreased likelihood that mutants will emerge which escape neutralization, and the potential for greater-than-additive therapeutic efficacy of non-competing antibodies (Einav and Bloom, 2020). A potential disadvantage is antagonism, for example by antibody interactions with overlapping epitopes.

To determine possible VHHs that can be used in cocktail therapeutics, we assessed the epitope classes recognized by exemplary disclosed VHHs, see **Figure 26**. The epitope classes are also compared with previously described VHHs, including VHH 72, Sb15, Sb45, and Sb68. We used BLI to evaluate binning competition to immobilized RBD in an epitope binning experiment. In general, most RBD binding and ACE2 competing VHHs cluster with Sb45 and compete with one another. This cluster (Cluster 1) is centered around PP758 and includes PP1148 and PP779. A unidirectional competition between Cluster 1 and PP917 was observed, where the unidirectional competition is only observed when PP917 is the secondary antibody in the competition. This unidirectional competition can be a function of the binding kinetics of PP917, where the VHH dimer exhibits a fast rate of association followed by a faster rate of dissociation.

[0227] Structural analysis done on RBD binding VHHs elucidate that Sb45-like VHHs bind to the class 2 neutralizing epitope on RBD. Cluster 1 VHHs, including PP758 and PP1148 share binding epitope with Sb45 and hence, we hypothesize these VHHs bind to the class 2 neutralizing epitopes. Antibodies that bind to this epitope class directly compete ACE2 binding and neutralize by sterilization. Structural analysis of human antibodies and VHHs using spike trimer complex structures indicate, the antibodies can bind RBD both in its "up" and "down" conformations. The epitope interacting paratope of these human antibodies heavy chain and VHHs exhibit long CDR3. Structural and sequence analysis of PP758 confirm binding to RBD in both "up" and "down" confirmation and the presence of long CDR3 at 14 amino acids. Given the observed unidirectional competition between PP917 and Cluster 2 VHHs, PP917 and some of the VHHs from cluster 1 can be binding to class-1 or class-2 epitope classes on RBD.

[0228] The binding experiment also delineated a second group of VHHs that competes with VHH-72 and Sb68 (Cluster 2). PP1146 clusters in this group. In addition to VHH-72 and Sb68, PP1146 also competes with Sb15. The bi-directional competition between PP1146 and Sb15 links the two clusters of VHHs, Cluster 1 and Cluster 2. VHHs in these cluster, including VHH-72 and Sb45 bind to RBD on the class 4 neutralizing epitope. These classes of antibodies do not directly inhibit ACE2 binding to RBD but cause steric hindrance that result in virus neutralization. Binning analysis groups PP1146 in the same epitope class as VHH-72 and Sb45, and so, we hypothesize that the mode of virus neutralization by PP1146 is not by direct competition with ACE2 for RBD binding. Taken together, VHHs from the two epitope classes are possible candidates for a cocktail mix to improve therapeutic potency and breadth of neutralization.

Example 13- Binding of Exemplary VHHs to SARS-CoV-2 RBD Variants by ELISA

[0229] To assess the cross-reactive nature of exemplary VHHs, binding of VHHs to RBD derived from spike protein of 5 SARS-CoV-2 variants was completed. In brief, high binding ELISA plates were coated with 1 $\mu\text{g/mL}$. VHHs were incubated in 5-fold dilution concentration series starting at 25 $\mu\text{g/mL}$, see **Figure 27** and **Table 13**.

Table 13:

	ELISA based binding EC50 (nM) NB = not binding, EC50 >1000 nM				
Protein Product	SARS-COV-2 Wuhan Reference RBD	SARS-CoV-2 Alpha Variant RBD	SARS-CoV-2 Beta Variant RBD	SARS-CoV-2 Gamma Variant RBD	SARS-CoV-2 Delta Variant RBD
PP755	3.41	4.41	544.50	778.70	NB
PP758	0.29	0.41	1.20	0.92	16.47
PP759	1.78	2.77	4.12	3.11	NB
PP760	0.28	0.30	NB	117.20	0.29
PP762	0.28	0.30	22.90	7.64	5.12
PP766	0.87	2.19	146.80	95.82	364.20
PP767	0.36	0.42	0.69	0.54	NB
PP768	0.38	0.41	1.83	0.76	8.40
PP769	0.40	0.44	6.80	3.56	0.45
PP770	0.54	0.83	261.20	126.90	NB
PP774	1.64	1.94	NB	NB	181.10
PP776	0.39	0.35	269.70	186.60	0.36
PP779	0.37	0.37	1.10	0.81	1.66
PP780	1.55	0.46	NB	NB	9.62
PP781	0.36	0.31	NB	NB	1.32
PP782	0.73	1.93	2.46	1.61	NB

PP783	0.32	0.27	1.38	0.68	0.42
PP784	0.26	0.26	255.00	165.10	NB
PP785	1.27	2.03	8.55	4.21	3.76
PP787	260.80	NB	NB	NB	NB
PP788	1.56	5.88	27.58	16.78	NB
PP790	NB	32.24	NB	NB	NB
PP799	0.54	0.56	579.80	147.20	0.61
PP800	1.06	1.19	NB	927.50	257.70
PP801	0.33	0.30	3.13	2.25	6.52
PP900	0.47	34.00	708.60	339.70	NB
PP901	NB	NB	NB	NB	NB
PP902	NB	123.20	NB	450.70	NB
PP903	0.27	0.28	NB	NB	0.26
PP904	1.06	12.98	138.70	34.65	NB
PP905	NB	447.20	NB	NB	NB
PP906	2.00	1.97	987.80	378.40	204.80
PP907	3.00	3.70	262.90	120.40	NB
PP908	1.59	1.74	7.42	3.75	2.15
PP909	0.62	0.44	8.10	5.37	81.40
PP911	2.36	2.19	545.00	316.80	NB
PP912	2.33	1.96	365.00	176.80	621.70
PP913	1.53	1.33	113.50	61.08	NB
PP914	3.24	2.94	19.36	10.48	NB
PP915	1.05	0.52	NB	NB	37.80
PP916	1.19	2.04	66.29	42.89	NB

PP917	0.98	0.77	NB	NB	8.47
PP927	1.43	2.22	NB	NB	94.20
PP928	NB	NB	NB	NB	NB
PP930	3.18	3.90	13.02	10.21	21.12
PP931	1.93	2.07	134.30	110.40	126.70
PP932	0.37	0.41	NB	NB	15.35
PP933	4.90	9.74	10.15	9.73	8.87
PP934	1.07	1.56	13.50	4.78	NB
PP935	0.47	0.47	343.40	166.60	151.10
PP1094	0.96	0.89	315.00	160.20	49.90
PP1095	0.78	1.18	192.90	90.92	16.94
PP1096	1.47	1.92	63.16	28.36	NB
PP1097	0.25	0.30	95.43	58.61	244.00
PP1098	0.83	0.97	24.81	11.23	NB
PP1099	4.24	4.95	193.50	160.40	NB
PP1100	0.31	0.35	NB	NB	46.52
PP1101	1.56	1.77	114.60	51.53	42.57
PP1102	0.97	1.55	2.00	1.26	NB
PP1103	2.40	1.45	226.60	92.75	2.69
PP1104	1.21	1.09	44.92	29.52	294.70
PP1105	1.24	1.41	26.57	15.42	36.61
PP1106	2.52	2.05	3.17	7.84	NB
PP1107	0.27	0.28	0.25	0.18	NB
PP1108	2.61	1.89	121.10	57.12	NB
PP1109	1.06	1.12	17.06	11.02	115.00

PP1110	3.50	4.98	17.00	8.98	407.00
PP1111	0.33	0.31	1.57	1.60	231.20
PP1112	1.36	1.76	4.77	3.01	15.09
PP1113	0.34	0.72	5.64	3.37	NB
PP1114	1.14	1.64	18.92	6.12	NB
PP1115	4.90	6.74	857.00	162.00	NB
PP1145	1.33	1.94	30.88	5.49	88.95
PP1147	0.36	0.33	NB	NB	0.49
PP1148	0.30	0.33	30.48	9.06	0.22
PP1149	2.50	3.01	57.98	133.50	NB
PP1150	0.64	20.13	NB	NB	0.86
PP1153	1.23	0.91	NB	NB	222.70
PP1154	2.42	2.37	24.72	15.85	221.20
PP1176	0.29	0.27	NB	NB	2.67
PP1181	NB	NB	NB	NB	NB

Example 14-Live Virus Assay

[0230] To test if exemplary VHHs identified through ELISA and pseudovirus assay can actively neutralize live Covid virus, the top 4 candidates, PP917, PP758, PP1146 and PP1148 were tested against 2019 Novel Coronavirus, Isolate USA-WA1/2020 (SARS-CoV-2) with African green monkey kidney (Vero E6) cells. The test was conducted by IIT Research Institute in Chicago.

[0231] Combinations of VHHs PP917, PP758, PP1146 and PP1148 were prepared in three forms in total of 31 samples (shown in **Table 14**). Samples #1-10 are purified proteins. Samples #11-20 are purified proteins mixed with 5mg/mL of cell extract from wild type cells SP003 (matrix). Samples #21-30 are cell extract from the corresponding strains SP1741, SP1641, SP1861 and SP1862.

Table 14: List of samples for Live virus assay

1. 917 @ IC ₈₀ (purified protein)
2. 758 @ IC ₈₀ (purified protein)

3. 1148 @ IC ₈₀ (purified protein)
4. 1146 @ IC ₈₀ (purified protein)
5. Isotype control (purified protein)
6. 917 and 758 each @ IC ₈₀ (purified protein)
7. 917 and 1146 each @ IC ₈₀ (purified protein)
8. 917 and 1148 each @ IC ₈₀ (purified protein)
9. 1148 and 1146 each @ IC ₈₀ (purified protein)
10. 917, 1146, 1148 each @ IC ₈₀ (purified protein)
11. 917 @ IC ₈₀ (purified protein + matrix)
12. 758 @ IC ₈₀ (purified protein + matrix)
13. 1148 @ IC ₈₀ (purified protein + matrix)
14. 1146 @ IC ₈₀ (purified protein + matrix)
15. Isotype control (purified protein + matrix)
16. 917 and 758 each @ IC ₈₀ (purified protein + matrix)
17. 917 and 1146 each @ IC ₈₀ (purified protein + matrix)
18. 917 and 1148 each @ IC ₈₀ (purified protein + matrix)
19. 1148 and 1146 each @ IC ₈₀ (purified protein + matrix)
20. 917, 1146, 1148 each @ IC ₈₀ (purified protein + matrix)
21. 917 @ IC ₈₀ (cell extract)
22. 758 @ IC ₈₀ (cell extract)
23. 1148 @ IC ₈₀ (cell extract)
24. 1146 @ IC ₈₀ (cell extract)
25. Isotype control (cell extract) (SP1182 extract)
26. 917 and 758 each @ IC ₈₀ (cell extract)
27. 917 and 1146 each @ IC ₈₀ (cell extract)
28. 917 and 1148 each @ IC ₈₀ (cell extract)
29. 1148 and 1146 each @ IC ₈₀ (cell extract)
30. 917, 1146, 1148 each @ IC ₈₀ (cell extract)
31. Matrix only (SP3 extract)

32. 917 (purified protein), 10x of sample #1.
33. 917 and 758 (purified protein), 10x of sample #6.
34. 917 (purified protein + matrix), 10x of sample #11
35. 917 and 758 (purified protein + matrix), 10x of sample #16

[0232] To prepare for the live virus assay, neutralizing potency of VHHs purified from freeze dried biomass was tested with pseudovirus assay. The results are shown in **Figure 28**. IC80 of each single VHH was estimated based on the measured neutralization curve and IC50. 31 samples were prepared with IC80 of each VHH and tested with pseudovirus assay. The % reduction of infectivity of each sample are shown in **Figure 29A**. All the VHH samples reduced 65-99.9% of the virus infectivity, which is within the expected range. As expected, the non-relevant isotype control PP322 (sample #5) and matrix (sample#31) didn't neutralize the virus. However, when PP322 was mixed with the matrix, ~30% of the virus infectivity was inhibited. It is not clear why this sample inhibit the infectivity. The experiment will be repeated with another set of samples. Another relevant control, cell extract from SP1182 which express PP322 will be included in the next round of experiment.

[0233] The set of 31 samples were prepared in a larger volume and divided into 2 aliquots. The aliquots were stored at -80C for 24 hours and one set of aliquots were tested with pseudovirus assay to assess the effect of freeze-thaw on the neutralizing potency of the VHHs. The results (**Figure 29B**) showed that PP917 lost ~30% of the potency in all three forms (sample #1, 11, and 21) after the process. Note that purified PP758 lost the majority of the potency (sample #2 and #12), while the cell extract from the corresponding strain (sample #22) was fine. Since PP917 is the top candidate, sample #1,6,11,16 which contain PP917 and PP758 were prepared with 10 times more VHHs (sample #32,33,34,35). These new samples reduced 78-99.7% of the virus infectivity (**Figure 29B**). Note that the non-relevant isotype control PP322 (sample #5, 15, 25) and matrix (sample#31) didn't neutralize the virus. Total of 35 samples were sent to IIRI for live virus assay.

[0234] For the live virus titer assay, all samples were serially diluted 2-fold for a total of 8 dilutions, mixed with 200 TCID 50 of virus, then transferred into 3 replicate wells/dilution to corresponding wells in 96-well plate which contains a monolayer of Vero E6 cells for titration. The 96-well plate was incubated in a humidified chamber at 37°C ± 2°C in 5 ± 2% CO₂. At 48 hours ± 4 hrs. post inoculation, wells were scored for virus replication by immunostaining with an antibody specific for the SARS-CoV-2 nucleoprotein. Results were recorded in **Figure 30A-Figure 30X**. As expected, the non-relevant

isotype control PP322 (sample #5, 15 and 25) and matrix (sample#31) didn't neutralize the virus. The positive control, Remdesivir, completely inhibits the infectivity at the first three dilutions. Some of Lumen VHH samples (sample #18, 33,34,35) show similar or stronger effect compared to Remdesivir.

[0235] The concentration of the VHHs at dilution factor 0.5 is the same with Lumen pseudovirus assay. The % reduction of infectivity at this dilution was plotted and compared with the results from pseudovirus assay (**Figure 29B** and **29C**). Most samples have similar potency in both assays with some exemptions. Purified PP917 (sample #1), PP1146 (sample #4) and PP917+PP758 (sample #6) and cell extract of PP1148 (sample #23) and PP1148 (sample #24) are significantly less potent in the live virus assay. The samples were stored at -80 for extended period of time before live virus assay, which possibly caused the reduced potency of those samples. Nonetheless most of the results from live virus assay conducted by IITRI is consistent with Lumen pseudovirus assay. This suggests the pseudovirus assay is an effective approach to screen and predict the Covid VHH candidates.

[0236] A more thorough analysis was conducted to confirm the correlation between the live virus assay and pseudovirus assay. After edge-effects correction was applied to the IITRI live virus assay data, the rescaled data were fit with a four-parameter model describing the inhibition of viral infection by VHH dilution (**Fig 31**). The response model estimated the dilution that that provided a 50% reduction in infectivity in the assay, Dil_{50} .

$$Y_i = \text{Top} - (\text{Top} - \text{Bottom}) \cdot \frac{\text{Dilution}^H}{Dil_{50}^H + \text{Dilution}^H}$$

[0237] All of the samples were provided at the IC80 concentration from the pseudovirus assay. As such, it is useful to reparametrize the model to instead estimate the dilution that provides 80% reduction in infectivity in the assay, Dil_{80} .

$$Dil_{50} = \left(\frac{Dil_{50}^H}{4} \right)^{1/H}$$

[0238] Thus, if a sample estimate of Dil_{80} is equal to 1.0, then the live virus assessment of potency is the same as the pseudovirus assessment. At values > 1.0 , the live virus assessment of potency is lower than the pseudovirus assessment. At values < 1.0 , the live virus assessment of potency is higher than the pseudovirus assessment. The analysis results are shown in **Figure 32**.

[0239] In conclusion, the live virus and pseudovirus estimates of potency (IC80) are in alignment. Initial purified protein (P; samples 1-10) showed the largest difference, where the potency according to the

live virus assay was 1-fold to 4-fold lower than in the pseudovirus assay. Initial purified protein plus matrix (P+M; samples 11-20) showed fair agreement, where the potency according to the live virus assay was 0.5-fold to 3-fold lower than in the pseudovirus assay. Cell-extract (C; samples 21-30) and follow-up purified protein (10x; samples 32-35) showed greater potency in the live virus assay, where the potency according to the live virus assay was 1-fold to 2-fold greater than in the pseudovirus assay. In conclusion, lysates from the biomass of Covid candidate strains effectively neutralize SARS-CoV-2 virus (USA-WA1/2020).

Example 15: Functional Assays

[0240] Expression of VHH antibodies in spirulina was evaluated. In brief, recombinant protein expression in spirulina was measured by capillary electrophoresis immunoassay (CEIA) using a Jess system (ProteinSimple). The Jess system was run as recommended by the manufacturer. Briefly, to determine the expression of heterologous protein as a percentage of total mass, dried biomass samples were diluted to a concentration of 0.1-1 mg/mL using water and a 5X master mix prepared from an EZ Standard Pack 1 (Bio-Techne). Similarly, to determine the proportion of heterologous protein in total soluble Spirulina extract, total soluble protein was extracted from dried spirulina biomass using bead beating extracting method. Total protein is determined using Pierce BCA Protein Assay Kit (Thermo Fisher). Jess samples were prepared as described above at concentrations of 0.1 -1 mg/mL. Purified protein controls used to generate standard curves were typically loaded at a range of concentrations from 0.25-8 µg/mL. A 12-230 kDa Jess/Wes Separation Module (ProteinSimple) was used for separation. A mouse anti-His-Tag antibody (GenScript) was diluted 1:100 and used as the primary antibody. An anti-mouse NIR fluorescence-conjugated secondary antibody (ProteinSimple) was primarily used for detection. Data analysis was performed using the Protein Simple Compass software and Microsoft Excel. Expression for exemplary VHHs is shown in **Table 15**.

Table 15: Spirulina Expression

	Spirulina Expression			
	% of Total Protein		% of Total Mass	
	Experiment1	Experiment2	Experiment1	Experiment2
PP661	1.4		0.5	
PP755	2	3	0.9	1.4
PP757	0.03	0.03	0.01	0.01
PP758	1.2	2.9		
PP759	10.8		4	
PP760	0.2		0.1	
PP762	0.1	0.1	0.03	0.04

PP764	0.4			
PP765	0.1	0.1	0.03	0.04
PP767	0.1	0.05	0.03	0.02
PP768	5.2	5.2	2	2
PP769	0.3	0.4	0.1	0.1
PP770	0.4	0.5	0.1	0.2
PP771	0.1			
PP772	0.4			
PP773	0.1	0.1	0.02	0.03
PP774	2.7	7.3	0.9	2.4
PP775	1.1			
PP776	0.1	0.1	0.03	0.04
PP777	0.1			
PP778	3.9	5.5	1.3	1.9
PP779	8.4	21.7	2.5	6.5
PP780	0.6	3.6	0.2	1
PP781	0.7	1.1	0.2	0.3
PP782	0.7	0.9	0.2	0.3
PP784	2.6	5.2	0.9	1.7
PP785	0.5	0.1	0.5	0.1
PP787	3			
PP788	0.1	0.1	0.02	0.03
PP789	0.1	0.1	0.05	0.05
PP790	5.6	5.4	1.6	1.5
PP791	0.9	0.6	0.3	0.2
PP793	0.01			
PP796	0.04	0.03	0.01	0.01
PP797	4	3.1	1.2	0.9
PP798	0.1			
PP799	0.5	0.5	0.1	0.1
PP800	15.7	11.7	5.4	4
PP801	9.9	10.4	3.8	4
PP899	0.02	0.05	0.01	0.03
PP900	8.3	4.6	5.2	2.9
PP901	6.6	5.5	2.4	2
PP902	2.6	3.4	1	1.3
PP903	0.2	0.2	0.1	0.1
PP904	3.3	4.9	0.8	1.2
PP905	4.6	1.14	2	
PP906	0.28	0.79	0.09	0.12
PP907	4.51	4.55	1.85	1.87
PP908	8.4	3.78	4	1.8

PP909	4.18	4.6	1.25	2
PP910	0.28	0.25		0.24
PP911	0.04	0.06	0.03	0.05
PP912	6.01	2.92	2.81	1.37
PP913	4.26	1.98	2.86	1.33
PP914	3.69	12.26	1.46	4.85
PP915	0.96	0.52	0.4	0.21
PP916	3.53	5.12	1.17	1.7
PP917	6.4	9.4		
PP927	0.45	0.94	0.22	0.47
PP928	3.73	4.6	2	2
PP929	0.03	8.73	0.01	2
PP930	3.91	5.87	1.27	1.91
PP931	4.95	4.95	2	2
PP932	0.69	0.84	0.3	0.36
PP933	0.21	0.04	0.06	0.01
PP934	1.94	1.92	0.49	0.48
PP935	12.85	15.16	8	9.44
PP1094	3.06	3.06	2	2
PP1095	0.05	0.07	0.02	0.03
PP1096	5.17	5.17	2	2
PP1097	29.85	29.85	8	8
PP1098	5.5	5.5	2	2
PP1099	0.71	1.54	0.22	0.48
PP1100	0.07	0.14	0.02	0.04
PP1101	6.68	6.68	2	2
PP1102	2.88	7.97	0.72	2
PP1104	7.11	7.11	2	2
PP1105	6.16	6.16	2	2
PP1106	0.12	0.33	0.05	0.14
PP1107	22.77	22.77	8	8
PP1108	5.78	5.78	2	2
PP1109	4.12	7.48	1.1	2
PP1110	5.24		2	
PP1111	6.47		2	
PP1112	5.69		2	
PP1113	2.95		1.4	
PP1114	0.33		0.12	
PP1115	4.15		2	
PP1116	16.33		8	
PP1117	3.32		1.24	
PP1118	4.18		1.59	

PP1144	0.03		0.01	
PP1145	14.61		8	
PP1146	1	1		
PP1147	0.05		0.03	
PP1148	0.7	1		
PP1149	0.7		0.24	
PP1150	0.22		0.07	
PP1151	0.06		0.02	
PP1152	0.02		0.01	
PP1153	2.48		1.11	
PP1176	0.01		0.01	

Infectivity Analysis

[0241] Virus neutralization potency by VHHs at single concentration was assessed following the neutralization protocol which the following modifications. Plates were seeded with 293-ACE2 cell line (Jessie Bloom lab, FHCRC) at a density of 1.25×10^4 cells per well in 100 μ L volume. 12-16 hours after seeding, VHHs were tested at 10 μ g/mL final concentration. Virus titer and controls were prepared as describe above. Virus infectivity was assessed by measuring luminescence as above. Luminescence RLUs from virus only wells were normalized as 100 % infectivity and RLUs from cells only were normalized as 0% infectivity. Pseudotyped virus infectivity were calculated as a proportion of the 100% and 0% infectivity, results are shown in **Table 16**.

Table 16

PP#	Description	Form	infectivity% under 10ug/mL VHH treatment
PP802	MBP-R2A-C5-H6	Monomer	87.79
PP803	MBP-R2A-F3-H6	Monomer	70.66
PP806	MBP-R2B-E6-H6	Monomer	86.46
PP807	MBP-R2C-B3-H6	Monomer	75.26
PP810	MBP-R2D-A6-H6	Monomer	77.48
PP811	MBP-R2D-D3-H6	Monomer	75.92
PP813	MBP-R2E-D2-H6	Monomer	75.51
PP814	MBP-R2E-F4-H6	Monomer	84.69
PP815	MBP-R5A-A1-H6	Monomer	84.41
PP817	MBP-R5A-B3-H6	Monomer	80.01
PP819	MBP-R5A-C5-H6	Monomer	54.15
PP821	MBP-R5A-D6-H6	Monomer	76.7
PP822	MBP-R5A-E5-H6	Monomer	71.62

PP823	MBP-R5B-C3-H6	Monomer	65.49
PP825	MBP-R5C-A1-H6	Monomer	56.52
PP826	MBP-R5C-A2-H6	Monomer	70.56
PP828	MBP-R5C-B2-H6	Monomer	73.37
PP831	MBP-R5C-F3-H6	Monomer	81.23
PP832	MBP-R5C-F5-H6	Monomer	75.46
PP833	MBP-R5C-F6-H6	Monomer	86.16
PP834	MBP-R5C-G2-H6	Monomer	89.22
PP836	MBP-R5D-A1-H6	Monomer	54.98
PP837	MBP-R5D-B2-H6	Monomer	77.62
PP846	MBP-R5D-G6-H6	Monomer	77.07
PP847	MBP-R5E-D1-H6	Monomer	77.14
PP848	MBP-R5E-G2-H6	Monomer	81.92
PP880	MBP-R5a-B5-H6	Monomer	57.96
PP881	MBP-R5a-F2-H6	Monomer	83.22
PP882	MBP-R1e-B2-H6	Monomer	84.01
PP883	MBP-R1e-D4-H6	Monomer	94.32
PP884	MBP-R1i-A11-H6	Monomer	91.32
PP885	MBP-R5b-C5-H6	Monomer	100.74
PP886	MBP-S1a-C4-H6	Monomer	84.79
PP887	MBP-S1b-E7-H6	Monomer	85.74
PP888	MBP-S1b-E11-H6	Monomer	79.99
PP889	MBP-S2a-C4-H6	Monomer	82.55
PP890	MBP-S2b-A7-H6	Monomer	89.97
PP891	MBP-S2b-C10-H6	Monomer	102.03
PP892	MBP-S2b-F7-H6	Monomer	83.18
PP893	MBP-S2b-G8-H6	Monomer	78.7
PP894	MBP-S3a-C5-H6	Monomer	76.11
PP895	<u>MBP-S3a-H1-H6</u>	Monomer	84.42
PP896	MBP-S3b-A7-H6	Monomer	82.75
PP897	MBP-S3b-A8-H6	Monomer	79.28
PP898	MBP-S3b-C8-H6	Monomer	62.37
PP918	MBP-R1i-C8-H6	Monomer	71.83
PP919	MBP-R1i-E7-H6	Monomer	79.31
PP921	MBP-R2b-D4-H6	Monomer	70.58
PP922	MBP-R5a-F5-H6	Monomer	67.74
PP923	MBP-R5d-E1-H6	Monomer	76.93
PP924	MBP-R5e-C1-H6	Monomer	102.23
PP925	MBP-S3a-H2-H6	Monomer	74.53
PP926	MBP-S3b-E11-H6	Monomer	67.58
PP899	<u>MBP-5HVZ-R5a-B5-H6</u>	Dimer	2.43
PP900	MBP-5HVZ-R5a-F2-H6	Dimer	18.27

PP901	MBP-5HVZ-R1e-B2-H6	Dimer	72.07
PP902	MBP-5HVZ-R1e-D4-H6	Dimer	72.4
PP903	MBP-5HVZ-R1i-A11-H6	Dimer	38.41
PP904	MBP-5HVZ-R5b-C5-H6	Dimer	77.02
PP904	MBP-5HVZ-R5b-C5-H6	Dimer	92.03
PP905	MBP-5HVZ-S1a-C4-H6	Dimer	75.66
PP906	MBP-5HVZ-S1b-E7-H6	Dimer	69.61
PP907	MBP-5HVZ-S1b-E11-H6	Dimer	76.34
PP908	MBP-5HVZ-S2a-C4-H6	Dimer	47.47
PP909	MBP-5HVZ-S2b-A7-H6	Dimer	67.07
PP909	MBP-5HVZ-S2b-A7-H6	Dimer	54.05
PP910	MBP-5HVZ-S2b-C10-H6	Dimer	65.07
PP911	MBP-5HVZ-S2b-F7-H6	Dimer	74.92
PP912	MBP-5HVZ-S2b-G8-H6	Dimer	73.24
PP913	MBP-5HVZ-S3a-C5-H6	Dimer	71.09
PP913	MBP-5HVZ-S3a-C5-H6	Dimer	62.68
PP914	MBP-5HVZ-S3a-H1-H6	Dimer	74.35
PP914	MBP-5HVZ-S3a-H1-H6	Dimer	77.51
PP915	MBP-5HVZ-S3b-A7-H6	Dimer	90.5
PP916	MBP-5HVZ-S3b-A8-H6	Dimer	70.45
PP916	MBP-5HVZ-S3b-A8-H6	Dimer	55.41
PP917	MBP-5HVZ-S3b-C8-H6	Dimer	6.45
PP927	MBP-5HVZ-R1i-C8-H6	Dimer	45.38
PP928	MBP-5HVZ-R1i-E7-H6	Dimer	78.55
PP930	<u>MBP-5HVZ-R2b-D4-H6</u>	Dimer	35.57
PP931	MBP-5HVZ-R5a-F5-H6	Dimer	57.5
PP933	MBP-5HVZ-R5e-C1-H6	Dimer	92.08
PP933	MBP-5HVZ-R5e-C1-H6	Dimer	73.99
PP934	MBP-5HVZ-S3a-H2-H6	Dimer	75.01
PP934	MBP-5HVZ-S3a-H2-H6	Dimer	84.12
PP935	MBP-5HVZ-S3b-E11-H6	Dimer	48.92
PP1094	MBP-5HVZ-PAPA-RN-1-H6	Dimer	83.42
PP1095	MBP-5HVZ-PAPA-RN-2-H6	Dimer	76.8
PP1096	MBP-5HVZ-PAPA-RN-3-H6	Dimer	86.48
PP1097	MBP-5HVZ-PAPA-RN-5-H6	Dimer	18.59
PP1098	MBP-5HVZ-PAPA-RN-9-H6	Dimer	58.26
PP1099	MBP-5HVZ-PAPA-RN-12-H6	Dimer	70.58
PP1100	MBP-5HVZ-PAPA-RN-14-H6	Dimer	17.22
PP1101	MBP-5HVZ-PAPA-RN-16-H6	Dimer	91.78
PP1102	MBP-5HVZ-PAPA-RN-17-H6	Dimer	40.91
PP1103	MBP-5HVZ-PAPA-RN-18-H6	Dimer	57.57
PP1104	MBP-5HVZ-PAPA-RN-19-H6	Dimer	73.81

PP1105	MBP-5HVZ-PAPA-RN-20-H6	Dimer	79.82
PP1106	MBP-5HVZ-PAPA-RN-21-H6	Dimer	76.58
PP1107	MBP-5HVZ-PAPA-RN-22-H6	Dimer	34.33
PP1108	MBP-5HVZ-PAPA-RN-23-H6	Dimer	93.09
PP1109	MBP-5HVZ-PAPA-RN-25-H6	Dimer	88.05
PP1110	MBP-5HVZ-PAPA-RN-26-H6	Dimer	57.89
PP1111	MBP-5HVZ-PAPA-RN-27-H6	Dimer	68.3
PP1112	MBP-5HVZ-PAPA-RN-28-H6	Dimer	70.44
PP1113	MBP-5HVZ-PAPA-RN-29-H6	Dimer	6.25
PP1114	MBP-5HVZ-PAPA-RN-30-H6	Dimer	76.26
PP1115	MBP-5HVZ-PAPA-RN-31-H6	Dimer	86.87
PP1116	MBP-5HVZ-PAPA-Sb-15-H6	Dimer	1.69
PP1117	MBP-5HVZ-PAPA-Sb-45-H6	Dimer	29.89
PP1118	MBP-5HVZ-PAPA-Sb-68-H6	Dimer	30.51
PP1143	MBP-5HVZ-PAPA-R2b-D5-H6	Dimer	0.09
PP1144	MBP-5HVZ-PAPA-R2c-F6-H6	Dimer	9.18
PP1145	MBP-5HVZ-PAPA-R2d-A6-H6	Dimer	65.24
PP1146	MBP-5HVZ-PAPA-R2d-D3-H6	Dimer	32.75
PP1147	MBP-5HVZ-PAPA-R5a-B7-H6	Dimer	1.79
PP1148	MBP-5HVZ-PAPA-R5a-C5-H6	Dimer	5.95
PP1149	MBP-5HVZ-PAPA-R5a-E5-H6	Dimer	25.51
PP1150	MBP-5HVZ-PAPA-R5b-G1-H6	Dimer	2.31
PP1151	MBP-5HVZ-PAPA-R5d-B5-H6	Dimer	35.62
PP1152	MBP-5HVZ-PAPA-R5d-B6-H6	Dimer	4.64
PP1153	MBP-5HVZ-PAPA-R5d-D4-H6	Dimer	81.66
PP1154	MBP-5HVZ-PAPA-R5d-G1-H6	Dimer	53.2
PP1176	MBP-5HVZ-PAPA-R2a-F3-H6	Dimer	58.44
PP1131	MBP-4B0F-PAPA-R2b-D5-H6	Heptamer	-0.01
PP1132	MBP-4B0F-PAPA-R2c-F6-H6	Heptamer	2.65
PP1133	MBP-4B0F-PAPA-R2d-A6-H6	Heptamer	48.14
PP1134	MBP-4B0F-PAPA-R2d-D3-H6	Heptamer	10.86
PP1135	MBP-4B0F-PAPA-R5a-B7-H6	Heptamer	-0.03
PP1136	MBP-4B0F-PAPA-R5a-C5-H6	Heptamer	1.53
PP1137	MBP-4B0F-PAPA-R5a-E5-H6	Heptamer	33.69
PP1138	MBP-4B0F-PAPA-R5b-G1-H6	Heptamer	31.54
PP1139	MBP-4B0F-PAPA-R5d-B5-H6	Heptamer	70.97
PP1140	MBP-4B0F-PAPA-R5d-B6-H6	Heptamer	2.47
PP1141	MBP-4B0F-PAPA-R5d-D4-H6	Heptamer	73.11
PP1142	MBP-4B0F-PAPA-R5d-G1-H6	Heptamer	35.91
PP1175	MBP-4B0F-PAPA-R2a-F3-H6	Heptamer	60.29
PP1155	MPB-scTRP-PAPA-R2b-D5-H6	Trimer	0.01
PP1156	MPB-scTRP-PAPA-R2c-F6-H6	Trimer	18.14

PP1157	MPB-scTRP-PAPA-R2d-A6-H6	Trimer	32.92
PP1158	MPB-scTRP-PAPA-R2d-D3-H6	Trimer	28.8
PP1159	MPB-scTRP-PAPA-R5a-B7-H6	Trimer	17.72
PP1160	MPB-scTRP-PAPA-R5a-C5-H6	Trimer	4.22
PP1161	MPB-scTRP-PAPA-R5a-E5-H6	Trimer	59.86
PP1162	MPB-scTRP-PAPA-R5b-G1-H6	Trimer	66.78
PP1165	MPB-scTRP-PAPA-R5d-D4-H6	Trimer	77.51
PP1166	MPB-scTRP-PAPA-R5d-G1-H6	Trimer	69.73
PP1177	MPB-scTRP-PAPA-R2a-F3-H6	Trimer	51.09
PP1181	R2d-D3-PP7-H6	Particle	1.57

Pseudovirus Neutralization

[0242] Constructs were evaluated for virus neutralization by way of a pseudovirus neutralization assay. Neutralization assays determine the ability of a VHH antibody to inhibit virus infection of cultured cells with SARS-CoV-2 variants Wuhan, Delta, or Gamma. If neutralizing antibodies are present, their levels can be measured by determining the threshold at which they are able to prevent viral replication in the infected cell cultures.

[0243] The SARS-CoV-2 pseudotyped lentiviral particle generation, titting and neutralization assays were performed following the protocol outline in Crawford K. H. D. et al., 2020 with minor modifications. In brief, poly-L-lysine coated clear bottom 96-well black plates (Thermo Scientific, 12-566-70) were seeded with 293-ACE2 cell line (Jessie Bloom lab, FHCRC) at a density of 1.25×10^4 cells per well in 100 μ L volume. 12-16 hours after seeding, 5-fold antibody dilutions, starting at 100 μ g/mL, were prepared. Control, virus only and cell only were prepared by as previously reported. 60 μ L of titrated virus was added and mixed with antibody dilutions and virus only wells. The mix was incubated at 37 °C for 1 hour. Gently, 100 μ L of growth media was removed from each well and 100 μ L of the mixture were added to corresponding well on 293-ACE2 cells seeded plates. Polybrene (Sigma Aldrich, P4707) was added to each well at a final concentration of 5 μ g/mL. Plates were incubated at 37 °C for 48-60-hour post infection. Virus neutralization was assessed by measuring luminescence. While incubation, Bright-Glo Luciferase reagents (Promega, E2610) were thawed, equilibrated at room temperature, and prepared following the manufacturer's recommendation. 100 μ L of growth media was removed from each well and 30 μ L per well of luciferase reagent added. Plates were incubated for 2 min at room temperature in the dark and luminescence was measured using M2 plate reader (Molecular Devices). Luminescence RLU from virus only wells were normalized as 100 % infectivity and RLUs from cells only were normalized as 0% infectivity. Infectivity and IC50 were calculated using Four Parameter Logistic Regression on GraphPad Prism (GraphPad Software), see **Table 17**.

Table 17

	Pseudovirus neutralization IC ₅₀ (nM) (NN = not neutralizing)			
PP#	SARS-COV-2 Wuhan Reference Pseudovirus	SARS-CoV-2 Delta Variant Pseudovirus	SARS-CoV-2 Gamma Variant Pseudovirus	Notes
PP756	210			
PP758	0.39	NN	3.73	
PP759	138.1			
PP760		0.8	NN	
PP762		39.28	70.91	
PP764	~ 25471			
PP767			40.71	
PP768		NN	NN	
PP769		35.18	NN	
PP772	42.73			
PP776		1.62	NN	
PP777	0.7873			
PP779		85.46	1.821	
PP780	NN	4.44E-17	NN	
PP781	NN		NN	
PP782	NN	NN	NN	
PP783		NN	NN	
PP785	NN	64.27	781.4	
PP787			NN	
PP799	NN	NN	NN	
PP801		NN	NN	
PP899	2.29			
PP900	29.63			
PP903	56.67	NN	NN	
PP908	1618	NN	63.13	
PP909		NN	NN	
PP913	NN			
PP915		NN	NN	
PP916			NN	
PP917	6.59	0.81	NN	
PP927	NN	NN	NN	
PP930	NN	NN	NN	
PP932		NN	NN	
PP933		NN	NN	

PP934			46310	
PP935	34.21			
PP1094		NN	NN	
PP1095		NN	NN	
PP1097	27.27	NN	NN	
PP1098			NN	
PP1099	NN			
PP1100	6.953	NN	NN	
PP1101		NN	NN	
PP1102	~ 5113		21.5	
PP1103		2.49	NN	
PP1105		NN	NN	
PP1106			63.41	
PP1107	32.86	NN	0.6491	
PP1112		NN	NN	
PP1113	7.09			
PP1131	0.93			
PP1132	4.14			
PP1133	186.29			
PP1134	3.97			
PP1135	0.5335			
PP1136	0.05			
PP1137	77.315			
PP1138	NN			
PP1140	2.07			
PP1142	107.485			
PP1143	0.3015		3.132	
PP1144	11.87			
PP1145	146.2	NN	NN	
PP1146	36.17	4.17	6.617	
PP1147	12.3	1.13	NN	
PP1148	15.67	0.53	NN	
PP1149	37.34			
PP1150	2.72	0.43	NN	
PP1151	15.2965			
PP1152	3.87			
PP1153			62.91	
PP1154	271.6			
PP1155	0.6337			
PP1156	36.8			
PP1157	18.95			
PP1157	19.75			

PP1158	27.41			
PP1159	31.9			
PP1160	2.41			
PP1176		NN	NN	
PP1181	6.95			
PP1816	0.4334			Cerberbody
PP1896		34.57		Cerberbody
PP1897		0.09		Cerberbody
PP1898		0.46		Cerberbody

Protease Resistance

[0244] Constructs were evaluated for protease resistance. A general strategy to rapidly screen VHHs for susceptibility to GI proteases is to evaluate VHH binding activity after treatment with an intestinal protease. Following protease incubation, protease is neutralized with inhibitors and VHH binding is activity is measured by capture ELISA using the target antigen. VHHs that functionally survive the protease incubations are able to bind the target antigen, which can then be detected with an anti-VHH or anti-VHH conjugate antibody. An EC₅₀ binding curve is calculated using a 4-parameter logistic curve fit of the results and the value is compared to an undigested control to calculate the percent activity remaining. The VHHs of the disclosure were incubated with trypsin, chymotrypsin, and elastase. Protease resistance results agreed well between the ELISA and neutralization assays. Protease resistance was observed with trypsin, chymotrypsin, and elastase. Binding activity after a 1 hour digestion with exemplary proteases is shown in Table 18.

Table 18

Protein Product	VHH	ELISA binding activity after 1 h digestion with the indicated protease					
		0.1 mg/mL trypsin	0.01 mg/mL trypsin	0.1 mg/mL chymotrypsin	0.01 mg/mL chymotrypsin	5 µg/mL elastase	0.5 µg/mL elastase
PP917	S3b-C8	84%	92%	111%	110%	118%	103%
PP1113	RN-29	100%	114%	6%	93%	29%	68%
PP758	R2B-D5	110%	107%	1%	57%	118%	72%
PP1146	R2d-D3	0%	0%	48%	66%	13%	90%
PP1148	R5a-C5	0%	0%	19%	71%	47%	86%
PP1107	RN-22	0%	7%	13%	132%	34%	103%
PP1144	R2c-F6	0%	43%	3%	6%	61%	61%
PP1150	R5b-G1	not tested	not tested	0%	0%	16%	56%
PP1147	R5a-B7	not tested	not tested	9%	21%	31%	64%
PP1100	RN-14	not tested	not tested	7%	112%	5%	47%

PP899	R5a-B5	not tested	not tested	0%	17%	0%	63%
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INCORPORATION BY REFERENCE

[0263] This patent application incorporates by reference in their entireties for all purposes the following patent publications and applications: US 10,131,870, US 62/672,891 filed May 17, 2018, and PCT/US2019/032998 filed May 17, 2019.

[0264] All references, articles, publications, patents, patent publications, and patent applications cited herein are incorporated by reference in their entireties for all purposes. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and

should not be taken as, an acknowledgment or any form of suggestion that they constitute valid prior art or form part of the common general knowledge in any country in the world.

CLAIMS

1. A VHH antibody that binds SARS-CoV-2, wherein the VHH antibody comprises three complementarity determining regions (CDR1, CDR2, and CDR3) each of which is selected from an amino acid sequence comprising at least about 85% identity to a sequence in Table 1.
2. The VHH antibody of claim 1, wherein the CDR1, CDR2, and CDR3 each comprise at least about 90%, 95%, 97%, or 99% identity to a sequence in Table 1.
3. The VHH antibody of claim 1, wherein CDR1, CDR2, and CDR3 correspond to SEQ ID NO: 610, 611, and 612.
4. The VHH antibody of claim 1, wherein the VHH antibody comprises at least 85% identity with SEQ ID NO: 365.
5. The VHH antibody of claim 1, wherein CDR1, CDR2, and CDR3 correspond to SEQ ID NO: 421, 422, and 423.
6. The VHH antibody of claim 1, wherein the VHH antibody comprises at least 85% identity with SEQ ID NO: 302.
7. A polypeptide comprising the VHH antibody of claim 1.
8. A polypeptide comprising at least two VHH antibodies of claim 1.
9. The polypeptide of claim 8, wherein the at least two VHH antibodies comprise SEQ ID NO: 365 or SEQ ID NO: 302.
10. The polypeptide of claim 8, wherein the at least two VHH antibodies are connected to each other via a linker.
11. The polypeptide of claim 10, wherein the linker comprises at least about 85% identity to SEQ ID NO: 390.
12. A polypeptide comprising at least three VHH antibodies of claim 1.
13. A polypeptide comprising at least four VHH antibodies of claim 1.
14. The polypeptide of claim 12 or 13, wherein the VHH antibodies are different.
15. The polypeptide of claim 12 or 13, wherein the VHH antibodies are the same.

16. A pharmaceutical composition comprising the VHH antibody of claim 1 and a pharmaceutically acceptable carrier.
17. A pharmaceutical composition comprising the polypeptide of claim 7, 12, or 13 and a pharmaceutically acceptable carrier.
18. A method of treating a disease or disorder related to SARS-CoV-2, the method comprising: administering the pharmaceutical composition of claim 16 to a subject in need thereof, thereby treating the disease or disorder related to SARS-CoV-2.
19. A method of treating a disease or disorder related to SARS-CoV-2, the method comprising: administering the pharmaceutical composition of claim 17 to a subject in need thereof, thereby treating the disease or disorder related to SARS-CoV-2.
20. The method of claim 18 or 19, wherein the subject in need thereof is infected with SARS-CoV-2 as determined by PCR assay.
21. The method of claim 18 or 19, wherein the subject in need thereof has been diagnosed as being infected with SARS-CoV-2.
22. The method of claim 18 or 19, wherein the subject in need thereof is at risk of developing Coronavirus Disease (COVID).
23. The method of claim 22, wherein the subject in need thereof is at risk of developing severe COVID.
24. The method of claim 18 or 19, wherein the subject in need thereof has one or more comorbidities.
25. The method of claim 24, wherein the subject in need thereof is treated for the one or more comorbidities.
26. The method of claim 18 or 19, wherein the pharmaceutical composition is delivered encapsulated in a spirulina cell.
27. The method of claim 26, wherein the spirulina cell is dried.
28. The method of claim 26 or 27, wherein the spirulina cell is of strain *A. platensis*.
29. The method of claim 28, wherein the pharmaceutical composition is delivered orally.
30. The method of claim 28, wherein the pharmaceutical composition is delivered via the airway.
31. The method of claim 30, wherein, the pharmaceutical composition is delivered via inhalation.
32. The method of claim 30 or 31, wherein the pharmaceutical composition is delivered nasally.
33. The method of claim 18 or 19, wherein the SARS-CoV-2 is a variant of concern.

34. The method of claim 33, wherein the variant of concern is selected from the group consisting of: Alpha, Beta, Gamma, Delta, and Omicron.
35. The method of claim 18 or 19, wherein the SARS-CoV-2 evades an immune response associated with vaccine therapy.
36. A method of treating a disease or disorder related to SARS-CoV-2, the method comprising: administering the pharmaceutical composition of claim 16 or 17 to a subject in need thereof thereby treating the disease or disorder related to SARS-CoV-2, wherein the disease or disorder is recalcitrant to anti-SARS-CoV-2 vaccine therapy.
37. The method of claim 36, wherein the subject in need thereof has previously been administered an anti-SARS-CoV-2 monoclonal therapy.
38. The method of claim 36, wherein the subject in need thereof has received a booster of the anti-SARS-CoV-2 vaccine therapy.
39. The method of claim 36, wherein the subject in need thereof has not received a booster of the anti-SARS-CoV-2 vaccine therapy.
40. The method of claim 36, wherein the administering is effective in reducing or eliminating a symptom of the disease or disorder related to the SARS-CoV-2.
41. The method of claim 40, wherein the symptom is a gastrointestinal symptom.
42. The method of claim 40, wherein the symptom is a respiratory symptom.
43. The method of claim 36, wherein the administering is effective in reducing a period of illness associated with the disease or disorder related to the SARS-CoV-2 by at least about 1-3 days.

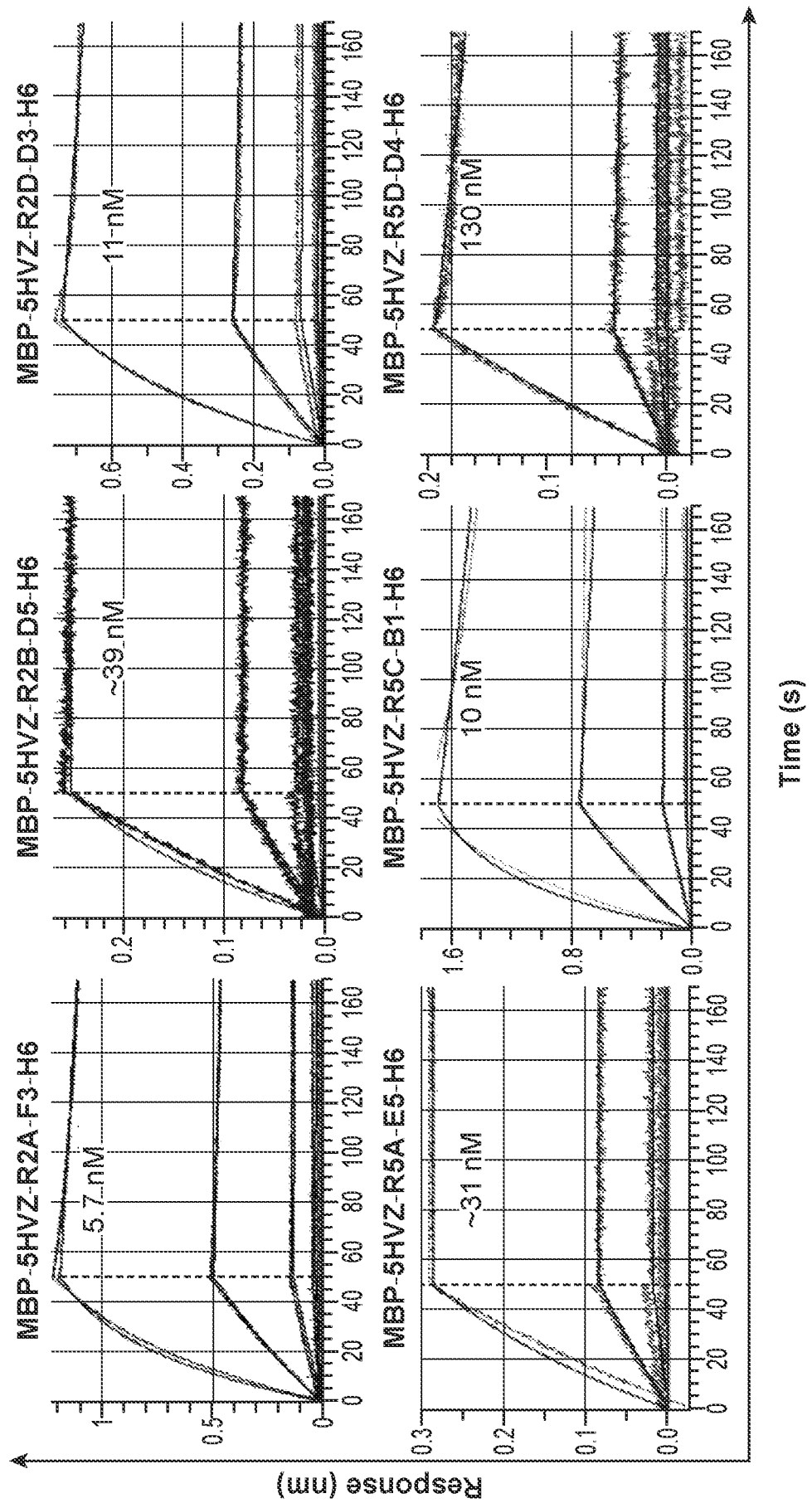


FIG. 1

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VHH	Scaffold	Name	% infectivity @ 10 µg/mL	Pseudovirus inhibition IC ₅₀ (nM)	ACE2-Fc competition IC ₅₀ (nM)	kD (nM)	% of total protein in spirulina ²
R2A-C5	monomer	MBP-R2A-C5-H6	87.8	NM	ND	BLD (23000)	NM
	Dimer	MBP-5HVZ-R2A-C5-H6	NM	ND	13.3	BLD (3250)	2
R2A-F3	monomer	MBP-R2A-F3-H6	70.7	NM	ND	OR (1900)	NM
	Dimer	MBP-5HVZ-R2A-F3-H6	NM	272.2	1.6	5.66	0.2
	Dimer-PAPA	MBP-5HVZ-PAPA-R2a-F3-H6	58.4	NM	10.4	NM	NM
	Trimer	MPB-scTRP-PAPA-R2a-F3-H6	51.1	NM	83.6	NM	NM
	Heptamer	MBP-480F-PAPA-R2a-F3-H6	60.3	NM	ND	NM	NM
R2B-D5	monomer	MBP-R2B-D5-H6	NM	175.7	NM	82	8
	Dimer	MBP-5HVZ-R2B-D5-H6	NM	36.47	27.7	39.3	4.1
	Dimer-PAPA	MBP-5HVZ-PAPA-R2b-D5-H6	0.1	0.6	8.4	NM	N/A
	Trimer	MPB-scTRP-PAPA-R2b-D5-H6	0.0	1.9	46.5	NM	N/A
	Heptamer	MBP-480F-PAPA-R2b-D5-H6	0.0	0.89	14.5	NM	NM
R2B-E6	monomer	MBP-R2B-E6-H6	86.5	NM	ND	OR (1400)	NM
	Dimer	MBP-5HVZ-R2B-E6-H6	NM	357.8	17.9	10.3	7.2
R2C-B3	monomer	MBP-R2C-B3-H6	75.3	NM	147.9	OR (950)	2
	Dimer	MBP-5HVZ-R2C-B3-H6	NM	ND	1.4	33	NM
R2C-F6	Monomer	MBP-R2C-F6-H6	NM	NM	NM	OR (3600)	0.7

FIG. 2

	Dimer	MBP-5HVZ-R2C-F6-H6	NM	92.39	6.5	OR (0.96)	0.2
	Dimer_P APA	MBP-5HVZ-PAPA- R2c-F6-H6	9.2	2.75	3.2	NM	NM
	Trimer	MPB-scTRP-PAPA- R2c-F6-H6	18.1	63.84	165.0	NM	NM
	Heptamer	MBP-4B0F-PAPA- R2c-F6-H6	2.7	5.66	158.3	NM	NM
R2C- G6	monomer	MBP-R2C-G6-H6	NM	NM	NM	OR (3900)	NM
	Dimer	MBP-5HVZ-R2C-G6- H6	NM	134.3	3.9	35.7	0.3
R2D- A6	monomer	MBP-R2D-A6-H6	77.5	NM	0.1	OR (4600)	8
	Dimer	MBP-5HVZ-R2D-A6- H6	NM	ND	6.3	19.6	NM
	Dimer- PAPA	MBP-5HVZ-PAPA- R2d-A6-H6	65.2	NM	3.8	NM	NM
	Trimer	MPB-scTRP-PAPA- R2d-A6-H6	24.3	54.4	4.7	NM	NM
	Heptamer	MBP-4B0F-PAPA- R2d-A6-H6	48.1	179.5	11.0	NM	NM
R2D- D3	monomer	MBP-R2D-D3-H6	75.9	NM	ND	OR (27000)	5.6
	Dimer	MBP-5HVZ-R2D-D3- H6	NM	116.8	2.7	10.9	0.5
	Dimer- PAPA	MBP-5HVZ-PAPA- R2d-D3-H6	32.8	76.98	2.1	NM	NM
	Trimer	MPB-scTRP-PAPA- R2d-D3-H6	28.8	123.3	43.4	NM	NM
	Heptamer	MBP-4B0F-PAPA- R2d-D3-H6	10.9	5.99	27.7	NM	NM
	Particle	R2d-D3-PP7-H6	1.6	5.61	282.3	NM	NM
R2E- D2	monomer	MBP-R2E-D2-H6	75.5	NM	ND	100	7
	Dimer	MBP-5HVZ-R2E-D2- H6	NM	ND	6.8	19.1	NM

FIG. 2 (Cont. 1)

R2E-F4	Monomer	MBP-R2E-F4-H6	84.7	NM	ND	OR (1600)	2
	Dimer	MBP-5HVZ-R2E-F4-H6	NM	ND	6.3	11.4	0.6
R5A-A1	Monomer	MBP-R5A-A1-H6	84.4	NM	ND	OR (2700)	NM
	Dimer	MBP-5HVZ-R5A-A1-H6	NM	ND	3.4	29	NM
R5A-A5	Monomer	MBP-R5A-A5-H6	NM	NM	NM	OR(920)	NM
	Dimer	MBP-5HVZ-R5A-A5-H6	NM	ND	4.4	24.7	NM
R5A-B3	Monomer	MBP-R5A-B3-H6	80.0	NM	ND	OR (5170)	NM
	Dimer	MBP-5HVZ-R5A-B3-H6	NM	ND	35.9	OR (1200)	NM
R5a-C5	Monomer	MBP-R5A-C5-H6	54.1	NM	71.9	42.1	NM
	Dimer	MBP-5HVZ-R5A-C5-H6	NM	ND	1.4	7.04	0.6
	Dimer-PAPA	MBP-5HVZ-PAPA-R5a-C5-H6	5.9	4.7	1.1	NM	NM
	Trimer	MPB-scTRP-PAPA-R5a-C5-H6	2.0	BLD	2.9	NM	NM
	Heptamer	MBP-4B0F-PAPA-R5a-C5-H6	1.5	0.35	1.8	NM	NM
R5A-D4	Monomer	MBP-R5A-D4-H6	NM	NM	NM	NM	NM
	Dimer	MBP-5HVZ-R5A-D4-H6	NM	ND	5.7	21.1	NM
R5A-D6	Monomer	MBP-R5A-D6-H6	76.7	NM	ND	100	NM
	Dimer	MBP-5HVZ-R5A-D6-H6	NM	ND	223.2	35	3.3
R5A-E5	Monomer	MBP-R5A-E5-H6	71.6	NM	182.9	6.97	6.1
	Dimer	MBP-5HVZ-R5A-E5-H6	NM	ND	17.7	31	1.1
	Dimer-PAPA	MBP-5HVZ-PAPA-R5a-E5-H6	25.5	74.68	14.2	NM	NM
	Trimer	MPB-scTRP-PAPA-R5a-E5-H6	54.4	NM	N	NM	NM

FIG. 2 (Cont. 2)

	Heptamer	MBP-4B0F-PAPA-R5a-E5-H6	33.7	370.4	ND	NM	NM
R5B-C3	Monomer	MBP-R5B-C3-H6	65.5	NM	ND	OR (17200)	1
	Dimer	MBP-5HVZ-R5B-C3-H6	NM	ND	1.6	13	0.5
R5B-G1	Monomer	MBP-R5B-G1-H6	NM	NM	NM	NM	1.6
	Dimer	MBP-5HVZ-R5B-G1-H6	NM	5.816	26.6	NM	0.1
	Dimer-PAPA	MBP-5HVZ-PAPA-R5b-G1-H6	2.3	2.33	5.2	NM	NM
	Trimer	MPB-scTRP-PAPA-R5b-G1-H6	66.8	NM	ND	NM	NM
	Heptamer	MBP-4B0F-PAPA-R5b-G1-H6	31.5	ND	ND	NM	NM
R5C-A1	Monomer	MBP-R5C-A1-H6	56.5	NM	ND	NM	0.2
	Dimer	MBP-5HVZ-R5C-A1-H6	NM	ND	10.3	379	4.9
R5C-A2	Monomer	MBP-R5C-A2-H6	70.6	NM	ND	420	NM
	Dimer	MBP-5HVZ-R5C-A2-H6	NM	ND	13.7	13.2	8
R5C-B1	Monomer	MBP-R5C-B1-H6	NM	NM	NM	55.3	8
	Dimer	MBP-5HVZ-R5C-B1-H6	NM	ND	0.3	9.98	0.7
R5C-B2	Monomer	MBP-R5C-B2-H6	73.4	NM	372.9	OR (1370)	5.3
	Dimer	MBP-5HVZ-R5C-B2-H6	NM	ND	5.9	57.3	1.4
R5C-E1	Monomer	MBP-R5C-E1-H6	NM	NM	NM	OR (2800)	NM
	Dimer	MBP-5HVZ-R5C-E1-H6	NM	ND	4.5	6.13	NM
R5C-E3	Monomer	MBP-R5C-E3-H6	NM	NM	NM	OR (1370)	8
	Dimer	MBP-5HVZ-R5C-E3-H6	NM	ND	9.2	OR	NM

FIG. 2 (Cont. 3)

R5C-F3	Monomer	MBP-R5C-F3-H6	81.2	NM	150.7	OR (5370)	8
	Dimer	MBP-5HVZ-R5C-F3-H6	NM	ND	11.9	11.3	5
R5C-F5	Monomer	MBP-R5C-F5-H6	75.5	NM	ND	100	NM
	Dimer	MBP-5HVZ-R5C-F5-H6	NM	ND	3.9	3.13	NM
R5C-F6	Monomer	MBP-R5C-F6-H6	86.2	NM	ND	100	NM
	Dimer	MBP-5HVZ-R5C-F6-H6	NM	ND	110.1	OR	NM
R5C-G2	Monomer	MBP-R5C-G2-H6	89.2	NM	184.5	OR (2120)	NM
	Dimer	MBP-5HVZ-R5C-G2-H6	NM	ND	120.6	29.6	NM
R5C-G6	Monomer	MBP-R5C-G6-H6	NM	NM	NM	NM	NM
	Dimer	MBP-5HVZ-R5C-G6-H6	NM	NM	8.7	NM	NM
R5D-A1	Monomer	MBP-R5D-A1-H6	55.0	NM	6.2	527	NM
	Dimer	MBP-5HVZ-R5D-A1-H6	NM	NM	12.6	NM	NM
R5D-B2	Monomer	MBP-R5D-B2-H6	77.6	NM	3.7	OR (2350)	NM
	Dimer	MBP-5HVZ-R5D-B2-H6	NM	ND	ND	1.21	8
R5D-B2	Monomer	MBP-R5D-B5-H6	NM	NM	NM	NM	0.2
	Dimer	MBP-5HVZ-R5D-B5-H6	NM	3.254	53.7	OR	0.1
	Dimer_P APA	MBP-5HVZ-PAPA-R5d-B5-H6	35.6	43.68	23.1	NM	NM
	Heptamer	MBP-4B0F-PAPA-R5d-B5-H6	71.0	NM	ND	NM	NM
R5D-B6	Monomer	MBP-R5D-B6-H6	NM	NM	NM	NM	0.2
	Dimer	MBP-5HVZ-R5D-B6-H6	NM	3.26	15.7	OR	0.1
	Dimer_P APA	MBP-5HVZ-PAPA-R5d-B6-H6	4.6	0.85	4.7	NM	NM

FIG. 2 (Cont. 4)

	Heptamer	MBP-4B0F-PAPA-R5d-B6-H6	2.5	7.04	105.9	NM	NM
R5D-D4	Monomer	MBP-R5D-D4-H6	NM	NM	NM	100	NM
	Dimer	MBP-5HVZ-R5D-D4-H6	NM	166.1	48.1	130	1.9
	Dimer_PAPA	MBP-5HVZ-PAPA-R5d-D4-H6	81.7	NM	ND	NM	NM
	Trimer	MPB-scTRP-PAPA-R5d-D4-H6	77.5	NM	ND	NM	NM
	Heptamer	MBP-4B0F-PAPA-R5d-D4-H6	73.1	NM	74.6	NM	NM
R5D-E4	Monomer	MBP-R5D-E4-H6	NM	NM	NM	NM	NM
	Dimer	MBP-5HVZ-R5D-E4-H6	NM	ND	13.7	9.34	0.1
R5D-F3	Monomer	MBP-R5D-F3-H6	NM	NM	NM	NM	NM
	Dimer	MBP-5HVZ-R5D-F3-H6	NM	ND	29.3	OR	2
R5D-G1	Monomer	MBP-R5D-G1-H6	NM	NM	NM	NM	NM
	Dimer	MBP-5HVZ-R5D-G1-H6	NM	ND	12.4	OR	0.1
	Dimer_PAPA	MBP-5HVZ-PAPA-R5d-G1-H6	53.2	ND	ND	NM	NM
	Trimer	MPB-scTRP-PAPA-R5d-G1-H6	69.7	ND	ND	NM	NM
	Heptamer	MBP-4B0F-PAPA-R5d-G1-H6	35.9	223.9	ND	NM	NM
R5D-G6	Monomer	MBP-R5D-G6-H6	77.1	NM	7.5	564	NM
	Dimer	MBP-5HVZ-R5D-G6-H6	NM	ND	3.2	31.3	NM
R5E-D1	Monomer	MBP-R5E-D1-H6	77.1	NM	2.9	NM	NM
	Dimer	MBP-5HVZ-R5E-D1-H6	NM	NM	NM	NM	3.5
R5E-G2	Monomer	MBP-R5E-G2-H6	81.9	NM	2.1	NM	NM
	Dimer	MBP-5HVZ-R5E-G2-H6	NM	NM	NM	3.14	7.2

FIG. 2 (Cont. 5)

R5A-B7	Monomer	MBP-R5A-B7-H6	NM	NM	NM	NM	2.7
	Dimer	MBP-5HVZ-R5A-B7-H6	NM	NM	NM	13.1	0.1
	Dimer_PAPA	MBP-5HVZ-PAPA-R5a-B7-H6	1.8	0.44	2.4	NM	NM
	Trimer	MPB-scTRP-PAPA-R5a-B7-H6	2.4	7.98	56.5	NM	NM
		MPB-scTRP-PAPA-R5a-B7-H6	NM	215.1	NM	NM	NM
	Heptamer	MBP-4B0F-PAPA-R5a-B7-H6	0.03	2.13	18.2	NM	NM
R5a-B5	Monomer	MBP-R5a-B5-H6	58.0	NM	43.9	60.8	0.1
	Dimer	MBP-5HVZ-R5a-B5-H6	2.4	5.25	0.9	NM	0.1
R5a-F2	Monomer	MBP-R5a-F2-H6	83.2	NM	ND	705	NM
	Dimer	MBP-5HVZ-R5a-F2-H6	18.3	59.27	1.7	9.13	2.8
R1e-B2	Monomer	MBP-R1e-B2-H6	84.0	NM	ND	OR	NM
	Dimer	MBP-5HVZ-R1e-B2-H6	72.1	NM	ND	197	NM
R1e-D4	Monomer	MBP-R1e-D4-H6	94.3	NM	ND	47	NM
	Dimer	MBP-5HVZ-R1e-D4-H6	72.4	NM	ND	OR	NM
R1i-A11	Monomer	MBP-R1i-A11-H6	91.3	NM	173.3	23.7	NM
	Dimer	MBP-5HVZ-R1i-A11-H6	38.4	50.4	1.5	6.98	0.7
R5b-C5	Monomer	MBP-R5b-C5-H6	100.7	NM	ND	88.5	NM
	Dimer	MBP-5HVZ-R5b-C5-H6	77.0	NM	2.5	11.8	NM
S1a-C4	Monomer	MBP-S1a-C4-H6	84.8	NM	ND	100	NM
	Dimer	MBP-5HVZ-S1a-C4-H6	75.7	NM	ND	167	NM
S1b-E7	Monomer	MBP-S1b-E7-H6	85.7	NM	ND	OR (6570)	NM
	Dimer	MBP-5HVZ-S1b-E7-H6	69.6	NM	35.7	15.5	NM

FIG. 2 (Cont. 6)

S1b-E11	Monomer	MBP-S1b-E11-H6	80.0	NM	ND	OR (5290)	NM
	Dimer	MBP-5HVZ-S1b-E11-H6	76.3	NM	15.9	OR	NM
S2a-C4	Monomer	MBP-S2a-C4-H6	82.6	NM	ND	NM	NM
	Dimer	MBP-5HVZ-S2a-C4-H6	47.5	ND	13.1	219	5.4
S2b-A7	Monomer	MBP-S2b-A7-H6	90.0	NM	ND	NM	NM
	Dimer	MBP-5HVZ-S2b-A7-H6	67.1	NM	5.9	22.5	NM
S2b-C10	Monomer	MBP-S2b-C10-H6	102.0	NM	ND	NM	3.1
	Dimer	MBP-5HVZ-S2b-C10-H6	65.1	NM	404.8	54	1.1
S2b-F7	Monomer	MBP-S2b-F7-H6	83.2	NM	ND	NM	NM
	Dimer	MBP-5HVZ-S2b-F7-H6	74.9	NM	43.6	44.3	NM
S2b-G8	Monomer	MBP-S2b-G8-H6	78.7	NM	ND	NM	NM
	Dimer	MBP-5HVZ-S2b-G8-H6	73.2	NM	ND	OR	NM
S3a-C5	Monomer	MBP-S3a-C5-H6	76.1	NM	ND	NM	NM
	Dimer	MBP-5HVZ-S3a-C5-H6	71.1	NM	2.8	1.42	NM
S3a-H1	Monomer	MBP-S3a-H1-H6	84.4	NM	ND	NM	8
	Dimer	MBP-5HVZ-S3a-H1-H6	74.3	NM	4.3	NM	6.1
S3b-A7	Monomer	MBP-S3b-A7-H6	82.7	NM	ND	NM	NM
	Dimer	MBP-5HVZ-S3b-A7-H6	90.5	NM	ND	2.63	NM
S3b-A8	Monomer	MBP-S3b-A8-H6	79.3	NM	ND	NM	NM
	Dimer	MBP-5HVZ-S3b-A8-H6	70.4	NM	2.0	12.1	NM
S3b-C8	Monomer	MBP-S3b-C8-H6	62.4	NM	ND	NM	NM
	Dimer	MBP-5HVZ-S3b-C8-H6	6.4	15.51	11.4	8.41	11.2
R1i-C8	Monomer	MBP-R1i-C8-H6	71.8	NM	ND	NM	NM

FIG. 2 (Cont. 7)

	Dimer	MBP-5HVZ-R1i-C8-H6	45.4	ND	111.2	25.2	1.2
R1i-E7	Monomer	MBP-R1i-E7-H6	79.3	NM	ND	NM	NM
	Dimer	MBP-5HVZ-R1i-E7-H6	78.5	NM	ND	OR	NM
R2b-D4	Monomer	MBP-R2b-D4-H6	70.6	NM	ND	NM	NM
	Dimer	MBP-5HVZ-R2b-D4-H6	35.6	ND	ND	NM	4.6
R5a-F5	Monomer	MBP-R5a-F5-H6	67.7	NM	97.9	NM	2
	Dimer	MBP-5HVZ-R5a-F5-H6	57.5	NM	5.6	11.9	NM
R5d-E1	Monomer	MBP-R5d-E1-H6	76.9	NM	ND	NM	NM
	Dimer	MBP-5HVZ-R5d-E1-H6	NM	NM	NM	7.98	NM
R5e-C1	Monomer	MBP-R5e-C1-H6	102.2	756	ND	NM	NM
	Dimer	MBP-5HVZ-R5e-C1-H6	92.1	NM	1.9	OR	0.2
S3a-H2	Monomer	MBP-S3a-H2-H6	74.5	NM	ND	NM	NM
	Dimer	MBP-5HVZ-S3a-H2-H6	75.0	NM	2.2	18.4	1.2
S3b-E11	Monomer	MBP-S3b-E11-H6	67.6	NM	ND	NM	NM
	Dimer	MBP-5HVZ-S3b-E11-H6	48.9	68.42	12.2	NM	3.9
RN-1	Dimer-PAPA	MBP-5HVZ-PAPA-RN-1-H6	83.4	NM	7.8	NM	NM
RN-2	Dimer-PAPA	MBP-5HVZ-PAPA-RN-2-H6	76.8	NM	3.2	NM	NM
RN-3	Dimer-PAPA	MBP-5HVZ-PAPA-RN-3-H6	86.5	NM	46.8	NM	NM
RN-5	Dimer-PAPA	MBP-5HVZ-PAPA-RN-5-H6	18.6	54.55	2.3	NM	NM
RN-9	Dimer-PAPA	MBP-5HVZ-PAPA-RN-9-H6	58.3	NM	2.3	NM	NM
RN-12	Dimer-PAPA	MBP-5HVZ-PAPA-RN-12-H6	70.6	NM	ND	NM	NM
RN-14	Dimer-PAPA	MBP-5HVZ-PAPA-RN-14-H6	17.2	13.91	3.2	NM	NM

FIG. 2 (Cont. 8)

RN-16	Dimer-PAPA	MBP-5HVZ-PAPA-RN-16-H6	91.8	NM	13.9	NM	NM
RN-17	Dimer-PAPA	MBP-5HVZ-PAPA-RN-17-H6	40.9	ND	2.4	NM	NM
RN-18	Dimer-PAPA	MBP-5HVZ-PAPA-RN-18-H6	57.6	NM	53.3	NM	NM
RN-19	Dimer-PAPA	MBP-5HVZ-PAPA-RN-19-H6	73.8	NM	3.5	NM	NM
RN-20	Dimer-PAPA	MBP-5HVZ-PAPA-RN-20-H6	79.8	NM	7.2	NM	NM
RN-21	Dimer-PAPA	MBP-5HVZ-PAPA-RN-21-H6	76.6	NM	9.7	NM	NM
RN-22	Dimer-PAPA	MBP-5HVZ-PAPA-RN-22-H6	34.3	110	3.9	NM	NM
RN-23	Dimer-PAPA	MBP-5HVZ-PAPA-RN-23-H6	93.1	NM	380.4	NM	NM
RN-25	Dimer-PAPA	MBP-5HVZ-PAPA-RN-25-H6	88.1	NM	29.6	NM	NM
RN-26	Dimer-PAPA	MBP-5HVZ-PAPA-RN-26-H6	57.9	NM	156.0	NM	NM
RN-27	Dimer-PAPA	MBP-5HVZ-PAPA-RN-27-H6	68.3	NM	9.4	NM	NM
RN-28	Dimer-PAPA	MBP-5HVZ-PAPA-RN-28-H6	70.4	NM	10.5	NM	NM
RN-29	Dimer-PAPA	MBP-5HVZ-PAPA-RN-29-H6	6.3	9.38	4.9	NM	NM
RN-30	Dimer-PAPA	MBP-5HVZ-PAPA-RN-30-H6	76.3	NM	3.4	NM	NM
RN-31	Dimer-PAPA	MBP-5HVZ-PAPA-RN-31-H6	86.9	NM	11.6	NM	NM
Sb-15	Dimer-PAPA	MBP-5HVZ-PAPA-Sb-15-H6	1.7	0.81	1.0	NM	NM
Sb-45	Dimer-PAPA	MBP-5HVZ-PAPA-Sb-45-H6	29.9	19.64	3.9	NM	NM
Sb-68	Dimer-PAPA	MBP-5HVZ-PAPA-Sb-68-H6	30.5	104	2.5	NM	NM

¹ ND: not detected; NM: not measured; OR: over-range; BLD: below limit of detection

² Preliminary assessment of protein expression under laboratory conditions
Characterization of VHHs through neutralization, binding, and competition assays.

FIG. 2 (Cont. 9)



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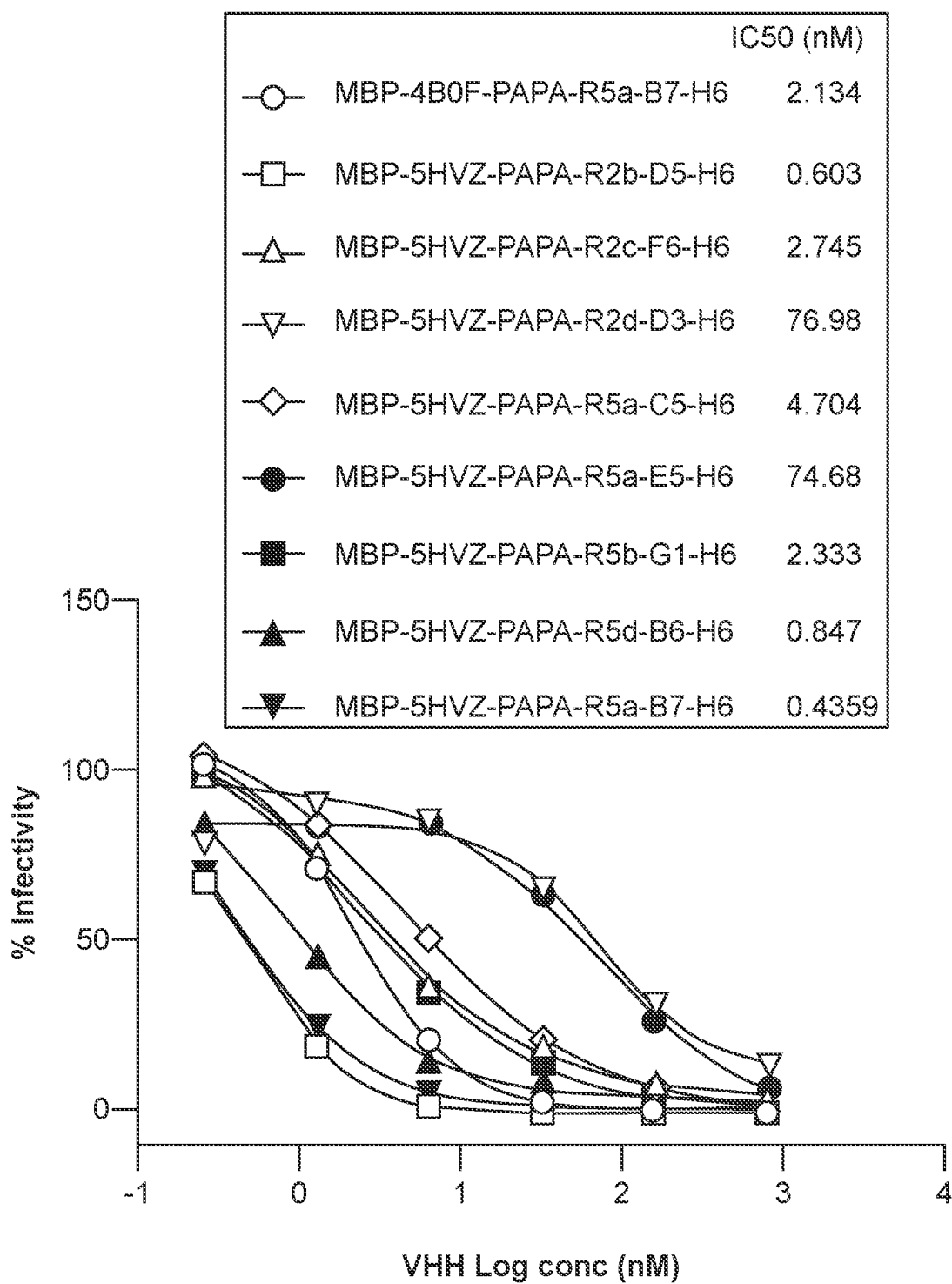


FIG. 4

[illegible]

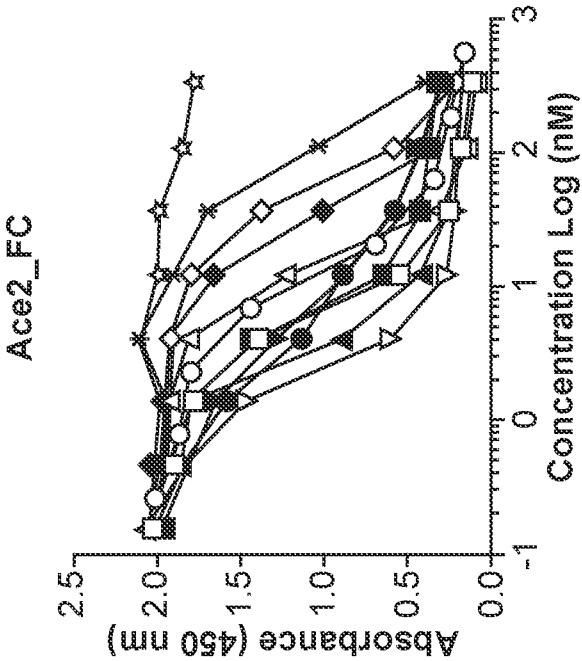


FIG. 6A

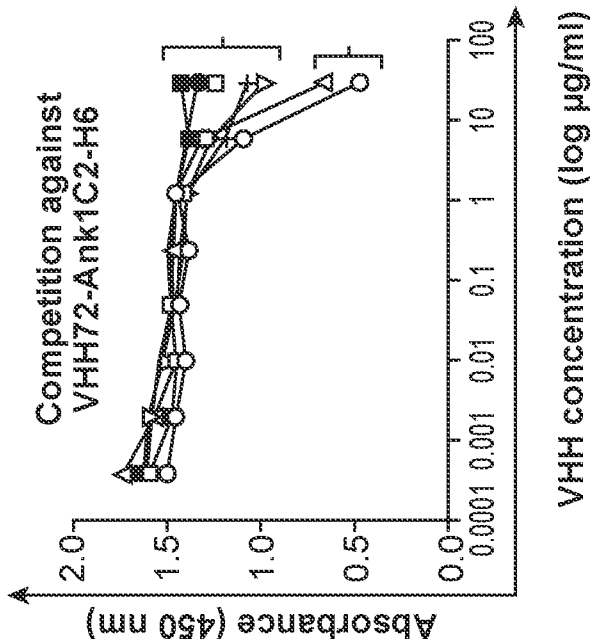


FIG. 6B

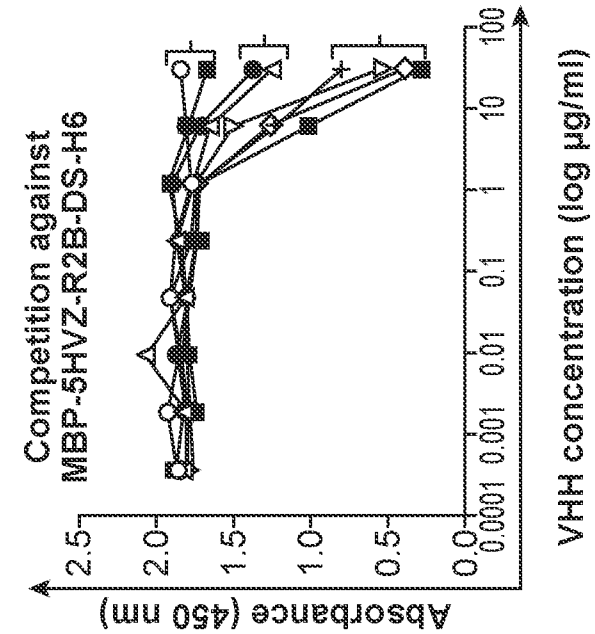


FIG. 6C

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Black line connects same VHH, different scaffolds

Construct:	Protein number:
MBF-5HVZ-PAPA-Sb-6B-H6	PP1118
MBF-5HVZ-PAPA-R2d-D3-H6	PP1146
MBF-5HVZ-PAPA-R5b-C5-H6	PP1148
MBF-5HVZ-R2B-D5-H6	PP758
MBF-5HVZ-PAPA-RN-22-H6	PP1107
MBF-5HVZ-PAPA-Sb-15-H6	PP1116
MBF-5HVZ-53b-C8-H6	PP917
MBF-5HVZ-R5C-G6-H6	PP788
MBF-5HVZ-R5a-B5-H6	PP899
MBF-5HVZ-R5A-C5-H6	PP772
MBF-5HVZ-R5B-G1-H6	PP777
VHH72-MBP-6His	PP555
MBF-5HVZ-PAPA-RN-29-H6	PP1113
MBF-5HVZ-PAPA-Sb-45-H6	PP1117
MBF-5HVZ-PAPA-RN-14-H6	PP1100
MBF-5HVZ-PAPA-R2c-F6-H6	PP1144
MBF-5HVZ-PAPA-R5a-B7-H6	PP1147
scTRP-PAPA-R2c-F6-H6	PP1156
MBF-480F-PAPA-R5a-C5-H6	PP1136
MBF-480F-PAPA-R5d-B6-H6	PP1140
MBF-5HVZ-PAPA-R5b-G1-H6	PP1150
MBF-5HVZ-RSD-A1-H6	PP789
MBF-480F-PAPA-R2c-F6-H6	PP1132
MBF-480F-PAPA-R2d-D3-H6	PP1134
R2d-D3-PP7-H6	PP1181
scTRP-PAPA-R5a-B7-H6	PP1159
scTRP-PAPA-R5a-C5-H6	PP1160
MBF-5HVZ-RSD-B6-H6	PP793
MBF-480F-PAPA-R5a-B7-H6	PP1135
MBF-5HVZ-PAPA-R5d-B6-H6	PP1152

FIG. 7

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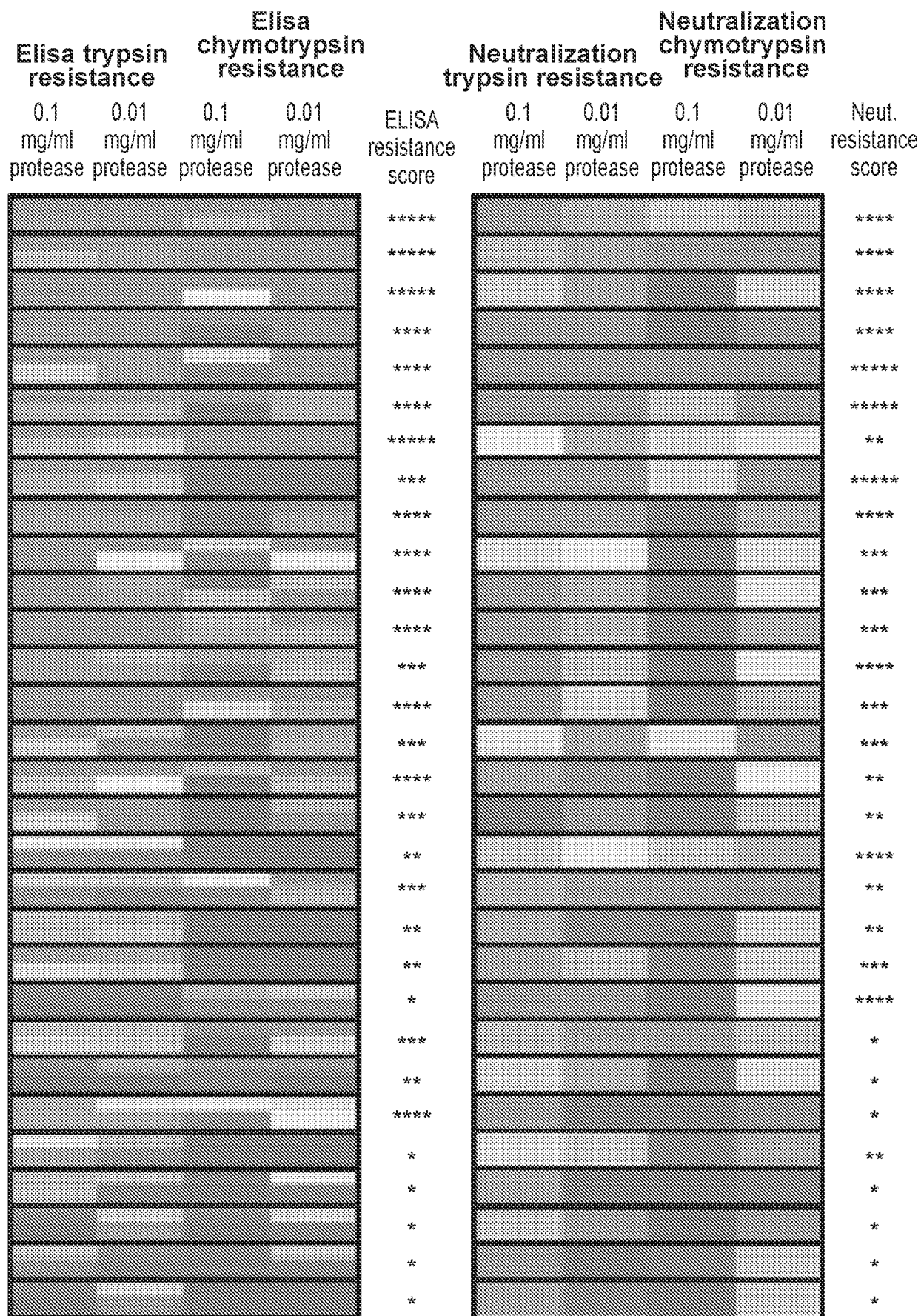


FIG. 7 (Cont.)

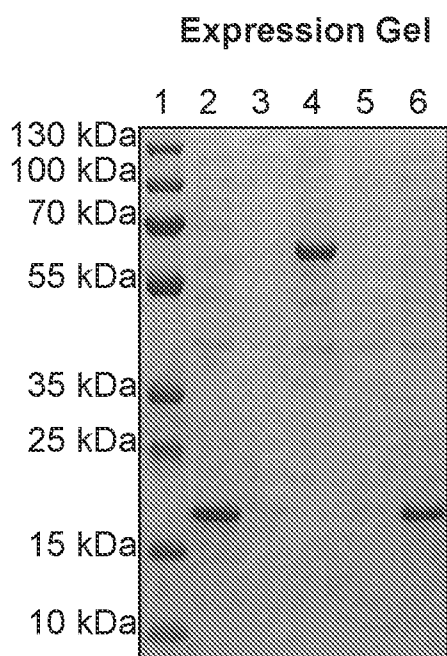


FIG. 8A

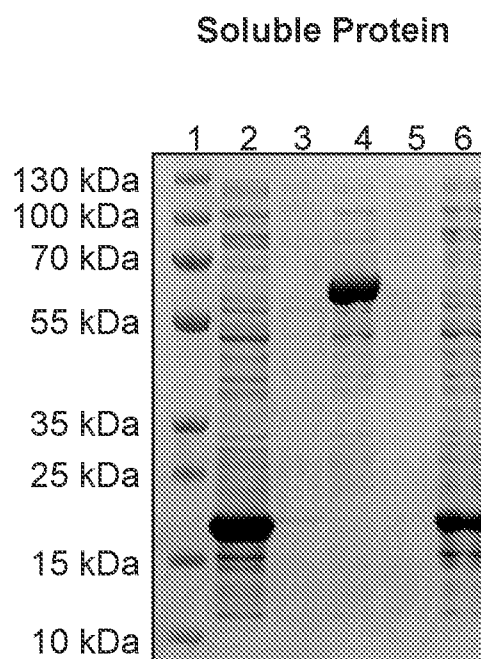


FIG. 8B

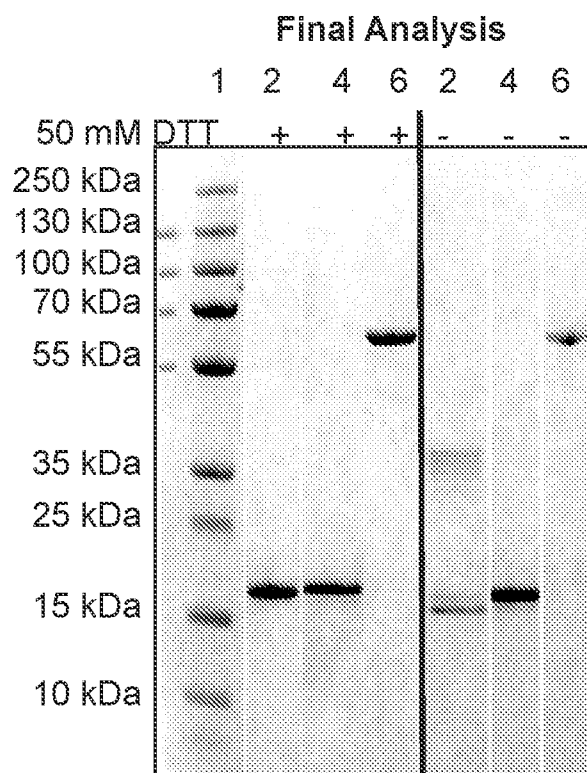


FIG. 8C

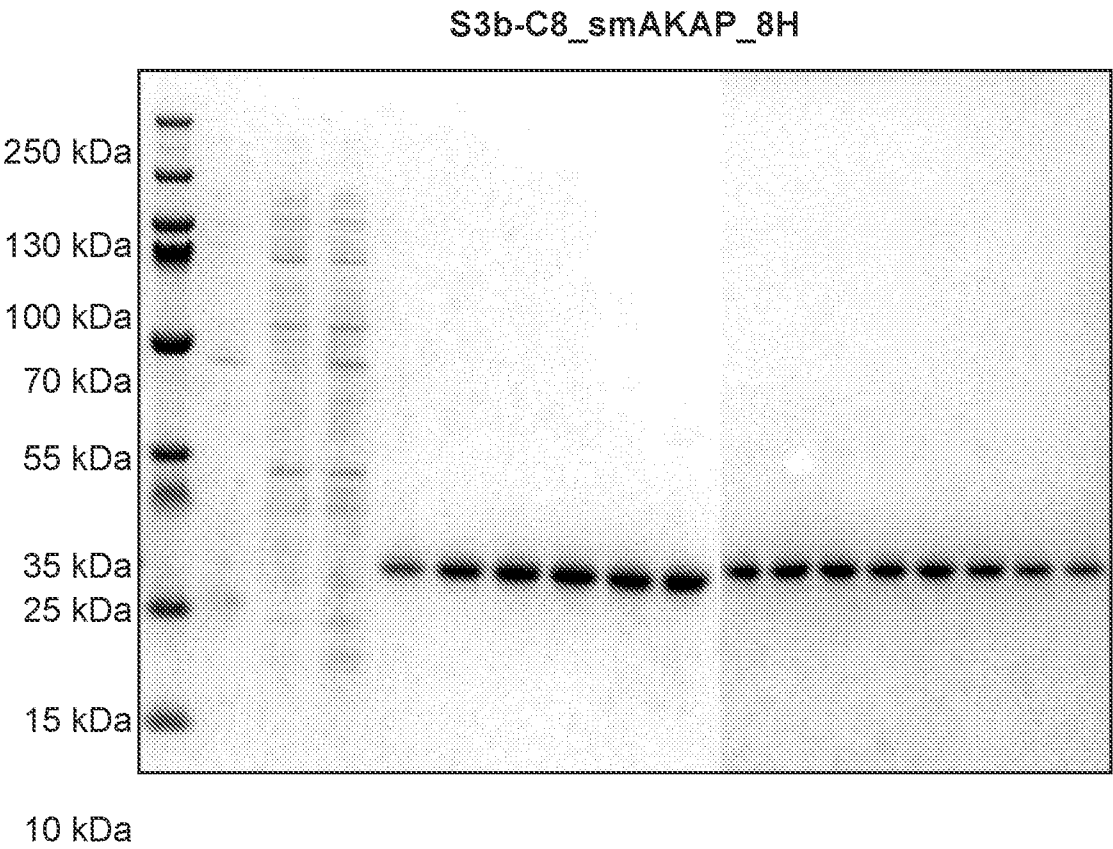


FIG. 9A

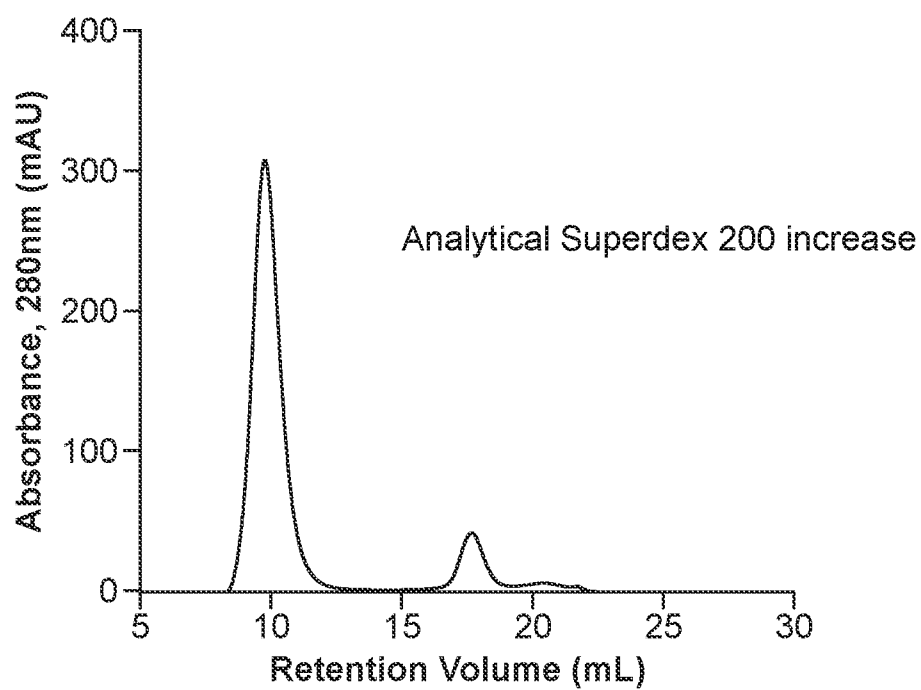


FIG. 9B

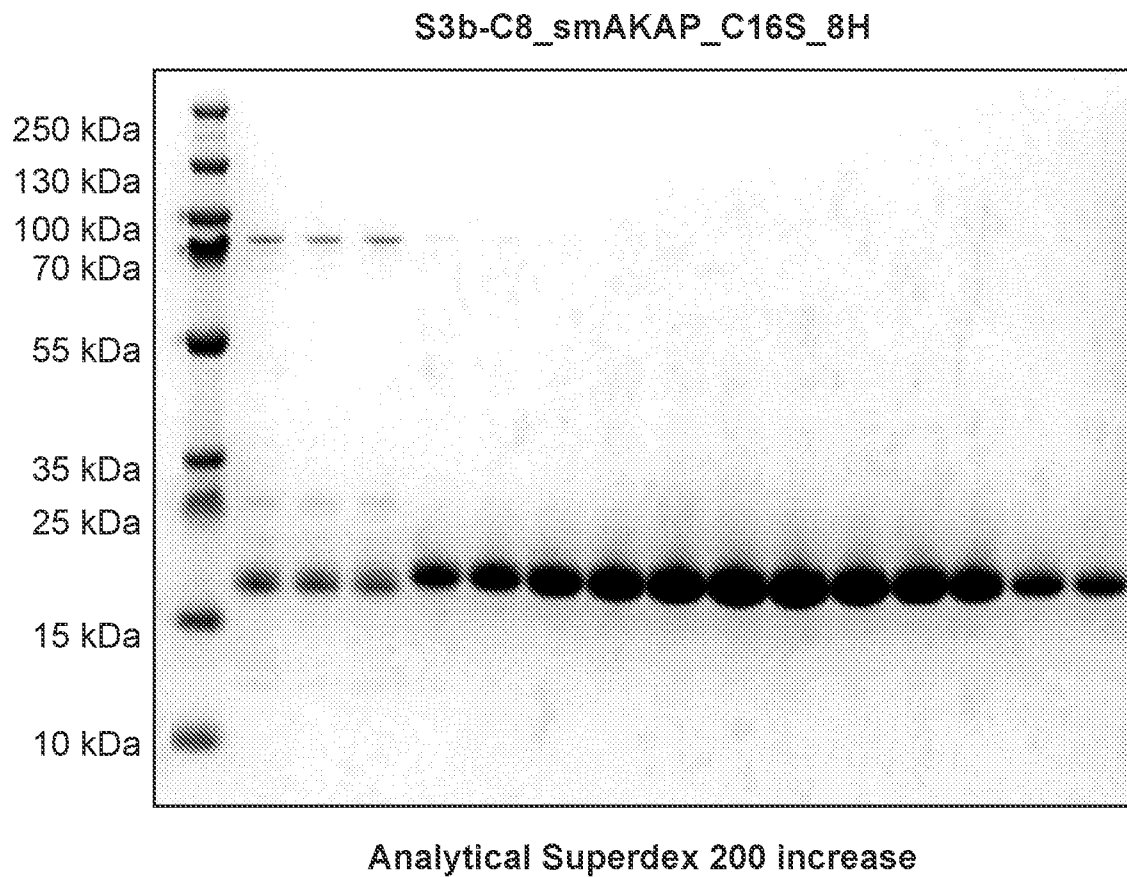
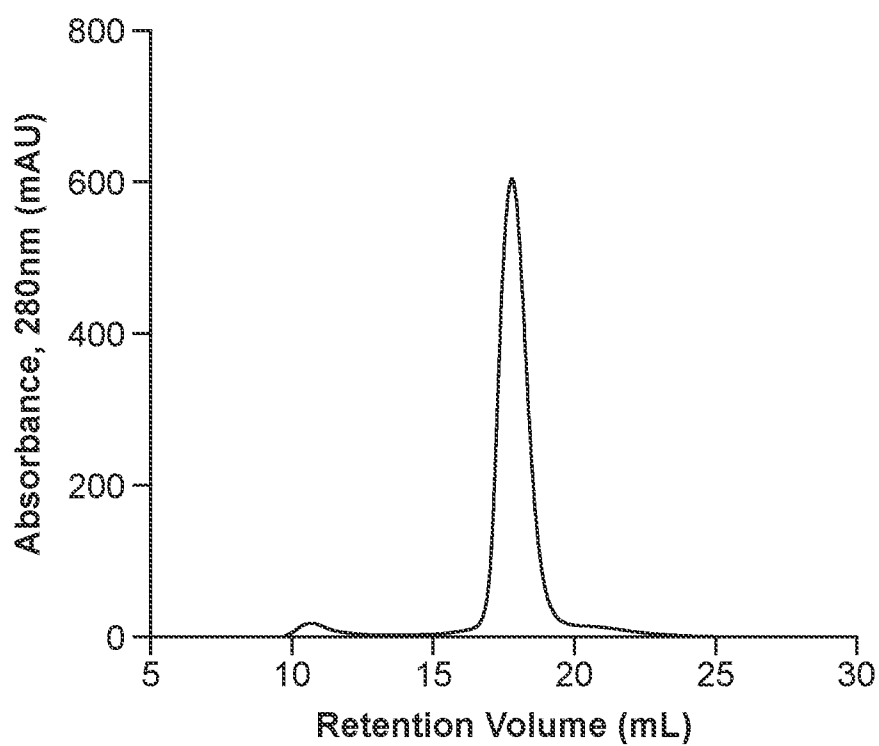


FIG. 10A

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Analytical Superdex 200 increase

FIG. 10B

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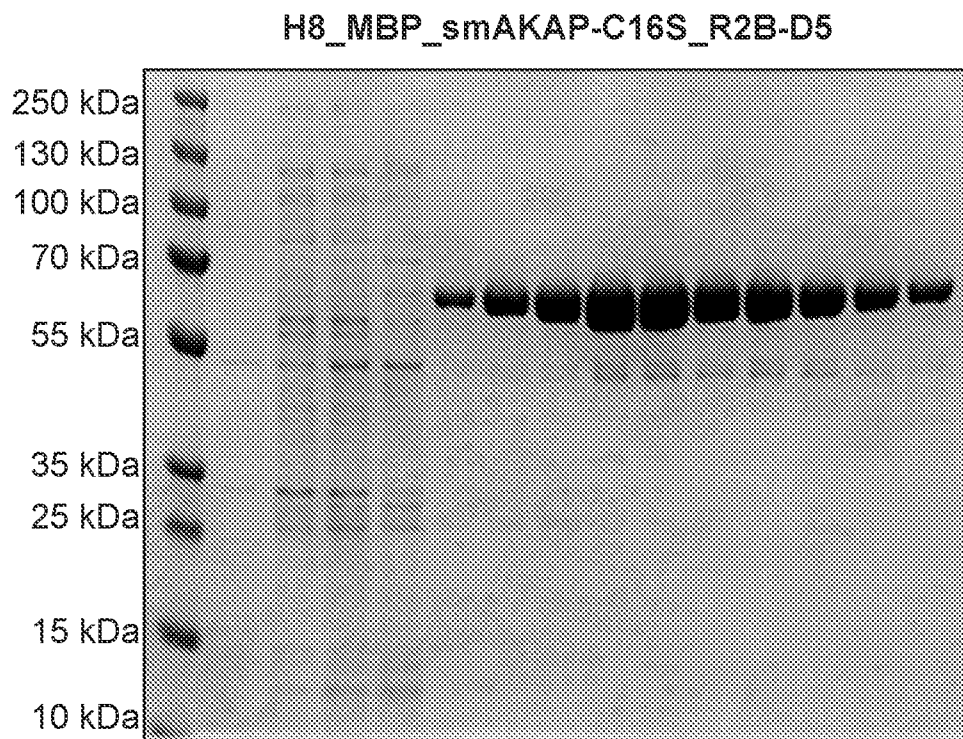
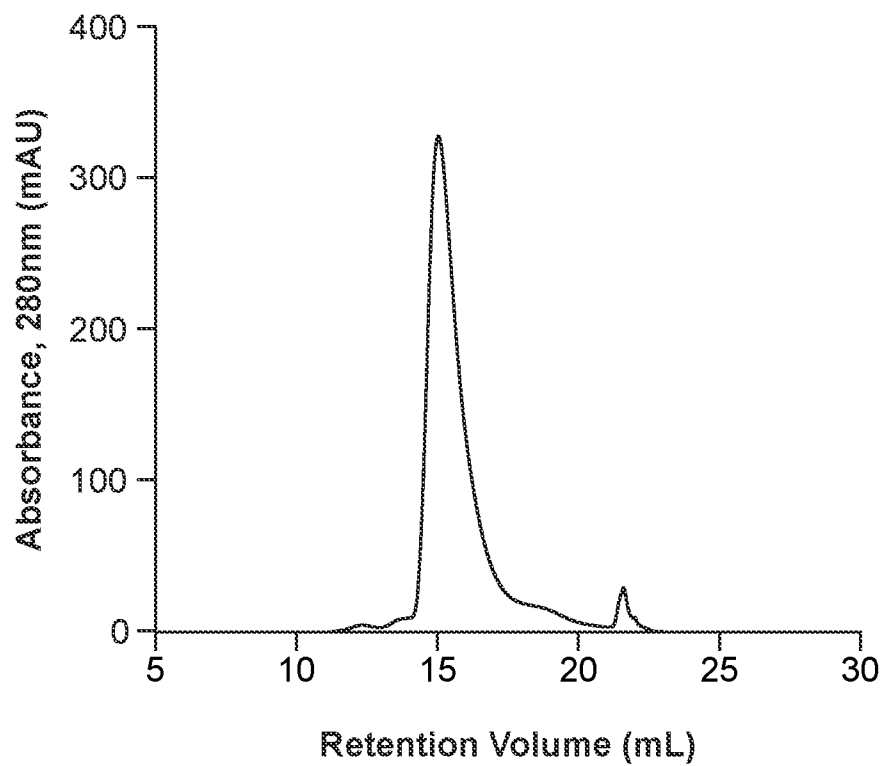


FIG. 11A

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Analytical Superdex 200 increase

FIG. 11B

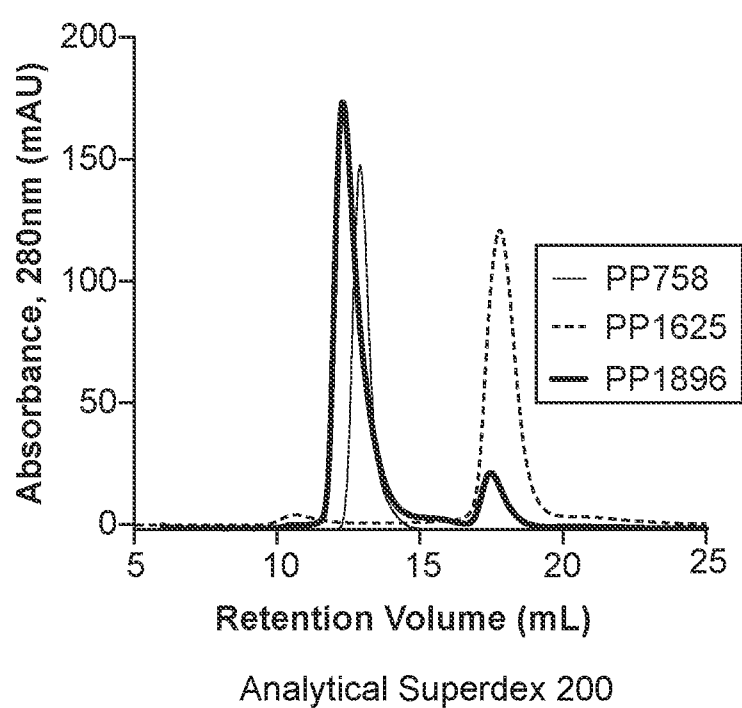


FIG. 12

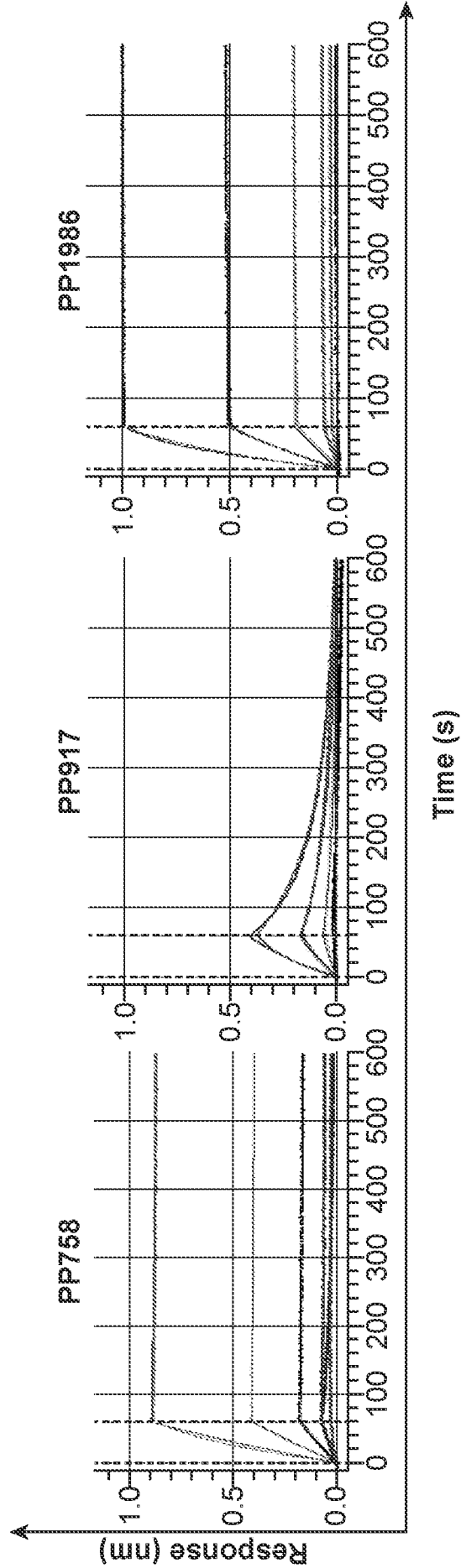


FIG. 13

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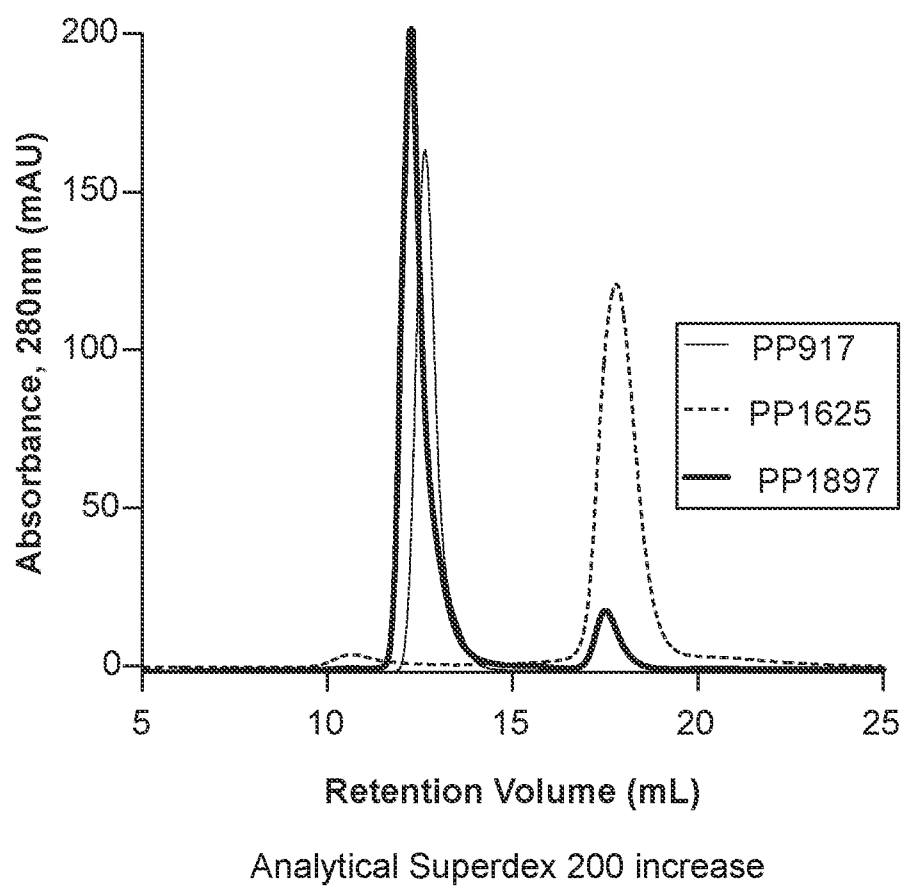


FIG. 14

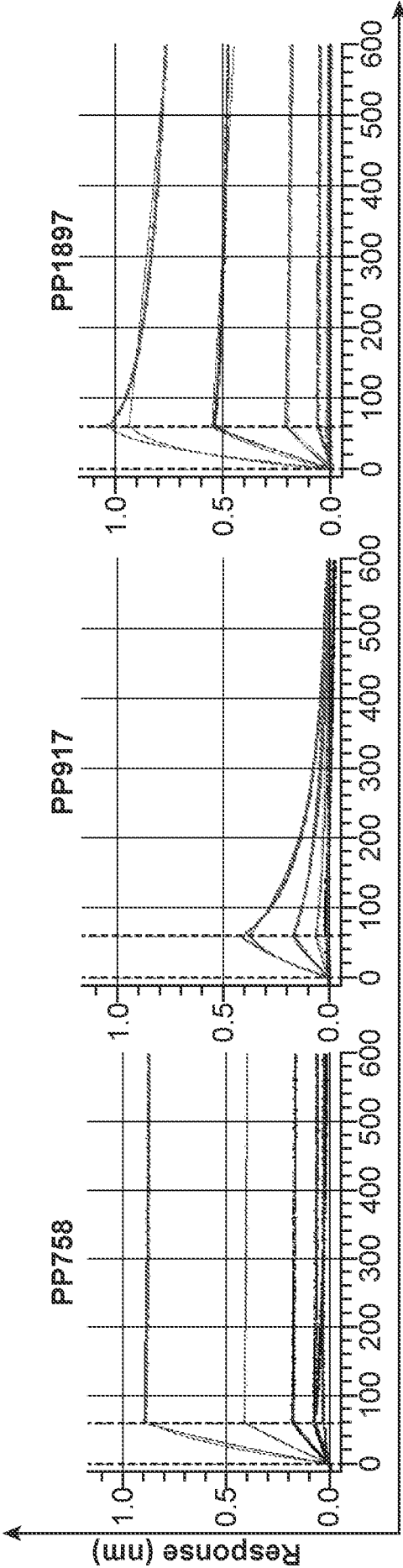


FIG. 15

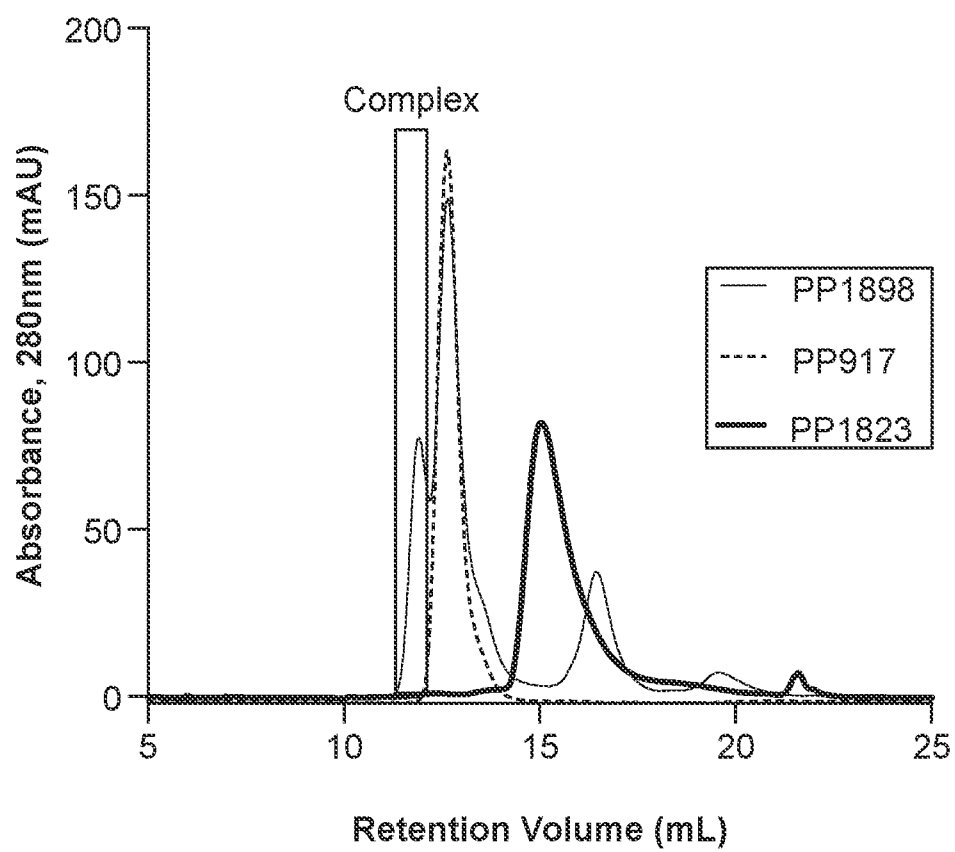


FIG. 16

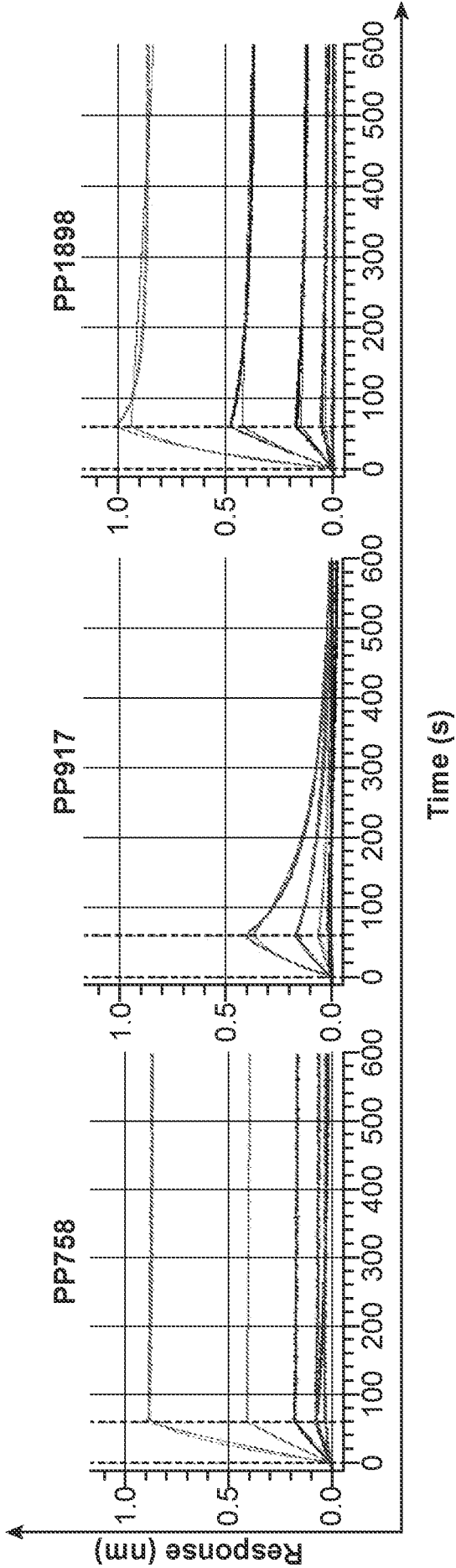


FIG. 17

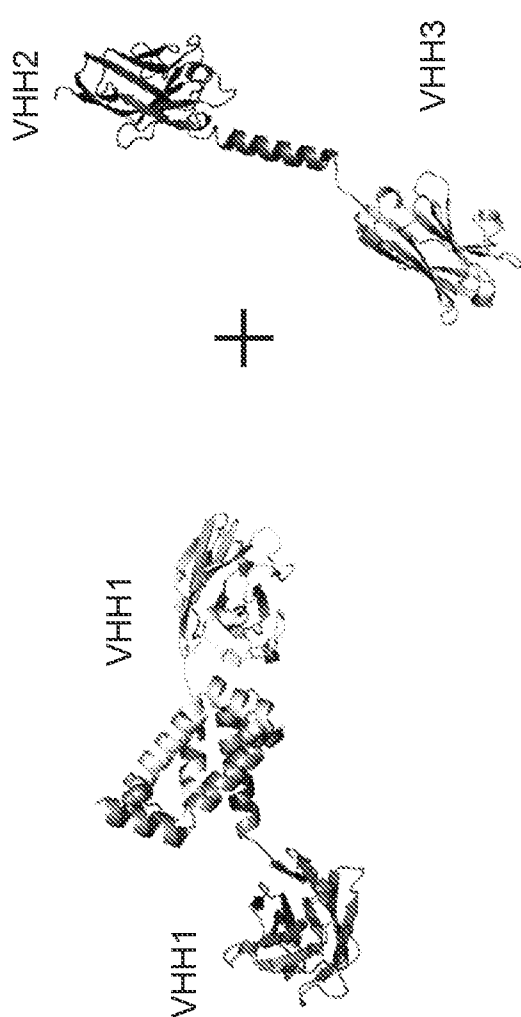
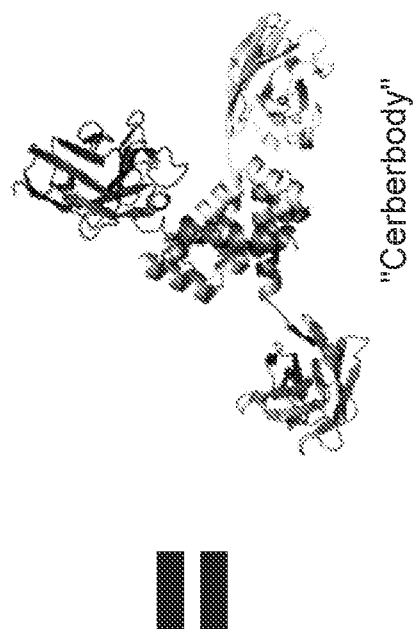
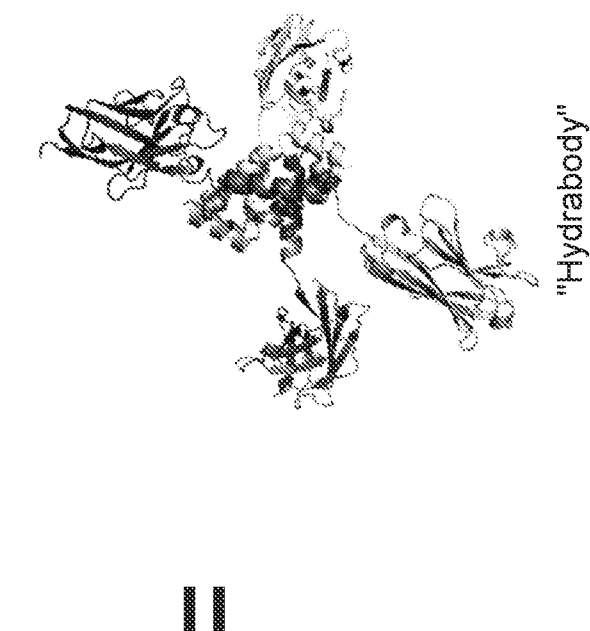


FIG. 18A

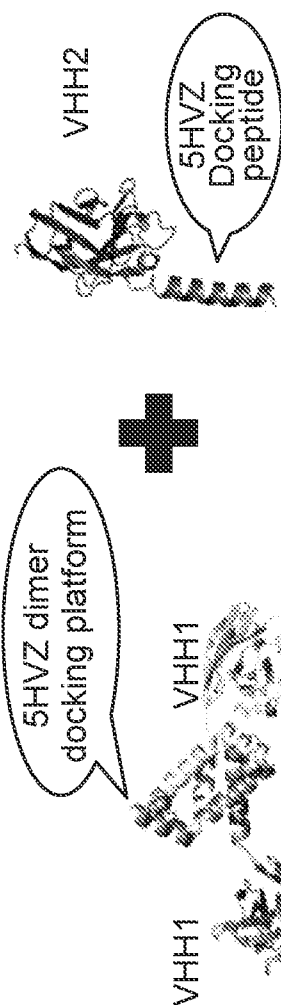


FIG. 18B

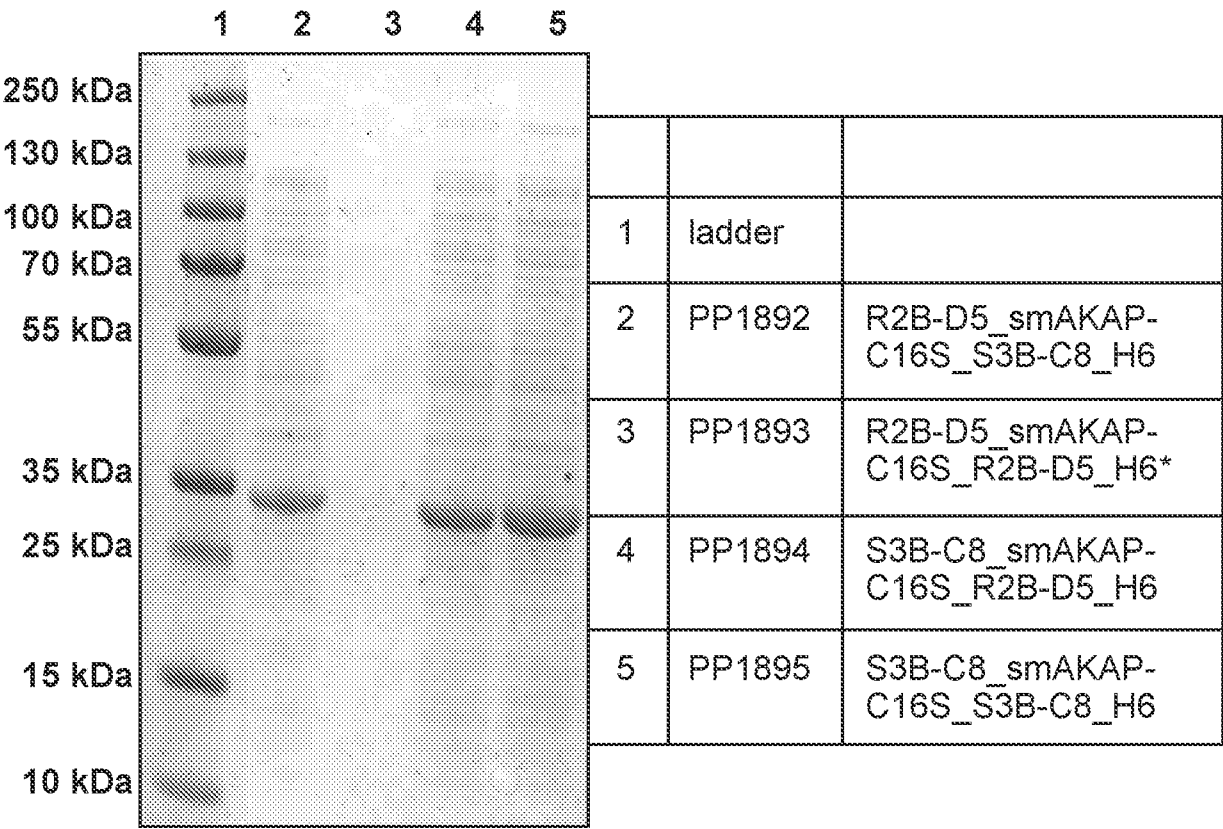


FIG. 19

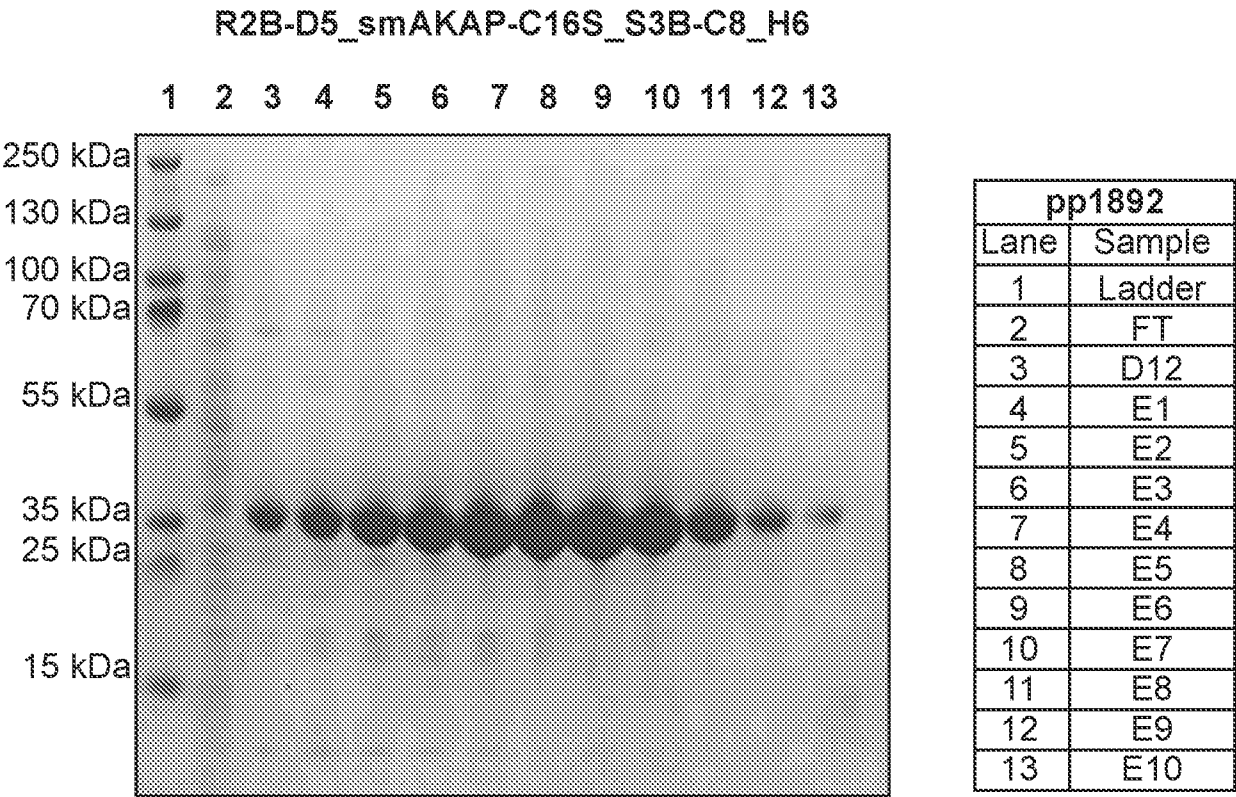
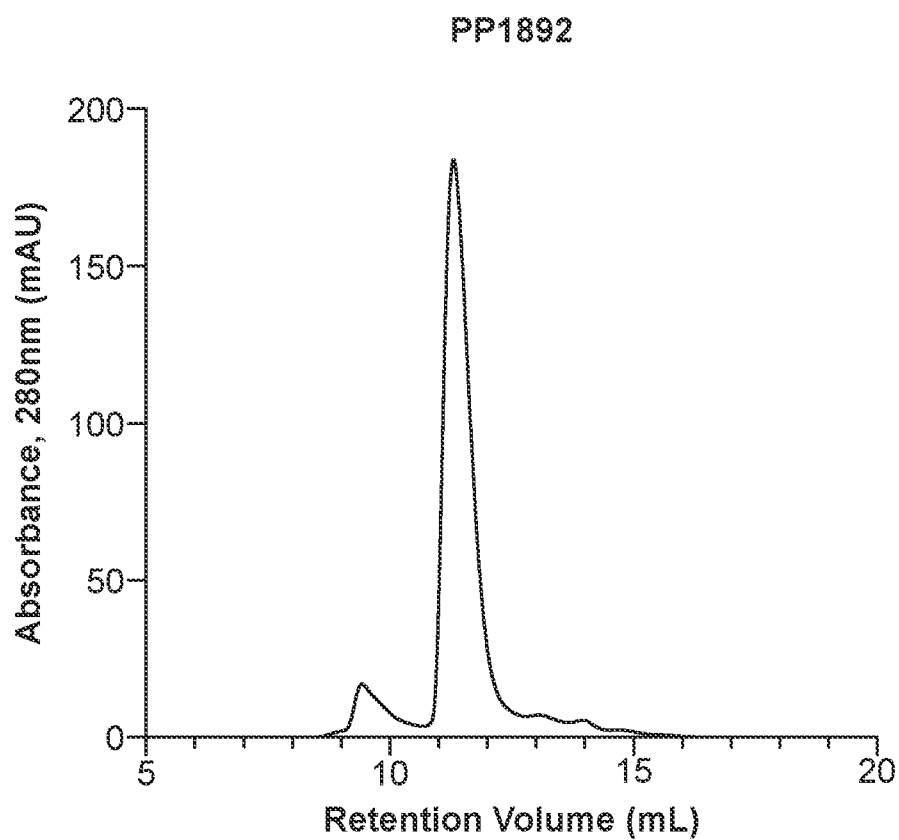


FIG. 20A

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Analytical Superdex 75 increase

FIG. 20B

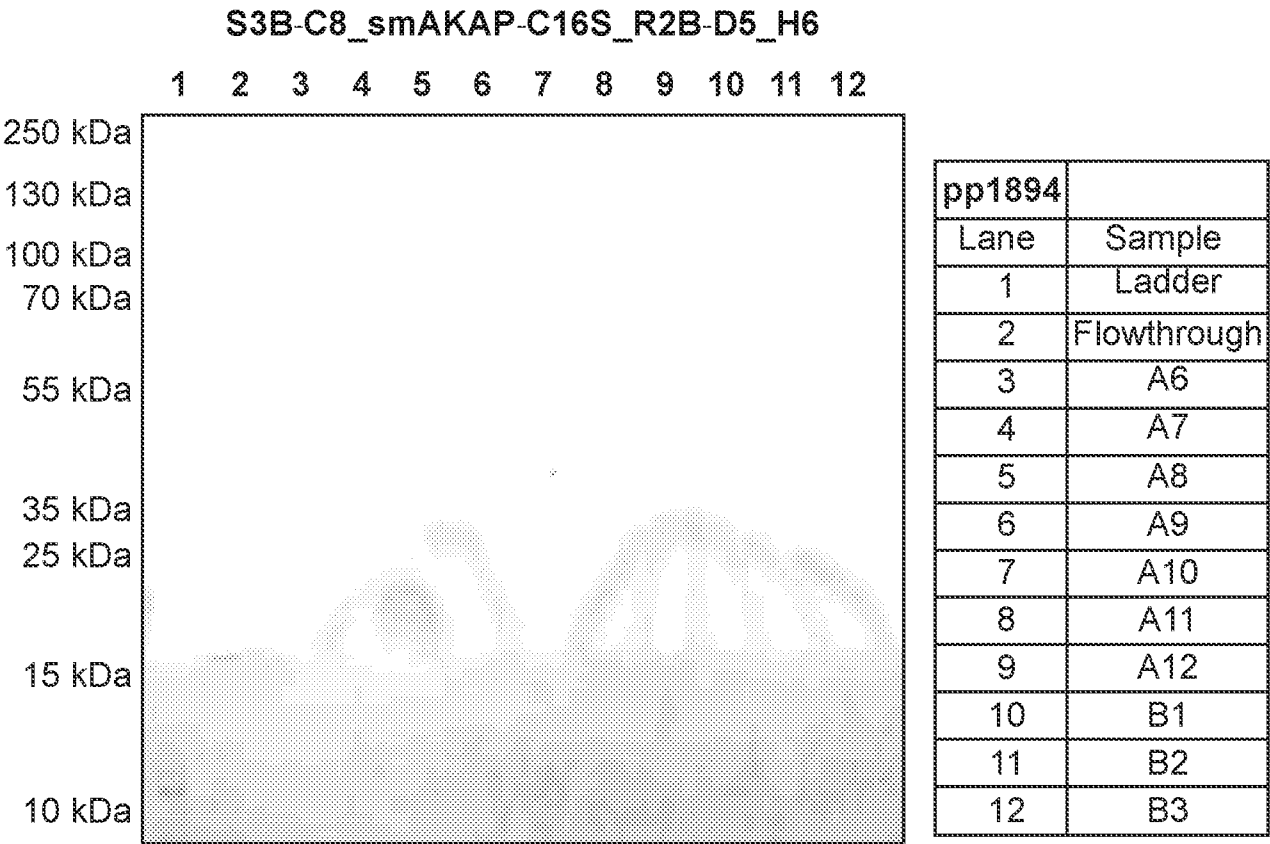


FIG. 21A

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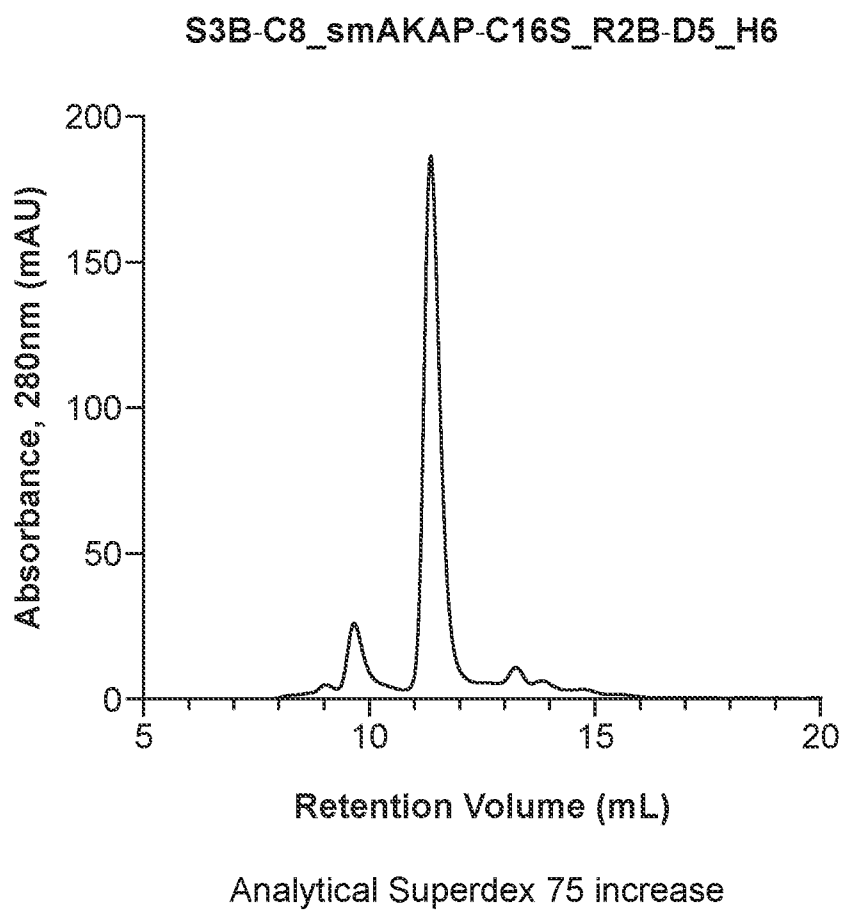
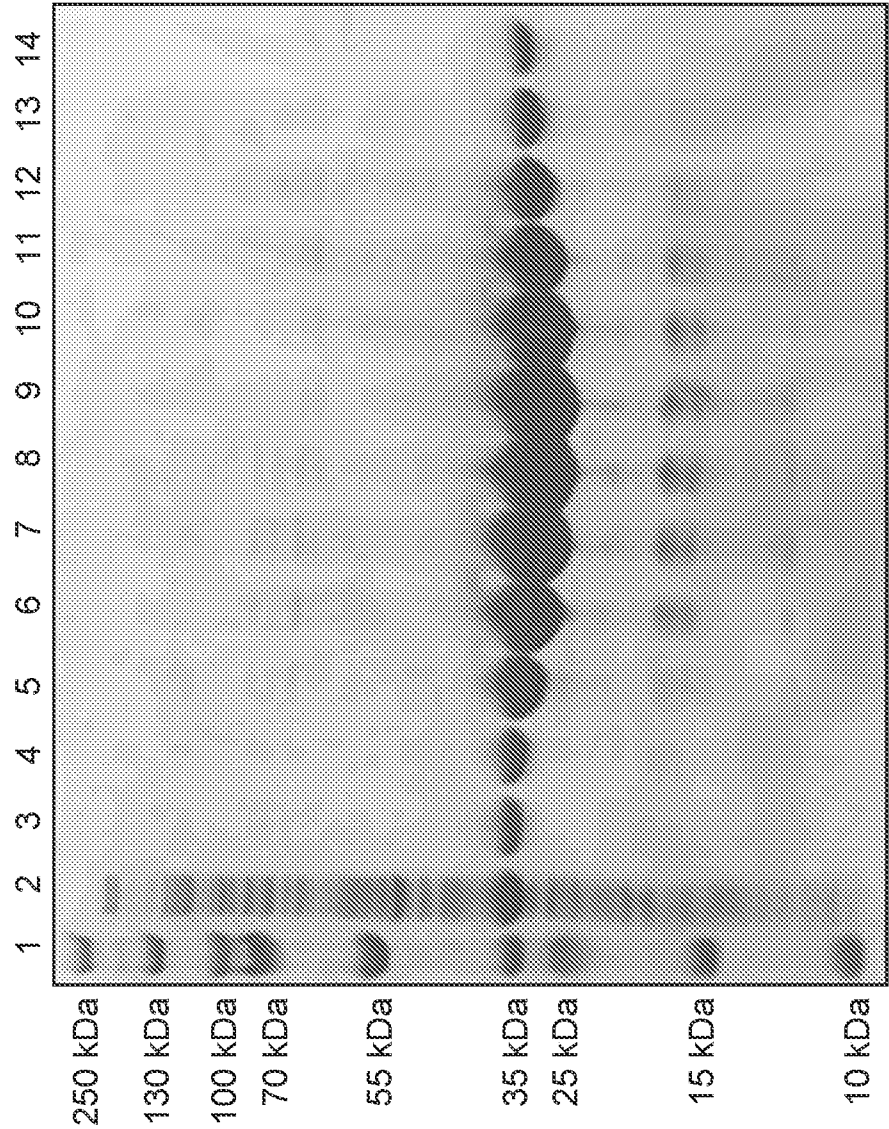


FIG. 21B

S3B-C8_smaKAP-C16S_S3B-C8_H6



pp1895	
Lane	Sample
1	Ladder
2	Flowthrough
3	B12
4	C1
5	C2
6	C3
7	C4
8	C5
9	C6
10	C7
11	C8
12	C9
13	C10
14	C11

FIG. 22A

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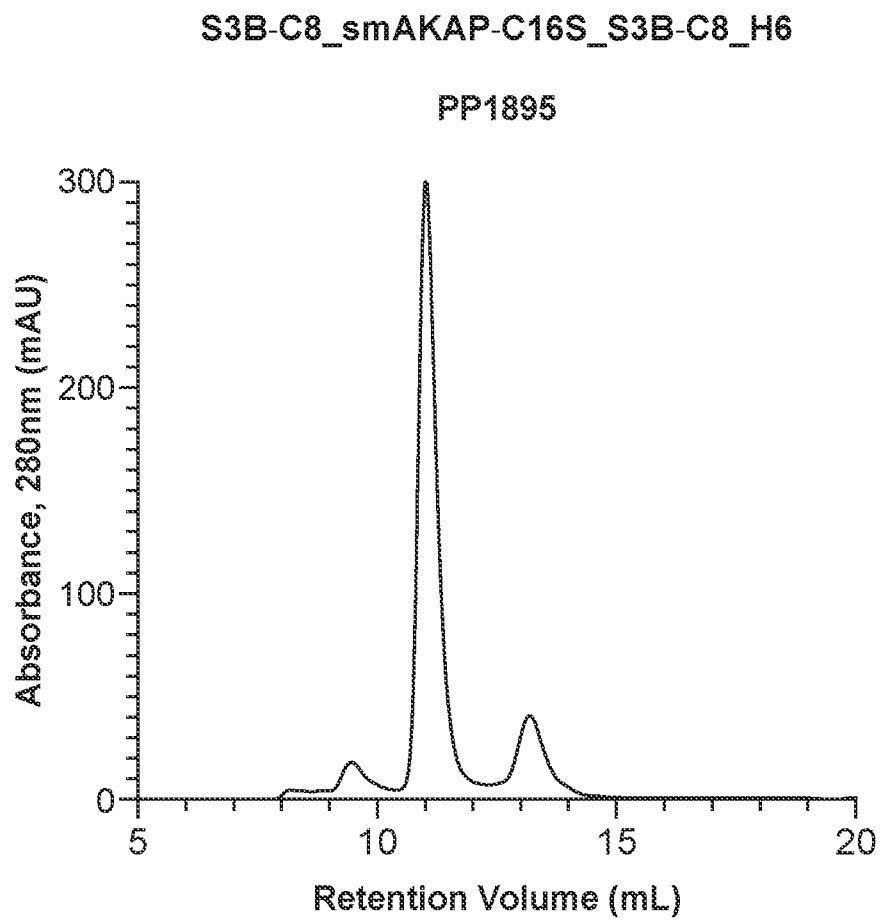


FIG. 22B

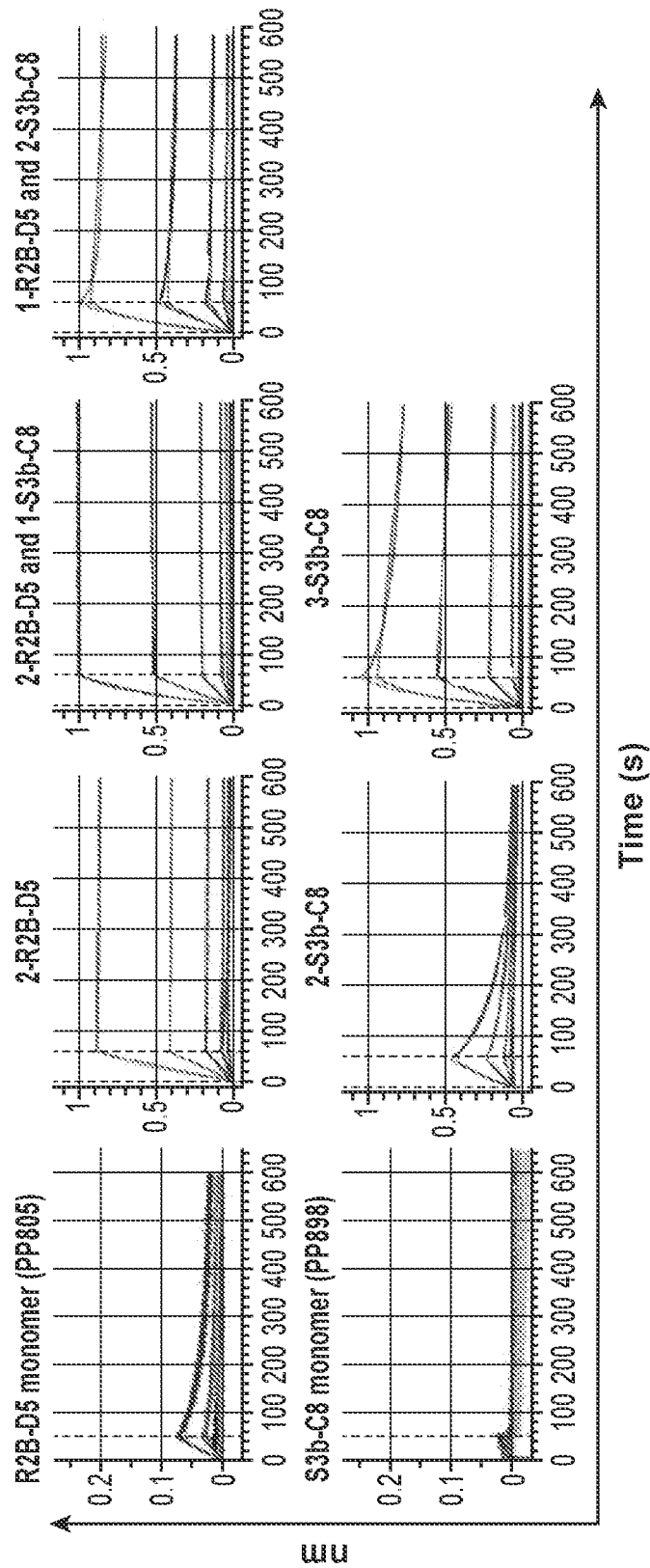


FIG. 23

Sample ID	Description	KD (nM)	ka (1/Ms)	kdis (1/s)
PP1896	2xR2B-D5 and 1xS3b-C8	<0.001	2.482E05	<1.0E-07
PP1897	3xS3b-C8	0.73	5.212E05	3.818E-04
PP1898	1xR2B-D5 and 2xS3b-C8	0.40	5.405E05	2.181E-04
PP917	2xS3b-C8	10.80	1.012E05	1.404E-03
PP758	2xR2B-D5	0.76	8.202E04	6.226E-05
PP898	1xS3b-C8			
PP805	1xR2B-D5	10.01	2.257E05	2.260E-03

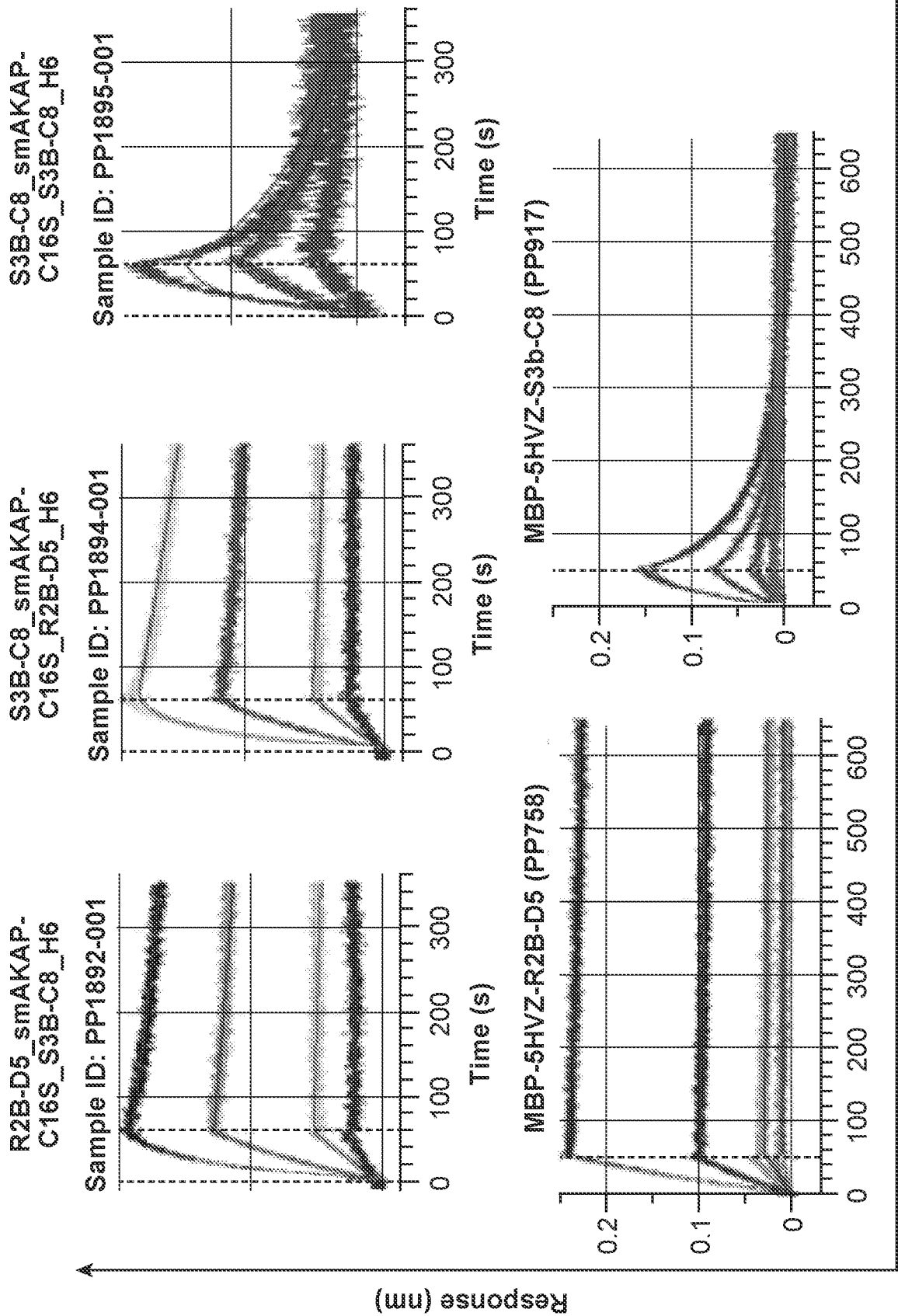


FIG. 24

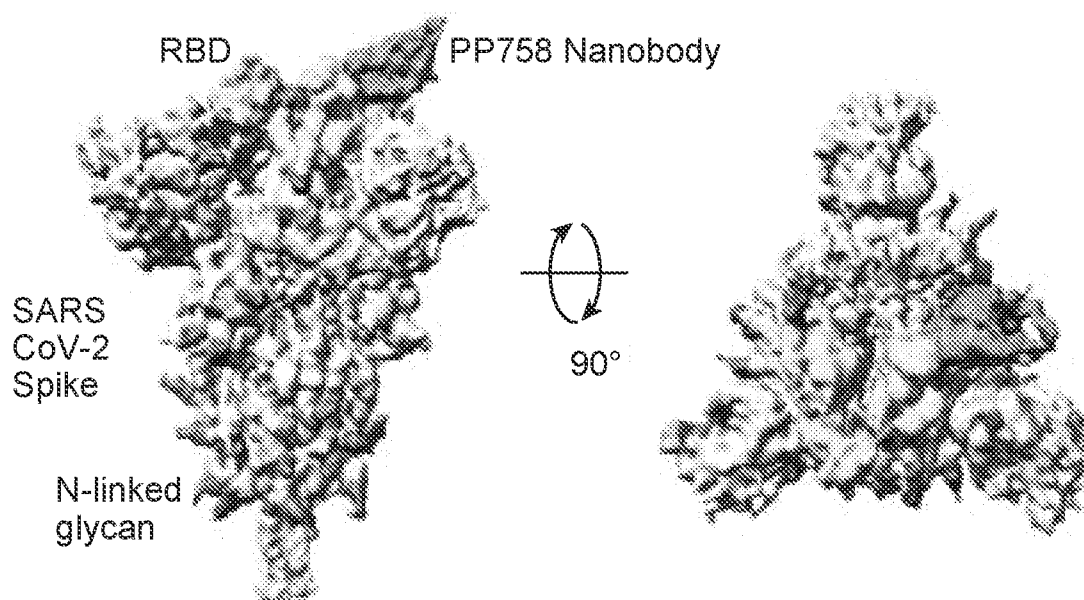


FIG. 25A

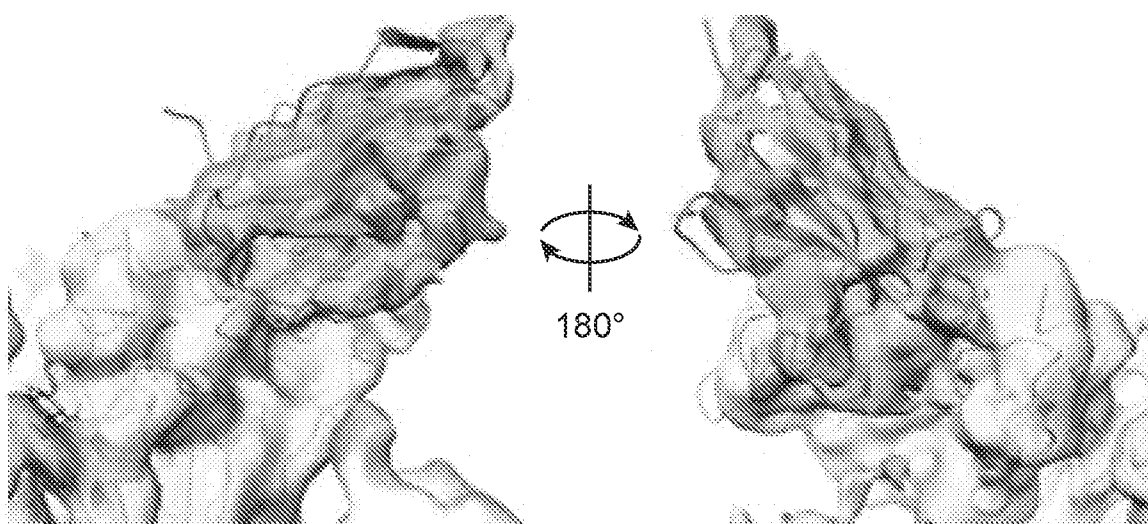


FIG. 25B

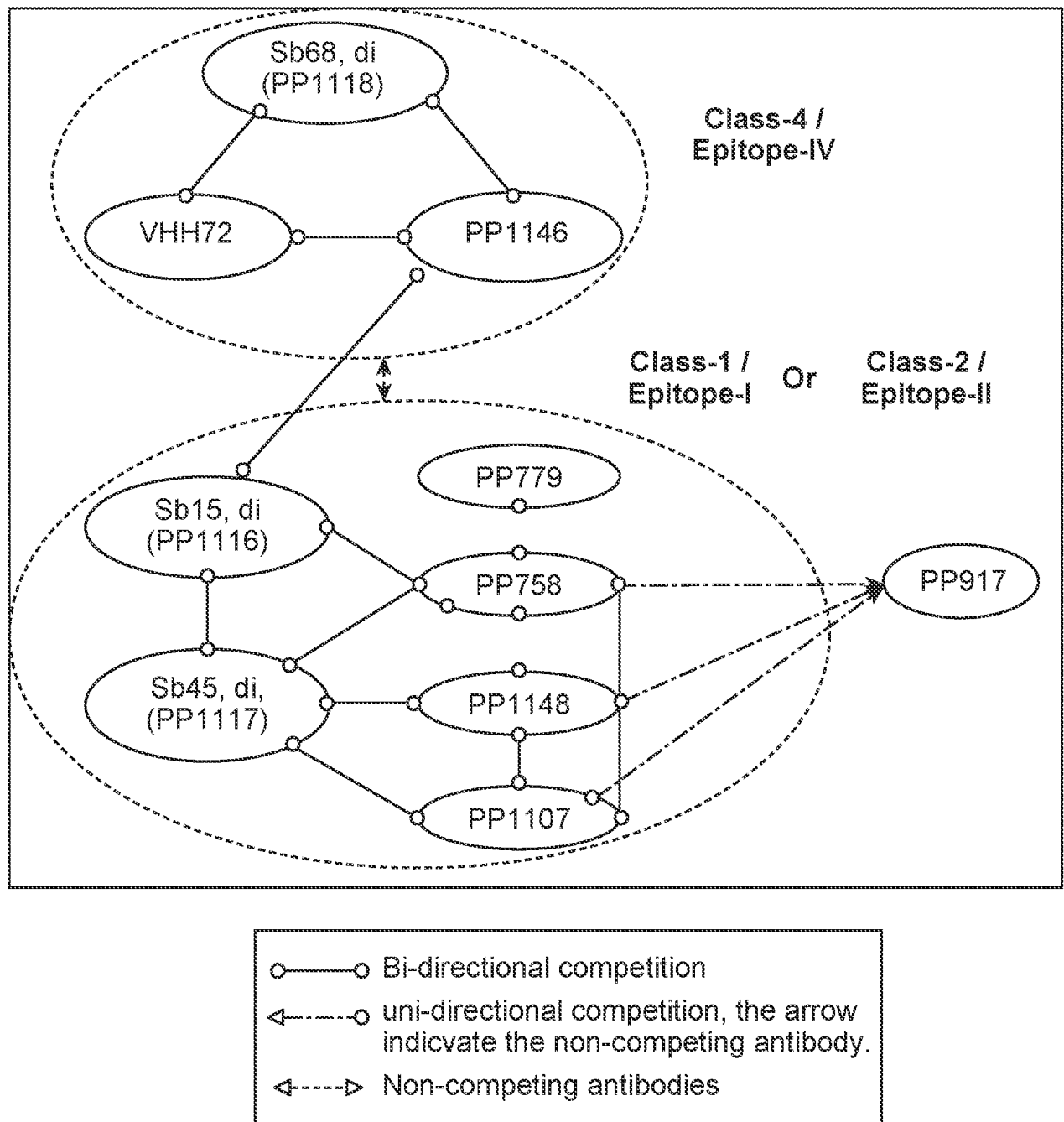


FIG. 26

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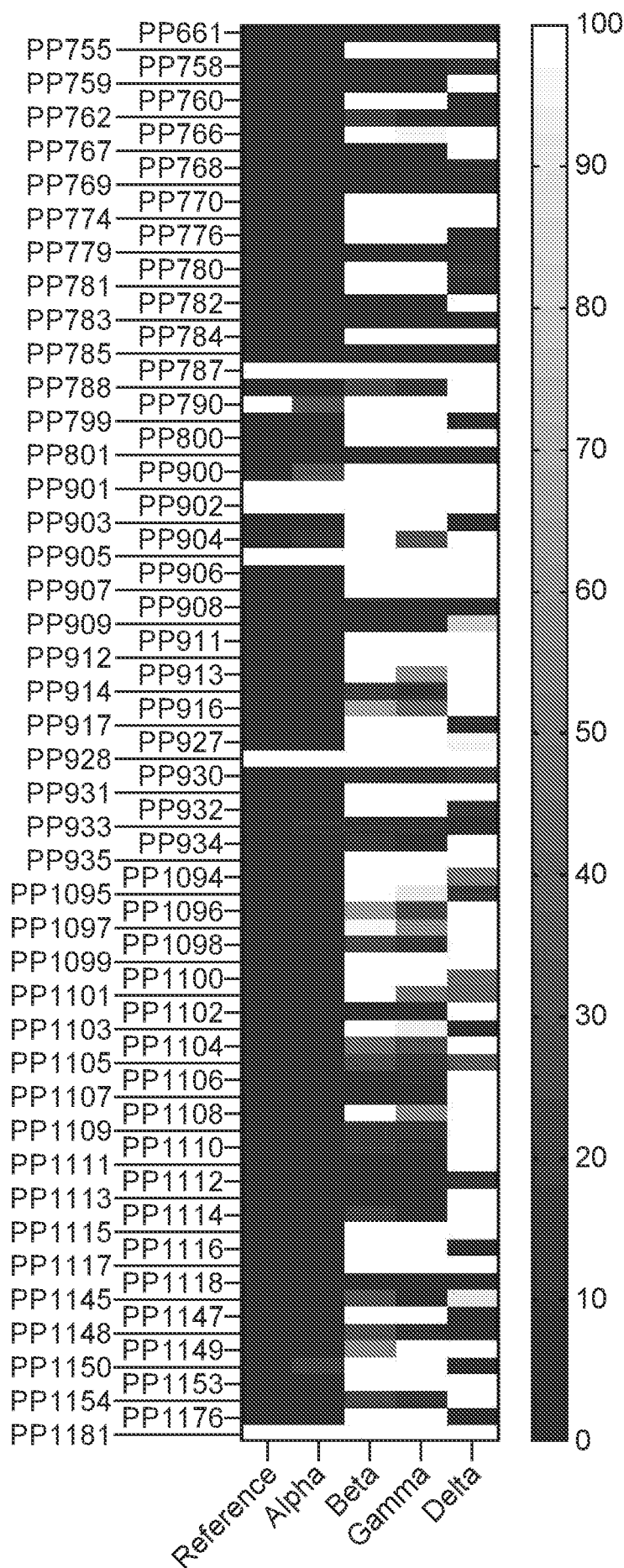


FIG. 27

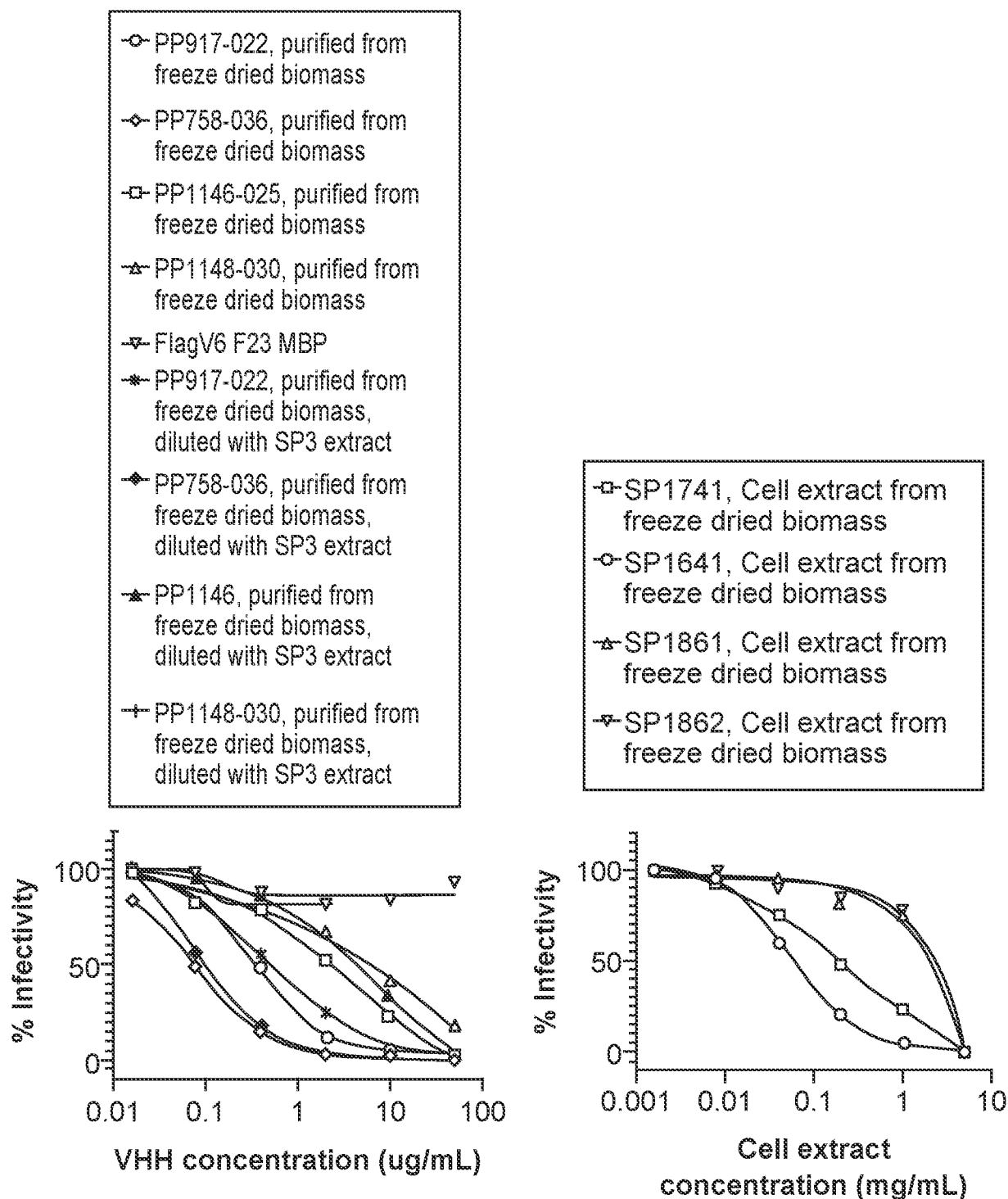


FIG. 28

% Reduction of infectivity, in-house pseudo-virus assay, initial test

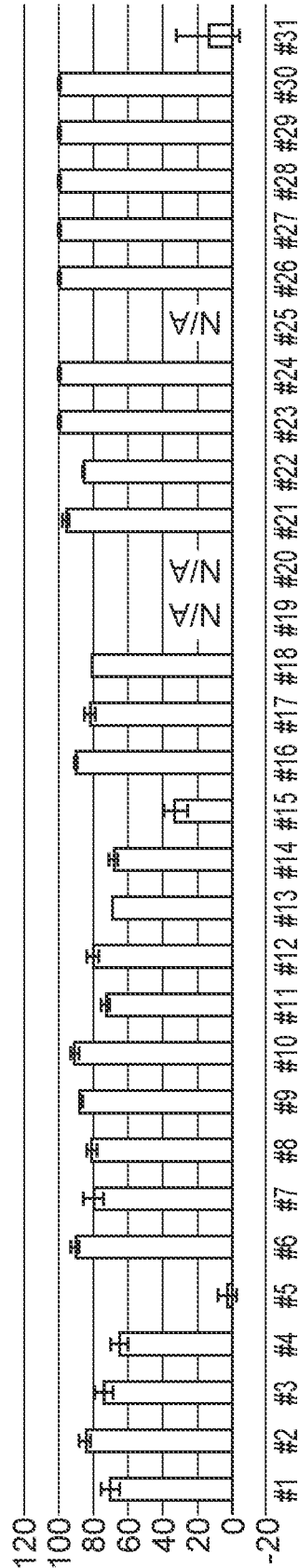


FIG. 29A

% Reduction of infectivity, in-house pseudo-virus assay, pre-live virus assay test

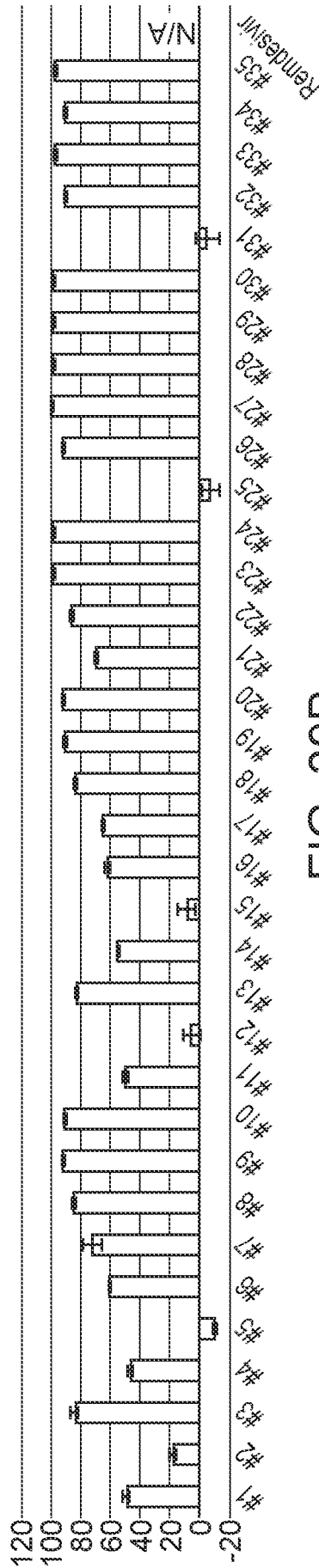


FIG. 29B

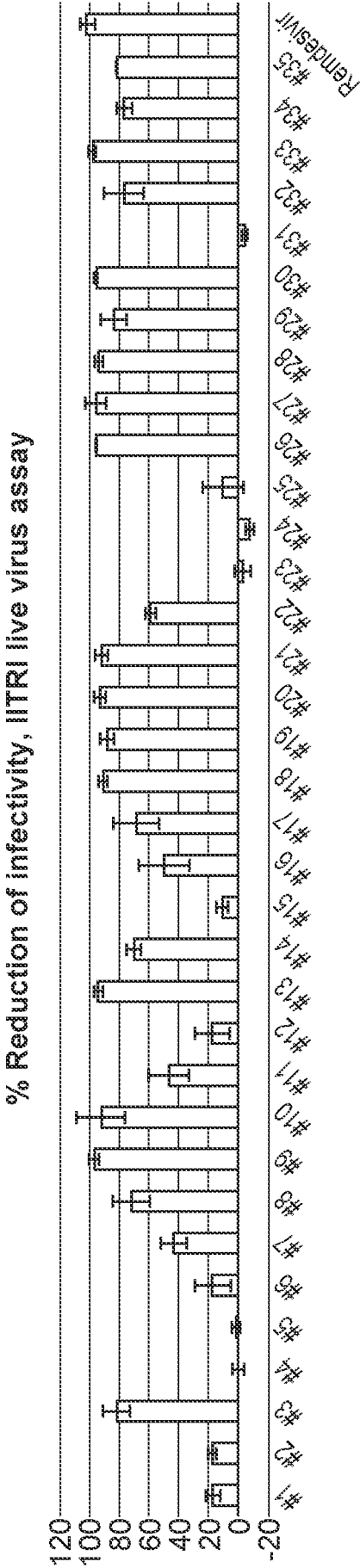


FIG. 29C

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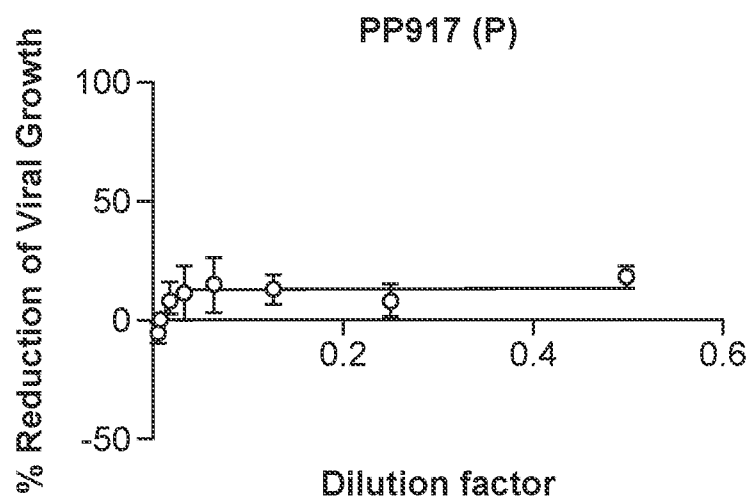


FIG. 30A

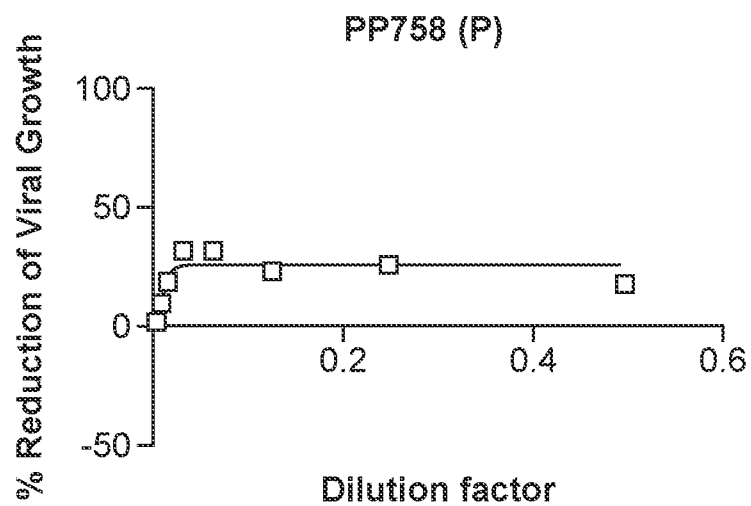


FIG. 30B

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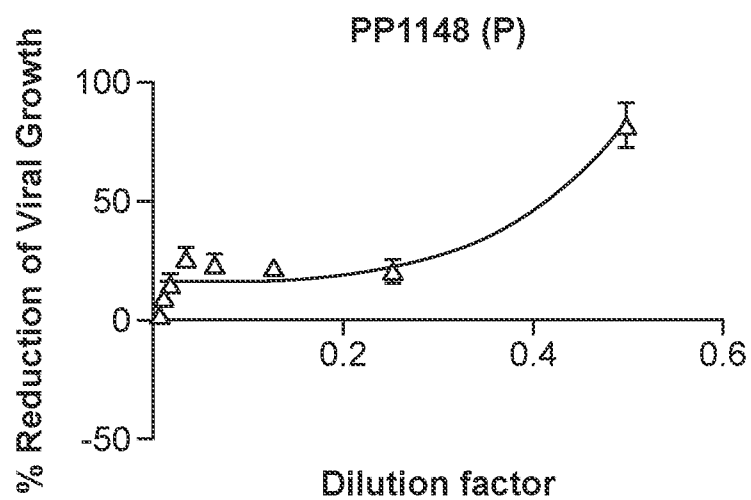


FIG. 30C

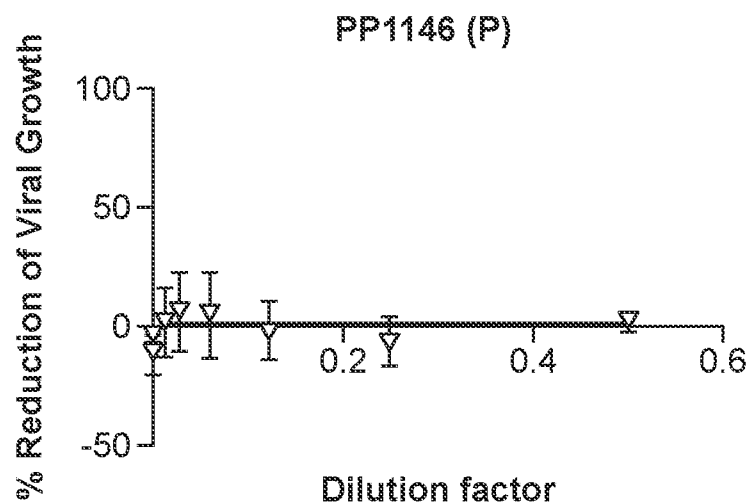


FIG. 30D

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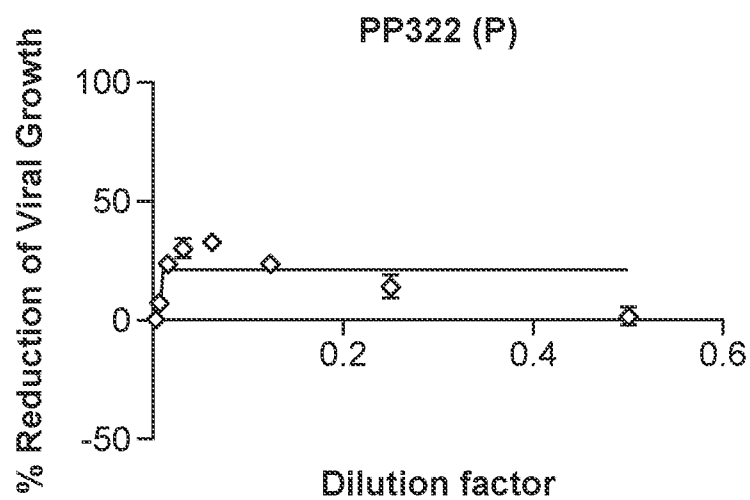


FIG. 30E

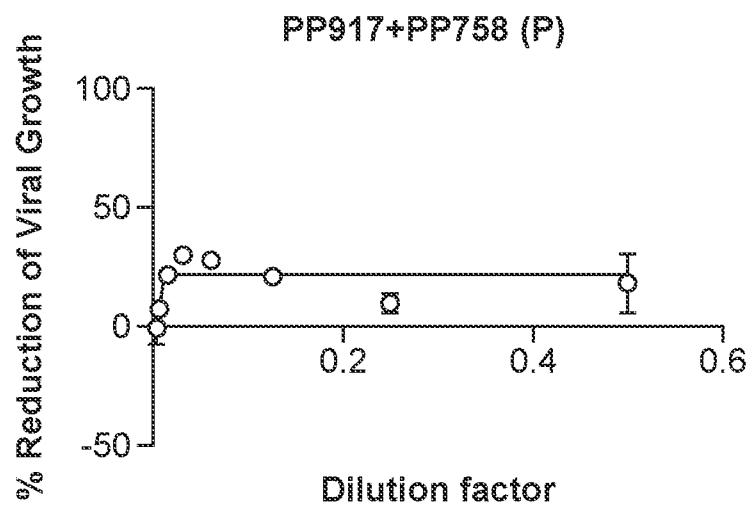


FIG. 30F

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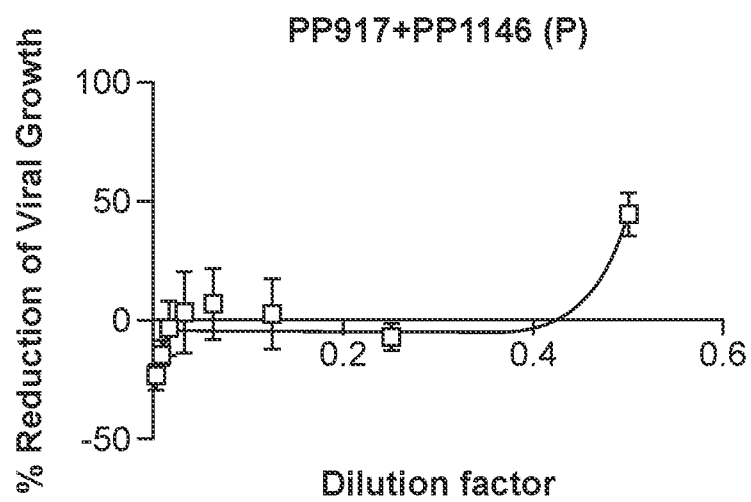


FIG. 30G

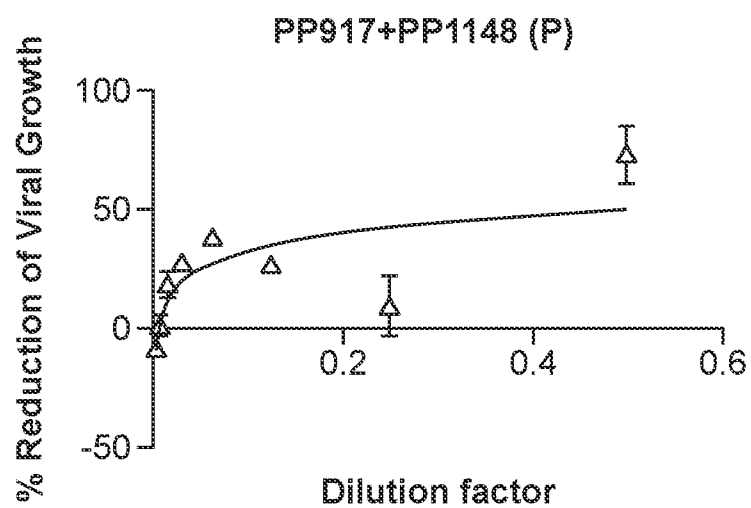


FIG. 30H

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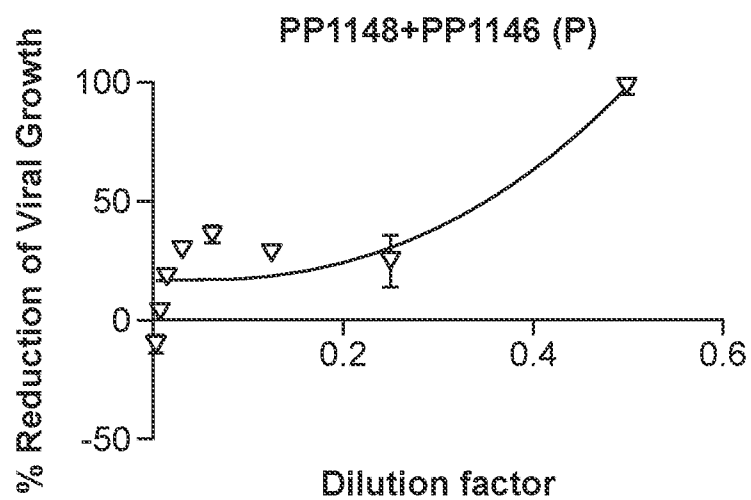


FIG. 30I

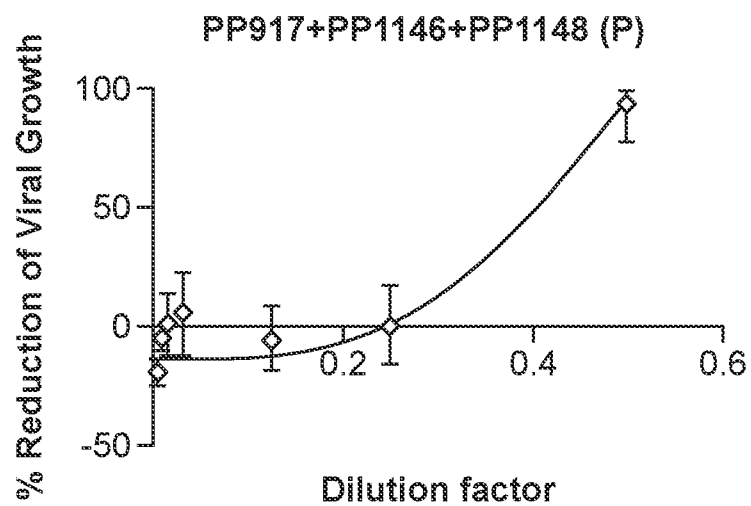


FIG. 30J

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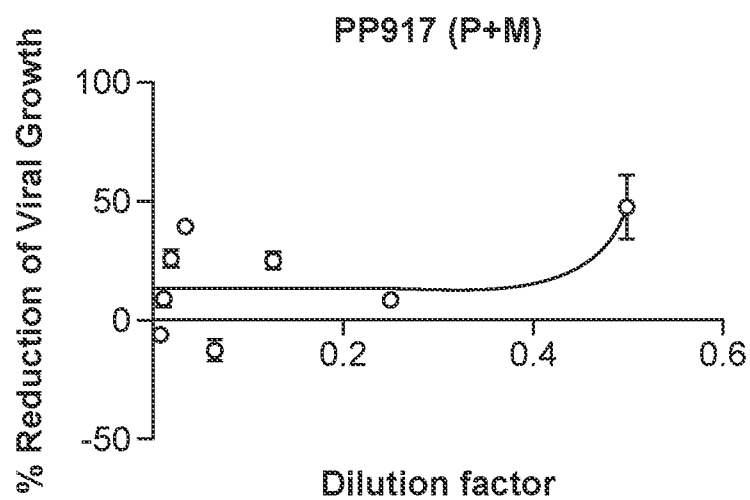


FIG. 30K

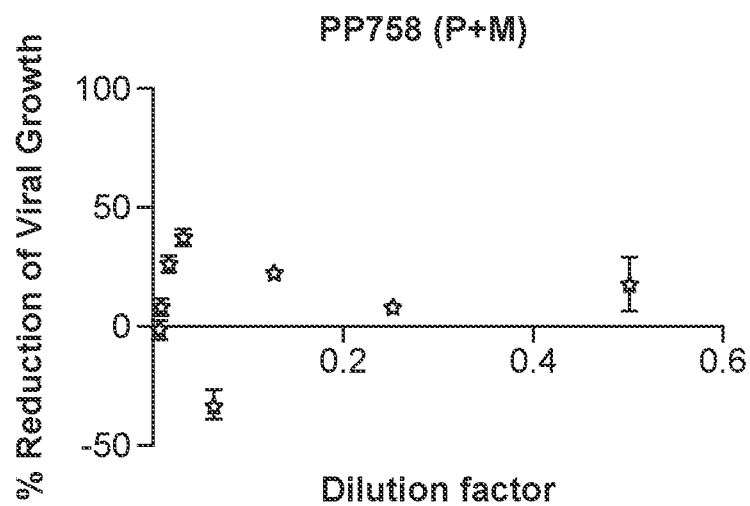


FIG. 30L

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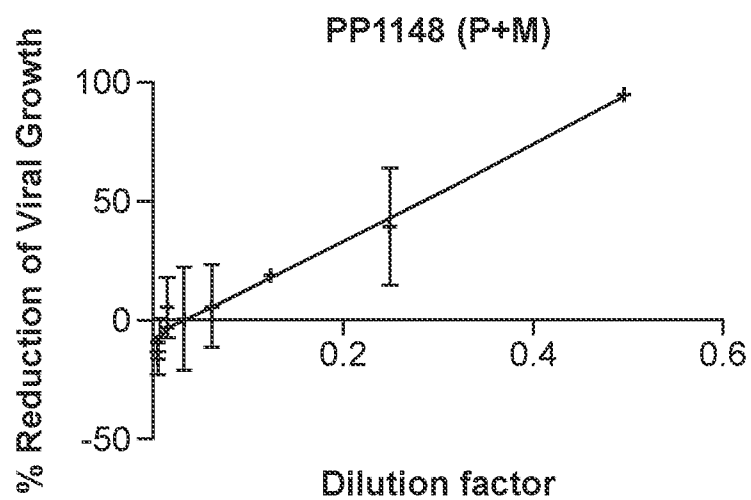


FIG. 30M

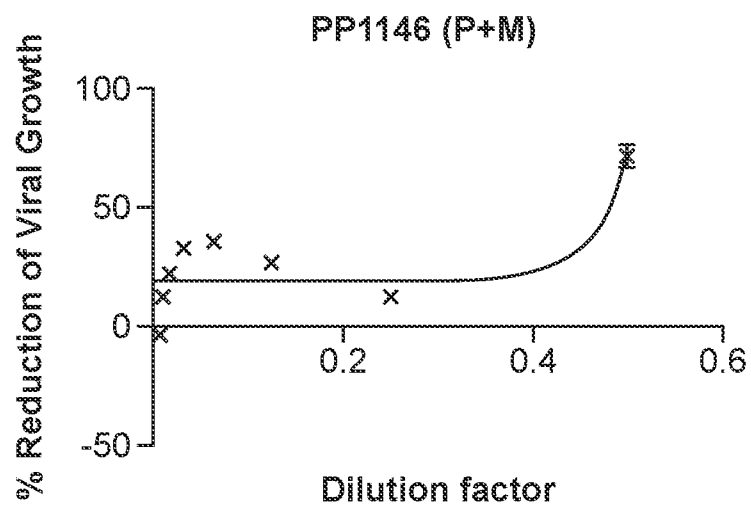


FIG. 30N

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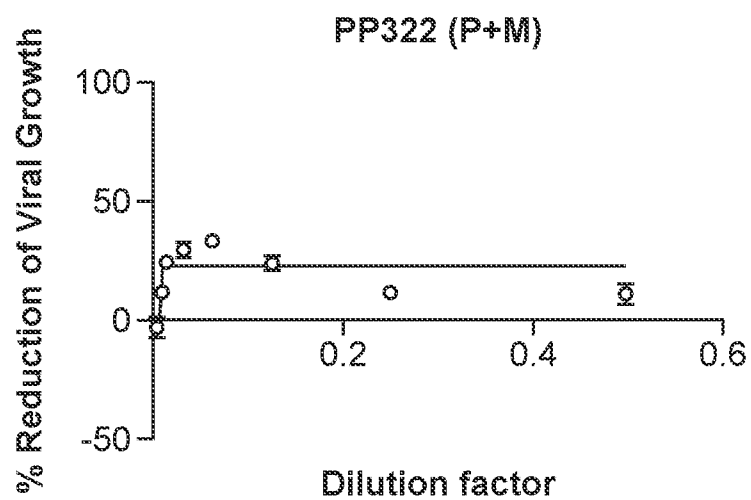


FIG. 30O

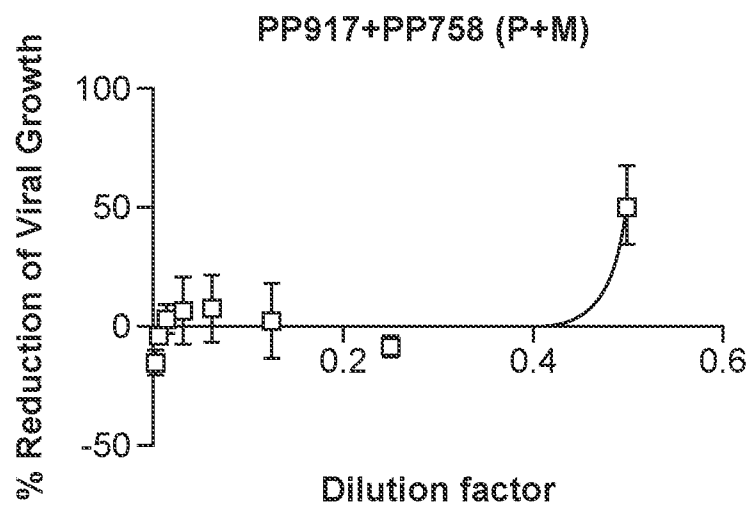


FIG. 30P

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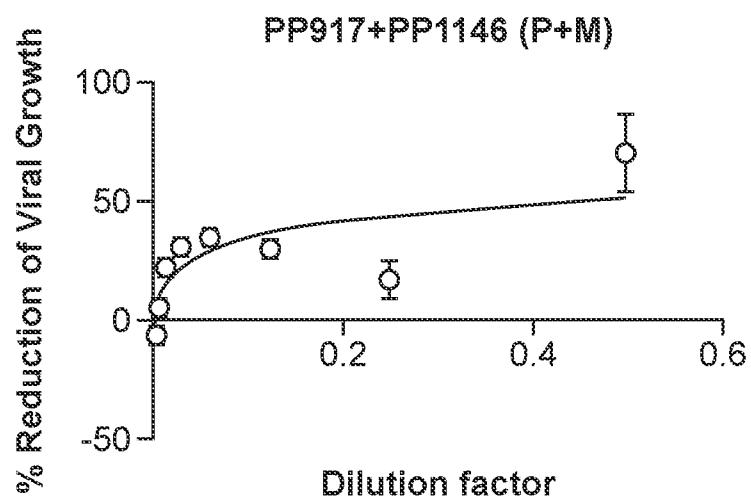


FIG. 30Q

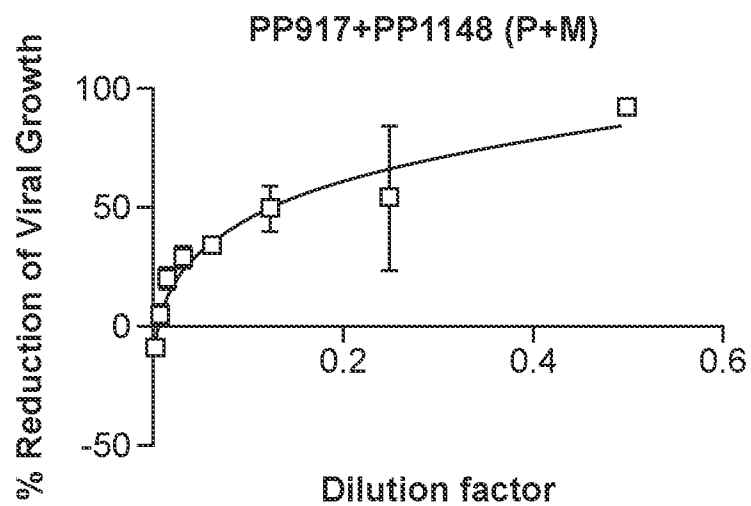


FIG. 30R

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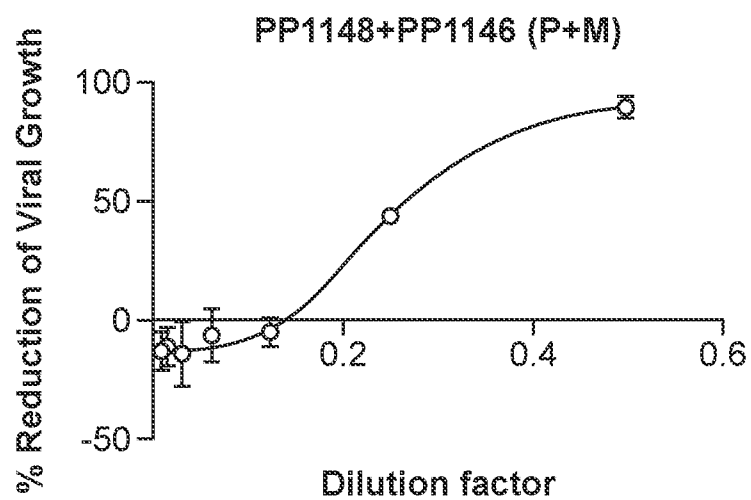


FIG. 30S

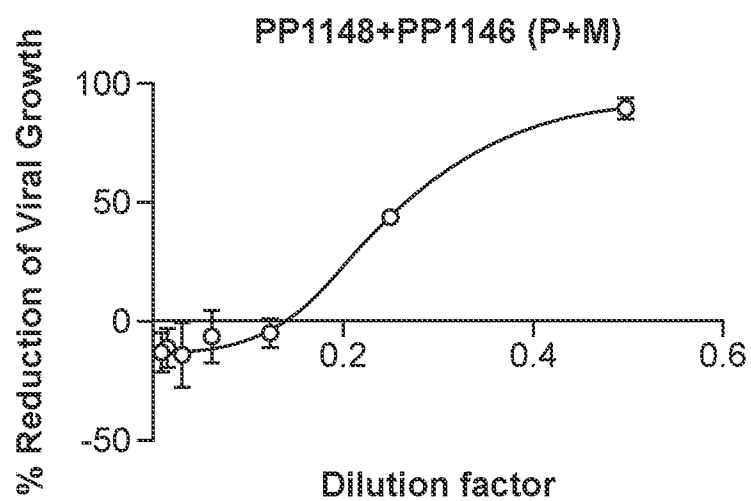


FIG. 30T

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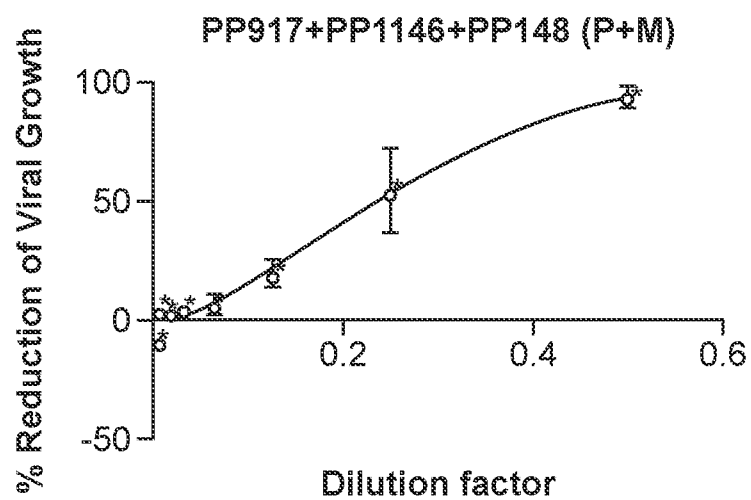


FIG. 30U

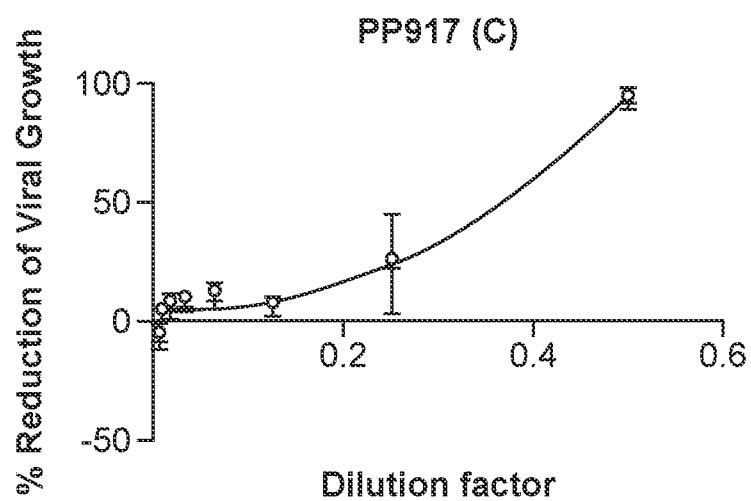


FIG. 30V

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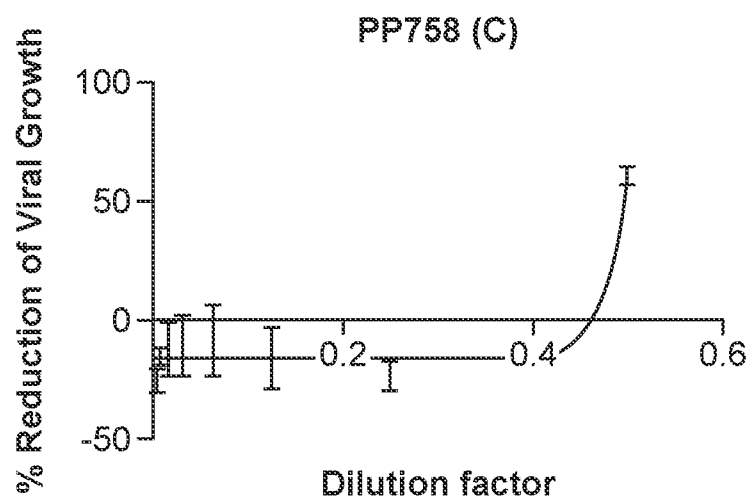


FIG. 30W

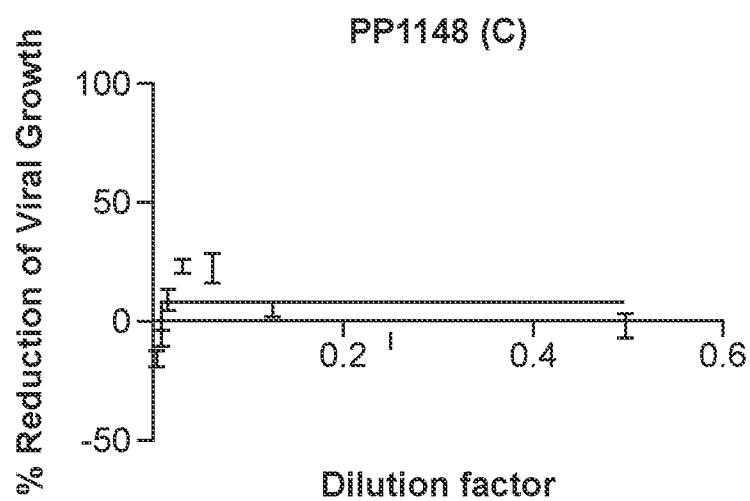


FIG. 30X

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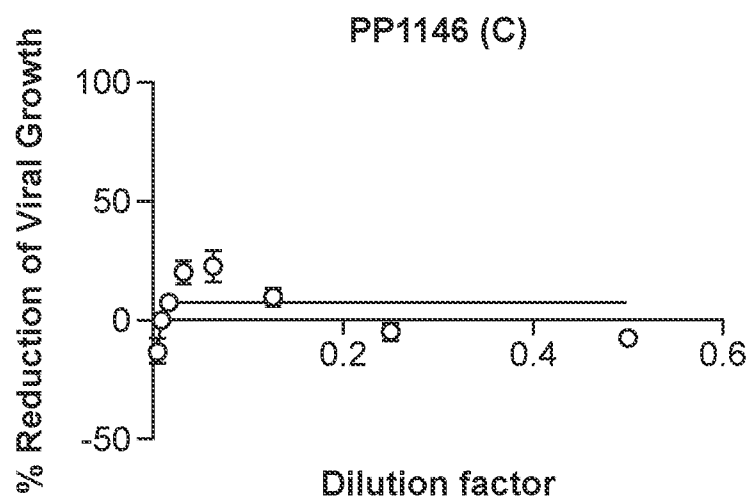


FIG. 30Y

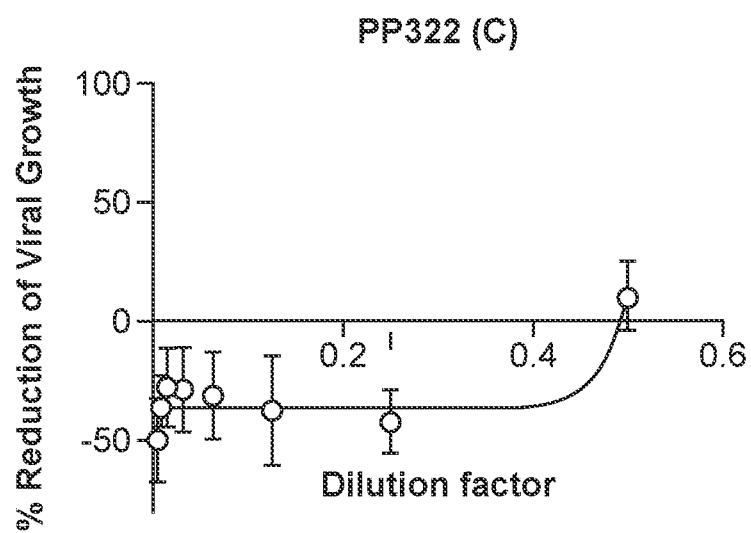


FIG. 30Z

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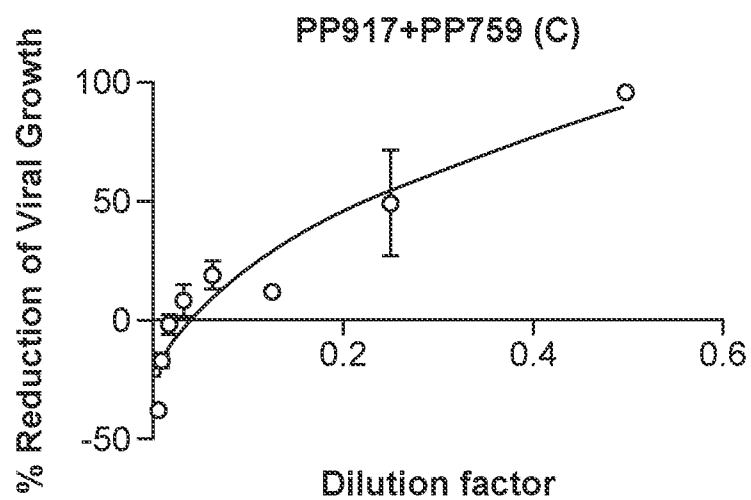


FIG. 30AA

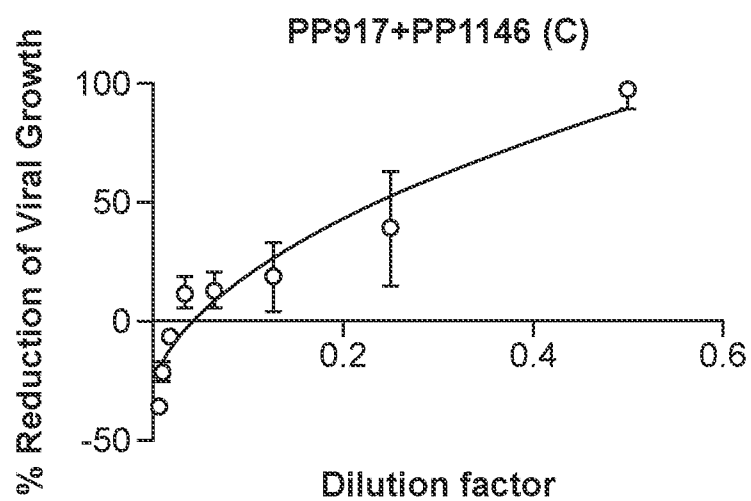


FIG. 30AB

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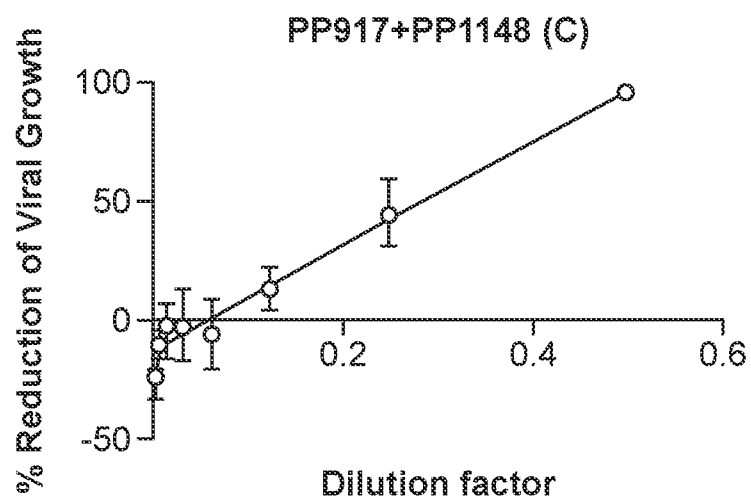


FIG. 30AC

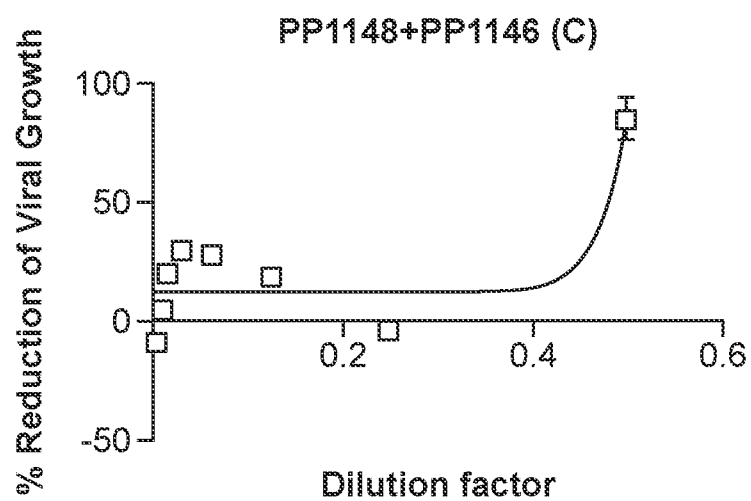


FIG. 30AD

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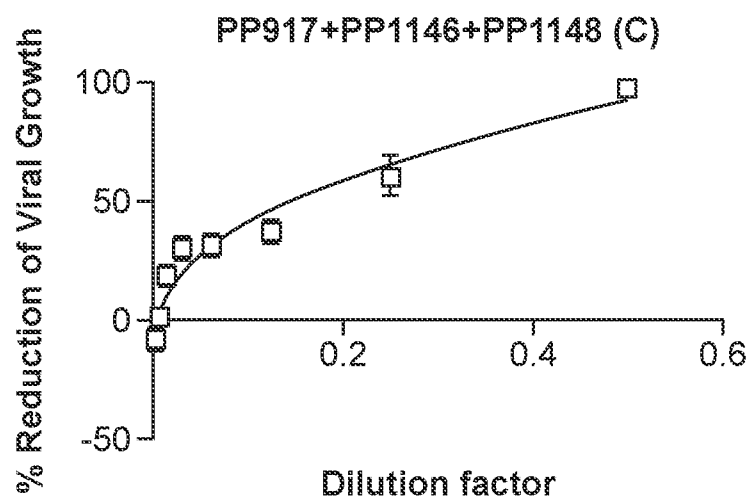


FIG. 30AE

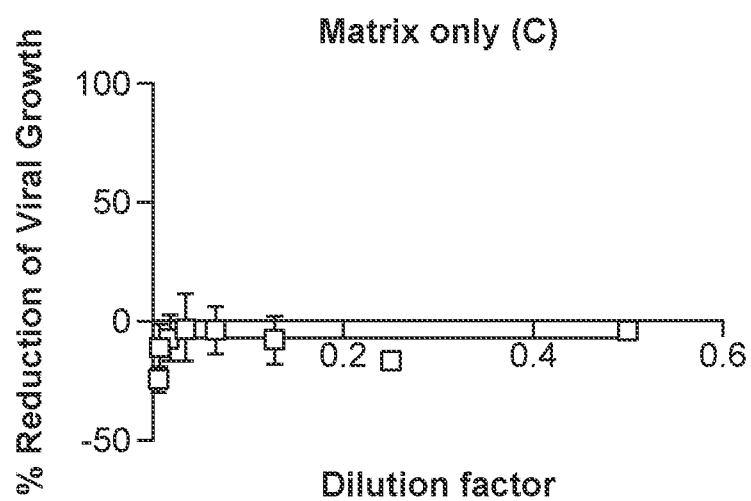


FIG. 30AF

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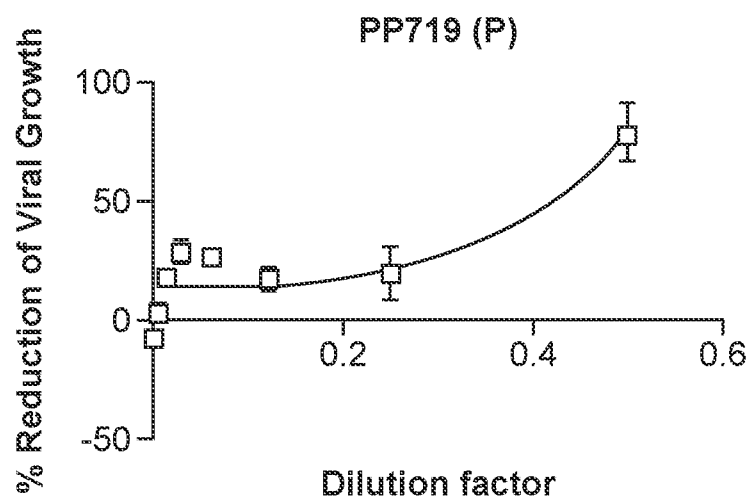


FIG. 30AG

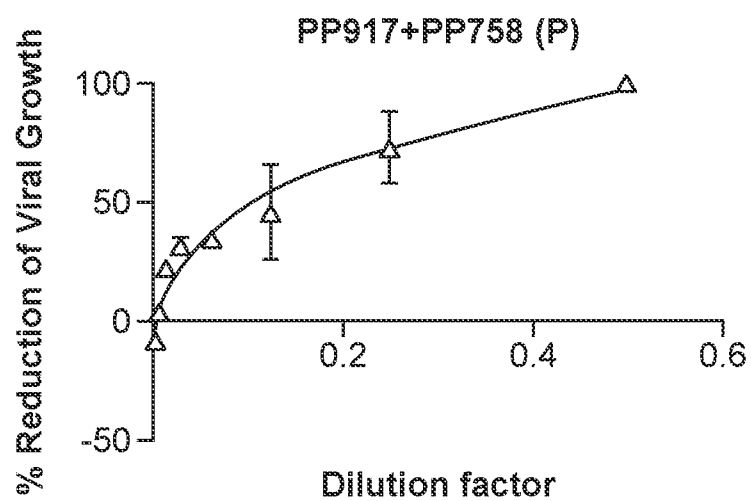


FIG. 30AH

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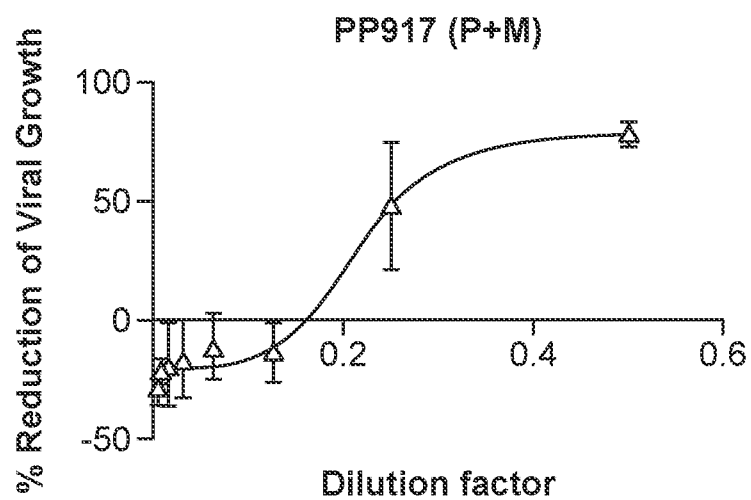


FIG. 30AI

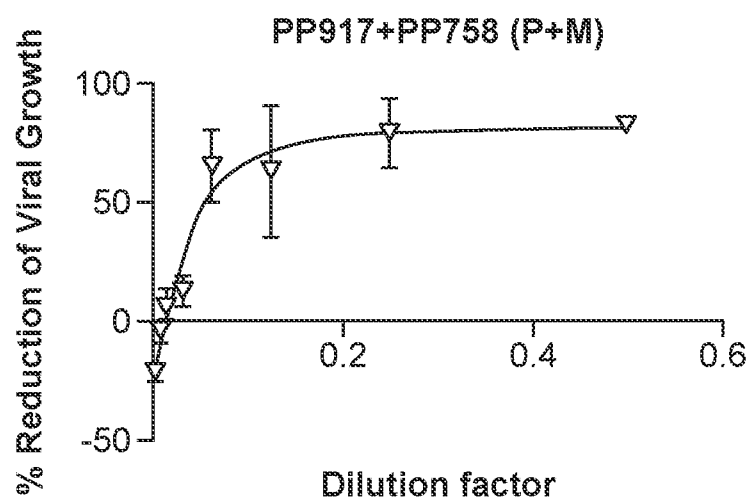


FIG. 30AJ

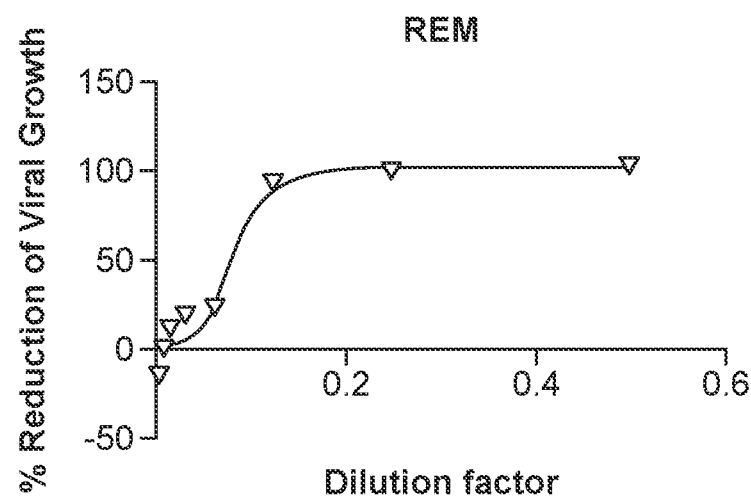
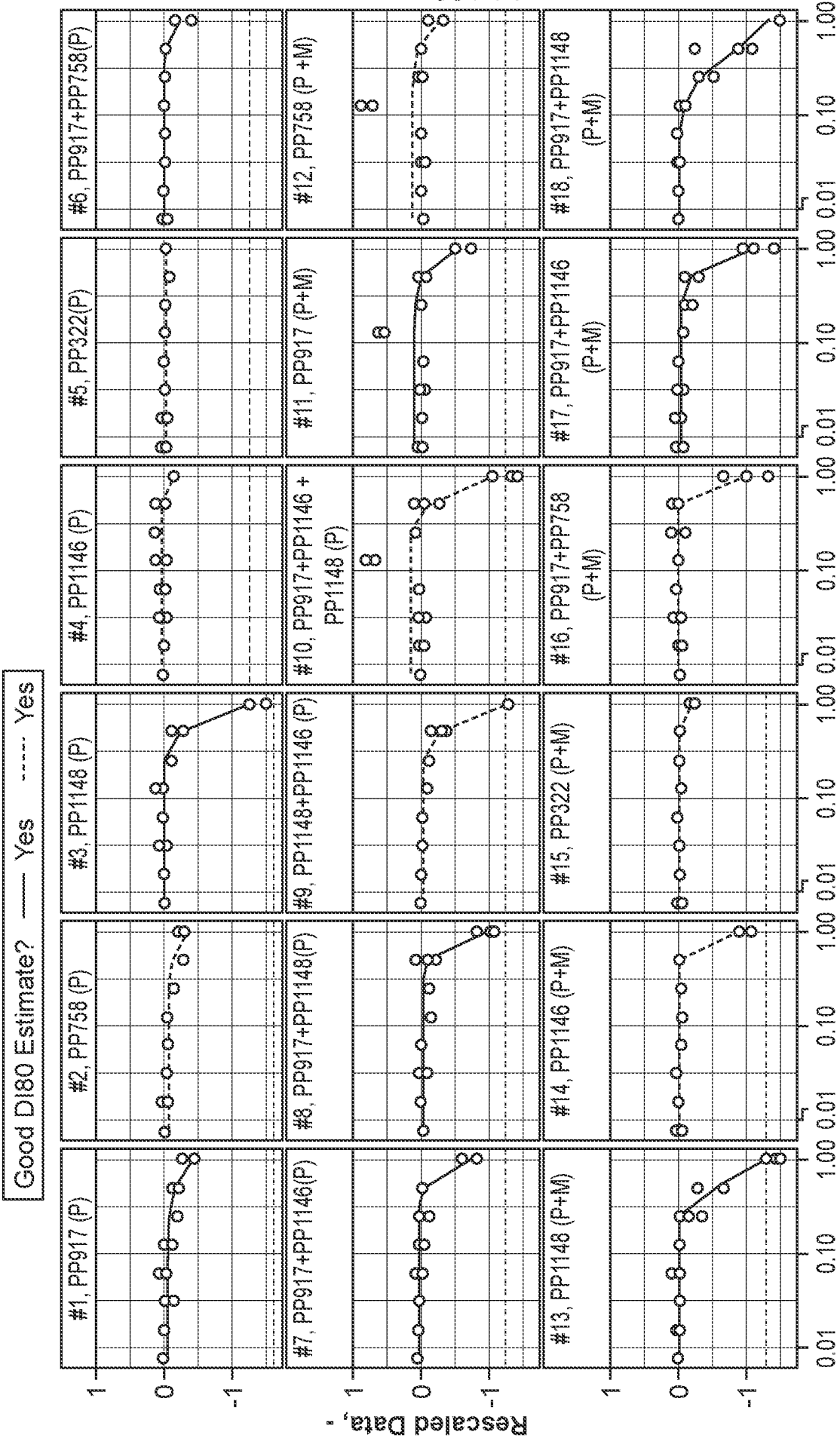
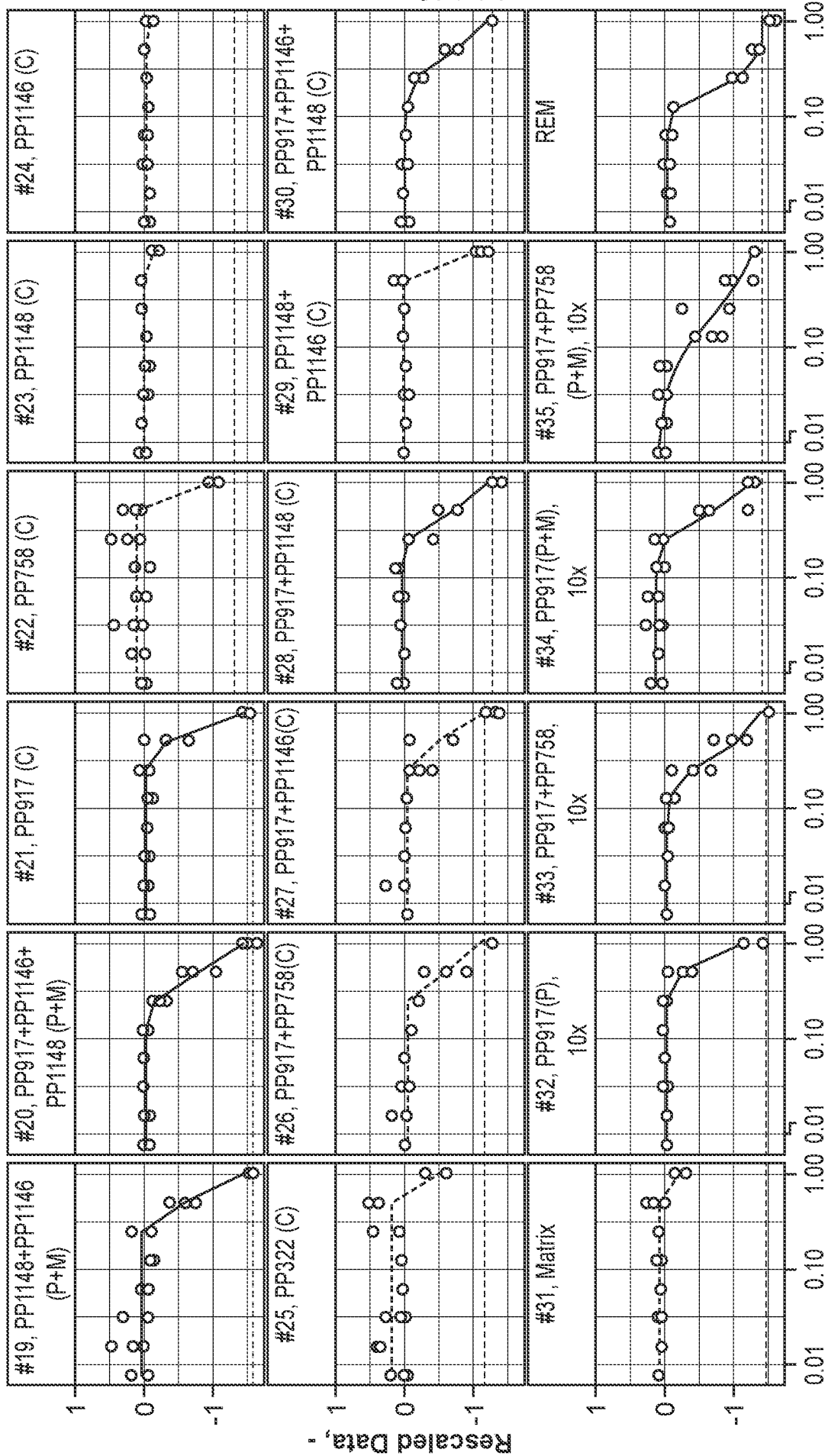


FIG. 30AK



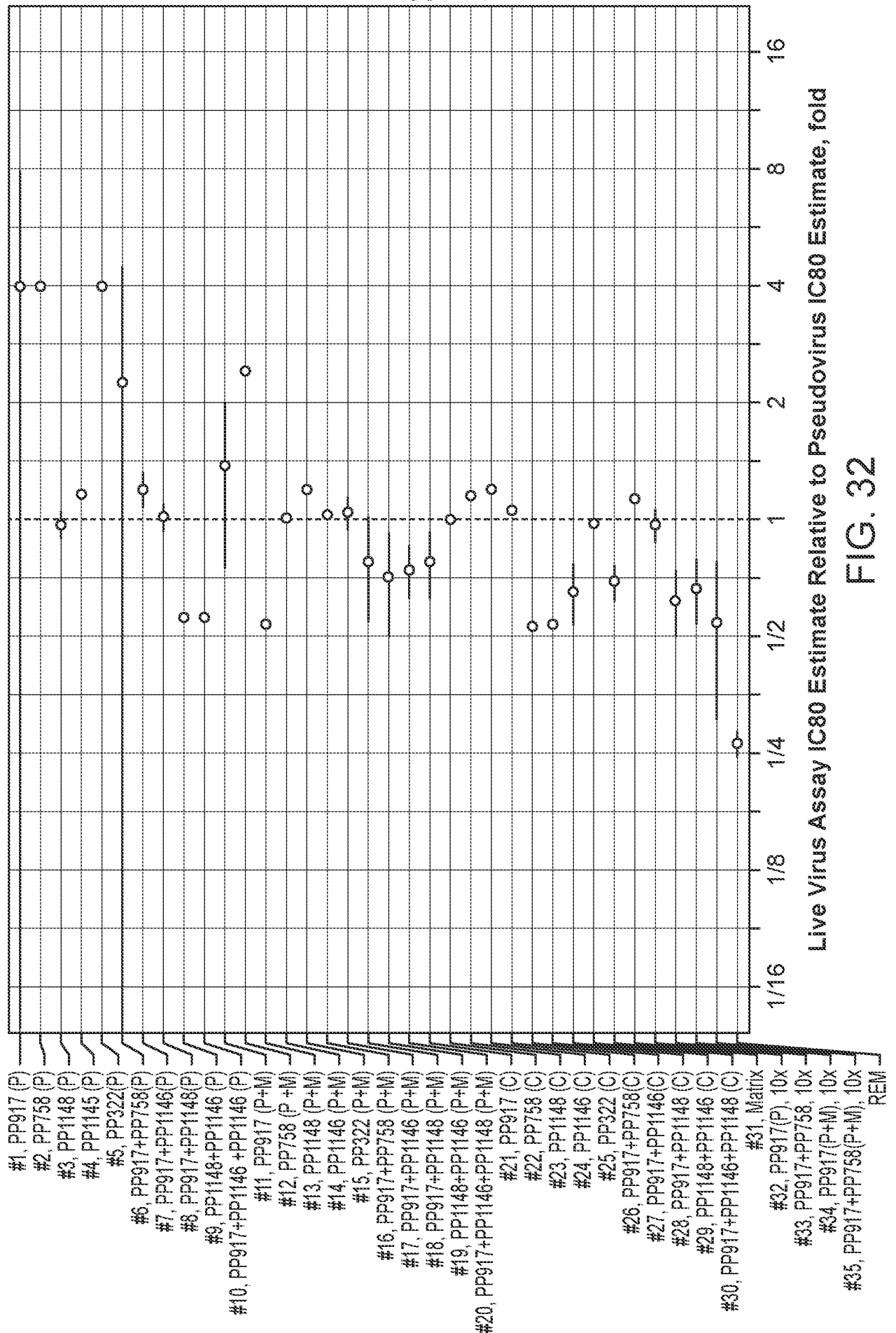
Concentration Relative to Neat Sample (IC80), fraction

FIG. 31



Concentration Relative to Neat Sample (IC80), fraction

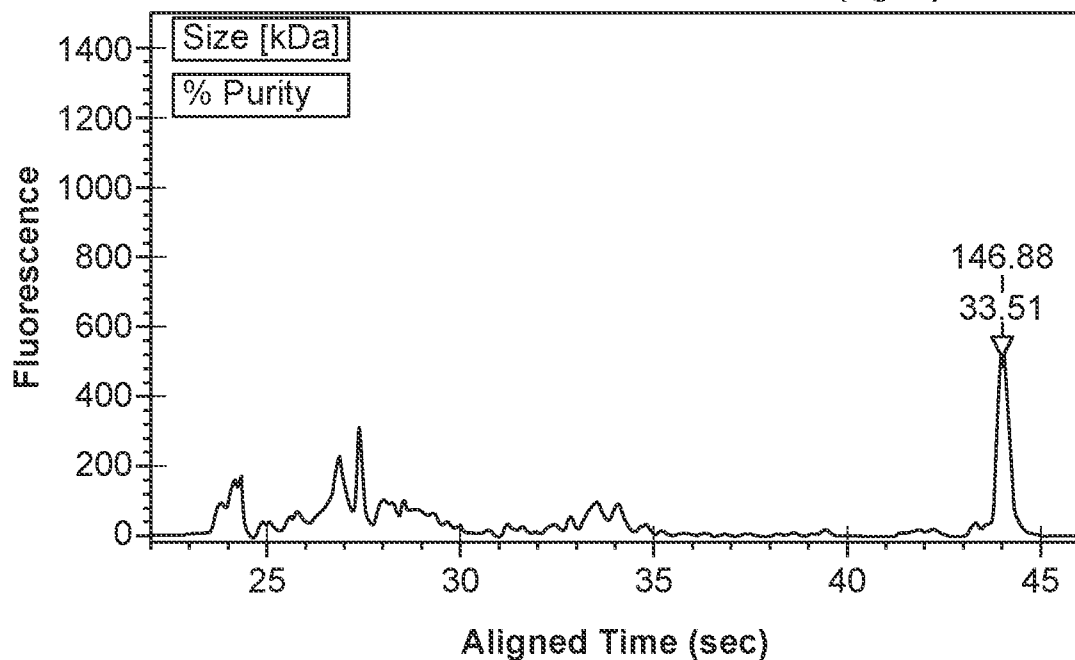
FIG. 31 (Cont.)



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[2021-11-03_Proj128 - purification_Proj128/B9] Proj113 R3 - NonReduced

Total Conc. (ng/ul) - 994.06



[2021-11-03_Proj128 - purification_Proj128/B8] Proj113 R3 - Reduced

Total Conc. (ng/ul) - 2711.05

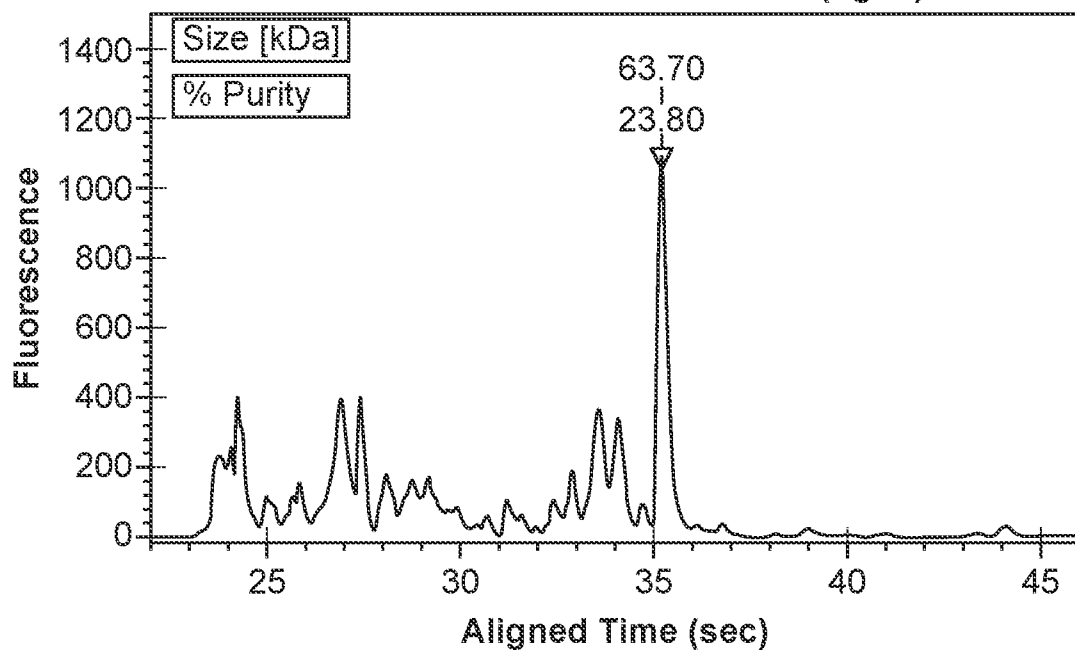


FIG. 33A

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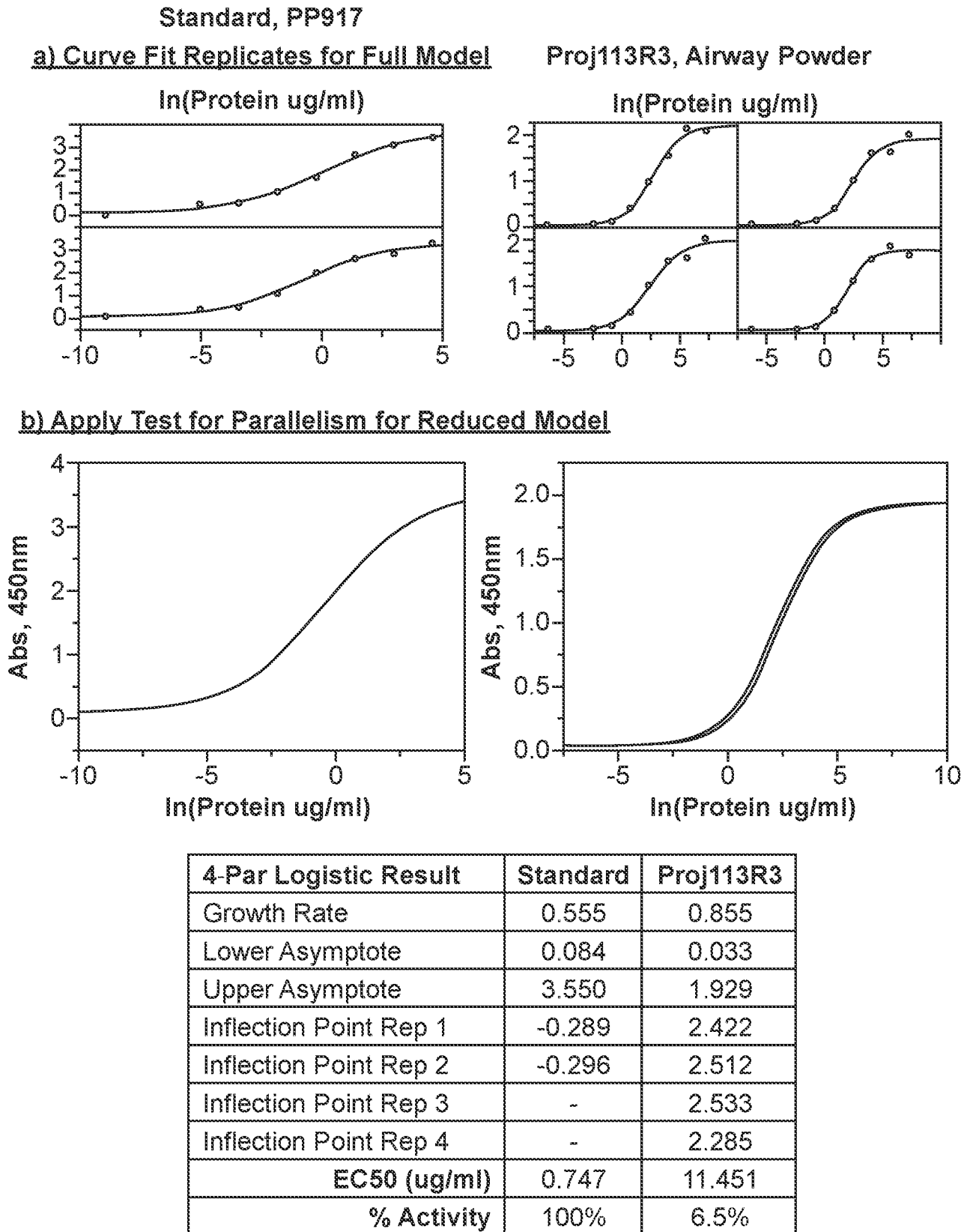
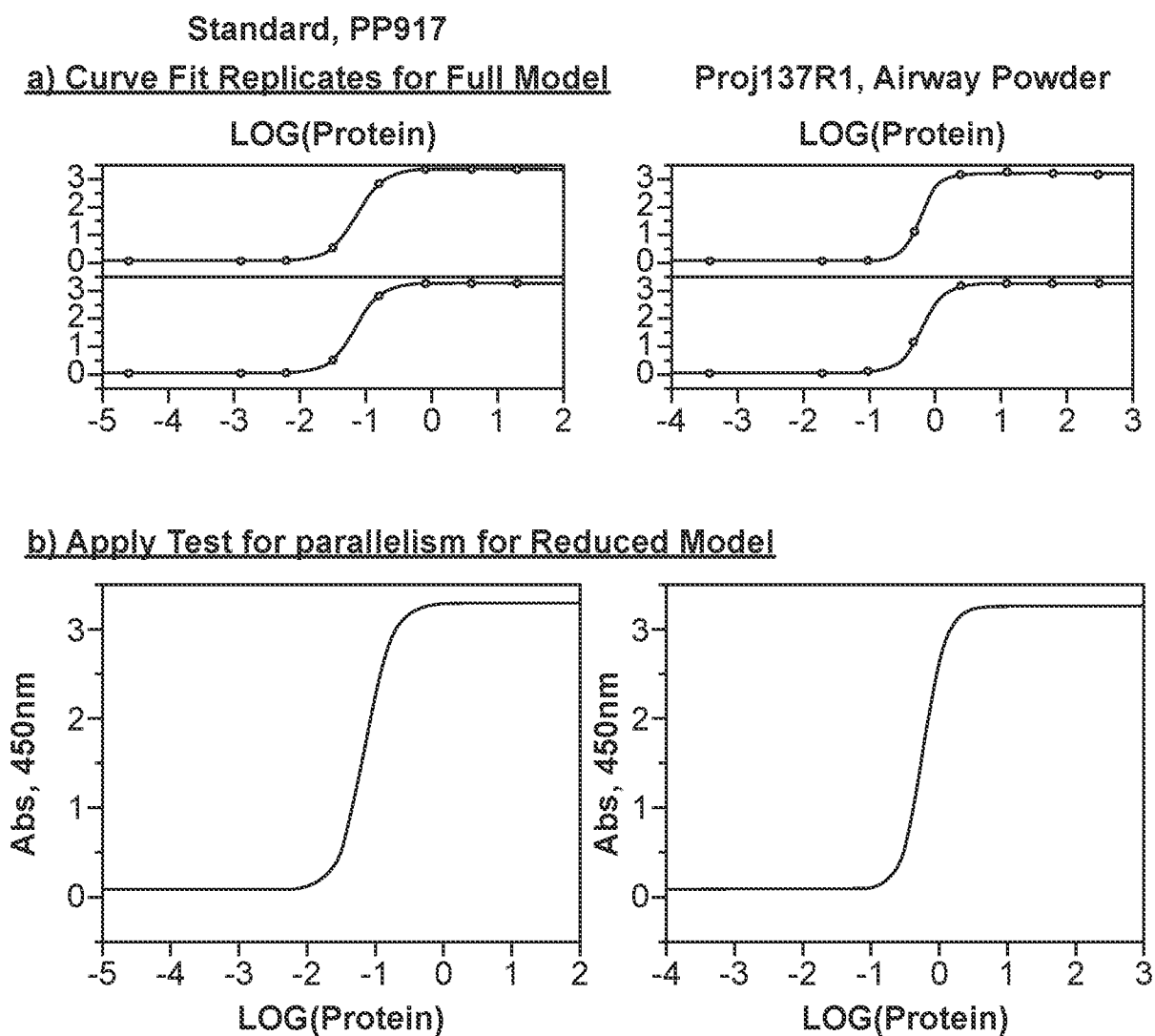


FIG. 33B

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Parameter	Standard	Proj137R1
Growth Rate	5.11	6.35
Lower Asymptote	0.06	0.08
Upper Asymptote	3.29	3.26
Inflection Point Rep 1	-1.14	-0.21
Inflection Point Rep 2	-1.14	-0.22
EC50 (ug/ml)	0.072	0.614
% Activity	100%	11.8%

FIG. 33C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/65138

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 39/42, C07K 16/10, A61P 31/12, A61K 35/748 (2022.01)

CPC - A61K 39/42, C07K 16/10, A61P 31/12, C07K 2317/569, C12N 2770/20011, A61K 2039/54, A61K 2039/505, A61K 2039/507, A61K 35/748

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	✓ CN 112094342 A (UNIVERSITY OF SCIENCE AND TECHNOLOGY OF CHINA) 18 December 2020 (18.12.2020) claim 1	1-2, 7-8, 10-13, 15-17
A	✓ A0A510UPM1, UniProt Accession No: A0A510UPM1 (A0A510UPM1_9CELL), 16 October 2019 [online]. [Retrieved on 6 April 2022]. Retrieved from the Internet <URL: https://www.uniprot.org/uniprot/A0A510UPM1 > Entire document	1-2, 7-8, 10-13, 15-17
A	US 2013/0230537 A1 (HUSSACK et al) 5 September 2013 (05.09.2013) claim 4; SEQ ID NO: 26	1-2, 7-8, 10-13, 15-17
A	✓ A0A0D2CT51, UniProt Accession No: A0A0D2CT51 (A0A0D2CT51_9EURO), 29 April 2015 [online]. [Retrieved on 6 April 2022]. Retrieved from the Internet <URL: https://www.uniprot.org/uniprot/A0A0D2CT51 > Entire document	1-2, 7-8, 10-13, 15-17
A	US 2011/0184152 A1 (ADAMS et al.) 28 July 2011 (28.07.2011) Abstract	1-2, 7-8, 10-13, 15-17

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"D" document cited by the applicant in the international application

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

6 April 2022

Date of mailing of the international search report

MAY 12 2022

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Kari Rodriguez

Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/65138

Box No. 1 **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed:
 - ☒ in the form of an Annex C/ST.25 text file.
 - ☐ on paper or in the form of an image file.
 - b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
 - ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
 - ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/65138

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 20-43
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

-----continued on supplemental sheet-----

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-2, 7-8, 10-13, 15-17 limited to CDR1-3 with SEQ ID Nos: 391, 392, and 393

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/65138

Box III: Observations where unity of invention is lacking:

Group I+: Claims 1-17 directed to a VHH antibody that binds SARS-CoV-2, wherein the VHH antibody comprises three complementarity determining regions (CDR1, CDR2, and CDR3). The VHH antibody will be searched to the extent wherein the VHH antibody comprises complementarity determining regions (CDR1, CDR2, and CDR3) with SEQ ID NOs: 391, 392, and 393. It is believed that claims 1-2, 7-8, 10-13 and 15-17 encompass this first named invention, and thus these claims will be searched without fee to the extent wherein the VHH antibody comprises complementarity determining regions (CDR1, CDR2, and CDR3) with SEQ ID NOs: 391, 392, and 393. Additional VHH antibodies will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected VHH antibodies. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be wherein the VHH antibody comprises three complementarity determining regions (CDR1, CDR2, and CDR3) with SEQ ID NOs: 610, 611, and 612 and VHH antibody comprises SEQ ID NO: 365 (claims 1-4, 7-13, 15-17).

Group II: Claims 18-19 directed to a method of treating a disease or disorder related to SARS-CoV-2.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I+ requires a VHH antibody that binds SARS-CoV-2, wherein the VHH antibody comprises three complementarity determining regions (CDR1, CDR2, and CDR3), not required by Group II.

Group II requires a method of treating a disease or disorder related to SARS-CoV-2, not required by Group I.

Common Technical Features

The features shared by the inventions listed as Group I+ and II are a VHH antibody that binds SARS-CoV-2 and use for treating disease related to SARS-CoV-2.

However, these shared technical features do not represent a contribution over prior art, because the shared technical features are taught by CN 112094342 A to University of Science and Technology of China (hereinafter 'USTA').

USTA teaches a VHH antibody that binds SARS-CoV-2 and use for treating disease related to SARS-CoV-2 (Abstract; [as interpreted by USTA translation at Abstract: "disclosure relates to alpaca-derived antibodies or antigen-binding fragments thereof that bind to SARS-CoV-2RBD, and in particular, to alpaca-derived nanobodies or dual epitope-specific antibodies or antigen-binding fragments thereof consisting of the same that can bind to the receptor-binding Region (RBD) of the novel coronavirus (SARS-CoV-2) with high affinity, which can be used for the prevention, treatment and/or diagnosis of SARS-CoV-2 infection"; claim 1; [as interpreted by USTA translation at claim 1- "alpaca-derived bi-epitopic specific antibody or antigen binding fragment thereof that binds to SARS-CoV-2RBD having VHH1"]]).

The features shared by the inventions listed as Group I+ are a VHH antibody that binds SARS-CoV-2, wherein the VHH antibody comprises three complementarity determining regions (CDR1, CDR2, and CDR3).

However, these shared technical features do not represent a contribution over prior art, because the shared technical features are taught by USTA.

USTA discloses (instant claim 1) a VHH antibody that binds SARS-CoV-2, wherein the VHH antibody comprises three complementarity determining regions (CDR1, CDR2, and CDR3) (claim 1; [as interpreted by USTA translation at claim 1- "alpaca-derived bi-epitopic specific antibody or antigen binding fragment thereof that binds to SARS-CoV-2RBD having VHH1...CDR1 shown in SEQ ID NO. 1, CDR2 as shown in SEQ ID NO. 2, and CDR3 shown..."]]).

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Groups I+ and II therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Item 4 (continued)

Claims 20-43 are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).