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(54) Title: "CHIMERIC MOLECULES AND USES THEREOF"

(57) Abstract: Disclosed are chimeric polypeptides based on viral membrane fusion proteins. More particularly, the present invention discloses chimeric polypeptides that comprise a virion surface exposed portion of a viral fusion protein and a heterologous structure-stabilizing moiety, and to complexes of those chimeric polypeptides. The present invention also discloses the use of these complexes in compositions and methods for eliciting an immune response to a fusion protein of an enveloped virus, or complex of the fusion protein, and/or for treating or preventing an enveloped virus infection. The present invention further discloses the use of the heterologous structure-stabilizing moiety for oligomerizing heterologous molecules of interest.

TITLE OF THE INVENTION

"CHIMERIC MOLECULES AND USES THEREOF"

FIELD OF THE INVENTION

[0001] This application claims priority to Australian Provisional Application No. 2017901152 entitled "Chimeric molecules and uses thereof" filed 30 March 2017, the contents of which are incorporated herein by reference in their entirety.

[0002] This invention relates generally to chimeric polypeptides based on viral membrane fusion proteins. More particularly, the present invention relates to chimeric polypeptides that comprise a virion surface exposed portion of a viral fusion protein and a heterologous structure-stabilizing moiety, and to complexes of those chimeric polypeptides. The present invention also relates to the use of these complexes in compositions and methods for eliciting an immune response to a fusion protein of an enveloped virus, or complex of the fusion protein, and/or for treating or preventing an enveloped virus infection. The present invention further relates to the use of the heterologous structure-stabilizing moiety for oligomerizing heterologous molecules of interest.

BACKGROUND OF THE INVENTION

[0003] Enveloped viruses, such as human immunodeficiency virus (HIV), influenza virus and respiratory syncytial virus (RSV), require fusion of viral membrane with the a host cell's membrane to enter and infect the host cell. Viral fusion proteins facilitate this process by undergoing energy favorable structural rearrangements from a metastable 'pre-fusion' conformation to a highly stable 'post-fusion' conformation. This structural change drives fusion of the virus and host cell membranes resulting in the release of viral genome into the cell.

[0004] Viral fusion proteins are currently classified into three classes based on their individual structural architecture and molecular features that drive the fusion process. Both class I and class III fusion proteins are trimeric in both their pre- and post-fusion conformations, while class II fusion proteins are dimeric in their pre-fusion conformation which is then rearranged into a trimeric post-fusion form. It is possible however that new classes of viral fusion proteins may be identified in the future that share some key features in common with these currently defined classes. Class I and class III fusion proteins share substantial structural features, including the N-terminal signal sequence and C-terminal transmembrane and cytoplasmic domain. They also share similar fusion mechanisms, with the initial pre-fusion trimer undergoing partial dissociation to allow the significant structural rearrangement required to form the post-fusion trimer.

[0005] Viral fusion proteins are excellent subunit vaccine candidates, as they are the primary targets of protective neutralizing antibody responses for many medically important enveloped viruses. The intrinsic metastable nature of fusion proteins, however, is a major obstacle for effective subunit vaccine design, as recent evidence has shown that broadly cross-reactive and potently neutralizing antibodies elicited during natural infection react primarily with the pre- and not post-fusion forms. In addition, the pre-fusion form of viral envelope fusion proteins contains epitopes that are not present on the post-fusion form (*e.g.*, Magro *et al.*, 2012. *Proc. Natl. Acad. Sci. USA* 109(8):3089-3094). Thus, for vaccines, the stabilized pre-fusion form is generally considered more desirable antigenically. However, traditional approaches to recombinant

expression of these proteins typically results in premature triggering and a conformational shift to the structurally more stable post-fusion form.

[0006] Consequently, there is a pressing need for new approaches to produce stabilized recombinant fusion proteins that remain substantially in their pre-fusion form to stimulate more efficacious immune responses against enveloped viruses.

SUMMARY OF THE INVENTION

[0007] The present invention arises from the determination that a pre-fusion conformation of a viral fusion protein can be mimicked by operably connecting downstream of the fusion protein virion surface exposed domain (also referred to herein as a "fusion ectodomain polypeptide" or "ectodomain") a heterologous moiety that comprises a pair of complementary heptad repeat regions that associate with one another to form an anti-parallel, two-helix bundle. This moiety acts as a kind of 'molecular clamp' that stabilizes the fusion ectodomain polypeptide, and inhibiting it from rearranging to a post-fusion conformation. The molecular clamp approach of the present invention has been used to produce chimeric polypeptides that mimic the pre-fusion conformations of influenza, RSV, HIV, measles virus and Ebola virus, respectively, and thus lends itself as a platform for producing mimetics of viral envelope fusion proteins in a pre-fusion conformation. The chimeric polypeptides so produced can self-assemble to form an artificial enveloped virus fusion protein complex that comprises an oligomer of the chimeric polypeptide and that mimics the pre-fusion conformation of a native enveloped virus fusion protein complex. This self-assembly permits facile production of the artificial complexes *inter alia* in recombinant expression systems. The artificial complexes produced using the chimeric polypeptides of the invention are useful in methods and compositions for stimulating an immune response, including the development of neutralizing antibodies, to the native enveloped virus fusion protein complex, as described hereafter.

[0008] Accordingly, the present invention provides, in one aspect, a chimeric polypeptide comprising an enveloped virus fusion ectodomain polypeptide operably connected downstream to a heterologous, structure-stabilizing moiety comprising complementary first heptad repeat (HR1) and second heptad repeat (HR2) regions that associate with each other under conditions suitable for their association (*e.g.*, in aqueous solution) to form an anti-parallel, two-helix bundle. The HR1 and HR2 regions typically lack complementarity to the ectodomain polypeptide, so that they preferentially form an anti-parallel, two-helix bundle with each other, rather than with structural elements of the ectodomain polypeptide. In some embodiments, each of the HR1 and HR2 regions is independently characterized by a n-times repeated 7-residue pattern of amino acid types, represented as $(a\text{-}b\text{-}c\text{-}d\text{-}e\text{-}f\text{-}g\text{-})_n$ or $(d\text{-}e\text{-}f\text{-}g\text{-}a\text{-}b\text{-}c\text{-})_n$, wherein the pattern elements 'a' to 'g' denote conventional heptad positions at which the amino acid types are located and n is a number equal to or greater than 2, and at least 50% (or at least 51% to at least 99% and all integer percentages in between) of the conventional heptad positions 'a' and 'd' are occupied by hydrophobic amino acid types and at least 50% (or at least 51% to at least 99% and all integer percentages in between) of the conventional heptad positions 'b', 'c', 'e', 'f' and 'g' are occupied by hydrophilic amino acid types, the resulting distribution between hydrophobic and hydrophilic amino acid types enabling the identification of the heptad repeat regions. In some embodiments, one or both of the HR1 and HR2 regions comprises, consists or consists essentially of an endogenous Class I enveloped virus fusion protein heptad repeat region amino acid

sequence. In representative examples of this type, the HR1 and HR2 regions comprise, consist or consist essentially of complementary endogenous heptad repeat A (HRA) and heptad repeat B (HRB) regions, respectively, of one or more Class I enveloped virus fusion proteins. In some embodiments, the HRA region amino acid sequence and the HRB region amino acid sequence are derived from the same Class I enveloped virus fusion protein. In other embodiments, the HRA region amino acid sequence and the HRB region amino acid sequence are derived from the different Class I enveloped virus fusion proteins. In representative examples, the HR1 and HR2 regions are independently selected from HRA and HRB regions of fusion proteins expressed by orthomyxoviruses, paramyxoviruses, retroviruses, coronaviruses, filoviruses and arenaviruses.

[0009] In some embodiments, the ectodomain polypeptide corresponds to a Class I enveloped virus fusion protein ectodomain. In representative examples of this type, the ectodomain polypeptide comprises one or both of an endogenous HRA region and an endogenous HRB region. Non-limiting enveloped viruses with Class I fusion proteins include orthomyxoviruses, paramyxoviruses, retroviruses, coronaviruses, filoviruses and arenaviruses.

[0010] In other embodiments, the ectodomain polypeptide corresponds to a Class III enveloped virus fusion protein ectodomain. Representative enveloped viruses with Class III fusion proteins include rhabdoviruses and herpesviruses.

[0011] The ectodomain polypeptide (*e.g.*, Class I or Class III) may comprise or consist of a whole precursor ectodomain polypeptide or a portion thereof. In some embodiments the ectodomain polypeptide lacks any one or more of an endogenous signal peptide, an endogenous head portion of an ectodomain, an endogenous stem portion of an ectodomain, an endogenous mucin-like domain, an endogenous membrane proximal external region and an endogenous fusion peptide. The ectodomain polypeptide suitably comprises at least one pre-fusion epitope that is not present in the post-fusion form of an enveloped virus fusion protein to which the ectodomain polypeptide corresponds.

[0012] In some embodiments, the HR1 and HR2 regions of the structure-stabilizing moiety are connected by a linker, which generally consists of about 1 to about 100 amino acid residues (and all integer amino acid residues therebetween), and typically of about 1 to about 100 amino acid residues (and all integer amino acid residues therebetween). The linker may comprise at least one moiety selected from a purification moiety that facilitates purification of the chimeric polypeptide, an immune-modulating moiety that modulates an immune response to the chimeric polypeptide, a cell targeting moiety that directs the chimeric polypeptide to a specific cell subtype and a structural flexibility-conferring moiety.

[0013] The chimeric polypeptide can be produced synthetically or by recombinant means. In embodiments in which the chimeric polypeptide is produced recombinantly, the present invention provides in another aspect a nucleic acid construct that comprises a coding sequence for a chimeric polypeptide as broadly described above and elsewhere herein, operably linked to a regulatory element that is operable in the host cell.

[0014] In a related aspect, the present invention provides a host cell that contains the nucleic acid construct broadly described above and elsewhere herein. The host cell may be a prokaryotic or eukaryotic host cell.

[0015] In addition to its utility in stabilizing the ectodomain polypeptide against rearrangement to a post-fusion conformation, the heterologous structure-stabilizing moiety is useful for oligomerizing any heterologous molecules of interest into oligomers, particularly trimers. Accordingly, in another aspect, the present invention provides a chimeric polypeptide comprising a proteinaceous molecule operably connected downstream to a heterologous, structure-stabilizing moiety comprising complementary first heptad repeat (HR1) and second heptad repeat (HR2) regions that associate with each other under conditions suitable for their association (*e.g.*, in aqueous solution) to form an anti-parallel, two-helix bundle, as broadly described above and elsewhere herein.

[0016] The chimeric polypeptides of the present invention can self-assemble under suitable conditions (*e.g.*, in aqueous solution) to form a chimeric polypeptide complex. Accordingly, in another aspect, the present invention provides a method of producing a chimeric polypeptide complex, wherein the method comprises: combining chimeric polypeptides as broadly defined above and elsewhere herein under conditions (*e.g.*, in aqueous solution) suitable for the formation of a chimeric polypeptide complex, whereby a chimeric polypeptide complex is produced that comprises three chimeric polypeptides and is characterized by a six-helix bundle formed by oligomerization of the two-helix bundles of the respective structure-stabilizing moieties of the chimeric polypeptides.

[0017] In a related aspect, the present invention provides a chimeric polypeptide complex that comprises three chimeric polypeptides as broadly described above and elsewhere herein and is characterized by a six-helix bundle formed by oligomerization of the two-helix bundles of the respective structure-stabilizing moieties of the chimeric polypeptides. In some embodiments in which the chimeric polypeptide comprises an enveloped virus fusion ectodomain polypeptide, the chimeric polypeptide complex formed by self-assembly of the chimeric polypeptide comprises at least one pre-fusion epitope of an enveloped virus fusion protein of interest (*e.g.*, a wild-type enveloped virus fusion protein), or complex thereof, which is not present on a post-fusion form of the enveloped virus fusion protein, or complex thereof.

[0018] The present invention in another related aspect provides a composition comprising a chimeric polypeptide or chimeric polypeptide complex as broadly described above and elsewhere herein, and a pharmaceutically acceptable carrier, diluent or adjuvant. In some embodiments, the composition is an immune-modulating composition.

[0019] In some embodiments in which the chimeric polypeptide comprises an enveloped virus fusion ectodomain polypeptide, the chimeric polypeptide complex of the present invention is useful for eliciting an immune response in subjects or production animals, to a fusion protein of an enveloped virus, or complex of the fusion protein. Accordingly, another aspect of the present invention provides a method of eliciting an immune response to a fusion protein of an enveloped virus, or complex of the fusion protein, in a subject, wherein the method comprises administering to the subject a chimeric polypeptide complex or composition, as broadly described above and elsewhere herein, wherein an ectodomain polypeptide of the chimeric polypeptide complex corresponds to the fusion protein of the enveloped virus.

[0020] In a related aspect, the present invention provides a method of identifying an agent (*e.g.*, a small molecule or macromolecule) that binds with a fusion protein of an enveloped

virus, or complex of the fusion protein, wherein the method comprises: contacting the candidate agent with an ectodomain polypeptide-containing chimeric polypeptide or chimeric polypeptide complex, as broadly described above and elsewhere herein, wherein the ectodomain polypeptide corresponds to the fusion protein of the enveloped virus, and detecting binding of the candidate agent to the chimeric polypeptide or chimeric polypeptide complex. In specific embodiments, the method further comprises contacting the candidate agent with the fusion protein or complex of the fusion protein and detecting binding of the candidate agent to the fusion protein or the complex. In specific embodiments, the candidate agent is part of a compound library (*e.g.*, small molecule or macromolecule library). In some of these embodiments, the method further comprises isolating the candidate agent from the library. Suitably, the candidate agent binds specifically to the chimeric polypeptide or chimeric polypeptide complex, and preferably to the fusion protein or complex of the fusion protein.

[0021] In another related aspect, the present invention provides a method of producing an antigen-binding molecule (*e.g.*, an antibody such as a neutralizing antibody) that is immuno-interactive with a fusion protein of an enveloped virus, or complex of the fusion protein, wherein the method comprises: (1) immunizing an animal with an ectodomain polypeptide-containing chimeric polypeptide or chimeric polypeptide complex, or composition as broadly described above and elsewhere herein, wherein the ectodomain polypeptide corresponds to the fusion protein of the enveloped virus; (2) identifying and/or isolating a B cell from the animal, which is immuno-interactive with the fusion protein or complex thereof; and (3) producing the antigen-binding molecule expressed by that B cell.

[0022] Also provided as a further aspect of the invention is an antigen-binding molecule produced by the immunizing methods broadly described above and elsewhere herein, or a derivative antigen-binding molecule with the same epitope-binding specificity as the antigen-binding molecule. The derivative antigen-binding molecule may be selected from antibody fragments (such as Fab, Fab', $F(ab')_2$, Fv), single chain (scFv) and domain antibodies (including, for example, shark and camelid antibodies), and fusion proteins comprising an antibody, and any other modified configuration of the immunoglobulin molecule that comprises an antigen binding/recognition site.

[0023] In a related aspect, the present invention provides an immune-modulating composition comprising an antigen-binding molecule as broadly described above and elsewhere herein, and a pharmaceutically acceptable carrier, diluent or adjuvant.

[0024] The subject enveloped virus fusion ectodomain polypeptide-containing chimeric polypeptide or complex thereof, as well as the composition and antigen-binding molecule, as broadly described above and elsewhere herein, are also useful for treating or preventing enveloped virus infections. Accordingly, in yet another aspect, the present invention provides a method for treating or preventing an enveloped virus infection in a subject, wherein the method comprises administering to the subject an effective amount of an enveloped virus fusion ectodomain polypeptide-containing chimeric polypeptide, or complex thereof, composition or antigen-binding molecule, as broadly described above and elsewhere herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] Figure 1 is a graphical representation showing ELISA RSV F reactivity with conformation specific monoclonal antibody. **A.** Prefusion specific monoclonal antibody D25 (Zhao *et al.*, *Proc. Natl. Acad. Sci. USA* 2000. 97(26): 14172-7) binds to chimeric clamp stabilized RSV F with an affinity of 23.4±12.5nM but does not bind to the corresponding unstabilized F ectodomain, F sol. **B.** Prefusion specific monoclonal antibody D25 binds to chimeric clamp stabilized RSV F DS cav mutant and the control chimeric foldon stabilized RSV F with comparable affinities (1.2±0.2nM and 1.3±0.2nM, respectively), but does not bind to the corresponding unstabilized F ectodomain, F sol. **C.** Broadly cross-neutralizing, prefusion specific monoclonal antibody MPE8 (Corti *et al.*, *Nature 2013*. 501(7467):439-43) binds to chimeric clamp stabilized RSV F DS cav mutant and the control chimeric foldon stabilized RSV F with comparable affinities (7.6±1.5nM and 10.8±2.4nM, respectively), but does not bind to the corresponding unstabilized F ectodomain, F sol.

[0026] Figure 2 is a graphical representation showing ELISA Influenza H3 reactivity with conformation specific monoclonal antibody. A. Head specific antibody C05 (Ekiert *et al.*, *Nature* 2012. 489(7417): 526-32) binds similarly to H3sol, H3 clamp and QIV), however the prefusion, stem, specific monoclonal antibody CR8043 (Friesen, *et al.*, *Proc. Natl. Acad. Sci. USA* 2014. 111(1): 445-50) binds to chimeric clamp stabilized Influenza HA with an affinity of 3.3 ± 0.8 nM but does not bind to the corresponding HA ectodomain or commercial QIV.

[0027] Figure 3 is a graphical representation showing size-exclusion chromatography separation of protein oligomeric state. Chimeric clamp stabilized influenza HA exists as a soluble trimer as seen *via* it's elution from a Superdex 200 Increase 10/300 GL column at approximately 11 mL. In comparison, the corresponding HA ectodomain expressed in isolation elutes from the column at approximately 8 mL and 12 mL consistent with a portion of the protein existing as aggregates and another portion as monomers. It has previously been shown that the post-fusion form of Influenza HA can dissociate into a monomeric form or aggregate *via* the exposure of the fusion peptide (Weldon *et al.*, *PLoS One* 2010. 5(9)). Commercial QIV eluted at 8 mL indicating high molecular weight aggregates.

[0028] Figure 4 is a graphical representation showing neutralizing immune response produced upon vaccination. Sera from mice vaccinated with commercial (QIV) vaccine showed neutralization against Influenza A/Hebei Baoding Anguo/51/2010 (H3N2) with an approximate IC50 value of 180. In comparison chimeric clamp stabilized Influenza HA showed a greatly increased level of virus neutralizing activity with an IC50 value of 1:14,000 (95%CI 11,000-17,000), while sera from mice vaccinated with the corresponding HA ectodomain showed no neutralizing activity even at the highest dose tested of 1:20. Pre-incubation with H3sol, QIV did not affect H3N2 neutralization however pre-incubation with H3clamp removed H3N2 neutralization.

[0029] Figure 5 is a graphical representation showing subdomain specificity of the induced immune response. Sera from mice vaccinated with H3sol, H3clamp or commercial vaccine (QIV) showed differential reactivity with the head and stem subdomains of H3. Sera from mice immunized with chimeric clamp stabilized Influenza HA showed the greatest reactivity to the stem domain (~25% of the total H3 specific response), while sera from mice immunized with H3sol and QIV this percentage was much lower (1% and 4%, respectively).

[0030] Figure 6 is a graphical representation showing H5 cross-reactivity. ELISA was used to measure reactivity with H5. Endpoint titers were calculated as the maximum dilution producing greater than background + 3 standard deviations reactivity. Sera from mice vaccinated with H3sol, H3clamp, commercial vaccine (QIV), H1 clamp and H5clamp showed differential reactivity with H5. Sera from mice immunized with H3clamp or H1clamp showed much greater cross reactivity with H5 than QIV (27-fold increase and 81-fold increase, respectively).

[0031] Figure 7 is a graphical representation showing subdomain responsible for H5 cross-reactivity. ELISA was used to measure reactivity with H5. Endpoint titres were calculated as the maximum dilution producing greater than background + 3 standard deviations reactivity. Total H5 reactivity for mouse sera is shown (grey bars). Pre-incubation of sera with H3stem, was used to pre-absorb stem specific antibodies prior to ELISA (white bars) or monoclonal antibody FI6v3 was added to outcompete stem specific antibodies (black bars).

[0032] Figure 8 is a graphical representation showing the purification of a soluble trimeric clamp stabilized MERS Spike protein. **A.** SDS-PAGE of clamp stabilized MERS Spike purified from CHO supernatant following expression. Protein band at approximately 200kDA is at the correct approximate size for the resulting monomeric MERS protein including associated glycans and is not visible in a control purification from CHO supernatant. **B.** Size exclusion chromatography of clamp stabilized MERS Spike protein on a Superdex 6 10/300 GL column. Elution of the major protein at 12.5ml is indicative of approximately 600kDA which is the correct size for non-aggregated, trimeric MERS protein including associated glycans.

[0033] Figure 9 is a graphical representation showing the purification of a clamp stabilized Ebola GP protein lacking the mucin-like domain from CHO supernatant. SDS-PAGE analysis without DTT shows a protein band at approximately 100kDA which is at the correct size for the resulting Ebola GP protein (GP1 and GP2 linked by the native disulfide bridge) including associated glycans. SDS-PAGE analysis with DTT shows two protein bands at approximately 60kDA and 30kDa which are the correct sizes for the Furin cleaved Ebola GP protein including associated glycans.

[0034] Figure 10 is a graphical representation showing ELISA Ebola GP clamp (lacking the mucin-like domain) reactivity with highly neutralizing monoclonal antibodies. Monoclonal antibodies Kz52, 1H3, 2G4, 4G7 and 13C6 (Murin *et al.*, PNAS. 2014 11(48):17182-7) all bind with high affinities to Ebola GP clamp (lacking the mucin-like domain).

[0035] Figure 11 is a graphical representation illustrating the thermal stability of Ebola GP clamp (lacking the mucin-like domain). Purified Ebola GP clamp (lacking the mucin-like domain) was bound to an ELISA plate and dried in the presence of 30% sucrose. Reactivity with highly neutralizing monoclonal antibodies Kz52, 4G7 and 13C6 (Murin *et al.*, PNAS. 2014 11(48):17182-7) was measure either straight away or after 14 days incubation at 37°C. No significant change in reactivity was seen indicating that the clamp stabilized protein is stable at high temperatures for extended periods.

[0036] Figure 12 is a graphical representation showing the immune response to Ebola GP clamp (lacking the mucin-like domain) following immunization of BALB/C mice. 3 groups of 5 mice were immunized intradermally with either 1 µg of Ebola GP clamp (lacking the mucin-like domain), 1 µg of Ebola GP clamp (lacking the mucin-like domain) + 3ug of Saponin adjuvant Quil A

or PBS as a negative control. Mice were immunized 3 times at day 0, 28 and 56 and blood was collected at day 27 (bleed1), day 55 (bleed2) and day 84 (bleed3). **A.** Antibody specific for Ebola GP clamp (lacking the mucin-like domain) within blood serum was quantified by ELISA. **B.** Endpoint titre of was calculated by calculating the ultimate dilution capable of producing a reading above the background + 3 standard deviations.

[0037] Figure 13 is a graphical representation illustrating the ability of the immune response to Ebola GP clamp (lacking the mucin-like domain) following immunization of BALB/C mice to neutralize live Ebola virus, Zaire strain. Sera from mice immunized with 1 µg of Ebola GP clamp (lacking the mucin-like domain) + 3 µg of Saponin adjuvant Quil A was capable of neutralizing live Ebola virus. The geometric mean titre producing 50% reduction in plaque forming units was calculated to be 52.8 (95%CI 24.5-114.0).

[0038] Figure 14 is a graphical representation showing immunosilencing of the clamp domain through the incorporation of N-linked glycosylation sites. Four separate mutations within the HRB of the clamp sequence based on the HIV GP160-based SSM were produced in the Ebola GP clamp (lacking the mucin-like domain). The reactivity of sera from mice immunized with the chimeric clamp stabilized influenza HA was tested against Ebola GP clamp (lacking the mucin-like domain) that was either stabilized with the identical clamp sequence or clamp sequences incorporating glycosylations at one of four potential sites. Reactivity was significantly reduced by glycosylation at each individual site supporting the hypothesis that this method can be used to reduce reactivity to the clamp domain. Correct folding of Ebola GP (lacking the mucin-like domain) incorporating mutated clamp sequences was confirmed by measuring Kz52 affinity.

[0039] Figure 15 is a photographic representation showing SDS-PAGE of purified clamp stabilized antigens from eight viruses. Bands designated by orange arrows indicate non-cleaved products and yellow arrows indicated cleaved products. The expected molecular weight of antigens are: Influenza HAclamp = ~85kDa, RSV Fclamp = ~65kDa, Ebola GPclamp = ~72kDa, Nipah Fclamp ~64, MERS Sclamp ~200kDA, Lassa GPCclamp = ~75kDA, Measles = ~65kDa and HSV2-Gbclamp = ~100kDa.

[0040] Figure 16 is a graphical representation showing the results of a mouse protection study following challenge with influenza virus H1N1pdm: (A/B) Mice (n=5) were vaccinated with either H1sol, H1foldon or H1 clamp derived from strain Cal/09 (H1N1pdm) and then challenged with the matched influenza strain. (C/D) Mice were vaccinated with either H3sol, H3foldon or H3clamp derived from strain Switz/13 (H3N2) and then challenged with divergent strain Cal/09 (H1N1pdm).

[0041] Figure 17 is a graphical representation showing thermal stability of clamp stabilized antigens at 43°C for 72 hrs. Clamp stabilized vaccine candidates and control proteins were incubated at 43°C for 72 hrs. and the reactivity with three well characterized mAbs for each antigen used as a measure of thermal stability **A.** Pre-fusion specific mAbs D25, AM22 and MPE8 were used to compare RSV Fclamp and the alternate approach utilizing the foldon trimerization domain and structure based stabilizing mutations (McLellan *et al.*, Science, 2013. 342(6158): p. 592-8). **B.** HA stem specific mAbs FI6V3 and CR6261 and HA head specific mAb 5J8 were used to compare Influenza H1clamp and alternate approach utilizing the foldon trimerization domain.

[0042] Figure 18 is a graphical representation showing immune response to clamp-stabilized subunit vaccines. Sera from mice immunized with RSV Fclamp, Fsol or Fdscav foldon (McLellan *et al.*, *Science*, 2013. 342(6158):592-598) was tested for its ability to neutralize RSV.

[0043] Figure 19 is a graphical and photographic representation showing that inclusion of the molecular clamp facilitates stabilization of the trimeric pre-fusion conformation. Clamp-stabilized Nipah virus fusion glycoprotein was analyzed by size exclusion chromatography. Elution volume on the superdex 200 column correlates with the expected molecular weight of the soluble trimeric protein. Negative stain electron microscopy (inset) confirms the presence of homogenous, pre-fusion protein conformation.

[0044] Figure 20 is a graphical representation showing immune response to clamp-stabilized subunit vaccines. Sera from mice immunized with Nipah Fclamp were tested for their ability to neutralize Nipah virus. In all panels, values shown are geometric mean of individual mice with error bars indicating geometric standard deviation.

[0045] Figure 21 is a graphical and photographic representation showing that inclusion of the molecular clamp facilitates stabilization of the trimeric structure of a class 3 viral fusion protein. Clamp-stabilized HSV2 Fusion glycoprotein B (gB) was analyzed by size exclusion chromatography. Elution volume on the superose 6 column correlates with the expected molecular weight of the soluble trimeric protein. Negative stain electron microscopy (inset) confirms the presence of homogenous, trimeric conformation similar to the published structure (Heldwein *et al.*, *Science*, 2006. 313(5784): 217-20). This conformation has been shown to bind most neutralizing antibodies (Cairns *et al.*, *JVi*, 2014. 88(5): 2677-2689).

DETAILED DESCRIPTION OF THE INVENTION

1. Definitions

[0046] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are described. For the purposes of the present invention, the following terms are defined below.

[0047] The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0048] As used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (or).

[0049] Further, the terms "about" and "approximate", as used herein when referring to a measurable value such as an amount, dose, time, temperature, activity, level, number, frequency, percentage, dimension, size, amount, weight, position, length and the like, is meant to encompass variations of ± 15%, ± 10%, ± 5%, ± 1%, ± 0.5%, or even ± 0.1% of the specified amount, dose, time, temperature, activity, level, number, frequency, percentage, dimension, size, amount, weight, position, length and the like. In instances in which the terms "about" and "approximate" are used in connection with the location or position of regions within a reference

polypeptide, these terms encompass variations of ± up to 20 amino acid residues, ± up to 15 amino acid residues, ± up to 10 amino acid residues, ± up to 5 amino acid residues, ± up to 4 amino acid residues, ± up to 3 amino acid residues, ± up to 2 amino acid residues, or even ± 1 amino acid residue.

[0050] The term "adjuvant" as used herein refers to a compound that, when used in combination with a specific immunogen (*e.g.*, a chimeric polypeptide or complex of the present invention) in a composition, will augment the resultant immune response, including intensification or broadening the specificity of either or both antibody and cellular immune responses.

[0051] The term "agent" is used interchangeably with "compound" herein to refer to any compound or substance such as, but not limited to, a small molecule, nucleic acid, polypeptide, peptide, drug, ion, etc. An "agent" can be any chemical, entity or moiety, including without limitation synthetic and naturally-occurring proteinaceous and non-proteinaceous entities. In some embodiments, an agent is nucleic acid, nucleic acid analogues, proteins, antibodies, peptides, aptamers, oligomer of nucleic acids, amino acids, or carbohydrates including without limitation proteins, oligonucleotides, ribozymes, DNAzymes, glycoproteins, siRNAs, lipoproteins, aptamers, and modifications and combinations thereof etc. In some embodiments, the nucleic acid is DNA or RNA, and nucleic acid analogues, for example can be PNA, pcPNA and LNA. A nucleic acid may be single or double stranded, and can be selected from a group comprising; nucleic acid encoding a protein of interest, oligonucleotides, PNA, *etc*. Such nucleic acid sequences include, for example, but not limited to, nucleic acid sequence encoding proteins that act as transcriptional repressors, antisense molecules, ribozymes, small inhibitory nucleic acid sequences, for example but not limited to RNAi, shRNAi, siRNA, micro RNAi (mRNAi), antisense oligonucleotides etc. A protein and/or peptide agent or fragment thereof, can be any protein of interest, for example, but not limited to; mutated proteins; therapeutic proteins; truncated proteins, wherein the protein is normally absent or expressed at lower levels in the cell. Proteins of interest can be selected from a group comprising; mutated proteins, genetically engineered proteins, peptides, synthetic peptides, recombinant proteins, chimeric proteins, antibodies, humanized proteins, humanized antibodies, chimeric antibodies, modified proteins and fragments thereof.

[0052] As used herein, the term "antigen" and its grammatically equivalents expressions (*e.g.*, "antigenic") refer to a compound, composition, or substance that may be specifically bound by the products of specific humoral or cellular immunity, such as an antibody molecule or T-cell receptor. Antigens can be any type of molecule including, for example, haptens, simple intermediary metabolites, sugars (*e.g.*, oligosaccharides), lipids, and hormones as well as macromolecules such as complex carbohydrates (*e.g.*, polysaccharides), phospholipids, and proteins. Common categories of antigens include, but are not limited to, viral antigens, bacterial antigens, fungal antigens, protozoa and other parasitic antigens, tumor antigens, antigens involved in autoimmune disease, allergy and graft rejection, toxins, and other miscellaneous antigens.

[0053] By "antigen-binding molecule" is meant a molecule that has binding affinity for a target antigen. It will be understood that this term extends to immunoglobulins, immunoglobulin fragments and non-immunoglobulin derived protein frameworks that exhibit antigen-binding activity. Representative antigen-binding molecules that are useful in the practice of the present invention include polyclonal and monoclonal antibodies as well as their fragments (such as Fab, Fab', $F(ab')_2$, Fv), single chain (scFv) and domain antibodies (including, for example, shark and

camelid antibodies), and fusion proteins comprising an antibody, and any other modified configuration of the immunoglobulin molecule that comprises an antigen binding/recognition site. An antibody includes an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant region of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant regions that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known. Antigen-binding molecules also encompass dimeric antibodies, as well as multivalent forms of antibodies. In some embodiments, the antigen-binding molecules are chimeric antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (see, for example, US Pat. No. 4,816,567; and Morrison *et al.*, 1984, *Proc. Natl. Acad. Sci. USA* 81:6851-6855). Also contemplated, are humanized antibodies, which are generally produced by transferring complementarity determining regions (CDRs) from heavy and light variable chains of a non-human (*e.g.*, rodent, preferably mouse) immunoglobulin into a human variable domain. Typical residues of human antibodies are then substituted in the framework regions of the non-human counterparts. The use of antibody components derived from humanized antibodies obviates potential problems associated with the immunogenicity of non-human constant regions. General techniques for cloning non-human, particularly murine, immunoglobulin variable domains are described, for example, by Orlandi *et al.* (1989, Proc. Natl. Acad. Sci. USA 86: 3833). Techniques for producing humanized monoclonal antibodies are described, for example, by Jones *et al.* (1986, *Nature* 321:522), Carter *et al.* (1992, *Proc. Natl. Acad. Sci. USA* 89: 4285), Sandhu (1992, *Crit. Rev. Biotech.* 12: 437), Singer *et al.* (1993, *J. Immun.* 150: 2844), Sudhir (ed., Antibody Engineering Protocols, Humana Press, Inc. 1995), Kelley ("Engineering Therapeutic Antibodies," in Protein Engineering: Principles and Practice Cleland *et al.* (eds.), pages 399-434 (John Wiley & Sons, Inc. 1996), and by Queen *et al.*, U.S. Pat. No. 5,693,762 (1997). Humanized antibodies include "primatized" antibodies in which the antigen-binding region of the antibody is derived from an antibody produced by immunizing macaque monkeys with the antigen of interest. Also contemplated as antigen-binding molecules are humanized antibodies.

[0054] The term "anti-parallel", as used herein, refers to a proteinaceous polymer in which regions or segments of the polymer are in a parallel orientation but have opposite polarities.

[0055] As used herein, the term "binds specifically" refers to a binding reaction which is determinative of the presence of a chimeric polypeptide or complex of the present invention in the presence of a heterogeneous population of molecules including macromolecules such as proteins and other biologics. In specific embodiments, the term "binds specifically" when referring to an antigen-binding molecule is used interchangeably with the term "specifically immuno-interactive" and the like to refer to a binding reaction which is determinative of the presence of a chimeric polypeptide or complex of the present invention in the presence of a heterogeneous population of

proteins and other biologics. Under designated assay conditions, a molecule binds specifically to a chimeric polypeptide or complex of the invention and does not bind in a significant amount to other molecules (*e.g.*, proteins or antigens) present in the sample. In antigen-binding molecule embodiments, a variety of immunoassay formats may be used to select antigen-binding molecules that are specifically immuno-interactive with a chimeric polypeptide or complex of the invention. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies that are specifically immuno-interactive with a protein. See Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity

[0056] The term "chimeric", when used in reference to a molecule, means that the molecule contains portions that are derived from, obtained or isolated from, or based upon two or more different origins or sources. Thus, a polypeptide is chimeric when it comprises two or more amino acid sequences of different origin and includes (1) polypeptide sequences that are not found together in nature (*i.e.*, at least one of the amino acid sequences is heterologous with respect to at least one of its other amino acid sequences), or (2) amino acid sequences that are not naturally adjoined.

[0057] By "coding sequence" is meant any nucleic acid sequence that contributes to the code for the polypeptide product of a gene or for the final mRNA product of a gene (*e.g.* the mRNA product of a gene following splicing). By contrast, the term "non-coding sequence" refers to any nucleic acid sequence that does not contribute to the code for the polypeptide product of a gene or for the final mRNA product of a gene.

[0058] The terms "coiled coil" or "coiled coil structure" are used interchangeably herein to refer to a structural motif in proteins, in which two or more α-helices (most often 2-7 α-helices) are coiled together like the strands of a rope (dimers and trimers are the most common types). Many coiled coil type proteins are involved in important biological functions such as the regulation of gene expression *e.g.*, transcription factors. Coiled coils often, but not always, contain a repeated pattern, hpphppp or hppphpp, of hydrophobic (h) and polar (p) amino-acid residues, referred to as a heptad repeat (see herein below). Folding a sequence with this repeating pattern into an α-helical secondary structure causes the hydrophobic residues to be presented as a ' stripe' that coils gently around the helix in left-handed fashion, forming an amphipathic structure. The most favorable way for two such helices to arrange themselves in a water-filled environment of is to wrap the hydrophobic strands against each other sandwiched between the hydrophilic amino acids. It is thus the burial of hydrophobic surfaces, which provides the thermodynamic driving force for oligomerization of the α-helices. The packing in a coiled-coil interface is exceptionally tight. The α-helices may be parallel or anti-parallel, and usually adopt a left-handed super-coil. Although disfavored, a few right-handed coiled coils have also been observed in nature and in designed proteins. The terms "coiled coil" or "coiled coil structure" will be clear to the person skilled in the art based on the common general knowledge. Particular reference in this regard is made to review papers concerning coiled coil structures, such as for example, Cohen and Parry (1990. *Proteins* 7:1-15); Kohn and Hodges (1998. *Trends Biotechnol* 16:379- 389); Schneider *et al.* (1998. *Fold Des* 3:R29-R40); Harbury *et al.* (1998. *Science* 282:1462-1467); Mason and Arndt (2004. *ChemBioChem* 5:170-176); Lupas and Gruber (2005. *Adv Protein Chem* 70:37-78); Woolfson (2005. *Adv*

Protein Chem 70:79-112); Parry *et al.* 2008. *J Struct Biol* 163:258-269); and Mcfarlane *et al.* (2009. *Eur J Pharmacol* 625:101-107)..

[0059] As used herein the term "complementary" and grammatically equivalent expressions thereof refer to the characteristic of two or more structural elements (*e.g.*, peptide, polypeptide, nucleic acid, small molecule, or portions thereof etc.) of being able to hybridize, oligomerize (*e.g.*, dimerize), interact or otherwise form a complex with each other. For example, "complementary regions of a polypeptide" are capable of coming together to form a complex, which is characterized in specific embodiments by an anti-parallel, two-helix bundle.

[0060] As used herein, the term "complex" refers to an assemblage or aggregate of molecules (*e.g.*, peptides, polypeptides, etc.) in direct and/or indirect contact with one another. In specific embodiments, "contact", or more particularly, "direct contact" means two or more molecules are close enough so that attractive noncovalent interactions, such as Van der Waal forces, hydrogen bonding, ionic and hydrophobic interactions, and the like, dominate the interaction of the molecules. In such embodiments, a complex of molecules (*e.g.*, a peptide and polypeptide) is formed under conditions such that the complex is thermodynamically favored (*e.g.*, compared to a non-aggregated, or non-complexed, state of its component molecules). As used herein the term "complex", unless described as otherwise, refers to the assemblage of two or more molecules (*e.g.*, peptides, polypeptides or a combination thereof). In specific embodiments, the term "complex" refers to the assemblage of three polypeptides.

[0061] The term "compound library" as used herein refers to any collection of compounds, which includes a plurality of molecules of different structure. Compound libraries may include combinatorial chemical libraries or natural products libraries. Any type of molecule that is capable of interacting, binding or has affinity for a chimeric polypeptide or complex of the present invention, through interactions inclusive of non-covalent interactions, such as, for example, through hydrogen bonds, ionic bonds, van der Waals attractions, or hydrophobic interactions, may be present in the compound library. For example, compound libraries encompasses by this invention may contain naturally-occurring molecules, such as carbohydrates, monosaccharides, oligosaccharides, polysaccharides, amino acids, peptides, oligopeptides, polypeptides, proteins, receptors, nucleic acids, nucleosides, nucleotides, oligonucleotides, polynucleotides, including DNA and DNA fragments, RNA and RNA fragments and the like, lipids, retinoids, steroids, glycopeptides, glycoproteins, proteoglycans and the like; or analogs or derivatives of naturally-occurring molecules, such as peptidomimetics and the like; and non-naturally occurring molecules, such as "small molecule" organic compounds generated, for example, using combinatorial chemistry techniques; and mixtures thereof.

[0062] Throughout this specification, unless the context requires otherwise, the words "comprise," "comprises" and "comprising" will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements. Thus, use of the term "comprising" and the like indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present. By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present. By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere

with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

[0063] As used herein, the terms "conjugated", "linked", "fused" or "fusion" and their grammatical equivalents, in the context of joining together of two more elements or components or domains by whatever means including chemical conjugation or recombinant means (*e.g.*, by genetic fusion) are used interchangeably. Methods of chemical conjugation (*e.g.*, using heterobifunctional crosslinking agents) are known in the art. More specifically, as used herein, an "enveloped virus fusion protein ectodomain" - "structure-stabilizing moiety" fusion or conjugate refers to the genetic or chemical conjugation of an enveloped virus fusion protein ectodomain, which is suitably in a metastable, pre-fusion conformation, to a structure-stabilizing moiety. In specific embodiments, the structure-stabilizing moiety is fused indirectly to a enveloped virus fusion protein ectodomain, *via* a linker, such as a glycine-serine (gly-ser) linker. In other embodiments, the structure-stabilizing moiety is fused directly to a enveloped virus fusion protein ectodomain

[0064] A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, which can be generally sub-classified as follows:

TABLE 1

AMINO ACID SUB-CLASSIFICATION

SUB-CLASSES	AMINO ACIDS
Acidic	Aspartic acid, Glutamic acid
Basic	Noncyclic: Arginine, Lysine; Cyclic: Histidine
Charged	Aspartic acid, Glutamic acid, Arginine, Lysine, Histidine
Small	Glycine, Serine, Alanine, Threonine, Proline
Polar/neutral	Asparagine, Histidine, Glutamine, Cysteine, Serine, Threonine
Polar/large	Asparagine, Glutamine
Hydrophobic	Tyrosine, Valine, Isoleucine, Leucine, Methionine, Phenylalanine, Tryptophan
Aromatic	Tryptophan, Tyrosine, Phenylalanine
Residues that influence chain orientation	Glycine and Proline

[0065] Conservative amino acid substitution also includes groupings based on side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. For example, it is reasonable to expect that replacement of a leucine with an isoleucine or valine, an aspartate

with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the properties of the resulting variant polypeptide. Whether an amino acid change results in a functional polypeptide can readily be determined by assaying its activity. Conservative substitutions are shown in Table 2 under the heading of exemplary and preferred substitutions. Amino acid substitutions falling within the scope of the invention, are, in general, accomplished by selecting substitutions that do not differ significantly in their effect on maintaining (a) the structure of the peptide backbone in the area of the substitution, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. After the substitutions are introduced, the variants are screened for biological activity.

TABLE 2

EXEMPLARY AND PREFERRED AMINO ACID SUBSTITUTIONS

ORIGINAL RESIDUE	EXEMPLARY SUBSTITUTIONS	PREFERRED SUBSTITUTIONS
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln, His, Lys, Arg	Gln
Asp	Glu	Glu
Cys	Ser	Ser
Gln	Asn, His, Lys,	Asn
Glu	Asp, Lys	Asp
Gly	Pro	Pro
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleu	Leu
Leu	Norleu, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, Gln, Asn	Arg
Met	Leu, Ile, Phe	Leu
Phe	Leu, Val, Ile, Ala	Leu
Pro	Gly	Gly
Ser	Thr	Thr
Thr	Ser	Ser
Trp	Tyr	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Leu, Met, Phe, Ala, Norleu	Leu

[0066] The term "construct" refers to a recombinant genetic molecule including one or more isolated nucleic acid sequences from different sources. Thus, constructs are chimeric molecules in which two or more nucleic acid sequences of different origin are assembled into a single nucleic acid molecule and include any construct that contains (1) nucleic acid sequences, including regulatory and coding sequences that are not found together in nature (*i.e.*, at least one of the nucleotide sequences is heterologous with respect to at least one of its other nucleotide sequences), or (2) sequences encoding parts of functional RNA molecules or proteins not naturally adjoined, or (3) parts of promoters that are not naturally adjoined. Representative constructs include any recombinant nucleic acid molecule such as a plasmid, cosmid, virus, autonomously

replicating polynucleotide molecule, phage, or linear or circular single stranded or double stranded DNA or RNA nucleic acid molecule, derived from any source, capable of genomic integration or autonomous replication, comprising a nucleic acid molecule where one or more nucleic acid molecules have been operably linked. Constructs of the present invention will generally include the necessary elements to direct expression of a nucleic acid sequence of interest that is also contained in the construct, such as, for example, a target nucleic acid sequence or a modulator nucleic acid sequence. Such elements may include control elements such as a promoter that is operably linked to (so as to direct transcription of) the nucleic acid sequence of interest, and often includes a polyadenylation sequence as well. Within certain embodiments of the invention, the construct may be contained within a vector. In addition to the components of the construct, the vector may include, for example, one or more selectable markers, one or more origins of replication, such as prokaryotic and eukaryotic origins, at least one multiple cloning site, and/or elements to facilitate stable integration of the construct into the genome of a host cell. Two or more constructs can be contained within a single nucleic acid molecule, such as a single vector, or can be containing within two or more separate nucleic acid molecules, such as two or more separate vectors. An "expression construct" generally includes at least a control sequence operably linked to a nucleotide sequence of interest. In this manner, for example, promoters in operable connection with the nucleotide sequences to be expressed are provided in expression constructs for expression in an organism or part thereof including a host cell. For the practice of the present invention, conventional compositions and methods for preparing and using constructs and host cells are well known to one skilled in the art, see for example, Molecular Cloning: A Laboratory Manual, 3rd edition Volumes 1, 2, and 3. J. F. Sambrook, D. W. Russell, and N. Irwin, Cold Spring Harbor Laboratory Press, 2000.

[0067] By "corresponds to" or "corresponding to" is meant an amino acid sequence that displays substantial sequence similarity or identity to a reference amino acid sequence. In general the amino acid sequence will display at least about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 97, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% or even up to 100% sequence similarity or identity to at least a portion of the reference amino acid sequence.

[0068] The term "domain", as used herein, refers to a part of a molecule or structure that shares common physicochemical features, such as, but not limited to, hydrophobic, polar, globular and helical domains or properties such as ligand-binding, membrane fusion, signal transduction, cell penetration and the like. Often, a domain has a folded protein structure which has the ability to retain its tertiary structure independently of the rest of the protein. Generally, domains are responsible for discrete functional properties of proteins, and in many cases may be added, removed or transferred to other proteins without loss of function of the remainder of the protein and/or of the domain. Domains may be co-extensive with regions or portions thereof; domains may also include distinct, non-contiguous regions of a molecule. Examples of protein domains include, but are not limited to, a cellular or extracellular localization domain (*e.g.*, signal peptide; SP), an immunoglobulin (Ig) domain, a membrane fusion (*e.g.*, fusion peptide; FP) domain, an ectodomain, a membrane proximal external region (MPER) domain, a transmembrane (TM) domain, and a cytoplasmic (C) domain.

[0069] By "effective amount," in the context of eliciting an immune response to a fusion protein of an enveloped virus, or complex of the fusion protein, or of treating or preventing a disease or condition, is meant the administration of an amount of agent to an individual in need

thereof, either in a single dose or as part of a series, that is effective for that elicitation, treatment or prevention. The effective amount will vary depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated, the formulation of the composition, the assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

[0070] The term "endogenous" refers to a polypeptide or part thereof that is present and/or naturally expressed within a host organism or cell thereof. For example, an "endogenous" ectodomain polypeptide or part thereof refers to an ectodomain polypeptide of an enveloped fusion protein or a part of that ectodomain that is naturally expressed in enveloped virus.

[0071] As used herein, the term "endogenous HRA region" refers to an HRA region that is present in a Class I ectodomain polypeptide at substantially the same position as the HRA region in the amino acid sequence of the fusion protein precursor form of the naturally occurring fusion protein. The approximate amino acid positions of endogenous HRA regions of non-limiting examples of class I fusion proteins are listed in Table 3.

TABLE 3

APPROXIMATE POSITIONS OF HRA REGIONS IN SELECTED CLASS I FUSION PROTEINS

Class I Fusion Protein	HRA region	
	Start position	End position
Influenza A hemagglutinin (HA)	356	390
Influenza B HA	383	416
RSV Fusion protein (F)	164	196
hMPV F	126	169
PIV F	136	168
Measles F	138	171
Hendra	136	169
Nipah F	136	169
HIV glycoprotein (GP) 160	539	587
Ebola GP	557	593
Marburg GP	582	598
SARS spike protein (S)	892	1013
MERS S	984	1105

[0072] As used herein, the term "endogenous HRB region" refers to an HRB region that is present in a Class I ectodomain polypeptide at substantially the same position as the HRB region in the amino acid sequence of the fusion protein precursor form of the naturally occurring fusion protein. The approximate amino acid positions of endogenous HRB regions of non-limiting examples of class I fusion proteins are listed Table 4.

TABLE 4

APPROXIMATE POSITIONS OF HRB REGIONS IN SELECTED CLASS I FUSION PROTEINS

Class I Fusion Protein	HRB region	
	Start position	End position
Influenza A HA	421	469
Influenza B HA	436	487
RSV F	488	524
hMPV F	456	490
PIV F	458	493
Measles F	454	493
Hendra	456	487
Nipah F	456	487
HIV GP160	631	667
Ebola GP	600	635
Marburg GP	611	627
SARS S	1145	1187
MERS S	1248	1291

[0073] The term "endogenous production" refers to expression of a nucleic acid in an organism and the associated production and/or secretion of an expression product of the nucleic acid in the organism. In specific embodiments, the organism is multicellular (*e.g.*, a vertebrate animal, preferably a mammal, more preferably a primate such as a human) and the nucleic acid is expressed within cells or tissues of the multicellular organism.

[0074] As used herein, "enveloped virus fusion ectodomain polypeptide" refers to a polypeptide that contains an virion surface exposed portion of a mature enveloped virus fusion protein, with or without the signal peptide but lacks the transmembrane domain and cytoplasmic tail of the naturally occurring enveloped virus fusion protein.

[0075] The terms "epitope" and "antigenic determinant" are used interchangeably herein to refer to an antigen, typically a protein determinant, that is capable of specific binding to an antibody (such epitopes are often referred to as "B cell epitopes") or of being presented by a Major Histocompatibility Complex (MHC) protein (*e.g.*, Class I or Class II) to a T-cell receptor (such epitopes are often referred to as "T cell epitopes"). Where a B cell epitope is a peptide or polypeptide, it typically comprises three or more amino acids, generally at least 5 and more usually at least 8 to 10 amino acids. The amino acids may be adjacent amino acid residues in the primary structure of the polypeptide (often referred to as contiguous peptide sequences), or may become spatially juxtaposed in the folded protein (often referred to as non-contiguous peptide sequences). T cell epitopes may bind to MHC Class I or MHC Class II molecules. Typically MHC Class I-binding T cell epitopes are 8 to 11 amino acids long. Class II molecules bind peptides that may be 10 to 30 residues long or longer, the optimal length being 12 to 16 residues. The ability of a putative T cell epitope to bind to an MHC molecule can be predicted and confirmed experimentally (Dimitrov *et al.*, 2010. Bioinformatics 26(16):2066-8).

[0076] The term "helix bundle" refers to a plurality of peptide helices that fold such that the helices are substantially parallel or anti-parallel to one another. A two-helix bundle has two helices folded such that they are substantially parallel or anti-parallel to one another. Likewise, a six-helix bundle has six helices folded such that they are substantially parallel or anti-parallel to one another. By "substantially parallel or anti-parallel" is meant that the helices are folded such that the side chains of the helices are able to interact with one another. For example, the hydrophobic side chains of the helices are able to interact with one another to form a hydrophobic core.

[0077] The term "heterologous" as used herein refers to any proteinaceous moiety whose sequence is chosen in such a way that the product of the fusion of this sequence with an ectodomain polypeptide has a sequence different from a precursor or mature form of a wild-type enveloped virus fusion protein.

[0078] The term "host" refers to any organism, or cell thereof, whether eukaryotic or prokaryotic into which a construct of the invention can be introduced, particularly, hosts in which RNA silencing occurs. In particular embodiments, the term "host" refers to eukaryotes, including unicellular eukaryotes such as yeast and fungi as well as multicellular eukaryotes such as animals non-limiting examples of which include invertebrate animals (*e.g.*, insects, cnidarians, echinoderms, nematodes, *etc.*); eukaryotic parasites (*e.g.*, malarial parasites, such as *Plasmodium falciparum*, helminths, *etc.*); vertebrate animals (*e.g.*, fish, amphibian, reptile, bird, mammal); and mammals (*e.g.*, rodents, primates such as humans and non-human primates). Thus, the term "host cell" suitably encompasses cells of such eukaryotes as well as cell lines derived from such eukaryotes.

[0079] Reference herein to "immuno-interactive" includes reference to any interaction, reaction, or other form of association between molecules and in particular where one of the molecules is, or mimics, a component of the immune system.

[0080] As use herein, the term "immunogenic composition" or "immunogenic formulation" refers to a preparation which, when administered to a vertebrate, especially an animal such as a mammal, will induce an immune response.

[0081] By "linker" is meant a molecule or group of molecules (such as a monomer or polymer) that connects two molecules and often serves to place the two molecules in a desirable configuration.

[0082] As used herein, the term "meta-stable", as used in the context of a protein (*e.g.*, an enveloped virus ectodomain polypeptide), refers to a labile conformational state that rapidly converts to a more stable conformational state upon a change in conditions. For example, an enveloped virus fusion protein in a pre-fusion form is in a labile, meta-stable conformation, and converts to the more stable post-fusion conformation upon, *e.g.*, fusion to a host cell.

[0083] As used herein, the term "moiety" refers to a portion of a molecule, which may be a functional group, a set of functional groups, and/or a specific group of atoms within a molecule, that is responsible for a characteristic chemical, biological, and/or medicinal property of the molecule.

[0084] The term "neutralizing antigen-binding molecule" refers to an antigen-binding molecule that binds to or interacts with a target molecule or ligand and prevents binding or association of the target antigen to a binding partner such as a receptor or substrate, thereby interrupting the biological response that otherwise would result from the interaction of the molecules. In the case of the instant invention a neutralizing antigen-binding molecule suitably associates with a metastable or pre-fusion form of an enveloped virus fusion protein and preferably interferes or reduces binding and/or fusion of the fusion protein to a cell membrane.

[0085] The term "oligomer" refers to a molecule that consists of more than one but a limited number of monomer units in contrast to a polymer that, at least in principle, consists of an unlimited number of monomers. Oligomers include, but are not limited to, dimers, trimers, tetramers, pentamers, hexamers, heptamers, octamers, nonamers, decamers and the like. An oligomer can be a macromolecular complex formed by non-covalent bonding of macromolecules like proteins. In this sense, a homo-oligomer would be formed by identical molecules and by contrast, a hetero-oligomer would be made of at least two different molecules. In specific embodiments, an oligomer of the invention is a trimeric polypeptide complex consisting of three polypeptide subunits. In these embodiments, the trimeric polypeptide may be a "homotrimeric polypeptide complex" consisting of three identical polypeptide subunits, or a "heterotrimeric polypeptide complex" consisting of three polypeptide subunits in which at least one subunit polypeptide is non-identical. A "polypeptide subunit" is a single amino acid chain or monomer that in combination with two other polypeptide subunits forms a trimeric polypeptide complex.

[0086] The term "operably connected" or "operably linked" as used herein refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For example, a regulatory sequence (*e.g.*, a promoter) "operably linked" to a nucleotide sequence of interest (*e.g.*, a coding and/or non-coding sequence) refers to positioning and/or orientation of the control sequence relative to the nucleotide sequence of interest to permit expression of that sequence under conditions compatible with the control sequence. The control sequences need not be contiguous with the nucleotide sequence of interest, so long as they function to direct its expression. Thus, for example, intervening non-coding sequences (*e.g.*, untranslated, yet transcribed, sequences) can be present between a promoter and a coding sequence, and the promoter sequence can still be considered "operably linked" to the coding sequence. Likewise, "operably connecting" an enveloped virus fusion ectodomain polypeptide to a heterologous, structure-stabilizing moiety encompasses positioning and/or orientation of the structure-stabilizing moiety such that the complementary HR1 and HR2 regions are permitted to associate with each other under conditions suitable for their association (*e.g.*, in aqueous solution) to form an anti-parallel, two-helix bundle.

[0087] The terms "patient", "subject", "host" or "individual" used interchangeably herein, refer to any subject, particularly a vertebrate subject, and even more particularly a mammalian subject, for whom therapy or prophylaxis is desired. Suitable vertebrate animals that fall within the scope of the invention include, but are not restricted to, any member of the subphylum Chordata including primates (*e.g.*, humans, monkeys and apes, and includes species of monkeys such from the genus *Macaca* (*e.g.*, cynomologus monkeys such as *Macaca fascicularis*, and/or rhesus monkeys (*Macaca mulatta*)) and baboon (*Papio ursinus*), as well as marmosets (species from the genus *Callithrix*), squirrel monkeys (species from the genus *Saimiri*) and

tamarins (species from the genus *Saguinus*), as well as species of apes such as chimpanzees (*Pan troglodytes*)), rodents (*e.g.*, mice rats, guinea pigs), lagomorphs (*e.g.*, rabbits, hares), bovines (*e.g.*, cattle), ovines (*e.g.*, sheep), caprines (*e.g.*, goats), porcines (*e.g.*, pigs), equines (*e.g.*, horses), canines (*e.g.*, dogs), felines (*e.g.*, cats), avians (*e.g.*, chickens, turkeys, ducks, geese, companion birds such as canaries, budgerigars *etc.*), marine mammals (*e.g.*, dolphins, whales), reptiles (snakes, frogs, lizards *etc.*), and fish. A preferred subject is a human in need of eliciting an immune response to a fusion protein of an enveloped virus, or complex of the fusion protein. However, it will be understood that the aforementioned terms do not imply that symptoms are present.

[0088] By "pharmaceutically acceptable carrier" is meant a solid or liquid filler, diluent or encapsulating substance that can be safely used in topical or systemic administration to an animal, preferably a mammal, including humans. Representative pharmaceutically acceptable carriers include any and all solvents, dispersion media, coatings, surfactants, antioxidants, preservatives (*e.g.*, antibacterial agents, antifungal agents), isotonic agents, absorption delaying agents, salts, preservatives, drugs, drug stabilizers, gels, binders, excipients, disintegration agents, lubricants, sweetening agents, flavoring agents, dyes, such like materials and combinations thereof, as would be known to one of ordinary skill in the art (see, for example, Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1990, pp. 1289-1329, incorporated herein by reference). Except insofar as any conventional carrier is incompatible with the active ingredient(s), its use in the pharmaceutical compositions is contemplated.

[0089] The term "polynucleotide" or "nucleic acid" as used herein designates mRNA, RNA, cRNA, cDNA or DNA. The term typically refers to polymeric forms of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

[0090] "Polypeptide", "peptide", "protein" and "proteinaceous molecule" are used interchangeably herein to refer to molecules comprising or consisting of a polymer of amino acid residues and to variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues are synthetic non-naturally occurring amino acids, such as a chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally-occurring amino acid polymers.

[0091] As used herein, the term "post-fusion conformation" of a fusion protein of an enveloped virus refers to the structure of an enveloped virus fusion protein, which is in a terminal conformation (i.e., formed at the end of the fusion process) and is the most energetically favorable state. In the post-fusion conformation, the fusion peptides or loops of the fusion protein are brought into close proximity with the fusion protein transmembrane domain. The specific structural elements that facilitate formation of the hairpin structure vary according to the class of enveloped fusion protein. For example, the post-fusion conformation of a Class I fusion protein is characterized by interaction between the endogenous HRA region and the endogenous HRB region of individual Class I fusion proteins to form a hairpin structure characterized by a six-helix bundle, comprising three endogenous HRB and three endogenous HRA regions. Alternatively, the post-fusion conformation of a Class III fusion protein is characterized by interaction between the internal fusion loops and the C-terminal transmembrane region which facilitates the formation of a hairpin structure. Post-fusion conformations of individual viral fusion proteins have been determined by

electron microscopy and/or x-ray crystallography, such structures are readily identifiable when viewed in negatively stained electron micrographs and/or by a lack of pre-fusion epitopes.

[0092] As used herein, the term "pre-fusion conformation" of a fusion protein of an enveloped virus refers to the structure of an enveloped virus fusion protein, which is in a meta-stable confirmation (*i.e.*, in a semi-stable conformation that is not the most energetically favorable terminal conformation) and upon appropriate triggering is able to undergo conformational rearrangement to the terminal post-fusion conformation. Typically pre-fusion conformations of viral fusion proteins contain an hydrophobic sequence, referred to as the fusion peptide or fusion loop, that is located internally within the pre-fusion conformation and cannot interact with either the viral or host cell membranes. Upon triggering this hydrophobic sequence is inserted into the host cell membrane and the fusion protein collapses into the post-fusion hairpin like conformation. The pre-fusion conformation of viral fusion proteins vary according to the class of enveloped fusion protein. Each class is characterized by non-interacting structural elements that subsequently associate in the energetically favorable post-fusion conformation. For example, the pre-fusion conformation of a Class I fusion protein is dependent on the endogenous HRA region not interacting with the endogenous HRB region of individual fusion proteins of the trimer, thereby not permitting formation of a hairpin structure characterized by a six-helix bundle. Alternatively, the pre-fusion conformation of a Class III fusion protein is dependent a central α-helical coiled coil not interacting with fusion loop(s) at the C-terminal region of individual fusion proteins of the trimer, thereby not permitting formation of a hairpin structure. Pre-fusion conformations of individual viral fusion proteins have been determined by electron microscopy and/or x-ray crystallography, such structures are readily identifiable when viewed in negatively stained electron micrographs and/or by pre-fusion epitopes that are not present on post-fusion conformations.

[0093] "Regulatory elements", "regulatory sequences", control elements", "control sequences" and the like are used interchangeably herein to refer to nucleotide sequences located upstream (5′ non-coding sequences), within, or downstream (3′ non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence, either directly or indirectly. Regulatory elements include enhancers, promoters, translation leader sequences, introns, Rep recognition element, intergenic regions and polyadenylation signal sequences. They include natural and synthetic sequences as well as sequences which may be a combination of synthetic and natural sequences.

[0094] The term "replicon" refers to any genetic element, *e.g.*, a plasmid, a chromosome, a virus, a cosmid, etc., that behaves as an autonomous unit of polynucleotide replication within a cell, *i.e.*, capable of replication under its own control.

[0095] "Self-assembly" refers to a process of spontaneous assembly of a higher order structure that relies on the natural attraction of the components of the higher order structure (*e.g.*, molecules) for each other. It typically occurs through random movements of the molecules and formation of bonds based on size, shape, composition, or chemical properties.

[0096] The term "sequence identity" as used herein refers to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions

at which the identical nucleic acid base (*e.g.*, A, T, C, G, I) or the identical amino acid residue (*e.g.*, Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (*i.e.*, the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The present invention contemplates the use in the methods and systems of the present invention of full-length IL-22 polypeptides as well as their biologically active fragments. Typically, biologically active fragments of a full-length IL-22 polypeptide may participate in an interaction, for example, an intra-molecular or an inter-molecular interaction.

[0097] "Similarity" refers to the percentage number of amino acids that are identical or constitute conservative substitutions as defined in Tables 1 and 2 *supra*. Similarity may be determined using sequence comparison programs such as GAP (Deveraux *et al.* 1984, *Nucleic Acids Research* 12: 387-395). In this way, sequences of a similar or substantially different length to those cited herein might be compared by insertion of gaps into the alignment, such gaps being determined, for example, by the comparison algorithm used by GAP.

[0098] Terms used to describe sequence relationships between two or more polynucleotides or polypeptides include "reference sequence," "comparison window", "sequence identity," "percentage of sequence identity" and "substantial identity". A "reference sequence" is at least 12 but frequently 15 to 18 and often at least 25 monomer units, inclusive of nucleotides and amino acid residues, in length. Because two polynucleotides may each comprise (1) a sequence (*i.e.*, only a portion of the complete polynucleotide sequence) that is similar between the two polynucleotides, and (2) a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of at least 6 contiguous positions, usually about 50 to about 100, more usually about 100 to about 150 in which a sequence is compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. The comparison window may comprise additions or deletions (*i.e.*, gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerized implementations of algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, WI, USA) or by inspection and the best alignment (*i.e.*, resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul *et al.*, 1997, *Nucl. Acids Res.* 25:3389. A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel *et al.*, "Current Protocols in Molecular Biology", John Wiley & Sons Inc, 1994-1998, Chapter 15.

[0099] As used herein, the term "single-chain" refers to a molecule comprising amino acid monomers linearly linked by peptide bonds.

[0100] As used herein a "small molecule" refers to a composition that has a molecular weight of less than 3 kilodaltons (kDa), and typically less than 1.5 kilodaltons, and more preferably less than about 1 kilodalton. Small molecules may be nucleic acids, peptides, polypeptides,

peptidomimetics, carbohydrates, lipids or other organic (carbon-containing) or inorganic molecules. As those skilled in the art will appreciate, based on the present description, extensive libraries of chemical and/or biological mixtures, often fungal, bacterial, or algal extracts, may be screened with any of the assays of the invention to identify compounds that modulate a bioactivity. A "small organic molecule" is an organic compound (or organic compound complexed with an inorganic compound (*e.g.*, metal)) that has a molecular weight of less than 3 kilodaltons, less than 1.5 kilodaltons, or even less than about 1 kDa.

[0101] As used herein, the terms "treatment", "treating", and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse affect attributable to the disease. "Treatment", as used herein, covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, *i.e.*, arresting its development; and (c) relieving the disease, *i.e.*, causing regression of the disease.

[0102] The terms "wild-type", "native" and "naturally occurring" are used interchangeably herein to refer to a gene or gene product that has the characteristics of that gene or gene product when isolated from a naturally occurring source. A wild type, native or naturally occurring gene or gene product (*e.g.*, a polypeptide) is that which is most frequently observed in a population and is thus arbitrarily designed the "normal" or "wild-type" form of the gene or gene product.

[0103] Each embodiment described herein is to be applied *mutatis mutandis* to each and every embodiment unless specifically stated otherwise.

2. Chimeric polypeptides

[0104] The present invention is predicated in part on a novel strategy for artificially stabilizing or 'clamping' an enveloped virus fusion protein ectodomain polypeptide in a pre-fusion conformation. This 'molecular clamping' strategy employs fusion or linkage of a structure-stabilizing moiety to an ectodomain polypeptide to form a chimeric polypeptide. The structure-stabilizing moiety is typically a single-chain polypeptide comprising complementary heptad repeats that lack complementarity to the ectodomain polypeptide and that therefore preferentially associate with each other rather than with structural elements of the ectodomain polypeptide. Association of the complementary heptad repeats to one another under conditions suitable for their association (*e.g.*, in aqueous solution) results in formation of an anti-parallel, two-helix bundle that inhibits rearrangement of the ectodomain polypeptide to a post-fusion conformation. The two-helix bundle of the structure-stabilizing moiety can trimerize to form a highly stable six-helix bundle, thus permitting self assembly of the chimeric polypeptide to form an artificial enveloped virus fusion protein complex. The complex so assembled can mimic the pre-fusion conformation of a native enveloped virus fusion protein complex and comprises three chimeric polypeptides, characterized by a six-helix bundle formed by the coiled coil structures of the respective structure-stabilizing moieties of the chimeric polypeptides.

2.1 Structure-stabilizing moieties

[0105] The present inventors have constructed a single-chain polypeptide moiety comprising complementary heptad repeats that fold into an anti-parallel configuration, forming an anti-parallel, two-helix bundle that stabilizes an operably connected enveloped virus ectodomain polypeptide in a pre-fusion conformation. The two-helix bundle suitably forms a coiled coil structure. The coiled coil fold occurs in a wide variety of proteins including motor proteins, DNA-binding proteins, extracellular proteins and viral fusion proteins (see, *e.g.*, Burkhard *et al.*, 2001. *Trends Cell Biol* 11:82-88). Coiled coils have been functionally characterized as folding (assembly, oligomerization) motifs, *i.e.*, formation of a coiled coil structure drives in many instances the non-covalent association of different protein chains. Coiled coils have been structurally characterized as 2-, 3-, 4- or 5 -stranded assemblies of α-helices arranged in parallel, anti-parallel or mixed topologies (see, *e.g.*, Lupas, 1996. *Trends Biochem Sci* 21:375- 382). Usually, the helices are slightly wrapped (coiled, wound) around each other in a left- or right-handed manner, termed supercoiling. It will be understood that the two-helix bundles of the present invention generally form coiled coil structures with a strong propensity to trimerize in order to form a highly stable six-helical coiled coil bundle.

2.1.1 Heptad repeats

[0106] Alpha-helical coiled coils have been characterized at the level of their amino acid sequences, in that, each helix is constituted of a series of heptad repeats. A heptad repeat (heptad unit, heptad) is a 7-residue sequence motif which can be encoded as hpphppp, and wherein each 'h' represents a hydrophobic residue and each 'p' is a polar residue. Occasionally, p-residues are observed at h-positions, and *vice versa*. A heptad repeat is also often encoded by the patterns a-b-c-d-e-f-g (abcdefg) or d-e-f -g-a-b-c (defgabc), in which case the indices 'a' to 'g' refer to the conventional heptad positions at which typical amino acid types are observed. By convention, indices 'a' and 'd' denote the positions of the core residues (central, buried residues) in a coiled coil. The typical amino acid types that are observed at core a- and d-positions are hydrophobic amino acid residue types; at all other positions (non-core positions) , predominantly polar (hydrophilic) residue types are observed. Thus, conventional heptad patterns 'hpphppp' match with the pattern notation 'abcdefg' ('hpppphpp' patterns match with the pattern notation 'defgabc', this notation being used for coiled coils starting with a hydrophobic residue at a d-position). The heptad repeat regions of the present invention include at least 2, and suitably 3 or more consecutive (uninterrupted) heptad repeats in individual α-helices of the coiled coil structure. Each series of consecutive heptad repeats in a helix is denoted a 'heptad repeat sequence' (HRS). The start and end of a heptad repeat sequence is preferably determined on the basis of the experimentally determined 3-dimensional (3-D) structure, if available. If a 3-D structure is not available, the start and end of a heptad repeat sequence is preferably determined on the basis of an optimal overlay of a $(hpphppp)_n$ or $(hpppphpp)_n$ pattern with the actual amino acid sequence, where 'h' and 'p' denote hydrophobic and polar residues, respectively, and where 'n' is a number equal to or greater than 2. The start and end of each heptad repeat sequence is taken to be the first and last hydrophobic residue at an a- or d-position, respectively. Conventional H-residues are preferably selected from the group consisting of valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, tryptophan, histidine, glutamine, threonine, serine and alanine, more preferably from the group consisting of valine, isoleucine, leucine and methionine, and most preferably isoleucine. Conventional p-residues are preferably selected from the group consisting of glycine, alanine, cysteine, serine, threonine,

histidine, asparagine, aspartic acid, glutamine, glutamic acid, lysine and arginine. In case this method does not permit unambiguous assignment of amino acid residues to a heptad repeat sequence, a more specialized analysis method can be applied, such as the COILS method of Lupas *et al.* (1991. *Science* 252:1162-1164; http://www.russell.embl-heidelberg.de/cgi -bin/coils-svr.pl).

[0107] In particular embodiments, each heptad repeat region (HR1, HR2) is independently characterized by a n-times repeated 7-residue pattern of amino acid types, represented as (a-b-c-d-e-f-g-)$_n$ or (d-e-f-g-a-b-c-)$_n$, as described for example in WO 2010/066740, the content of which is incorporated by reference herein in its entirety, wherein the pattern elements 'a' to 'g' denote conventional heptad positions at which the amino acid types are located and n is a number equal to or greater than 2, and at least 50% (or at least 51% to at least 99% and all integer percentages in between) of the conventional heptad positions 'a' and 'd are occupied by hydrophobic amino acid types and at least 50% (or at least 51% to at least 99% and all integer percentages in between) of the conventional heptad positions 'b', 'c', 'e', 'f' and 'g' are occupied by hydrophilic amino acid types, the resulting distribution between hydrophobic and hydrophilic amino acid types enabling the identification of the heptad repeat regions. In specific embodiments, at least 50%, 70%, 90%, or 100% of the conventional heptad positions 'a' and 'd' are occupied by amino acids selected from the group consisting of valine, isoleucine, leucine, methionine or non-natural derivatives thereof. Since the latter amino acids correspond to more standard (more frequently observed) coiled coil core residues. In other embodiments, at least 50%, 70%, 90%, or 100% of the conventional heptad positions 'a' and 'd' are occupied by isoleucines. In some embodiments, at least 50%, 70%, 90%, or 100% of the conventional heptad positions 'b', 'c', 'e', 'f' and 'g' are occupied by amino acids selected from the group consisting of glycine, alanine, cysteine, serine, threonine, histidine, asparagine, aspartic acid, glutamine, glutamic acid, lysine, arginine or non-natural derivatives thereof. In illustrative examples of this type, the HR1 and HR2 regions comprise, consist or consist essentially of the sequence: IEEIQKQIAAIQKQIAAIQKQIYRM [SEQ ID NO: 1]

[0108] In particular embodiments, the HR1 and HR2 regions of the structure-stabilizing moiety (also referred to herein as "SSM") comprise at least one endogenous heptad repeat of a Class I enveloped virus fusion protein. Suitably, the HR1 and HR2 regions are formed largely by complementary HRA and HRB regions, respectively, of one or more Class I enveloped virus fusion proteins. The HRA region amino acid sequence and the HRB region amino acid sequence may be derived from the same Class I enveloped virus fusion protein. Alternatively, they may be derived from the different Class I enveloped virus fusion proteins. In representative examples, the HR1 and HR2 regions are independently selected from HRA and HRB regions of orthomyxoviruses (*e.g.*, Influenza A (Inf A), Influenza B (Inf B), Influenza C (Inf C)), paramyxoviruses (*e.g.*, Measles (MeV), Rinderpest virus (RPV), Canine distemper virus (CDV), RSV, Human Metapneumovirus (HMPV), Parainfluenza virus (PIV), Mumps virus (MuV), Hendra virus (HeV), Nipah virus (NiV), Newcastle disease virus (NDV)), retroviruses (*e.g.*, Human T cell leukemia virus type 1 (HTLV-1), HTLV-2, HTLV-3, HIV-1, HIV-2), filoviruses (*e.g.*, Ebola virus (EBOV) including Zaire (ZEBOV), Reston (REBOV) and Sudan (SEBOV) strains, Marburg virus (MARV)), arenaviruses (*e.g.*, Lassa virus (LASV), Lymphocytic choriomeningitis virus (LCMV), Junín virus (JUNV)), and coronaviruses (*e.g.*, Human Coronavirus (HCoV), including HCoV 229E, HCoV OC43, HCoV HKU1, HCoV EMC,

Human Torovirus (HToV), Middle East Respiratory Syndrome virus (MERS-CoV), Severe Acute Respiratory Syndrome virus (SARS-CoV)).

[0109] Exemplary HRA region amino acid sequences include, but are not limited to, those in Table 5:

TABLE 5

HRA REGION SEQUENCES OF SELECTED CLASS I FUSION PROTEINS

VIRUS	HRA REGION SEQUENCE
HIV GP160	SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILA [SEQ ID NO: 2] (GenPept dbjBAF31430.1)
RSV F	LHLEGEVNKIKSALLSTNKAVVSLGNGVSVLTSKVLDLK [SEQ ID NO: 3] (GenPept gbAHL84194.1)
HMPV F	IRLESEVTAIKNALKKTNEAVSTLGNGVRVLATAVRELK [SEQ ID NO: 4] (GenPept gbAAN52913.1)
PIV F	KQARSDIEKLKEAIRDTNKAVQSVQSSIGNLIVAIKSVQ [SEQ ID NO: 5] (GenPept gbAAB21447.1)
MeV F	MLNSQAIDNLRASLETTNQAIEAIRQAGQEMILAVQGVQ [SEQ ID NO: 6] (GenPept dbjBAB60865.1)
HeV F	MKNADNINKLKSSIESTNEAVVKLQETAEKTVYVLTALQ [SEQ ID NO: 7] (GenPept NP_047111.2)
Inf A HA	ENGWEGMVDGWYGFRHQNSEGTGQAADLKSTQAAI [SEQ ID NO: 8] (GenPept gbAEC23340.1)
Inf B HA	HGYTSHGAHGVAVAADLKSTQEAINKITKNLNYL [SEQ ID NO: 9] (GenPept gbAFH57854.1)
EBOV GP	GLRQLANETTQALQLFLRATTELRTFSILNRKAIDFL [SEQ ID NO: 10] (GenPept NP_066246.1)
MARV GP	LANQTAKSLELLLRVTTEERTFSLINRHAIDFLLTRWG [SEQ ID NO: 11] (GenPept YP_001531156.1)
MERS S	ISASIGDIIQRLDVLEQDAQIDRLINGRLTTLNAFVAQQLVRSESAALSAQLAKDKVNE [SEQ ID NO: 12] (GenPept gbAHX00711.1)
SARS S	ISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSE [SEQ ID NO: 13] (GenPept gbAAR86788.1]

[0110] Exemplary HRB region amino acid sequences include, but are not limited to, those in Table 6:

TABLE 6

HRB REGION SEQUENCES OF SELECTED CLASS I FUSION PROTEINS

VIRUS	HRB REGION SEQUENCE
HIV GP160	HTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 14] (GenPept dbjBAF31430.1)
RSV F	FDASISQVNEKINQSLAFIRKSDELLHNVNAGKSTTN [SEQ ID NO: 15] (GenPept gbAHL84194.1)
hMPV F	FNVALDQVFESIENSQALVDQSNRILSSAEKGNTG [SEQ ID NO: 16] (GenPept gbAAN52913.1]
PIV F	IELNKAKSDLEESKEWIRRSNQKLDSIGNWHQSSTT [SEQ ID NO: 17] (GenPept gbAAB21447.1)
MeV F	LERLDVGTNLGNAIAKLEDAKELLESSDQILRSMKGLSST [SEQ ID NO: 18] (GenPept dbjBAB60865.1)
HeV F	ISSQISSMNQSLQQSKDYIKEAQKILDTVNPS [SEQ ID NO: 19] (GenPept NP_047111.2)
Inf A HA	RIQDLEKYVEDTKIDLWSYNAELVLALENQHTIDLTDSEMSKLFERTRR [SEQ ID NO: 20] (GenPept gbAEC23340.1]

Inf B HA	DEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKML [SEQ ID NO: 21] (GenPept gbAFH57854.1]
EBOV GP	HDWTKNITDKIDQIIHDFVDKTL [SEQ ID NO: 22] (GenPept NP_066246.1]
MARV GP	IGIEDLSKNISEQIDQI [SEQ ID NO: 23] (GenPept YP_001531156.1]
MERS S	NFGSLTQINTTLLDLTYEMLSLQQVVKALNESYIDLKELGNYTY [SEQ ID NO: 24] (GenPept gbAHX00711.1]
SARS S	DVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGK [SEQ ID NO: 25] (GenPept gbAAR86788.1]

[0111] The HR1 and HR2 regions are capable of coming together to form an oligomer, typically a hexamer composed of three HR1 regions and three HR2 regions, which is thermodynamically stable and typifies the post-fusion conformation of class I viral fusion proteins. HR1 and HR2 regions with a strong propensity to oligomerize are referred to herein as "complementary" heptad repeat regions. Non-limiting examples of such heptad repeat regions those listed in Table 7.

[0112] In particular embodiments, the structure-stabilizing moiety, including one or both of the heptad repeat regions, includes an immune-silencing or suppressing moiety that inhibits elicitation or production of an immune response to the structure-stabilizing moiety, particularly when folded into an anti-parallel, two-helix bundle. These embodiments are advantageous as they can permit the generation of a stronger or enhanced immune response to the ectodomain polypeptide or complex thereof. The immune-silencing moiety can be a glycosylation site that is specifically recognized and glycosylated by a glycosylation enzyme, in particular a glycosyltransferase. Glycosylations can be N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences N-X-S and N-X-T, where X is any amino acid except P, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain, and these sequences are commonly referred to as 'glycosylation sites'. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used. The immune-silencing moiety may be inserted into the structure stabilizing moiety, including one or both of the heptad repeat regions.

[0113] In other embodiments, unnatural or nonnative amino acids can be incorporated into one or both of the heptad repeat regions using an expanded genetic code. The nonnative amino acids are biosynthetically incorporated into a desired location using tyrosyl-tRNA/aminoacyl-tRNA synthetase orthogonal pair and a nonsense codon at the desired site. The nonnative or unnatural amino acids are supplied to cells expressing a construct from which the chimeric polypeptide is expressible, from an external source and this strategy can incorporate side chains with a wide range of physical attributes including, but not limited to, chemical crosslinking group (*e.g.*, azide or haloalkane), a trackable maker (*e.g.*, fluorescent or radioactive) and photosensitive groups to enable temporally controlled modifications. To these unnatural amino acids various moieties can be covalently linked by chemical addition to the structure-stabilizing moiety to provide advantageous properties.

TABLE 7

COMPLEMENTARY HEPTAD REPEAT REGION SEQUENCES

VIRUS	HR1 REGION SEQUENCE	HR2 REGION SEQUENCE
Synthetic	IEEIQKQIAAIQKQIAAIQKQIYRM [SEQ ID NO: 26]	IEEIQKQIAAIQKQIAAIQKQIYRM [SEQ ID NO: 27]
HIV GP160	SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILA [SEQ ID NO: 28] (GenPept dbjBAF31430.1)	HTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 29] (GenPept dbjBAF31430.1)
RSV F	LHLEGEVNKIKSALLSTNKAVVSLGNGVSVLTSKVLDLK [SEQ ID NO: 30] (GenPept gbAHL84194.1]	FDASISQVNEKINQSLAFIRKSDELLHNVNAGKSTTN [SEQ ID NO: 31] (GenPept gbAHL84194.1]
hMPV F	IRLESEVTAIKNALKKTNEAVSTLGNGVRVLATAVRELK [SEQ ID NO: 32] (GenPept gbAAN52913.1]	FNVALDQVFESIENSQALVDQSNRILSSAEKGNTG [SEQ ID NO: 33] (GenPept gbAAN52913.1)
PIV F	KQARSDIEKLKEAIRDTNKAVQSVQSSIGNLIVAIKSVQ [SEQ ID NO: 34] (GenPept gbAAB21447.1)	IELNKAKSDLEESKEWIRRSNQKLDSIGNWHQSSTT [SEQ ID NO: 35] (GenPept gbAAB21447.1]
MeV F	MLNSQAIDNLRASLETTNQAIEAIRQAGQEMILAVQGVQ [SEQ ID NO: 36 dbjBAB60865.1]	LERLDVGTNLGNAIAKLEDAKELLESSDQILRSMKGLSST [SEQ ID NO: 37] (GenPept dbjBAB60865.1)
HeV F	MKNADNINKLKSSIESTNEAVVKLQETAEKTVYVLTALQ [SEQ ID NO: 38] (GenPept NP_047111.2)	ISSQISSMNQSLQQSKDYIKEAQKILDTVNPS [SEQ ID NO: 39] (GenPept NP_047111.2]
Inf A HA	ENGWEGMVDGWYGFRHQNSEGTGQAADLKSTQAAI [SEQ ID NO: 40] (GenPept gbAEC23340.1)	RIQDLEKYVEDTKIDLWSYNAELVLALENQHTIDLTDSEMSKLFERTRR [SEQ ID NO: 41] (GenPept gbAEC23340.1)
Inf B HA	HGYTSHGAHGVAVAADLKSTQEAINKITKNLNYL [SEQ ID NO: 42] (GenPept gbAFH57854.1)	DEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKML [SEQ ID NO: 43] (GenPept gbAFH57854.1)
EBOV GP	GLRQLANETTQALQLFLRATTELRTFSILNRKAIDFL [SEQ ID NO: 44] (GenPept NP_066246.1)	HDWTKNITDKIDQIIHDFVDKTL [SEQ ID NO: 45] (GenPept NP_066246.1)
MARV GP	LANQTAKSLELLLRVTTEERTFSLINRHAIDFLLTRW [SEQ ID NO: 46] (GenPept YP_001531156.1)	IGIEDLSKNISEQIDQI [SEQ ID NO: 47] (GenPept YP_001531156.1)
MERS S	ISASIGDIIQRLDVLEQDAQIDRLINGRLTTLNAFVAQQLVRSESAALSAQLAKDKV NEG [SEQ ID NO: 48] (GenPept gbAHX00711.1)	GSGGNFGSLTQINTTLLDLTYEMLSLQQVVKALNESYIDLKELGNYTY [SEQ ID NO: 49] (GenPept gbAHX00711.1)
SARS S	ISSVLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMS EG [SEQ ID NO: 50] (GenPept gbAAR86788.1)	GSGGDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGK [SEQ ID NO: 51] (GenPept gbAAR86788.1)

[0114] Further embodiments may include any possible combination of the above examples, or additional unnatural chemical addition, covalently linked to the structure-stabilizing moiety.

[0115] Optionally, one or more additional cysteine residues may be inserted into the HR1 and/or HR2 regions to form disulfide bonds and further stabilize the anti-parallel, α-helical coiled coil structure of the structure stabilizing moiety.

2.1.2 Linkers

[0116] The structure-stabilizing moiety of the present invention suitably comprises a linker that spaces the heptad repeat regions (also referred to herein as HR1 and HR2). The linker generally includes any amino acid residue that cannot be unambiguously assigned to a heptad repeat sequence. Linkers are frequently used in the field of protein engineering to interconnect different functional units, *e.g.*, in the creation of single-chain variable fragment (scFv) constructs derived from antibody variable light (VL) and variable heavy (VH) chains. They are generally conformationally flexible in solution, and are suitably and predominantly composed of polar amino acid residue types. Typical (frequently used) amino acids in flexible linkers are serine and glycine. Less preferably, flexible linkers may also include alanine, threonine and proline. Thus, an intervening linker of the structure-stabilizing moiety is preferably flexible in conformation to ensure relaxed (unhindered) association of HR1 and HR2 as two-helix bundle that suitably adopts an α-helical coiled coil structure. Suitable linkers for use in the polypeptides envisaged herein will be clear to the skilled person, and may generally be any linker used in the art to link amino acid sequences, as long as the linkers are structurally flexible, in the sense that they permit, and suitably do not impair, assembly of the characteristic two-helix bundle structure of the structure-stabilizing moiety.

[0117] The skilled person will be able to determine the optimal linkers, optionally after performing a limited number of routine experiments. The intervening linker is suitably an amino acid sequence generally consisting of at least 1 amino acid residue and usually consisting of at least 2 amino acid residues, with a non-critical upper limit chosen for reasons of convenience being about 100 amino acid residues. In particular embodiments, the linker consists of about 1 to about 50 amino acid residues, or about 50 to about 100 amino acid residues, usually about 1 to about 40 amino acid residues, typically about 1 to about 30 amino acid residues. In non-limiting examples, the linker has about the same number of amino acids as the number of amino acids connecting complementary HRA and HRB regions of a Class I enveloped virus fusion protein. In particular, non-limiting embodiments, at least 50% of the amino acid residues of a linker sequence are selected from the group proline, glycine, and serine. In further non-limiting embodiments, at least 60%, such as at least 70%, such as for example 80% and more particularly 90% of the amino acid residues of a linker sequence are selected from the group proline, glycine, and serine. In other particular embodiments, the linker sequences essentially consist of polar amino acid residues; in such particular embodiments, at least 50%, such as at least 60%, such as for example 70% or 80% and more particularly 90% or up to 100% of the amino acid residues of a linker sequence are selected from the group consisting of glycine, serine, threonine, alanine, proline, histidine, asparagine, aspartic acid, glutamine, glutamic acid, lysine and arginine. In specific embodiments, linker sequences may include $[GGSG]_nGG$, $[GGGGS]_n$, $[GGGGG]_n$, $[GGGKGGGG]_n$, $[GGGNGGGG]_n$, $[GGGCGGGG]_n$, wherein n is an integer from 1 to 10, suitably 1 to 5, more suitably 1 to 3.

[0118] In specific embodiments in which the heptad repeat regions comprise, consist or consist essentially of complementary HRA and HRB regions, respectively, of a Class I enveloped virus fusion protein, the linker comprises, consists or consists essentially of an intervening naturally-occurring amino acid sequence, which connects the HRA and HRB regions. The intervening sequence can be full-length, or about full-length or can comprise, consist or consist essentially of one or more portions of a full-length intervening naturally-occurring amino acid sequence. In other embodiments, the linker lacks a naturally-occurring amino acid sequence interposed between the HRA and HRB regions of a wild-type Class I enveloped virus fusion protein. In any of the above embodiments, the linker may comprise one or more non-naturally-occurring amino acid sequences.

[0119] In addition to spacing the heptad repeat regions of the structure-stabilizing moiety and preferably introducing structural flexibility to facilitate anti-parallel association of those regions, the linker may comprise one or more ancillary functionalities. For example, the linker may comprise a purification moiety that facilitates purification of the chimeric polypeptide and/or at least one immune-modulating moiety that modulates an immune response to the chimeric polypeptide.

[0120] Purification moieties typically comprise a stretch of amino acids that enables recovery of the chimeric polypeptide through affinity binding. Numerous purification moieties or 'tags' are known in the art, illustrative examples of which include biotin carboxyl carrier protein-tag (BCCP-tag), Myc-tag (c-myc-tag), Calmodulin-tag, FLAG-tag, HA-tag, His-tag (Hexahistidine-tag, His6, 6H), Maltose binding protein-tag (MBP-tag), Nus-tag, Chitin-binding protein-tag (CBP-tag) Glutathione-S-transferase-tag (GST-tag), Green fluorescent protein-tag (GFP-tag), Polyglutamate-tag, Amyloid beta-tag, Thioredoxin-tag, S-tag, Softag 1, Softag 3, Strep-tag, Streptavidin-binding peptide-tag (SBP-tag), biotin-tag, streptavidin-tag and V5-tag.

[0121] Immune-modulating moieties can be introduced into the linker to modulate the immune response elicited by the chimeric polypeptide or complex thereof. Non-limiting examples of such moieties include immune-silencing or suppressing moieties as described for example above, antigenic moieties, including antigenic moieties derived from pathogenic organisms, or other disease associated antigenic moieties such as cancer or tumor associated antigens. Exemplary pathogenic organisms include, but are not limited to, viruses, bacteria, fungi parasites, algae and protozoa and amoebae. In specific embodiments, the antigenic moieties are derived from antigens of pathogenic viruses. Illustrative viruses responsible for diseases including, but not limited to, measles, mumps, rubella, poliomyelitis, hepatitis A, B (*e.g.*, GenBank Accession No. E02707), and C (*e.g.*, GenBank Accession No. E06890), as well as other hepatitis viruses, influenza, adenovirus (*e.g.*, types 4 and 7), rabies (*e.g.*, GenBank Accession No. M34678), yellow fever, Epstein-Barr virus and other herpesviruses such as papillomavirus, Ebola virus, influenza virus, Japanese encephalitis (*e.g.*, GenBank Accession No. E07883), dengue (*e.g.*, GenBank Accession No. M24444), hantavirus, Sendai virus, respiratory syncytial virus, orthomyxoviruses, vesicular stomatitis virus, visna virus, cytomegalovirus and human immunodeficiency virus (HIV) (*e.g.*, GenBank Accession No. U18552). Any suitable antigen derived from such viruses are useful in the practice of the present invention. For example, illustrative retroviral antigens derived from HIV include, but are not limited to, antigens such as gene products of the gag, pol, and env genes, the Nef protein, reverse transcriptase, and other HIV components. Illustrative examples of hepatitis

viral antigens include, but are not limited to, antigens such as the S, M, and L proteins of hepatitis B virus, the pre-S antigen of hepatitis B virus, and other hepatitis, *e.g.*, hepatitis A, B, and C. Illustrative examples of influenza viral antigens include; but are not limited to, antigens such as hemagglutinin and neuraminidase and other influenza viral components. Illustrative examples of measles viral antigens include, but are not limited to, antigens such as the measles virus fusion protein and other measles virus components. Illustrative examples of rubella viral antigens include, but are not limited to, antigens such as proteins E1 and E2 and other rubella virus components; rotaviral antigens such as VP7sc and other rotaviral components. Illustrative examples of Cytomegaloviral antigens include, but are not limited to, antigens such as envelope glycoprotein B and other Cytomegaloviral antigen components. Non-limiting examples of respiratory syncytial viral antigens include antigens such as the RSV fusion protein, the M2 protein and other respiratory syncytial viral antigen components. Illustrative examples of herpes simplex viral antigens include, but are not limited to, antigens such as immediate early proteins, glycoprotein D, and other herpes simplex viral antigen components. Non-limiting examples of varicella zoster viral antigens include antigens such as 9PI, gpII, and other varicella zoster viral antigen components. Non-limiting examples of Japanese encephalitis viral antigens include antigens such as proteins E, M-E, M-E-NS 1, NS 1, NS 1-NS2A, 80% E, and other Japanese encephalitis viral antigen components. Representative examples of rabies viral antigens include, but are not limited to, antigens such as rabies glycoprotein, rabies nucleoprotein and other rabies viral antigen components. Illustrative examples of papillomavirus antigens include, but are not limited to, the L1 and L2 capsid proteins as well as the E6/E7 antigens associated with cervical cancers, See Fundamental Virology, Second Edition, eds. Fields, B. N. and Knipe, D. M., 1991, Raven Press, New York, for additional examples of viral antigens. In particular embodiments, the viral antigen is an antigen of an enveloped virus to which the ectodomain polypeptide corresponds. In other embodiments, the viral antigen is an antigen of a different enveloped virus to which the ectodomain polypeptide corresponds.

[0122] In some embodiments, one or more cancer- or tumor-associated antigens are inserted into the linker. Such antigens include, but are not limited to, MAGE-2, MAGE-3, MUC-1, MUC-2, HER-2, high molecular weight melanoma-associated antigen MAA, GD2, carcinoembryonic antigen (CEA), TAG-72, ovarian-associated antigens OV-TL3 and MOV 18, TUAN, alpha-feto protein (AFP), OFP, CA-125, CA-50, CA-19-9, renal tumor-associated antigen G250, EGP-40 (also known as EpCAM), S100 (malignant melanoma-associated antigen), p53, prostate tumor-associated antigens (*e.g.*, PSA and PSMA), p21ras, Her2/neu, EGFR, EpCAM, VEGFR, FGFR, MUC-I, CA 125, CEA, MAGE, CD20, CD19, CD40, CD33, A3, antigen specific to A33 antibodies, BrE3 antigen, CD1, CD1a, CD5, CD8, CD14, CD15, CD16, CD21, CD22, CD23, CD30, CD33, CD37, CD38, CD40, CD45, CD46, CD52, CD54, CD74, CD79a, CD126, CD138, CD154, B7, Ia, Ii, HMl.24, HLA-DR (*e.g.*, HLA-DR10), NCA95, NCA90, HCG and sub-units, CEA (CEACAM5), CEACAM-6, CSAp, EGP-I, EGP-2, Ba 733, KC4 antigen, KS-I antigen, KS1-4, Le-Y, MUC2, MUC3, MUC4, PlGF, ED-B fibronectin, NCA 66a-d, PAM-4 antigen, PSA, PSMA, RS5, SlOO, TAG-72, TlOl, TAG TRAIL-Rl, TRAIL-R2, p53, tenascin, insulin growth factor-1 (IGF-I), Tn antigen *etc*.

[0123] The antigenic moiety or moieties included in the linker may correspond to full-length antigens or part antigens. When part antigens are employed, the part antigens may comprise one or more epitopes of an antigen of interest, including B cell epitopes and/or T cell epitopes (*e.g.*, cytotoxic T lymphocyte (CTL) epitopes and/or T helper (Th) epitopes). Epitopes of numerous antigens are known in the literature or can be determined using routine techniques

known to persons of skill in the art. In other embodiments the linker may include another cell targeting moiety which can provide delivery to a specific cell type within the immunized individual. Cell populations of interest include, but are not limited to, B-cells, Microfold cells and antigen-presenting cells (APC). In the later example the targeting moiety facilitates enhanced recognition of the chimeric polypeptide or complex thereof to an APC such as a dendritic cell or macrophage. Such targeting sequences can enhance APC presentation of epitopes of an associated ectodomain polypeptide, which can in turn augment the resultant immune response, including intensification or broadening the specificity of either or both of antibody and cellular immune responses to the ectodomain polypeptide. Non-limiting examples of APC-targeting moieties include ligands that bind to APC surface receptors such as, but not limited to, mannose-specific lectin (mannose receptor), IgG Fc receptors, DC-SIGN, BDCA3 (CD141), 33D1, SIGLEC-H, DCIR, CD11c, heat shock protein receptors and scavenger receptors. In particular embodiments, the APC-targeting moiety is a dendritic cell targeting moiety, which comprises, consists or consists essentially of the sequence FYPSYHSTPQRP (Uriel, *et al.*, *J. Immunol.* 2004 172: 7425–7431) or NWYLPWLGTNDW (Sioud, *et al.*, *FASEB J* 2013 27(8): 3272-83).

2.2 Enveloped virus fusion proteins and ectodomain polypeptides

[0124] The molecular clamping strategy of the present invention is useful for stabilizing a range of ectodomain polypeptides whose wild-type counterparts assemble into trimers in their pre-fusion forms, including Class I and Class III fusion proteins. Non-limiting Class I fusion proteins include the fusion proteins of orthomyxoviruses (*e.g.*, the HA proteins of Inf A, Inf B and Inf C), paramyxoviruses (*e.g.*, the F and proteins of MeV, RPV, CDV, RSV, HMPV, PIV, MuV, HeV, NiV and NDV), retroviruses (*e.g.*, the envelope glycoproteins of HTLV-1, HTLV-2, HTLV-3, HIV-1, HIV-2), filoviruses (*e.g.*, the glycoproteins of EBOV, ZEBOV, REBOV, SEBOV and MARV), arenaviruses (*e.g.*, the glycoproteins and stable signal peptides (SSP) of LASV, LCMV and JUNV), and coronaviruses (*e.g.*, the S proteins of HCoV, HToV, SARS-CoV and MERS-CoV). Representative Class III fusion proteins include the fusion proteins of rhabdoviruses (*e.g.*, the glycoproteins (G) of Rabies virus (RABV), Australian Bat Lyssavirus (ABLV), Bovine ephemeral fever virus (BEFV) and Vesicular stomatitis virus (VSV)) and herpesviruses (*e.g.*, the glycoproteins (gB, gD, gH/L) of Human herpes virus type 1 (HHV-1; also known as Herpes simplex virus type 1 (HSV-1)), HHV-2 (also known as HSV-2), HHV-3 (also known as Varicella zoster virus (VZV)), HHV-4 (also known as Epstein Barr virus (EBV)) and HHV-5 (also known as Cytomegalovirus (CMV)).

[0125] The ectodomain polypeptide may comprise or consist of a full-length precursor ectodomain polypeptide or a portion thereof. In some embodiments, the ectodomain polypeptide lacks any one or more of an endogenous signal peptide, an endogenous head portion of an ectodomain, an endogenous stem portion of an ectodomain, an endogenous mucin-like domain, an endogenous membrane proximal external region (MPER) and an endogenous fusion peptide. Alternatively, or in addition, one or more endogenous proteolytic cleavage sites (*e.g.*, one or more furin cleavage sites) of a wild-type or reference fusion protein may be altered or deleted to render the ectodomain polypeptide less susceptible to proteolytic cleavage by a protease (*e.g.*, a cellular protease such as furin).

[0126] The ectodomain polypeptides of the invention can be constructed with knowledge of the locations of various structural and functional moieties or domains that are present in a full-length enveloped virus precursor fusion protein. Non-limiting examples of such

precursor proteins and their associated domains are discussed below with reference to the construction of illustrative ectodomain polypeptide embodiments.

2.2.1 Inf A HA

[0127] An exemplary Inf A HA precursor has the following amino acid sequence: MKTIIALSYIFCLALGQDLPGNDNSTATLCLGHHAVPNGTLVKTITDDQIEVTNATELVQSSSTGKIC NNPHRILDGIDCTLIDALLGDPHCDVFQNETWDLFVERSKAFSNCYPYDVPDYASLRSLVASSGTLEFITEGFTW TGVTQNGGSNACKRGPGSGFFSRLNWLTKSGSTYPVLNVTMPNNDNFDKLYIWGIHHPSTNQEQTSLYVQASG RVTVSTRRSQQTIIPNIGSRPWVRGLSSRISIYWTIVKPGDVLVINSNGNLIAPRGYFKMRTGKSSIMRSDAPIDT CISECITPNGSIPNDKPFQNVNKITYGACPKYVKQNTLKLATGMRNVPEKQTRGLFGAIAGFIENGWEGMIDGWY GFRHQNSEGTGQAADLKSTQAAIDQINGKLNRVIEKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAE LLVALENQHTIDLTDSEMNKLFEKTRRQLRENAEEMGNGCFKIYHKCDNACIESIRNGTYDHDVYRDEALNNRF QIKGVELKSGYKDWILWISFAISCFLLCVVLLGFIMWACQRGNIRCNICI [SEQ ID NO: 52] (GenPept gbAEC23340.1). This sequence comprises the following domains/moieties:

[0128] SP = 1 – 16

[0129] Ectodomain = 17 – 529

[0130] Furin cleavage sites = 345-346

[0131] FP = 346-355

[0132] HRA region = 356 – 390

[0133] HRB region = 421 – 470

[0134] MPER = 470-529

[0135] TM = 530-553

[0136] C =534-556

[0137] Head region = 51-328, 403-444,

[0138] Stem region = 17-58, 327-401, 442-509.

[0139] Non-limiting examples of Inf A HA ectodomain polypeptides include:

Ectodomain 1 – 529:

[0140] MKTIIAFSCILCLIFAQKLPGSDNSMATLCLGHHAVPNGTLVKTITDDQIEVTNATELVQSS STGRICNSPHQILDGKNCTLIDALLGDPHCDDFQNKEWDLFVERSTAYSNCYPYYVPDYATLRSLVASSGNLEFT QESFNWTGVAQDGSSYACRRGSVNSFFSRLNWLYNLNYKYPEQNVTMPNNDKFDKLYIWGVHHPGTDKDQTN LYVQASGRVIVSTKRSQQTVIPNIGSRPWVRGVSSIISIYWTIVKPGDILLINSTGNLIAPRGYFKIQSGKSSIMRS DAHIDECNSECITPNGSIPNDKPFQNVNKITYGACPRYVKQNTLKLATGMRNVPEKQTRGIFGAIAGFIENGWEG MVDGWYGFRHQNSEGTGQAADLKSTQAAINQITGKLNRVIKKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKID LWSYNAELLVALENQHTIDLTDSEMSKLFERTRRQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHDIYRN EALNNRFQIKGVQLKSGYKD [SEQ ID NO: 53] (GenPept gbAEC23340.1).

Ectodomain minus SP 18 – 529:

[0141] KLPGSDNSMATLCLGHHAVPNGTLVKTITDDQIEVTNATELVQSSSTGRICNSPHQILDGK NCTLIDALLGDPHCDDFQNKEWDLFVERSTAYSNCYPYYVPDYATLRSLVASSGNLEFTQESFNWTGVAQDGSS YACRRGSVNSFFSRLNWLYNLNYKYPEQNVTMPNNDKFDKLYIWGVHHPGTDKDQTNLYVQASGRVIVSTKRS

QQTVIPNIGSRPWVRGVSSIISIYWTIVKPGDILLINSTGNLIAPRGYFKIQSGKSSIMRSDAHIDECNSECITPNG
SIPNDKPFQNVNKITYGACPRYVKQNTLKLATGMRNVPEKQTRGIFGAIAGFIENGWEGMVDGWYGFRHQNSE
GTGQAADLKSTQAAINQITGKLNRVIKKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQ
HTIDLTDSEMSKLFERTRRQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHDIYRNEALNNRFQIKGVQLK
SGYKD [SEQ ID NO: 54] (GenPept gbAEC23340.1).

Ectodomain minus SP minus, MPER18 – 469:

[0142] KLPGSDNSMATLCLGHHAVPNGTLVKTITDDQIEVTNATELVQSSSTGRICNSPHQILDGK
NCTLIDALLGDPHCDDFQNKEWDLFVERSTAYSNCYPYYVPDYATLRSLVASSGNLEFTQESFNWTGVAQDGSS
YACRRGSVNSFFSRLNWLYNLNYKYPEQNVTMPNNDKFDKLYIWGVHHPGTDKDQTNLYVQASGRVIVSTKRS
QQTVIPNIGSRPWVRGVSSIISIYWTIVKPGDILLINSTGNLIAPRGYFKIQSGKSSIMRSDAHIDECNSECITPNG
SIPNDKPFQNVNKITYGACPRYVKQNTLKLATGMRNVPEKQTRGIFGAIAGFIENGWEGMVDGWYGFRHQNSE
GTGQAADLKSTQAAINQITGKLNRVIKKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALENQ
HTIDLTDSEMSKLFERTRR [SEQ ID NO: 55] (GenPept gbAEC23340.1).

Ectodomain 18 – 341, 346 - 529 plus altered furin cleavage sites:

[0143] KLPGSDNSMATLCLGHHAVPNGTLVKTITDDQIEVTNATELVQSSSTGRICNSPHQILDGK
NCTLIDALLGDPHCDDFQNKEWDLFVERSTAYSNCYPYYVPDYATLRSLVASSGNLEFTQESFNWTGVAQDGSS
YACRRGSVNSFFSRLNWLYNLNYKYPEQNVTMPNNDKFDKLYIWGVHHPGTDKDQTNLYVQASGRVIVSTKRS
QQTVIPNIGSRPWVRGVSSIISIYWTIVKPGDILLINSTGNLIAPRGYFKIQSGKSSIMRSDAHIDECNSECITPNG
SIPNDKPFQNVNKITYGACPRYVKQNTLKLATGMRNVPE*RRRKKR*GIFGAIAGFIENGWEGMVDGWYGFRHQN
SEGTGQAADLKSTQAAINQITGKLNRVIKKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELLVALEN
QHTIDLTDSEMSKLFERTRRQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHDIYRNEALNNRFQIKGVQL
KSGYKD [SEQ ID NO: 56] (GenPept gbAEC23340.1).

Stem domain 1-58, 327-401, 442-509 plus linker regions:

[0144] MKTIIALSYILCLVFAQKLPGNDNSTATLCLGHHAVPNGTIVKTITNDQIEVTNATEL*GFG*Q
NTLKLATGMRNVPEKQTRGIFGAIAGFIENGWEGMLDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGMLNRL
I*GSGGSG*ELLVALLNQHTIDLTDSEMNKLFEKTKKQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHDVYR
DEALNNRFQIKGVELKSGYKD [SEQ ID NO: 57] (GenPept gbAEC23340.1).

Head domain 1-18, 51-328, 403-444 plus linker regions:

[0145] MKTIIALSYILCLVFAQKEVTNATELVQNSSTGGICDSPHQILDGENCTLIDALLGDPQCDG
FQNKKWDLFVERSKAYSNCYPYDVPDYASLRSLVASSGTLEFNNESFNWTGVTQNGTSSACKRGSNNSFFSRL
NWLTHSKFKYPALNVTMPNNEEFDKLYIWGVHHPGTDNDQIFLYAQASGRITVSTKRSQQTVIPNIGSRPRVRNI
PSRISIYWTIVKPGDILLINSTGNLIAPRGYFKIRSGKSSIMRSDAPIGKCNSECITPNGSIPNDKPFQNVNRITYG
ACPRYVKQN*GSGGSG*KTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELL [SEQ ID NO: 58]
(GenPept gbAEC23340.1).

2.2.2 Inf B HA

[0146] A representative Inf B HA precursor has the following amino acid
sequence: MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGTQTRGK
LCPNCFNCTDLDVALGRPKCMGNTPSAKVSILHEVKPATSGCFPIMHDRTKIRQLPNLLRGYENIRLSTSNVINTE
TAPGGPYKVGTSGSCPNVANGNGFFNTMAWVIPKDNNKTAINPVTVEVPYICSEGEDQITVWGFHSDDKTQME
RLYGDSNPQKFTSSANGVTTHYVSQIGGFPNQTEDEGLKQSGRIVVDYMVQKPGKTGTIVYQRGILLPQKVWCA
SGRSKVIKGSLPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPAKLLKERGFFGA

IAGFLEGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNYLSELEVKNLQRLSGAMNELHDEILE LDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFETKHKCNQTCLDRIAAG TFNAGDFSLPTFDSLNITAASLNDDGLDNHTILLYYSTAASSLAVTLMIAIFIVYMVSRDNVSCSICL [SEQ ID NO: 59] (GenPept gbAFH57854.1].

[0147] This sequence comprises the following domains/moieties:

[0148] SP = 1-16

[0149] Ectodomain = 17-547

[0150] Furin cleavage sites = 361-362

[0151] FP = 362-382

[0152] HRA region = 383-416

[0153] HRB region = 436-487

[0154] MPER = 488-547

[0155] TM = 548-573

[0156] C = 574-584

[0157] Head region = 48-344, 418-456

[0158] Stem region = 17-47, 345-417, 457-547

[0159] Non-limiting examples of Inf B HA ectodomain polypeptides include:

Ectodomain 1 – 547:

[0160] MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGT QTRGKLCPNCFNCTDLDVALGRPKCMGNTPSAKVSILHEVKPATSGCFPIMHDRTKIRQLPNLLRGYENIRLSTS NVINTETAPGGPYKVGTSGSCPNVANGNGFFNTMAWVIPKDNNKTAINPVTVEVPYICSEGEDQITVWGFHSDD KTQMERLYGDSNPQKFTSSANGVTTHYVSQIGGFPNQTEDEGLKQSGRIVVDYMVQKPGKTGTIVYQRGILLPQ KVWCASGRSKVIKGSLPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPAKLLKE RGFFGAIAGFLEGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNYLSELEVKNLQRLSGAMNEL HDEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFETKHKCNQTCL DRIAAGTFNAGDFSLPTFDSLNITAASLNDDGLDNHT [SEQ ID NO: 60] (GenPept gbAFH57854.1).

Ectodomain minus SP 17-547:

[0161] RICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGTQTRGKLCPNCFNCTD LDVALGRPKCMGNTPSAKVSILHEVKPATSGCFPIMHDRTKIRQLPNLLRGYENIRLSTSNVINTETAPGGPYKVG TSGSCPNVANGNGFFNTMAWVIPKDNNKTAINPVTVEVPYICSEGEDQITVWGFHSDDKTQMERLYGDSNPQK FTSSANGVTTHYVSQIGGFPNQTEDEGLKQSGRIVVDYMVQKPGKTGTIVYQRGILLPQKVWCASGRSKVIKGS LPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWE GMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNYLSELEVKNLQRLSGAMNELHDEILELDEKVDDLRA DTISSQIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFETKHKCNQTCLDRIAAGTFNAGDFSLP TFDSLNITAASLNDDGLDNHT [SEQ ID NO: 61] (GenPept gbAFH57854.1).

Ectodomain minus SP, minus MPER 17-487:

[0162] RICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGTQTRGKLCPNCFNCTD LDVALGRPKCMGNTPSAKVSILHEVKPATSGCFPIMHDRTKIRQLPNLLRGYENIRLSTSNVINTETAPGGPYKVG

TSGSCPNVANGNGFFNTMAWVIPKDNNKTAINPVTVEVPYICSEGEDQITVWGFHSDDKTQMERLYGDSNPQK
FTSSANGVTTHYVSQIGGFPNQTEDEGLKQSGRIVVDYMVQKPGKTGTIVYQRGILLPQKVWCASGRSKVIKGS
LPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWE
GMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNYLSELEVKNLQRLSGAMNELHDEILELDEKVDDLRA
DTISSQIELAVLLSNEGIINSEDEHLLALERKLKKML [SEQ ID NO: 62] (GenPept gbAFH57854.1).

Ectodomain minus SP plus altered furin cleavage sites 17-355, 362-547:

[0163] RICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGTQTRGKLCPNCFNCTD
LDVALGRPKCMGNTPSAKVSILHEVKPATSGCFPIMHDRTKIRQLPNLLRGYENIRLSTSNVINTETAPGGPYKVG
TSGSCPNVANGNGFFNTMAWVIPKDNNKTAINPVTVEVPYICSEGEDQITVWGFHSDDKTQMERLYGDSNPQK
FTSSANGVTTHYVSQIGGFPNQTEDEGLKQSGRIVVDYMVQKPGKTGTIVYQRGILLPQKVWCASGRSKVIKGS
LPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPA*RRRKKR*AGFFGAIAGFLEGG
WEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNYLSELEVKNLQRLSGAMNELHDEILELDEKVDDL
RADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFETKHKCNQTCLDRIAAGTFNAGDFS
LPTFDSLNITAASLNDDGLDNHT [SEQ ID NO: 63] (GenPept gbAFH57854.1).

Stem domain 1-47, 345-417, 457-547 plus linker regions:

[0164] MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPL*GSG*LANGTKYRPPA
KLLKERGFFGAIAGFLEGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNYLS*GSGGSG*IELAVL
LSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFETKHKCNQTCLDRIAAGTFNAGDFSLPTFDSLNITAASL
NDDGLDNHT [SEQ ID NO: 64] (GenPept gbAFH57854.1).

Head domain 1-17, 48-344, 418-456 plus linker regions:

[0165] MKAIIVLLMVVTSNADRTTTPTKSHFANLKGTQTRGKLCPNCFNCTDLDVALGRPKCMGN
TPSAKVSILHEVKPATSGCFPIMHDRTKIRQLPNLLRGYENIRLSTSNVINTETAPGGPYKVGTSGSCPNVANGNG
FFNTMAWVIPKDNNKTAINPVTVEVPYICSEGEDQITVWGFHSDDKTQMERLYGDSNPQKFTSSANGVTTHYVS
QIGGFPNQTEDEGLKQSGRIVVDYMVQKPGKTGTIVYQRGILLPQKVWCASGRSKVIKGSLPLIGEADCLHEKY
GGLNKSKPYYTGEHAKAIGNCPIWVKTPLK*GSGGSG*ELEVKNLQRLSGAMNELHDEILELDEKVDDLRADTISS
Q [SEQ ID NO: 65] (GenPept gbAFH57854.1).

2.2.3 RSV F

[0166] A non-limiting RSF F precursor has the following amino acid sequence:
MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKKNKCNGTDAKVK
LIKQELDKYKNAVTELQLLMQSTQATNNRARRELPRFMNYTLNNAKKTNVTLSKKRKRRFLGFLLGVGSAIASGV
AVSKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSISNIETVIEFQQKNN
RLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIIKEEVLAYVVQLPL
YGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEV
NLCNVDIFNPKYDCEIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCDYVSNKGVDTVSVGN
TLYYVNKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVNAGKSTTNIMITTIII
VIIVILLSLIAVGLLLYCKARSTPVTLSKDQLSGINNIAFSN [SEQ ID NO: 66] (GenPept gbAHL84194.1).

[0167] This sequence comprises the following domains/moieties:

[0168] SP = 1-23

[0169] Ectodomain = 24-524

[0170] Furin cleavage sites = 109-110, 136-137

[0171] FP =137-163

[0172] HRA region = 164-196

[0173] HRB region = 488-524

[0174] TM = 525-548

[0175] C = 549-574

[0176] D25 interaction domain = 61-97, 193-240

[0177] Non-limiting examples of RSV F ectodomain polypeptides include:

[0178] Ectodomain 1 – 524:

[0179] MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNI KKNKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTQATNNRARRELPRFMNYTLNNAKKTNVTLSKKRKRRFL GFLLGVGSAIASGVAVSKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSI SNIETVIEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMS IIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRV FCDTMNSLTLPSEVNLCNVDIFNPKYDCEIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCDY VSNKGVDTVSVGNTLYYVNKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVN AGKSTTN [SEQ ID NO: 67] (GenPept gbAHL84194.1).

Ectodomain of RSV F (1-520):

[0180] MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNI KKNKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTQATNNRARRELPRFMNYTLNNAKKTNVTLSKKRKRRFL GFLLGVGSAIASGVAVSKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSI SNIETVIEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMS IIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRV FCDTMNSLTLPSEVNLCNVDIFNPKYDCEIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCDY VSNKGVDTVSVGNTLYYVNKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVN AGK [SEQ ID NO: 146].

Ectodomain minus SP 24-524:

[0181] SGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKKNKCNGTDAKVKLIKQELD KYKNAVTELQLLMQSTQATNNRARRELPRFMNYTLNNAKKTNVTLSKKRKRRFLGFLLGVGSAIASGVAVSKVLH LEGEVNKIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSISNIETVIEFQQKNNRLLEITRE FSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIIKEEVLAYVVQLPLYGVIDTP CWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNVDI FNPKYDCEIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVNK QEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVNAGKSTTN [SEQ ID NO: 68] (GenPept gbAHL84194.1).

Ectodomain minus SP plus altered furin cleavage sites 24-524:

[0182] SGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNIKKNKCNGTDAKVKLIKQELD KYKNAVTELQLLMQSTQATNN*NA**NN*ELPRFMNYTLNNAKKTNVTLS*NNNNN*FLGFLLGVGSAIASGVAVSKVL HLEGEVNKIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSISNIETVIEFQQKNNRLLEITR EFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIIKEEVLAYVVQLPLYGVIDT PCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNVD

IFNPKYDCEIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVN KQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVNAGKSTTN [SEQ ID NO: 69] (GenPept gbAHL84194.1).

D25 interaction domain 61-97, 193-240 plus linker regions:

[0183] LSNIKKNKCNGTDAKVKLIKQELDKYKNAVTELQLLM*GG*LDLKNYIDKQLLPIVNKQSCSI SNIETVIEFQQKNNRLLEITREFSVN [SEQ ID NO: 70] (GenPept gbAHL84194.1).

2.2.4 hMPV F

[0184] An illustrative hMPV F precursor has the following amino acid sequence: MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIK TELDLTKSALRELRTVSADQLAREEQIENPRQSRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTN EAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEK KGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRH PISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVS SSFDPVKFPEDQFNVALDQVFESIENSQALVDQSNRILSSAEKGNTGFIIVIILIAVLGSTMILVSVFIIIKKTKKPT GAPPELSGVTNNGFIPHN [SEQ ID NO: 71] (GenPept gbAAN52913.1).

[0185] This sequence comprises the following domains/moieties:

[0186] SP = 1-19

[0187] Ectodomain = 1-490

[0188] Furin cleavage sites = 102-103

[0189] FP = 103-125

[0190] HRA region = 126-169

[0191] HRB region = 456-490

[0192] TM = 491-514

[0193] C = 515-539

[0194] Non-limiting examples of hMPV F ectodomain polypeptides include:

Ectodomain 1 – 490:

[0195] MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCAD GPSLIKTELDLTKSALRELRTVSADQLAREEQIENPRQSRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKN ALKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGIT PAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAP SCSEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIK GRPVSSSFDPVKFPEDQFNVALDQVFESIENSQALVDQSNRILSSAEKGNTG [SEQ ID NO: 72] (GenPept gbAAN52913.1).

Ectodomain minus SP 20-490:

[0196] KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALREL RTVSADQLAREEQIENPRQSRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNGVRV

LATAVRELKDFVSKNLTRAINKNKCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVS
NMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEKKGNYACLLREDQ
GWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPISMVALSPLGAL
VACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPVKFPEDQF
NVALDQVFESIENSQALVDQSNRILSSAEKGNTG [SEQ ID NO: 73] (GenPept gbAAN52913.1).

Ectodomain minus SP plus altered furin cleavage sites 20-490:

[0197] KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCADGPSLIKTELDLTKSALREL
RTVSADQLAREEQIENP*A*QS*A*FVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNGVRV
LATAVRELKDFVSKNLTRAINKNKCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVS
NMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEKKGNYACLLREDQ
GWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPISMVALSPLGAL
VACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPVKFPEDQF
NVALDQVFESIENSQALVDQSNRILSSAEKGNTG [SEQ ID NO: 74] (GenPept gbAAN52913.1).

2.2.5 PIV F

[0198] An exemplary PIV F precursor has the following amino acid sequence: MPTSILLIITTMIMASFCQIDITKLQHVGVLVNSPKGMKISQNFETRYLILSLIPKIEDSNSCGDQQIK
QYKRLLDRLIIPLYDGLRLQKDVIVSNQESNENTDPRTKRFFGGVIGTIALGVATSAQITAAVALVEAKQARSDIE
KLKEAIRDTNKAVQSVQSSIGNLIVAIKSVQDYVNKEIVPSIARLGCEAAGLQLGIALTQHYSELTNIFGDNIGSL
QEKGIKLQGIASLYRTNITEIFTTSTVDKYDIYDLLFTESIKVRVIDVDLNDYSITLQVRLPLLTRLLNTQIYKVDSIS
YNIQNREWYIPLPSHIMTKGAFLGGADVKECIEAFSSYICPSDPGFVLNHEMESCLSGNISQCPRTVVTSDIVPRY
AFVNGGVVANCITTTCTCNGIGNRINQPPDQGVKIITHKECNTIGINGMLFNTNKEGTLAFYTPNDITLNNSVALD
PIDISIELNKAKSDLEESKEWIRRSNQKLDSIGNWHQSSTTIIIVLIMIIILFIINVTIIIIAVKYYRIQKRNRVDQND
KPYVLTNK [SEQ ID NO: 75] (GenPept gbAAB21447.1).

[0199] This sequence comprises the following domains/moieties:

[0200] SP = 1-19

[0201] Ectodomain = 1-493

[0202] Furin cleavage sites = 109-110

[0203] FP = 110-135

[0204] HRA region = 136-168

[0205] HRB region = 458-493

[0206] TM = 494-516

[0207] C = 517-536

[0208] Non-limiting examples of PIV F ectodomain polypeptides include:

Ectodomain 1 – 493:

[0209] MPTSILLIITTMIMASFCQIDITKLQHVGVLVNSPKGMKISQNFETRYLILSLIPKIEDSNSC
GDQQIKQYKRLLDRLIIPLYDGLRLQKDVIVSNQESNENTDPRTKRFFGGVIGTIALGVATSAQITAAVALVEAKQ
ARSDIEKLKEAIRDTNKAVQSVQSSIGNLIVAIKSVQDYVNKEIVPSIARLGCEAAGLQLGIALTQHYSELTNIFGD
NIGSLQEKGIKLQGIASLYRTNITEIFTTSTVDKYDIYDLLFTESIKVRVIDVDLNDYSITLQVRLPLLTRLLNTQIYK
VDSISYNIQNREWYIPLPSHIMTKGAFLGGADVKECIEAFSSYICPSDPGFVLNHEMESCLSGNISQCPRTVVTSD

IVPRYAFVNGGVVANCITTTCTCNGIGNRINQPPDQGVKIITHKECNTIGINGMLFNTNKEGTLAFYTPNDITLNN SVALDPIDISIELNKAKSDLEESKEWIRRSNQKLDSIGNWHQSSTT [SEQ ID NO: 76] (GenPept gbAAB21447.1).

Ectodomain minus SP 20-493:

[0210] IDITKLQHVGVLVNSPKGMKISQNFETRYLILSLIPKIEDSNSCGDQQIKQYKRLLDRLIIPL YDGLRLQKDVIVSNQESNENTDPRTKRFFGGVIGTIALGVATSAQITAAVALVEAKQARSDIEKLKEAIRDTNKA VQSVQSSIGNLIVAIKSVQDYVNKEIVPSIARLGCEAAGLQLGIALTQHYSELTNIFGDNIGSLQEKGIKLQGIASL YRTNITEIFTTSTVDKYDIYDLLFTESIKVRVIDVDLNDYSITLQVRLPLLTRLLNTQIYKVDSISYNIQNREWYIPLP SHIMTKGAFLGGADVKECIEAFSSYICPSDPGFVLNHEMESCLSGNISQCPRTVVTSDIVPRYAFVNGGVVANCI TTTCTCNGIGNRINQPPDQGVKIITHKECNTIGINGMLFNTNKEGTLAFYTPNDITLNNSVALDPIDISIELNKAKS DLEESKEWIRRSNQKLDSIGNWHQSSTT [SEQ ID NO: 77] (GenPept gbAAB21447.1).

Ectodomain minus SP plus altered furin cleavage sites 20-493:

[0211] IDITKLQHVGVLVNSPKGMKISQNFETRYLILSLIPKIEDSNSCGDQQIKQYKRLLDRLIIPL YDGLRLQKDVIVSNQESNENTDP*M*TK*M*FFGGVIGTIALGVATSAQITAAVALVEAKQARSDIEKLKEAIRDTNKA VQSVQSSIGNLIVAIKSVQDYVNKEIVPSIARLGCEAAGLQLGIALTQHYSELTNIFGDNIGSLQEKGIKLQGIASL YRTNITEIFTTSTVDKYDIYDLLFTESIKVRVIDVDLNDYSITLQVRLPLLTRLLNTQIYKVDSISYNIQNREWYIPLP SHIMTKGAFLGGADVKECIEAFSSYICPSDPGFVLNHEMESCLSGNISQCPRTVVTSDIVPRYAFVNGGVVANCI TTTCTCNGIGNRINQPPDQGVKIITHKECNTIGINGMLFNTNKEGTLAFYTPNDITLNNSVALDPIDISIELNKAKS DLEESKEWIRRSNQKLDSIGNWHQSSTT [SEQ ID NO: 78] (GenPept gbAAB21447.1).

2.2.6 MeV F

[0212] A representative MeV F precursor has the following amino acid sequence: MGLKVNVSAIFMAVLLTLQTPTGQIHWGNLSKIGVVGIGSASYKVMTRSSHQSLVIKLMPNITLLNN CTRVEIAEYRRLLRTVLEPIRDALNAMTQNIRPVQSVASSRRHKRFAGVVLAGAALGVATAAQITAGIALHQSML NSQAIDNLRASLETTNQAIEAIRQAGQEMILAVQGVQDYINNELIPSMNQLSCDLIGQKLGLKLLRYYTEILSLFG PSLRDPISAEISIQALSYALGGDINKVLEKLGYSGGDLLGILESRGIKARITHVDTESYLIVLSIAYPTLSEIKGVIVH RLEGVSYNIGSQEWYTTVPKYVATQGYLISNFDESSCTFMPEGTVCSQNALYPMSPLLQECLRGSTKSCARTLVS GSFGNRFILSQGNLIANCASILCKCYTTGTIINQDPDKILTYIAADHCPVVEVNGVTIQVGSRRYPDAVYLHRIDLG PPILLERLDVGTNLGNAIAKLEDAKELLESSDQILRSMKGLSSTCIVYILIAVCLGGLIGIPALICCCRGRCNKKGEQ VGMSRPGLKPDLTGTSKSYVRSL [SEQ ID NO: 79] (GenPept dbjBAB60865.1).

[0213] This sequence comprises the following domains/moieties:

[0214] SP = 1-24

[0215] Ectodomain = 1-493

[0216] Furin cleavage sites = 112-113

[0217] FP = 113-137

[0218] HRA region = 138-171

[0219] HRB region = 454-493

[0220] TM = 494-517

[0221] C = 518-550

[0222] Non-limiting examples of MeV F ectodomain polypeptides include:

Ectodomain 1-493:

[0223] MGLKVNVSAIFMAVLLTLQTPTGQIHWGNLSKIGVVGIGSASYKVMTRSSHQSLVIKLMP NITLLNNCTRVEIAEYRRLLRTVLEPIRDALNAMTQNIRPVQSVASSRRHKRFAGVVLAGAALGVATAAQITAGIA LHQSMLNSQAIDNLRASLETTNQAIEAIRQAGQEMILAVQGVQDYINNELIPSMNQLSCDLIGQKLGLKLLRYYT EILSLFGPSLRDPISAEISIQALSYALGGDINKVLEKLGYSGGDLLGILESRGIKARITHVDTESYLIVLSIAYPTLSE IKGVIVHRLEGVSYNIGSQEWYTTVPKYVATQGYLISNFDESSCTFMPEGTVCSQNALYPMSPLLQECLRGSTKS CARTLVSGSFGNRFILSQGNLIANCASILCKCYTTGTIINQDPDKILTYIAADHCPVVEVNGVTIQVGSRRYPDAV YLHRIDLGPPILLERLDVGTNLGNAIAKLEDAKELLESSDQILRSMKGLSST [SEQ ID NO: 80] (GenPept dbjBAB60865.1).

Ectodomain minus SP 25-493:

[0224] IHWGNLSKIGVVGIGSASYKVMTRSSHQSLVIKLMPNITLLNNCTRVEIAEYRRLLRTVLEP IRDALNAMTQNIRPVQSVASSRRHKRFAGVVLAGAALGVATAAQITAGIALHQSMLNSQAIDNLRASLETTNQAI EAIRQAGQEMILAVQGVQDYINNELIPSMNQLSCDLIGQKLGLKLLRYYTEILSLFGPSLRDPISAEISIQALSYAL GGDINKVLEKLGYSGGDLLGILESRGIKARITHVDTESYLIVLSIAYPTLSEIKGVIVHRLEGVSYNIGSQEWYTTV PKYVATQGYLISNFDESSCTFMPEGTVCSQNALYPMSPLLQECLRGSTKSCARTLVSGSFGNRFILSQGNLIANC ASILCKCYTTGTIINQDPDKILTYIAADHCPVVEVNGVTIQVGSRRYPDAVYLHRIDLGPPILLERLDVGTNLGNAI AKLEDAKELLESSDQILRSMKGLSST [SEQ ID NO: 81] (GenPept dbjBAB60865.1)

Ectodomain minus SP plus altered furin cleavage sites:

[0225] IHWGNLSKIGVVGIGSASYKVMTRSSHQSLVIKLMPNITLLNNCTRVEIAEYRRLLRTVLEP IRDALNAMTQNIRPVQSVASS*NN*HK*M*FAGVVLAGAALGVATAAQITAGIALHQSMLNSQAIDNLRASLETTNQA IEAIRQAGQEMILAVQGVQDYINNELIPSMNQLSCDLIGQKLGLKLLRYYTEILSLFGPSLRDPISAEISIQALSYAL GGDINKVLEKLGYSGGDLLGILESRGIKARITHVDTESYLIVLSIAYPTLSEIKGVIVHRLEGVSYNIGSQEWYTTV PKYVATQGYLISNFDESSCTFMPEGTVCSQNALYPMSPLLQECLRGSTKSCARTLVSGSFGNRFILSQGNLIANC ASILCKCYTTGTIINQDPDKILTYIAADHCPVVEVNGVTIQVGSRRYPDAVYLHRIDLGPPILLERLDVGTNLGNAI AKLEDAKELLESSDQILRSMKGLSST [SEQ ID NO: 82] (GenPept dbjBAB60865.1).

2.2.7 HeV F

[0226] A non-limiting HeV F precursor has the following amino acid sequence: MATQEVRLKCLLCGIIVLVLSLEGLGILHYEKLSKIGLVKGITRKYKIKSNPLTKDIVIKMIPNVSNVSKCTGTVME NYKSRLTGILSPIKGAIELYNNNTHDLVGDVKLAGVVMAGIAIGIATAAQITAGVALYEAMKNADNINKLKSSIES TNEAVVKLQETAEKTVYVLTALQDYINTNLVPTIDQISCKQTELALDLALSKYLSDLLFVFGPNLQDPVSNSMTIQA ISQAFGGNYETLLRTLGYATEDFDDLLESDSIAGQIVYVDLSSYYIIVRVYFPILTEIQQAYVQELLPVSFNNDNSE WISIVPNFVLIRNTLISNIEVKYCLITKKSVICNQDYATPMTASVRECLTGSTDKCPRELVVSSHVPRFALSGGVLF ANCISVTCQCQTTGRAISQSGEQTLLMIDNTTCTTVVLGNIIISLGKYLGSINYNSESIAVGPPVYTDKVDISSQIS SMNQSLQQSKDYIKEAQKILDTVNPSLISMLSMIILYVLSIAALCIGLITFISFVIVEKKRGNYSRLDDRQVRPVSN GDLYYIGT [SEQ ID NO: 83] (GenPept NP_047111.2).

[0227] This sequence comprises the following domains/moieties:

[0228] SP = 1-20

[0229] Ectodomain = 1-487

[0230] Furin cleavage sites = 109-110

[0231] FP = 110-135

[0232] HRA region = 136-169

[0233] HRB region = 456-587

[0234] TM = 488-518

[0235] C = 519-546

[0236] Non-limiting examples of HeV F ectodomain polypeptides include:

Ectodomain 1-487:

[0237] MATQEVRLKCLLCGIIVLVLSLEGLGILHYEKLSKIGLVKGITRKYKIKSNPLTKDIVIKMIPN VSNVSKCTGTVMENYKSRLTGILSPIKGAIELYNNNTHDLVGDVKLAGVVMAGIAIGIATAAQITAGVALYEAMK NADNINKLKSSIESTNEAVVKLQETAEKTVYVLTALQDYINTNLVPTIDQISCKQTELALDLALSKYLSDLLFVFGP NLQDPVSNSMTIQAISQAFGGNYETLLRTLGYATEDFDDLLESDSIAGQIVYVDLSSYYIIVRVYFPILTEIQQAYV QELLPVSFNNDNSEWISIVPNFVLIRNTLISNIEVKYCLITKKSVICNQDYATPMTASVRECLTGSTDKCPRELVVS SHVPRFALSGGVLFANCISVTCQCQTTGRAISQSGEQTLLMIDNTTCTTVVLGNIIISLGKYLGSINYNSESIAVGP PVYTDKVDISSQISSMNQSLQQSKDYIKEAQKILDTVNPS [SEQ ID NO: 84] (GenPept NP_047111.2).

Ectodomain minus SP 21-487:

[0238] SLEGLGILHYEKLSKIGLVKGITRKYKIKSNPLTKDIVIKMIPNVSNVSKCTGTVMENYKSRL TGILSPIKGAIELYNNNTHDLVGDVKLAGVVMAGIAIGIATAAQITAGVALYEAMKNADNINKLKSSIESTNEAVV KLQETAEKTVYVLTALQDYINTNLVPTIDQISCKQTELALDLALSKYLSDLLFVFGPNLQDPVSNSMTIQAISQAFG GNYETLLRTLGYATEDFDDLLESDSIAGQIVYVDLSSYYIIVRVYFPILTEIQQAYVQELLPVSFNNDNSEWISIVPN FVLIRNTLISNIEVKYCLITKKSVICNQDYATPMTASVRECLTGSTDKCPRELVVSSHVPRFALSGGVLFANCISVT CQCQTTGRAISQSGEQTLLMIDNTTCTTVVLGNIIISLGKYLGSINYNSESIAVGPPVYTDKVDISSQISSMNQSL QQSKDYIKEAQKILDTVNPS [SEQ ID NO: 85] (GenPept NP_047111.2).

Ectodomain minus SP plus altered furin cleavage sites 21-487:

[0239] SLEGLGILHYEKLSKIGLVKGITRKYKIKSNPLTKDIVIKMIPNVSNVSKCTGTVMENYKSRL TGILSPIKGAIELYNNNTHDLVGDV*M*LAGVVMAGIAIGIATAAQITAGVALYEAMKNADNINKLKSSIESTNEAVV KLQETAEKTVYVLTALQDYINTNLVPTIDQISCKQTELALDLALSKYLSDLLFVFGPNLQDPVSNSMTIQAISQAFG GNYETLLRTLGYATEDFDDLLESDSIAGQIVYVDLSSYYIIVRVYFPILTEIQQAYVQELLPVSFNNDNSEWISIVPN FVLIRNTLISNIEVKYCLITKKSVICNQDYATPMTASVRECLTGSTDKCPRELVVSSHVPRFALSGGVLFANCISVT CQCQTTGRAISQSGEQTLLMIDNTTCTTVVLGNIIISLGKYLGSINYNSESIAVGPPVYTDKVDISSQISSMNQSL QQSKDYIKEAQKILDTVNPS [SEQ ID NO: 86] (GenPept NP_047111.2).

2.2.8 NiV F

[0240] A representative NiV F precursor has the following amino acid sequence: MVVILDKRCYCNLLILILMISECSVGILHYEKLSKIGLVKGVTRKYKIKSNPLTKDIVIKMIPNVSNMS QCTGSVMENYKTRLNGILTPIKGALEIYKNNTHDLVGDVRLAGVIMAGVAIGIATAAQITAGVALYEAMKNADNI NKLKSSIESTNEAVVKLQETAEKTVYVLTALQDYINTNLVPTIDKISCKQTELSLDLALSKYLSDLLFVFGPNLQDP VSNSMTIQAISQAFGGNYETLLRTLGYATEDFDDLLESDSITGQIIYVDLSSYYIIVRVYFPILTEIQQAYIQELLPVS FNNDNSEWISIVPNFILVRNTLISNIEIGFCLITKRSVICNQDYATPMTNNMRECLTGSTEKCPRELVVSSHVPRFA LSNGVLFANCISVTCQCQTTGRAISQSGEQTLLMIDNTTCPTAVLGNVIISLGKYLGSVNYNSEGIAIGPPVFTDK VDISSQISSMNQSLQQSKDYIKEAQRLLDTVNPSLISMLSMIILYVLSIASLCIGLITFISFIIVEKKRNTYSRLEDR RVRPTSSGDLYYIGT [SEQ ID NO: 87] (GenPept NP 112026).

[0241] This sequence comprises the following domains/moieties:

[0242] SP = 1-20

[0243] Ectodomain = 1-487

[0244] Furin cleavage sites = 109-110

[0245] FP = 110-135

[0246] HRA region = 136-169

[0247] HRB region = 456-487

[0248] TM = 488-518

[0249] C = 519-546

[0250] Non-limiting examples of NiV F ectodomain polypeptides include:

Ectodomain 1-487:

[0251] MVVILDKRCYCNLLILILMISECSVGILHYEKLSKIGLVKGVTRKYKIKSNPLTKDIVIKMIPN VSNMSQCTGSVMENYKTRLNGILTPIKGALEIYKNNTHDLVGDVRLAGVIMAGVAIGIATAAQITAGVALYEAMK NADNINKLKSSIESTNEAVVKLQETAEKTVYVLTALQDYINTNLVPTIDKISCKQTELSLDLALSKYLSDLLFVFGP NLQDPVSNSMTIQAISQAFGGNYETLLRTLGYATEDFDDLLESDSITGQIIYVDLSSYYIIVRVYFPILTEIQQAYIQ ELLPVSFNNDNSEWISIVPNFILVRNTLISNIEIGFCLITKRSVICNQDYATPMTNNMRECLTGSTEKCPRELVVSS HVPRFALSNGVLFANCISVTCQCQTTGRAISQSGEQTLLMIDNTTCPTAVLGNVIISLGKYLGSVNYNSEGIAIGP PVFTDKVDISSQISSMNQSLQQSKDYIKEAQRLLDTVNPS [SEQ ID NO: 88] (GenPept NP 112026).

Ectodomain minus SP:

[0252] SECSVGILHYEKLSKIGLVKGVTRKYKIKSNPLTKDIVIKMIPNVSNMSQCTGSVMENYKT RLNGILTPIKGALEIYKNNTHDLVGDVRLAGVIMAGVAIGIATAAQITAGVALYEAMKNADNINKLKSSIESTNEA VVKLQETAEKTVYVLTALQDYINTNLVPTIDKISCKQTELSLDLALSKYLSDLLFVFGPNLQDPVSNSMTIQAISQA FGGNYETLLRTLGYATEDFDDLLESDSITGQIIYVDLSSYYIIVRVYFPILTEIQQAYIQELLPVSFNNDNSEWISIVP NFILVRNTLISNIEIGFCLITKRSVICNQDYATPMTNNMRECLTGSTEKCPRELVVSSHVPRFALSNGVLFANCISV TCQCQTTGRAISQSGEQTLLMIDNTTCPTAVLGNVIISLGKYLGSVNYNSEGIAIGPPVFTDKVDISSQISSMNQS LQQSKDYIKEAQRLLDTVNPS [SEQ ID NO: 89] (GenPept NP 112026).

Ectodomain minus SP plus altered furin cleavage sites:

[0253] SECSVGILHYEKLSKIGLVKGVTRKYKIKSNPLTKDIVIKMIPNVSNMSQCTGSVMENYKT RLNGILTPIKGALEIYKNNTHDLVGDV*M*LAGVIMAGVAIGIATAAQITAGVALYEAMKNADNINKLKSSIESTNEA VVKLQETAEKTVYVLTALQDYINTNLVPTIDKISCKQTELSLDLALSKYLSDLLFVFGPNLQDPVSNSMTIQAISQA FGGNYETLLRTLGYATEDFDDLLESDSITGQIIYVDLSSYYIIVRVYFPILTEIQQAYIQELLPVSFNNDNSEWISIVP NFILVRNTLISNIEIGFCLITKRSVICNQDYATPMTNNMRECLTGSTEKCPRELVVSSHVPRFALSNGVLFANCISV TCQCQTTGRAISQSGEQTLLMIDNTTCPTAVLGNVIISLGKYLGSVNYNSEGIAIGPPVFTDKVDISSQISSMNQS LQQSKDYIKEAQRLLDTVNPS [SEQ ID NO: 90] (GenPept NP 112026).

2.2.9 HIV GP160

[0254] An illustrative HIV GP160 precursor has the following amino acid sequence: MRVKGTRKNYWWRWGTMLLGMLMICSAAEQLWVTVYYGVPVWKEATTTLFCASDAKAVNTEVH NVWATHACVPTDPNPQEVVLENVTENFNMWKNDMVEQMQEDIISLWDQSLKPCVKLTPLCVTLNCTNWDGRN GTMNTTSTRNTTTANISRWEMEGEIKNCSFNVTTSIRNKMHKEYALFYKLDVMPIDNGSSYTLINCNTSVITQAC PKVSFEPIPIHYCTPAGFALLKCNDKKFNGTGPCKNVSTVQCTHGIRPVVSTQLLLNGSLAEEEIVIRSENLTDNA

KTIIVQLNETVVINCTRPGNNTRKSIHIGPGRAFYATGDIIGDIRQAHCNLSEASWNKTLKQIATKLREQFVNKTII
FNQSSGGDPEIVMHSFNCGGEFFYCDTTQLFNSAWFSNNTGLNYNNGSNTGGNITLPCRIKQIVNRWQEVGKA
MYAPPIRGNITCSSNITGLLLTRDGGNNVTNESEIFRPGGGNMKDNWRSELYKYKVVKIEPLGVAPTRAKRRVVQ
REKRAVGTIGAMFLGFLGAAGSTMGAASLTLTVQARQLLSGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARV
LAVERYLKDQQLLGIWGCSGRLICTTAVPWNASWSNKSLDDIWNNMTWMQWEKEIDNYTGLIYRLIEESQTQQ
EKNEQDLLQLDTWASLWNWFSISNWLWYIKIFIMIVAGLVGLRIFFAVLSIVNRVRQGYSPLSFQTHLPAQRGPD
RPGGIEEEGGERDNGRSIRLVDGFLALIWDDLRSLCLFSYHRLRDLLLLVKRVVELLGHRGWEILKYWWNLLQY
WSQELKNSAVSLFNAIAIAVAEGTDRVIEGIQRIGRGFLHIPRRIRQGLERALL [SEQ ID NO: 91] (GenPept dbjBAF31430.1).

[0255] This sequence comprises the following domains/moieties:

[0256] SP = 1-28

[0257] Ectodomain = 1-688

[0258] Furin cleavage sites = 508-509

[0259] FP = 509-538

[0260] HRA region = 539-587

[0261] HRB region = 631-667

[0262] MPER = 668-688

[0263] TM = 689-711

[0264] C = 712-861

[0265] GP41 = 509-861

[0266] GP120 = 1-508

[0267] Non-limiting examples of HIV GP160 ectodomain polypeptides include:

Ectodomain 1-688:

[0268] MRVKGTRKNYWWRWGTMLLGMLMICSAAEQLWVTVYYGVPVWKEATTTLFCASDAKAV
NTEVHNVWATHACVPTDPNPQEVVLENVTENFNMWKNDMVEQMQEDIISLWDQSLKPCVKLTPLCVTLNCTN
WDGRNGTMNTTSTRNTTTANISRWEMEGEIKNCSFNVTTSIRNKMHKEYALFYKLDVMPIDNGSSYTLINCNTS
VITQACPKVSFEPIPIHYCTPAGFALLKCNDKKFNGTGPCKNVSTVQCTHGIRPVVSTQLLLNGSLAEEEIVIRSEN
LTDNAKTIIVQLNETVVINCTRPGNNTRKSIHIGPGRAFYATGDIIGDIRQAHCNLSEASWNKTLKQIATKLREQF
VNKTIIFNQSSGGDPEIVMHSFNCGGEFFYCDTTQLFNSAWFSNNTGLNYNNGSNTGGNITLPCRIKQIVNRWQ
EVGKAMYAPPIRGNITCSSNITGLLLTRDGGNNVTNESEIFRPGGGNMKDNWRSELYKYKVVKIEPLGVAPTRAK
RRVVQREKRAVGTIGAMFLGFLGAAGSTMGAASLTLTVQARQLLSGIVQQQNNLLRAIEAQQHLLQLTVWGIKQ
LQARVLAVERYLKDQQLLGIWGCSGRLICTTAVPWNASWSNKSLDDIWNNMTWMQWEKEIDNYTGLIYRLIEE
SQTQQEKNEQDLLQLDTWASLWNWFSISNWLWYIK [SEQ ID NO: 92] (GenPept dbjBAF31430.1).

Ectodomain minus SP:

[0269] EQLWVTVYYGVPVWKEATTTLFCASDAKAVNTEVHNVWATHACVPTDPNPQEVVLENVT
ENFNMWKNDMVEQMQEDIISLWDQSLKPCVKLTPLCVTLNCTNWDGRNGTMNTTSTRNTTTANISRWEMEGE
IKNCSFNVTTSIRNKMHKEYALFYKLDVMPIDNGSSYTLINCNTSVITQACPKVSFEPIPIHYCTPAGFALLKCNDK
KFNGTGPCKNVSTVQCTHGIRPVVSTQLLLNGSLAEEEIVIRSENLTDNAKTIIVQLNETVVINCTRPGNNTRKSI

HIGPGRAFYATGDIIGDIRQAHCNLSEASWNKTLKQIATKLREQFVNKTIIFNQSSGGDPEIVMHSFNCGGEFFY
CDTTQLFNSAWFSNNTGLNYNNGSNTGGNITLPCRIKQIVNRWQEVGKAMYAPPIRGNITCSSNITGLLLTRDG
GNNVTNESEIFRPGGGNMKDNWRSELYKYKVVKIEPLGVAPTRAKRRVVQREKRAVGTIGAMFLGFLGAAGST
MGAASLTLTVQARQLLSGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARVLAVERYLKDQQLLGIWGCSGRLI
CTTAVPWNASWSNKSLDDIWNNMTWMQWEKEIDNYTGLIYRLIEESQTQQEKNEQDLLQLDTWASLWNWFSI
SNWLWYIK [SEQ ID NO: 93] (GenPept dbjBAF31430.1).

Ectodomain minus SP, minus MPER:

[0270] EQLWVTVYYGVPVWKEATTTLFCASDAKAVNTEVHNVWATHACVPTDPNPQEVVLENVT
ENFNMWKNDMVEQMQEDIISLWDQSLKPCVKLTPLCVTLNCTNWDGRNGTMNTTSTRNTTTANISRWEMEGE
IKNCSFNVTTSIRNKMHKEYALFYKLDVMPIDNGSSYTLINCNTSVITQACPKVSFEPIPIHYCTPAGFALLKCNDK
KFNGTGPCKNVSTVQCTHGIRPVVSTQLLLNGSLAEEEIVIRSENLTDNAKTIIVQLNETVVINCTRPGNNTRKSI
HIGPGRAFYATGDIIGDIRQAHCNLSEASWNKTLKQIATKLREQFVNKTIIFNQSSGGDPEIVMHSFNCGGEFFY
CDTTQLFNSAWFSNNTGLNYNNGSNTGGNITLPCRIKQIVNRWQEVGKAMYAPPIRGNITCSSNITGLLLTRDG
GNNVTNESEIFRPGGGNMKDNWRSELYKYKVVKIEPLGVAPTRAKRRVVQREKRAVGTIGAMFLGFLGAAGST
MGAASLTLTVQARQLLSGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARVLAVERYLKDQQLLGIWGCSGRLI
CTTAVPWNASWSNKSLDDIWNNMTWMQWEKEIDNYTGLIYRLIEESQTQQEKNEQDLLQ [SEQ ID NO: 94]
(GenPept dbjBAF31430.1).

Ectodomain minus SP plus altered furin cleavage sites:

[0271] EQLWVTVYYGVPVWKEATTTLFCASDAKAVNTEVHNVWATHACVPTDPNPQEVVLENVT
ENFNMWKNDMVEQMQEDIISLWDQSLKPCVKLTPLCVTLNCTNWDGRNGTMNTTSTRNTTTANISRWEMEGE
IKNCSFNVTTSIRNKMHKEYALFYKLDVMPIDNGSSYTLINCNTSVITQACPKVSFEPIPIHYCTPAGFALLKCNDK
KFNGTGPCKNVSTVQCTHGIRPVVSTQLLLNGSLAEEEIVIRSENLTDNAKTIIVQLNETVVINCTRPGNNTRKSI
HIGPGRAFYATGDIIGDIRQAHCNLSEASWNKTLKQIATKLREQFVNKTIIFNQSSGGDPEIVMHSFNCGGEFFY
CDTTQLFNSAWFSNNTGLNYNNGSNTGGNITLPCRIKQIVNRWQEVGKAMYAPPIRGNITCSSNITGLLLTRDG
GNNVTNESEIFRPGGGNMKDNWRSELYKYKVVKIEPLGVAPT*RA*N*NR*VVQREKRAVGTIGAMFLGFLGAAGST
MGAASLTLTVQARQLLSGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARVLAVERYLKDQQLLGIWGCSGRLI
CTTAVPWNASWSNKSLDDIWNNMTWMQWEKEIDNYTGLIYRLIEESQTQQEKNEQDLLQLDTWASLWNWFSI
SNWLWYIK [SEQ ID NO: 95] (GenPept dbjBAF31430.1).

GP41 ectodomain 509-688:

[0272] VVQREKRAVGTIGAMFLGFLGAAGSTMGAASLTLTVQARQLLSGIVQQQNNLLRAIEAQQ
HLLQLTVWGIKQLQARVLAVERYLKDQQLLGIWGCSGRLICTTAVPWNASWSNKSLDDIWNNMTWMQWEKEI
DNYTGLIYRLIEESQTQQEKNEQDLLQLDTWASLWNWFSISNWLWYIK [SEQ ID NO: 96] (GenPept
dbjBAF31430.1).

2.2.10 EBOV GP

[0273] A representative EBOV GP precursor has the following amino acid sequence: MGVTGILQLPRDRFKRTSFFLWVIILFQRTFSIPLGVIHNSTLQVSDVDKLVCRDKLSSTNQLRSVG
LNLEGNGVATDVPSATKRWGFRSGVPPKVVNYEAGEWAENCYNLEIKKPDGSECLPAAPDGIRGFPRCRYVHKV
SGTGPCAGDFAFHKEGAFFLYDRLASTVIYRGTTFAEGVVAFLILPQAKKDFFSSHPLREPVNATEDPSSGYYSTTI
RYQATGFGTNETEYLFEVDNLTYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKVNPEIDTTIGEWAFWETK
KNLTRKIRSEELSFTVVSNGAKNISGQSPARTSSDPGTNTTTEDHKIMASENSSAMVQVHSQGREAAVSHLTTL
ATISTSPQSLTTKPGPDNSTHNTPVYKLDISEATQVEQHHRRTDNDSTASDTPSATTAAGPPKAENTNTSKSTDF
LDPATTTSPQNHSETAGNNNTHHQDTGEESASSGKLGLITNTIAGVAGLITGGRRTRREAIVNAQPKCNPNLHY

WTTQDEGAAIGLAWIPYFGPAAEGIYIEGLMHNQDGLICGLRQLANETTQALQLFLRATTELRTFSILNRKAIDFLL QRWGGTCHILGPDCCIEPHDWTKNITDKIDQIIHDFVDKTLPDQGDNDNWWTGWRQWIPAGIGVTGVIIAVIA LFCICKFVF [SEQ ID NO: 97] (GenPept NP_066246.1).

[0274] This sequence comprises the following domains/moieties:

[0275] SP = 1-27

[0276] Ectodomain = 1-650

[0277] Furin cleavage sites = 501-502

[0278] Cathepsin cleavage sites = 191-192, 201-202

[0279] FP = 511-556

[0280] HRA region = 557-593

[0281] HRB region = 600-635

[0282] MPER = 636-650

[0283] TM = 651-669

[0284] C = 670-676

[0285] Mucin-like domain = 312-461

[0286] Non-limiting examples of EBOV GP ectodomain polypeptides include:

Ectodomain 1-650:

[0287] MGVTGILQLPRDRFKRTSFFLWVIILFQRTFSIPLGVIHNSTLQVSDVDKLVCRDKLSSTNQ LRSVGLNLEGNGVATDVPSATKRWGFRSGVPPKVVNYEAGEWAENCYNLEIKKPDGSECLPAAPDGIRGFPRCR YVHKVSGTGPCAGDFAFHKEGAFFLYDRLASTVIYRGTTFAEGVVAFLILPQAKKDFFSSHPLREPVNATEDPSSG YYSTTIRYQATGFGTNETEYLFEVDNLTYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKVNPEIDTTIGEWA FWETKKNLTRKIRSEELSFTVVSNGAKNISGQSPARTSSDPGTNTTTEDHKIMASENSSAMVQVHSQGREAAVS HLTTLATISTSPQSLTTKPGPDNSTHNTPVYKLDISEATQVEQHHRRTDNDSTASDTPSATTAAGPPKAENTNTS KSTDFLDPATTTSPQNHSETAGNNNTHHQDTGEESASSGKLGLITNTIAGVAGLITGGRRTRREAIVNAQPKCNP NLHYWTTQDEGAAIGLAWIPYFGPAAEGIYIEGLMHNQDGLICGLRQLANETTQALQLFLRATTELRTFSILNRKA IDFLLQRWGGTCHILGPDCCIEPHDWTKNITDKIDQIIHDFVDKTLPDQGDNDNWWTGWRQ [SEQ ID NO: 98] (GenPept NP_066246.1)

Ectodomain minus SP:

[0288] QRTFSIPLGVIHNSTLQVSDVDKLVCRDKLSSTNQLRSVGLNLEGNGVATDVPSATKRWG FRSGVPPKVVNYEAGEWAENCYNLEIKKPDGSECLPAAPDGIRGFPRCRYVHKVSGTGPCAGDFAFHKEGAFFLY DRLASTVIYRGTTFAEGVVAFLILPQAKKDFFSSHPLREPVNATEDPSSGYYSTTIRYQATGFGTNETEYLFEVDNL TYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKVNPEIDTTIGEWAFWETKKNLTRKIRSEELSFTVVSNGA KNISGQSPARTSSDPGTNTTTEDHKIMASENSSAMVQVHSQGREAAVSHLTTLATISTSPQSLTTKPGPDNSTH NTPVYKLDISEATQVEQHHRRTDNDSTASDTPSATTAAGPPKAENTNTSKSTDFLDPATTTSPQNHSETAGNNN THHQDTGEESASSGKLGLITNTIAGVAGLITGGRRTRREAIVNAQPKCNPNLHYWTTQDEGAAIGLAWIPYFGPA AEGIYIEGLMHNQDGLICGLRQLANETTQALQLFLRATTELRTFSILNRKAIDFLLQRWGGTCHILGPDCCIEPHD WTKNITDKIDQIIHDFVDKTLPDQGDNDNWWTGWRQ [SEQ ID NO: 99] (GenPept NP_066246.1).

Ectodomain minus SP, minus MPER:

[0289] QRTFSIPLGVIHNSTLQVSDVDKLVCRDKLSSTNQLRSVGLNLEGNGVATDVPSATKRWG FRSGVPPKVVNYEAGEWAENCYNLEIKKPDGSECLPAAPDGIRGFPRCRYVHKVSGTGPCAGDFAFHKEGAFFLY DRLASTVIYRGTTFAEGVVAFLILPQAKKDFFSSHPLREPVNATEDPSSGYYSTTIRYQATGFGTNETEYLFEVDNL TYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKVNPEIDTTIGEWAFWETKKNLTRKIRSEELSFTVVSNGA KNISGQSPARTSSDPGTNTTTEDHKIMASENSSAMVQVHSQGREAAVSHLTTLATISTSPQSLTTKPGPDNSTH NTPVYKLDISEATQVEQHHRRTDNDSTASDTPSATTAAGPPKAENTNTSKSTDFLDPATTTSPQNHSETAGNNN THHQDTGEESASSGKLGLITNTIAGVAGLITGGRRTRREAIVNAQPKCNPNLHYWTTQDEGAAIGLAWIPYFGPA AEGIYIEGLMHNQDGLICGLRQLANETTQALQLFLRATTELRTFSILNRKAIDFLLQRWGGTCHILGPDCCIEPHD WTKNITDKIDQIIHDFVDKTL [SEQ ID NO: 100] (GenPept NP_066246.1).

Ectodomain minus SP plus altered furin cleavage sites:

[0290] QRTFSIPLGVIHNSTLQVSDVDKLVCRDKLSSTNQLRSVGLNLEGNGVATDVPSATKRWG FRSGVPPKVVNYEAGEWAENCYNLEIKKPDGSECLPAAPDGIRGFPRCRYVHKVSGTGPCAGDFAFHKEGAFFLY DRLASTVIYRGTTFAEGVVAFLILPQAKKDFFSSHPLREPVNATEDPSSGYYSTTIRYQATGFGTNETEYLFEVDNL TYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKVNPEIDTTIGEWAFWETKKNLTRKIRSEELSFTVVSNGA KNISGQSPARTSSDPGTNTTTEDHKIMASENSSAMVQVHSQGREAAVSHLTTLATISTSPQSLTTKPGPDNSTH NTPVYKLDISEATQVEQHHRRTDNDSTASDTPSATTAAGPPKAENTNTSKSTDFLDPATTTSPQNHSETAGNNN THHQDTGEESASSGKLGLITNTIAGVAGLITGG*NNTNN*EAIVNAQPKCNPNLHYWTTQDEGAAIGLAWIPYFGP AAEGIYIEGLMHNQDGLICGLRQLANETTQALQLFLRATTELRTFSILNRKAIDFLLQRWGGTCHILGPDCCIEPH DWTKNITDKIDQIIHDFVDKTL [SEQ ID NO: 101] (GenPept NP_066246.1).

Ectodomain minus SP, minus mucin-like domain:

[0291] QRTFSIPLGVIHNSTLQVSDVDKLVCRDKLSSTNQLRSVGLNLEGNGVATDVPSATKRWG FRSGVPPKVVNYEAGEWAENCYNLEIKKPDGSECLPAAPDGIRGFPRCRYVHKVSGTGPCAGDFAFHKEGAFFLY DRLASTVIYRGTTFAEGVVAFLILPQAKKDFFSSHPLREPVNATEDPSSGYYSTTIRYQATGFGTNETEYLFEVDNL TYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKVNPEIDTTIGEWAFWETKKNLTRKIRSEELSFTVV*GG*NN THHQDTGEESASSGKLGLITNTIAGVAGLITGGRRTRREAIVNAQPKCNPNLHYWTTQDEGAAIGLAWIPYFGPA AEGIYIEGLMHNQDGLICGLRQLANETTQALQLFLRATTELRTFSILNRKAIDFLLQRWGGTCHILGPDCCIEPHD WTKNITDKIDQIIHDFVDKTLPDQGDNDNWWTGWRQ [SEQ ID NO: 102] (GenPept NP_066246.1).

Ectodomain minus mucin-like domain:

[0292] MGVTGILQLPRDRFKRTSFFLWVIILFQRTFSIPLGVIHNSTLQVSDVDKLVCRDKLSSTNQ LRSVGLNLEGNGVATDVPSATKRWGFRSGVPPKVVNYEAGEWAENCYNLEIKKPDGSECLPAAPDGIRGFPRCR YVHKVSGTGPCAGDFAFHKEGAFFLYDRLASTVIYRGTTFAEGVVAFLILPQAKKDFFSSHPLREPVNATEDPSSG YYSTTIRYQATGFGTNETEYLFEVDNLTYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKVNPEIDTTIGEWA FWETKKNLTRKIRSEESASSGKLGLITNTIAGVAGLITGGRRTRREAIVNAQPKCNPNLHYWTTQDEGAAIGLAW IPYFGPAAEGIYIEGLMHNQDGLICGLRQLANETTQALQLFLRATTELRTFSILNRKAIDFLLQRWGGTCHILGPDC CIEPHDWTKNITDKIDQIIHDFVDKTL [SEQ ID NO: 154]

2.2.11 MARV GP

[0293] A non-limiting MARV GP precursor has the following amino acid sequence: MKTTCFLISLILIQGTKNLPILEIASNNQPQNVDSVCSGTLQKTEDVHLMGFTLSGQKVADSPLEAS KRWAFRTGVPPKNVEYTEGEEAKTCYNISVTDPSGKSLLLDPPTNIRDYPKCKTIHHIQGQNPHAQGIALHLWGA FFLYDRIASTTMYRGKVFTEGNIAAMIVNKTVHKMIFSRQGQGYRHMNLTSTNKYWTSSNGTQTNDTGCFGAL QEYNSTKNQTCAPSKIPPPLPTARPEIKLTSTPTDATKLNTTDPSSDDEDLATSGSGSGEREPHTTSDAVTKQGL

SSTMPPTPSPQPSTPQQGGNNTNHSQDAVTELDKNNTTAQPSMPPHNTTTISTNNTSKHNFSTLSAPLQNTTND
NTQSTITENEQTSAPSITTLPPTGNPTTAKSTSSKKGPATTAPNTTNEHFTSPPPTPSSTAQHLVYFRRKRSILWRE
GDMFPFLDGLINAPIDFDPVPNTKTIFDESSSSGASAEEDQHASPNISLTLSYFPNINENTAYSGENENDCDAELR
IWSVQEDDLAAGLSWIPFFGPGIEGLYTAVLIKNQNNLVCRLRRLANQTAKSLELLLRVTTEERTFSLINRHAIDFL
LTRWGGTCKVLGPDCCIGIEDLSKNISEQIDQIKKDEQKEGTGWGLGGKWWTSDWGVLTNLGILLLLSIAVLIA
LSCICRIFTKYIG [SEQ ID NO: 103] (GenPept YP_001531156.1).

[0294] This sequence comprises the following domains/moieties:

[0295] SP = 1-19

[0296] Ectodomain = 1-650

[0297] Furin cleavage sites = 434-435

[0298] FP = 526-549

[0299] HRA region = 582-598

[0300] HRB region = 611-627

[0301] MPER = 628-650

[0302] TM = 651-669

[0303] C = 670-681

[0304] Mucin-like domain = 244-425

[0305] Non-limiting examples of MARV GP ectodomain polypeptides include:

Ectodomain 1 – 650:

[0306] MKTTCFLISLILIQGTKNLPILEIASNNQPQNVDSVCSGTLQKTEDVHLMGFTLSGQKVAD
SPLEASKRWAFRTGVPPKNVEYTEGEEAKTCYNISVTDPSGKSLLLDPPTNIRDYPKCKTIHHIQGQNPHAQGIAL
HLWGAFFLYDRIASTTMYRGKVFTEGNIAAMIVNKTVHKMIFSRQGQGYRHMNLTSTNKYWTSSNGTQTNDTG
CFGALQEYNSTKNQTCAPSKIPPPLPTARPEIKLTSTPTDATKLNTTDPSSDDEDLATSGSGSGEREPHTTSDAVT
KQGLSSTMPPTPSPQPSTPQQGGNNTNHSQDAVTELDKNNTTAQPSMPPHNTTTISTNNTSKHNFSTLSAPLQN
TTNDNTQSTITENEQTSAPSITTLPPTGNPTTAKSTSSKKGPATTAPNTTNEHFTSPPPTPSSTAQHLVYFRRKRSI
LWREGDMFPFLDGLINAPIDFDPVPNTKTIFDESSSSGASAEEDQHASPNISLTLSYFPNINENTAYSGENENDC
DAELRIWSVQEDDLAAGLSWIPFFGPGIEGLYTAVLIKNQNNLVCRLRRLANQTAKSLELLLRVTTEERTFSLINR
HAIDFLLTRWGGTCKVLGPDCCIGIEDLSKNISEQIDQIKKDEQKEGTGWGLGGKWWTSDWG [SEQ ID NO:
104] (GenPept YP_001531156.1).

Ectodomain minus SP 20-650:

[0307] PILEIASNNQPQNVDSVCSGTLQKTEDVHLMGFTLSGQKVADSPLEASKRWAFRTGVPPK
NVEYTEGEEAKTCYNISVTDPSGKSLLLDPPTNIRDYPKCKTIHHIQGQNPHAQGIALHLWGAFFLYDRIASTTMY
RGKVFTEGNIAAMIVNKTVHKMIFSRQGQGYRHMNLTSTNKYWTSSNGTQTNDTGCFGALQEYNSTKNQTCAP
SKIPPPLPTARPEIKLTSTPTDATKLNTTDPSSDDEDLATSGSGSGEREPHTTSDAVTKQGLSSTMPPTPSPQPST
PQQGGNNTNHSQDAVTELDKNNTTAQPSMPPHNTTTISTNNTSKHNFSTLSAPLQNTTNDNTQSTITENEQTSA
PSITTLPPTGNPTTAKSTSSKKGPATTAPNTTNEHFTSPPPTPSSTAQHLVYFRRKRSILWREGDMFPFLDGLINAP
IDFDPVPNTKTIFDESSSSGASAEEDQHASPNISLTLSYFPNINENTAYSGENENDCDAELRIWSVQEDDLAAGL
SWIPFFGPGIEGLYTAVLIKNQNNLVCRLRRLANQTAKSLELLLRVTTEERTFSLINRHAIDFLLTRWGGTCKVLGP

DCCIGIEDLSKNISEQIDQIKKDEQKEGTGWGLGGKWWTSDWG [SEQ ID NO: 105] (GenPept YP_001531156.1).

Ectodomain minus SP, minus MPER 20-627:

[0308] PILEIASNNQPQNVDSVCSGTLQKTEDVHLMGFTLSGQKVADSPLEASKRWAFRTGVPPK NVEYTEGEEAKTCYNISVTDPSGKSLLLDPPTNIRDYPKCKTIHHIQGQNPHAQGIALHLWGAFFLYDRIASTTMY RGKVFTEGNIAAMIVNKTVHKMIFSRQGQGYRHMNLTSTNKYWTSSNGTQTNDTGCFGALQEYNSTKNQTCAP SKIPPPLPTARPEIKLTSTPTDATKLNTTDPSSDDEDLATSGSGSGEREPHTTSDAVTKQGLSSTMPPTPSPQPST PQQGGNNTNHSQDAVTELDKNNTTAQPSMPPHNTTTISTNNTSKHNFSTLSAPLQNTTNDNTQSTITENEQTSA PSITTLPPTGNPTTAKSTSSKKGPATTAPNTTNEHFTSPPPTPSSTAQHLVYFRRKRSILWREGDMFPFLDGLINAP IDFDPVPNTKTIFDESSSSGASAEEDQHASPNISLTLSYFPNINENTAYSGENENDCDAELRIWSVQEDDLAAGL SWIPFFGPGIEGLYTAVLIKNQNNLVCRLRRLANQTAKSLELLLRVTTEERTFSLINRHAIDFLLTRWGGTCKVLGP DCCIGIEDLSKNISEQIDQI [SEQ ID NO: 106] (GenPept YP_001531156.1).

Ectodomain minus SP plus altered furin cleavage sites:

[0309] PILEIASNNQPQNVDSVCSGTLQKTEDVHLMGFTLSGQKVADSPLEASKRWAFRTGVPPK NVEYTEGEEAKTCYNISVTDPSGKSLLLDPPTNIRDYPKCKTIHHIQGQNPHAQGIALHLWGAFFLYDRIASTTMY RGKVFTEGNIAAMIVNKTVHKMIFSRQGQGYRHMNLTSTNKYWTSSNGTQTNDTGCFGALQEYNSTKNQTCAP SKIPPPLPTARPEIKLTSTPTDATKLNTTDPSSDDEDLATSGSGSGEREPHTTSDAVTKQGLSSTMPPTPSPQPST PQQGGNNTNHSQDAVTELDKNNTTAQPSMPPHNTTTISTNNTSKHNFSTLSAPLQNTTNDNTQSTITENEQTSA PSITTLPPTGNPTTAKSTSSKKGPATTAPNTTNEHFTSPPPTPSSTAQHLVYF*NNNN*SILWREGDMFPFLDGLINA PIDFDPVPNTKTIFDESSSSGASAEEDQHASPNISLTLSYFPNINENTAYSGENENDCDAELRIWSVQEDDLAAG LSWIPFFGPGIEGLYTAVLIKNQNNLVCRLRRLANQTAKSLELLLRVTTEERTFSLINRHAIDFLLTRWGGTCKVLG PDCCIGIEDLSKNISEQIDQIKKDEQKEGTGWGLGGKWWTSDWG [SEQ ID NO: 107] (GenPept YP_001531156.1).

Ectodomain minus SP, minus mucin-like domain:

[0310] PILEIASNNQPQNVDSVCSGTLQKTEDVHLMGFTLSGQKVADSPLEASKRWAFRTGVPPK NVEYTEGEEAKTCYNISVTDPSGKSLLLDPPTNIRDYPKCKTIHHIQGQNPHAQGIALHLWGAFFLYDRIASTTMY RGKVFTEGNIAAMIVNKTVHKMIFSRQGQGYRHMNLTSTNKYWTSSNGTQTNDTGCFGALQEYNSTKNQTCAP SKIPPPLPTARPEIKL*GG*AQHLVYFRRKRSILWREGDMFPFLDGLINAPIDFDPVPNTKTIFDESSSSGASAEEDQ HASPNISLTLSYFPNINENTAYSGENENDCDAELRIWSVQEDDLAAGLSWIPFFGPGIEGLYTAVLIKNQNNLVCR LRRLANQTAKSLELLLRVTTEERTFSLINRHAIDFLLTRWGGTCKVLGPDCCIGIEDLSKNISEQIDQIKKDEQKEG TGWGLGGKWWTSDWG [SEQ ID NO: 108] (GenPept YP_001531156.1).

2.2.12 SARS-CoV S

[0311] An illustrative SARS-CoV S precursor has the following amino acid sequence: MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTQDLFLPFYSNVT GFHTINHTFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQSVIIINNSTNVVIRACNFELCDNPFFAVSKP MGTQTHTMIFDNAFNCTFEYISDAFSLDVSEKSGNFKHLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNTLKPI FKLPLGINITNFRAILTAFSPAQDIWGTSAAAYFVGYLKPTTFMLKYDENGTITDAVDCSQNPLAELKCSVKSFEID KGIYQTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKISNCVADYSVLYNSTFFSTFKCYGVSAT KLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRH GKLRPFERDISNVPFSPDGKPCTPPALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLIK NQCVNFNFNGLTGTGVLTPSSKRFQPFQQFGRDVSDFTDSVRDPKTSEILDISPCAFGGVSVITPGTNASSEVAV LYQDVNCTNVSAAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEHVDTSYECDIPIGAGICASYHTVSLLRSTSQ

KSIVAYTMSLGADSSIAYSNNTIAIPTNFSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNR
ALSGIAAEQDRNTREVFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQYGECL
GDINARDLICAQKFNGLTVLPPLLTDDMIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVL
YENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEV
QIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGVVFLHVT
YVPSQERNFTTAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFSPQIITTDNTFVSGNCDVVIGIINNTVYDPLQ
PELDSFKGELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYVW
LGFIAGLIAIVMVTILLCCMTSCCSCLKGACSCGSCCKFDEDDSEPVLKGVKLHYT [SEQ ID NO: 109]
(GenPept gbAAR86788.1).

[0312] This sequence comprises the following domains/moieties:

[0313] SP = 1-13

[0314] Ectodomain = 1-1199?

[0315] human airway trypsin-like protease cleavage sites = 667-668

[0316] FP = 770-788

[0317] HRA region = 892-1013

[0318] HRB region = 1145-1187

[0319] MPER = 1188-1199

[0320] TM = 1200-1216

[0321] C = 1217-1255

[0322] Non-limiting examples of SARS-CoV S ectodomain polypeptides include:

Ectodomain 1 – 1199:

[0323] MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTQDLFLPF
YSNVTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQSVIIINNSTNVVIRACNFELCDNPFF
AVSKPMGTQTHTMIFDNAFNCTFEYISDAFSLDVSEKSGNFKHLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGF
NTLKPIFKLPLGINITNFRAILTAFSPAQDIWGTSAAAYFVGYLKPTTFMLKYDENGTITDAVDCSQNPLAELKCSV
KSFEIDKGIYQTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKISNCVADYSVLYNSTFFSTFKC
YGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYK
YRYLRHGKLRPFERDISNVPFSPDGKPCTPPALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKL
STDLIKNQCVNFNFNGLTGTGVLTPSSKRFQPFQQFGRDVSDFTDSVRDPKTSEILDISPCAFGGVSVITPGTNA
SSEVAVLYQDVNCTNVSAAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEHVDTSYECDIPIGAGICASYHTVSL
LRSTSQKSIVAYTMSLGADSSIAYSNNTIAIPTNFSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSF
CTQLNRALSGIAAEQDRNTREVFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMK
QYGECLGDINARDLICAQKFNGLTVLPPLLTDDMIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIG
VTQNVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLD
KVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGV
VFLHVTYVPSQERNFTTAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFSPQIITTDNTFVSGNCDVVIGIINNTV
YDPLQPELDSFKGELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWP
WYVW [SEQ ID NO: 110] (GenPept gbAAR86788.1).

Ectodomain minus SP:

[0324] SDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTQDLFLPFYSNVTGFHTINH TFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQSVIIINNSTNVVIRACNFELCDNPFFAVSKPMGTQTH TMIFDNAFNCTFEYISDAFSLDVSEKSGNFKHLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNTLKPIFKLPLGI NITNFRAILTAFSPAQDIWGTSAAAYFVGYLKPTTFMLKYDENGTITDAVDCSQNPLAELKCSVKSFEIDKGIYQT SNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKISNCVADYSVLYNSTFFSTFKCYGVSATKLNDL CFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPF ERDISNVPFSPDGKPCTPPALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLIKNQCVNF NFNGLTGTGVLTPSSKRFQPFQQFGRDVSDFTDSVRDPKTSEILDISPCAFGGVSVITPGTNASSEVAVLYQDVN CTNVSAAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEHVDTSYECDIPIGAGICASYHTVSLLRSTSQKSIVAYT MSLGADSSIAYSNNTIAIPTNFSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRALSGIAA EQDRNTREVFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQYGECLGDINAR DLICAQKFNGLTVLPPLLTDDMIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQK QIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLI TGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGVVFLHVTYVPSQ ERNFTTAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFSPQIITTDNTFVSGNCDVVIGIINNTVYDPLQPELDSF KGELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYVW [SEQ ID NO: 111] (GenPept gbAAR86788.1).

Ectodomain minus SP, minus MPER:

[0325] SDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTQDLFLPFYSNVTGFHTINH TFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQSVIIINNSTNVVIRACNFELCDNPFFAVSKPMGTQTH TMIFDNAFNCTFEYISDAFSLDVSEKSGNFKHLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGFNTLKPIFKLPLGI NITNFRAILTAFSPAQDIWGTSAAAYFVGYLKPTTFMLKYDENGTITDAVDCSQNPLAELKCSVKSFEIDKGIYQT SNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKISNCVADYSVLYNSTFFSTFKCYGVSATKLNDL CFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYKYRYLRHGKLRPF ERDISNVPFSPDGKPCTPPALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKLSTDLIKNQCVNF NFNGLTGTGVLTPSSKRFQPFQQFGRDVSDFTDSVRDPKTSEILDISPCAFGGVSVITPGTNASSEVAVLYQDVN CTNVSAAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEHVDTSYECDIPIGAGICASYHTVSLLRSTSQKSIVAYT MSLGADSSIAYSNNTIAIPTNFSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSFCTQLNRALSGIAA EQDRNTREVFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMKQYGECLGDINAR DLICAQKFNGLTVLPPLLTDDMIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQK QIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLI TGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGVVFLHVTYVPSQ ERNFTTAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFSPQIITTDNTFVSGNCDVVIGIINNTVYDPLQPELDSF KGELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGK [SEQ ID NO: 112] (GenPept gbAAR86788.1).

2.2.13 MERS-CoV S

[0326] An exemplary MERS-CoV S precursor has the following amino acid sequence: MIHSVFLLMFLLTPTESYVDVGPDSVKSACIEVDIQQTFFDKTWPRPIDVSKADGIIYPQGRTYSNIT ITYQGLFPYQGDHGDMYVYSAGHATGTTPQKLFVANYSQDVKQFANGFVVRIGAAANSTGTVIISPSTSATIRKI YPAFMLGSSVGNFSDGKMGRFFNHTLVLLPDGCGTLLRAFYCILEPRSGNHCPAGNSYTSFATYHTPATDCSDG NYNRNASLNSFKEYFNLRNCTFMYTYNITEDEILEWFGITQTAQGVHLFSSRYVDLYGGNMFQFATLPVYDTIKYY SIIPHSIRSIQSDRKAWAAFYVYKLQPLTFLLDFSVDGYIRRAIDCGFNDLSQLHCSYESFDVESGVYSVSSFEAK

PSGSVVEQAEGVECDFSPLLSGTPPQVYNFKRLVFTNCNYNLTKLLSLFSVNDFTCSQISPAAIASNCYSSLILDYF
SYPLSMKSDLGVSSAGPISQFNYKQSFSNPTCLILATVPHNLTTITKPLKYSYINKCSRLLSDDRTEVPQLVNANQ
YSPCVSIVPSTVWEDGDYYRKQLSPLEGGGWLVASGSTVAMTEQLQMGFGITVQYGTDTNSVCPKLEFANDTKI
ASQLGNCVEYSLYGVSGRGVFQNCTAVGVRQQRFVYDAYQNLVGYYSDDGNYYCLRACVSVPVSVIYDKETKT
HATLFGSVACEHISSTMSQYSRSTRSMLKRRDSTYGPLQTPVGCVLGLVNSSLFVEDCKLPLGQSLCALPDTPST
LTPRSVRSVPGEMRLASIAFNHPIQVDQFNSSYFKLSIPTNFSFGVTQEYIQTTIQKVTVDCKQYICNGFQKCEQL
LREYGQFCSKINQALHGANLRQDDSVRNLFASVKSSQSSPIIPGFGGDFNLTLLEPVSISTGSRSARSAIEDLLFD
KVTIADPGYMQGYDDCMQQGPASARDLICAQYVAGYKVLPPLMDVNMEAAYTSSLLGSIAGVGWTAGLSSFAA
IPFAQSIFYRLNGVGITQQVLSENQKLIANKFNQALGAMQTGFTTTNEAFRKVQDAVNNNAQALSKLASELSNTF
GAISASIGDIIQRLDVLEQDAQIDRLINGRLTTLNAFVAQQLVRSESAALSAQLAKDKVNECVKAQSKRSGFCGQ
GTHIVSFVVNAPNGLYFMHVGYYPSNHIEVVSAYGLCDAANPTNCIAPVNGYFIKTNNTRIVDEWSYTGSSFYSP
EPITSLNTKYVAPQVTYQNISTNLPPPLLGNSTGIDFQDELDEFFKNVSTSIPNFGSLTQINTTLLDLTYEMLSLQQ
VVKALNESYIDLKELGNYTYYNKWPWYIWLGFIAGLVALALCVFFILCCTGCGTNCMGKLKCNRCCDRYEEYDLE
PHKVHVH [SEQ ID NO: 113] (GenPept gbAHX00711.1).

[0327] This sequence comprises the following domains/moieties:

[0328] SP = 1-21

[0329] Ectodomain = 1-1301

[0330] Furin cleavage sites = 751-752, 887-888

[0331] FP = 888-891, 951-980

[0332] HRA region = 984-1105

[0333] HRB region = 1248-1291

[0334] MPER = 1292-1301

[0335] TM = 1302-1318

[0336] C = 1319-1353

[0337] Non-limiting examples of MERS-CoV S ectodomain polypeptides include:

Ectodomain 1 – 1301:

[0338] MIHSVFLLMFLLTPTESYVDVGPDSVKSACIEVDIQQTFFDKTWPRPIDVSKADGIIYPQGR
TYSNITITYQGLFPYQGDHGDMYVYSAGHATGTTPQKLFVANYSQDVKQFANGFVVRIGAAANSTGTVIISPSTS
ATIRKIYPAFMLGSSVGNFSDGKMGRFFNHTLVLLPDGCGTLLRAFYCILEPRSGNHCPAGNSYTSFATYHTPATD
CSDGNYNRNASLNSFKEYFNLRNCTFMYTYNITEDEILEWFGITQTAQGVHLFSSRYVDLYGGNMFQFATLPVYD
TIKYYSIIPHSIRSIQSDRKAWAAFYVYKLQPLTFLLDFSVDGYIRRAIDCGFNDLSQLHCSYESFDVESGVYSVSS
FEAKPSGSVVEQAEGVECDFSPLLSGTPPQVYNFKRLVFTNCNYNLTKLLSLFSVNDFTCSQISPAAIASNCYSSLI
LDYFSYPLSMKSDLGVSSAGPISQFNYKQSFSNPTCLILATVPHNLTTITKPLKYSYINKCSRLLSDDRTEVPQLVN
ANQYSPCVSIVPSTVWEDGDYYRKQLSPLEGGGWLVASGSTVAMTEQLQMGFGITVQYGTDTNSVCPKLEFAN
DTKIASQLGNCVEYSLYGVSGRGVFQNCTAVGVRQQRFVYDAYQNLVGYYSDDGNYYCLRACVSVPVSVIYDKE
TKTHATLFGSVACEHISSTMSQYSRSTRSMLKRRDSTYGPLQTPVGCVLGLVNSSLFVEDCKLPLGQSLCALPDT
PSTLTPRSVRSVPGEMRLASIAFNHPIQVDQFNSSYFKLSIPTNFSFGVTQEYIQTTIQKVTVDCKQYICNGFQKC
EQLLREYGQFCSKINQALHGANLRQDDSVRNLFASVKSSQSSPIIPGFGGDFNLTLLEPVSISTGSRSARSAIEDL
LFDKVTIADPGYMQGYDDCMQQGPASARDLICAQYVAGYKVLPPLMDVNMEAAYTSSLLGSIAGVGWTAGLSS
FAAIPFAQSIFYRLNGVGITQQVLSENQKLIANKFNQALGAMQTGFTTTNEAFRKVQDAVNNNAQALSKLASELS

NTFGAISASIGDIIQRLDVLEQDAQIDRLINGRLTTLNAFVAQQLVRSESAALSAQLAKDKVNECVKAQSKRSGF
CGQGTHIVSFVVNAPNGLYFMHVGYYPSNHIEVVSAYGLCDAANPTNCIAPVNGYFIKTNNTRIVDEWSYTGSSF
YSPEPITSLNTKYVAPQVTYQNISTNLPPPLLGNSTGIDFQDELDEFFKNVSTSIPNFGSLTQINTTLLDLTYEMLSL
QQVVKALNESYIDLKELGNYTYYNKWPWYIWL [SEQ ID NO: 114] (GenPept gbAHX00711.1).

Ectodomain minus SP 22-1301:

[0339] GPDSVKSACIEVDIQQTFFDKTWPRPIDVSKADGIIYPQGRTYSNITITYQGLFPYQGDHG
DMYVYSAGHATGTTPQKLFVANYSQDVKQFANGFVVRIGAAANSTGTVIISPSTSATIRKIYPAFMLGSSVGNFS
DGKMGRFFNHTLVLLPDGCGTLLRAFYCILEPRSGNHCPAGNSYTSFATYHTPATDCSDGNYNRNASLNSFKEYF
NLRNCTFMYTYNITEDEILEWFGITQTAQGVHLFSSRYVDLYGGNMFQFATLPVYDTIKYYSIIPHSIRSIQSDRKA
WAAFYVYKLQPLTFLLDFSVDGYIRRAIDCGFNDLSQLHCSYESFDVESGVYSVSSFEAKPSGSVVEQAEGVECD
FSPLLSGTPPQVYNFKRLVFTNCNYNLTKLLSLFSVNDFTCSQISPAAIASNCYSSLILDYFSYPLSMKSDLGVSSA
GPISQFNYKQSFSNPTCLILATVPHNLTTITKPLKYSYINKCSRLLSDDRTEVPQLVNANQYSPCVSIVPSTVWED
GDYYRKQLSPLEGGGWLVASGSTVAMTEQLQMGFGITVQYGTDTNSVCPKLEFANDTKIASQLGNCVEYSLYG
VSGRGVFQNCTAVGVRQQRFVYDAYQNLVGYYSDDGNYYCLRACVSVPVSVIYDKETKTHATLFGSVACEHISS
TMSQYSRSTRSMLKRRDSTYGPLQTPVGCVLGLVNSSLFVEDCKLPLGQSLCALPDTPSTLTPRSVRSVPGEMRL
ASIAFNHPIQVDQFNSSYFKLSIPTNFSFGVTQEYIQTTIQKVTVDCKQYICNGFQKCEQLLREYGQFCSKINQAL
HGANLRQDDSVRNLFASVKSSQSSPIIPGFGGDFNLTLLEPVSISTGSRSARSAIEDLLFDKVTIADPGYMQGYD
DCMQQGPASARDLICAQYVAGYKVLPPLMDVNMEAAYTSSLLGSIAGVGWTAGLSSFAAIPFAQSIFYRLNGVGI
TQQVLSENQKLIANKFNQALGAMQTGFTTTNEAFRKVQDAVNNNAQALSKLASELSNTFGAISASIGDIIQRLDV
LEQDAQIDRLINGRLTTLNAFVAQQLVRSESAALSAQLAKDKVNECVKAQSKRSGFCGQGTHIVSFVVNAPNGL
YFMHVGYYPSNHIEVVSAYGLCDAANPTNCIAPVNGYFIKTNNTRIVDEWSYTGSSFYSPEPITSLNTKYVAPQVT
YQNISTNLPPPLLGNSTGIDFQDELDEFFKNVSTSIPNFGSLTQINTTLLDLTYEMLSLQQVVKALNESYIDLKELG
NYTYYNKWPWYIWL [SEQ ID NO: 115] (GenPept gbAHX00711.1).

Ectodomain minus SP, minus MPER 22-1291:

[0340] GPDSVKSACIEVDIQQTFFDKTWPRPIDVSKADGIIYPQGRTYSNITITYQGLFPYQGDHG
DMYVYSAGHATGTTPQKLFVANYSQDVKQFANGFVVRIGAAANSTGTVIISPSTSATIRKIYPAFMLGSSVGNFS
DGKMGRFFNHTLVLLPDGCGTLLRAFYCILEPRSGNHCPAGNSYTSFATYHTPATDCSDGNYNRNASLNSFKEYF
NLRNCTFMYTYNITEDEILEWFGITQTAQGVHLFSSRYVDLYGGNMFQFATLPVYDTIKYYSIIPHSIRSIQSDRKA
WAAFYVYKLQPLTFLLDFSVDGYIRRAIDCGFNDLSQLHCSYESFDVESGVYSVSSFEAKPSGSVVEQAEGVECD
FSPLLSGTPPQVYNFKRLVFTNCNYNLTKLLSLFSVNDFTCSQISPAAIASNCYSSLILDYFSYPLSMKSDLGVSSA
GPISQFNYKQSFSNPTCLILATVPHNLTTITKPLKYSYINKCSRLLSDDRTEVPQLVNANQYSPCVSIVPSTVWED
GDYYRKQLSPLEGGGWLVASGSTVAMTEQLQMGFGITVQYGTDTNSVCPKLEFANDTKIASQLGNCVEYSLYG
VSGRGVFQNCTAVGVRQQRFVYDAYQNLVGYYSDDGNYYCLRACVSVPVSVIYDKETKTHATLFGSVACEHISS
TMSQYSRSTRSMLKRRDSTYGPLQTPVGCVLGLVNSSLFVEDCKLPLGQSLCALPDTPSTLTPRSVRSVPGEMRL
ASIAFNHPIQVDQFNSSYFKLSIPTNFSFGVTQEYIQTTIQKVTVDCKQYICNGFQKCEQLLREYGQFCSKINQAL
HGANLRQDDSVRNLFASVKSSQSSPIIPGFGGDFNLTLLEPVSISTGSRSARSAIEDLLFDKVTIADPGYMQGYD
DCMQQGPASARDLICAQYVAGYKVLPPLMDVNMEAAYTSSLLGSIAGVGWTAGLSSFAAIPFAQSIFYRLNGVGI
TQQVLSENQKLIANKFNQALGAMQTGFTTTNEAFRKVQDAVNNNAQALSKLASELSNTFGAISASIGDIIQRLDV
LEQDAQIDRLINGRLTTLNAFVAQQLVRSESAALSAQLAKDKVNECVKAQSKRSGFCGQGTHIVSFVVNAPNGL
YFMHVGYYPSNHIEVVSAYGLCDAANPTNCIAPVNGYFIKTNNTRIVDEWSYTGSSFYSPEPITSLNTKYVAPQVT
YQNISTNLPPPLLGNSTGIDFQDELDEFFKNVSTSIPNFGSLTQINTTLLDLTYEMLSLQQVVKALNESYIDLKELG
NYTY [SEQ ID NO: 116] (GenPept gbAHX00711.1).

Ectodomain minus SP plus altered furin cleavage sites:

[0341] GPDSVKSACIEVDIQQTFFDKTWPRPIDVSKADGIIYPQGRTYSNITITYQGLFPYQGDHG DMYVYSAGHATGTTPQKLFVANYSQDVKQFANGFVVRIGAAANSTGTVIISPSTSATIRKIYPAFMLGSSVGNFS DGKMGRFFNHTLVLLPDGCGTLLRAFYCILEPRSGNHCPAGNSYTSFATYHTPATDCSDGNYNRNASLNSFKEYF NLRNCTFMYTYNITEDEILEWFGITQTAQGVHLFSSRYVDLYGGNMFQFATLPVYDTIKYYSIIPHSIRSIQSDRKA WAAFYVYKLQPLTFLLDFSVDGYIRRAIDCGFNDLSQLHCSYESFDVESGVYSVSSFEAKPSGSVVEQAEGVECD FSPLLSGTPPQVYNFKRLVFTNCNYNLTKLLSLFSVNDFTCSQISPAAIASNCYSSLILDYFSYPLSMKSDLGVSSA GPISQFNYKQSFSNPTCLILATVPHNLTTITKPLKYSYINKCSRLLSDDRTEVPQLVNANQYSPCVSIVPSTVWED GDYYRKQLSPLEGGGWLVASGSTVAMTEQLQMGFGITVQYGTDTNSVCPKLEFANDTKIASQLGNCVEYSLYG VSGRGVFQNCTAVGVRQQRFVYDAYQNLVGYYSDDGNYYCLRACVSVPVSVIYDKETKTHATLFGSVACEHISS TMSQYSRSTRSMLKRRDSTYGPLQTPVGCVLGLVNSSLFVEDCKLPLGQSLCALPDTPSTLTP*N*SV*N*SVPGEMRL ASIAFNHPIQVDQFNSSYFKLSIPTNFSFGVTQEYIQTTIQKVTVDCKQYICNGFQKCEQLLREYGQFCSKINQAL HGANLRQDDSVRNLFASVKSSQSSPIIPGFGGDFNLTLLEPVSISTGS*N*SA*N*SAIEDLLFDKVTIADPGYMQGYD DCMQQGPASARDLICAQYVAGYKVLPPLMDVNMEAAYTSSLLGSIAGVGWTAGLSSFAAIPFAQSIFYRLNGVGI TQQVLSENQKLIANKFNQALGAMQTGFTTTNEAFRKVQDAVNNNAQALSKLASELSNTFGAISASIGDIIQRLDV LEQDAQIDRLINGRLTTLNAFVAQQLVRSESAALSAQLAKDKVNECVKAQSKRSGFCGQGTHIVSFVVNAPNGL YFMHVGYYPSNHIEVVSAYGLCDAANPTNCIAPVNGYFIKTNNTRIVDEWSYTGSSFYSPEPITSLNTKYVAPQVT YQNISTNLPPPLLGNSTGIDFQDELDEFFKNVSTSIPNFGSLTQINTTLLDLTYEMLSLQQVVKALNESYIDLKELG NYTYYNKWPWYIWL [SEQ ID NO: 117] (GenPept gbAHX00711.1).

2.2.14 VSV G

[0342] An exemplary VSV G precursor has the following amino acid sequence: MKCLLYLAFLFIGVNCKFTIVFPHNQKGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPKSHK AIQADGWMCHASKWVTTCDFRWYGPKYITQSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAE AVIVQVTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSEDGELSS LGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEGSSISAPSQTSVDVS LIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIINGTLKYFETRYIRVDIAAPILSRMVGMIS GTTTERELWDDWAPYEDVEIGPNGVLRTSSGYKFPLYMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDES LFFGDTGLSKNPIELVEGWFSSWKSSIASFFFIIGLIIGLFLVLRVGIHLCIKLKHTKKRQIYTDIEMNRLG [SEQ ID NO: 118] (GenPept gbADX53329.1).

[0343] This sequence comprises the following domains/moieties:

[0344] SP = 1-17

[0345] Ectodomain = 1-462

[0346] MPER = 421-462

[0347] TM = 462-483

[0348] C = 484-510

[0349] Non-limiting examples of VSV G ectodomain polypeptides include:

Ectodomain 1 – 462:

[0350] MKCLLYLAFLFIGVNCKFTIVFPHNQKGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVK MPKSHKAIQADGWMCHASKWVTTCDFRWYGPKYITQSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYA TVTDAEAVIVQVTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSE

DGELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEGSSISAPSQ TSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIINGTLKYFETRYIRVDIAAPILSR MVGMISGTTTERELWDDWAPYEDVEIGPNGVLRTSSGYKFPLYMIGHGMLDSDLHLSSKAQVFEHPHIQDAAS QLPDDESLFFGDTGLSKNPIELVEGWFSSWK [SEQ ID NO: 119] (GenPept gbADX53329.1).

Ectodomain minus SP:

[0351] FTIVFPHNQKGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPKSHKAIQADGWMC HASKWVTTCDFRWYGPKYITQSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQVTPHH VLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSEDGELSSLGKEGTGFRS NYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEGSSISAPSQTSVDVSLIQDVERILDY SLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIINGTLKYFETRYIRVDIAAPILSRMVGMISGTTTERELWD DWAPYEDVEIGPNGVLRTSSGYKFPLYMIGHGMLDSDLHLSSKAQVFEHPHIQDAASQLPDDESLFFGDTGLSK NPIELVEGWFSSWK [SEQ ID NO: 120] (gbADX53329.1).

Ectodomain minus SP, minus MPER:

[0352] FTIVFPHNQKGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVKMPKSHKAIQADGWMC HASKWVTTCDFRWYGPKYITQSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQVTPHH VLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSEDGELSSLGKEGTGFRS NYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEGSSISAPSQTSVDVSLIQDVERILDY SLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIINGTLKYFETRYIRVDIAAPILSRMVGMISGTTTERELWD DWAPYEDVEIGPNGVLRTSSGYKFPLYMIGHGMLDSDLHLSSKAQVF [SEQ ID NO: 121] (GenPept gbADX53329.1).

2.2.15 RABV GP

[0353] An exemplary RABV GP precursor has the following amino acid sequence: MIPQTLLFVPLLVFSLCFGKFPIYTIPDKLGPWSPIDIHHLSCPNNLVVEDEGCTNLSGFSYMELKVGY ISAIKVNGFTCTGVVTEAETYTNFVGYVTTTFKRKHFRPTPDACRAAYNWKMAGDPRYEESLHNPYPDYHWLRTV KTTKESLVIISPSVSDLDPYDKSLHSRVFPSGKCSGITVSSTYCPTNHDYTIWMPENPRLGTSCDIFTNSRGKRAS KGSKTCGFVDERGLYKSLKGACKLKLCGVLGLRLMDGTWAAIQTSDEAKWCPPDQLVNIHDFRSDEIEHLVVEE LVKKREECLDALESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLMEADAHYKSVRTWNEIIPSKGCLRVGGRC HPHVNGVFFNGIILGPDGHVLIPEMQSSLLQQHMELLESSVIPLMHPLADPSTVFKDGDEAEDFVEVHLPDVHKQ VSGVDLGLPSWGKYVLMSVGTLIALMLMILLTTCCRKANGAESIQHRLGETGRKVSVTSQNGRVISSWESYKSG GETKL [SEQ ID NO: 122] (GenPept gbAFM52658.1).

[0354] This sequence comprises the following domains/moieties:

[0355] SP = 1-20

[0356] Ectodomain = 1-458

[0357] TM = 459-478

[0358] C = 479-524

[0359] Non-limiting examples of RABV GP ectodomain polypeptides include:

Ectodomain 1-458:

[0360] MIPQTLLFVPLLVFSLCFGKFPIYTIPDKLGPWSPIDIHHLSCPNNLVVEDEGCTNLSGFSYM ELKVGYISAIKVNGFTCTGVVTEAETYTNFVGYVTTTFKRKHFRPTPDACRAAYNWKMAGDPRYEESLHNPYPDY HWLRTVKTTKESLVIISPSVSDLDPYDKSLHSRVFPSGKCSGITVSSTYCPTNHDYTIWMPENPRLGTSCDIFTNS

RGKRASKGSKTCGFVDERGLYKSLKGACKLKLCGVLGLRLMDGTWAAIQTSDEAKWCPPDQLVNIHDFRSDEI EHLVVEELVKKREECLDALESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLMEADAHYKSVRTWNEIIPSKGC LRVGGRCHPHVNGVFFNGIILGPDGHVLIPEMQSSLLQQHMELLESSVIPLMHPLADPSTVFKDGDEAEDFVEVH LPDVHKQVSGVDLGLPSWGK [SEQ ID NO: 123] (GenPept gbAFM52658.1).

Ectodomain minus SP:

[0361] FPIYTIPDKLGPWSPIDIHHLSCPNNLVVEDEGCTNLSGFSYMELKVGYISAIKVNGFTCTG VVTEAETYTNFVGYVTTTFKRKHFRPTPDACRAAYNWKMAGDPRYEESLHNPYPDYHWLRTVKTTKESLVIISPS VSDLDPYDKSLHSRVFPSGKCSGITVSSTYCPTNHDYTIWMPENPRLGTSCDIFTNSRGKRASKGSKTCGFVDE RGLYKSLKGACKLKLCGVLGLRLMDGTWAAIQTSDEAKWCPPDQLVNIHDFRSDEIEHLVVEELVKKREECLDA LESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLMEADAHYKSVRTWNEIIPSKGCLRVGGRCHPHVNGVFFN GIILGPDGHVLIPEMQSSLLQQHMELLESSVIPLMHPLADPSTVFKDGDEAEDFVEVHLPDVHKQVSGVDLGLPS WGK [SEQ ID NO: 124] (GenPept gbAFM52658.1).

2.2.16 HSV1 Gb

[0362] An exemplary HSV1 Gb precursor has the following amino acid sequence: MRQGAPARGCRWFVVWALLGLTLGVLVASAAPSSPGTPGVAAATQAANGGPATPAPPALGAAPTG DPKPKKNKKPKNPTPPRPAGDNATVAAGHATLREHLRDIKAESTDANFYVCPPPTGATVVQFEQPRRCPTRPEGQ NYTEGIAVVFKENIAPYKFKATMYYKDVTVSQVWFGHRYSQFMGIFEDRAPVPFEEVIDKINAKGVCRSTAKYVR NNLETTAFHRDDHETDMELKPANAATRTSRGWHTTDLKYNPSRVEAFHRYGTTVNCIVEEVDARSVYPYDEFVL ATGDFVYMSPFYGYREGSHTEHTSYAADRFKQVDGFYARDLTTKARATAPTTRNLLTTPKFTVAWDWVPKRPSV CTMTKWQEVDEMLRSEYGGSFRFSSDAISTTFTTNLTEYPLSRVDLGDCIGKDARDAMDRIFARRYNATHIKVG QPQYYLANGGFLIAYQPLLSNTLAELYVREHLREQSRKPPNPTPPPPGASANASVERIKTTSSIEFARLQFTYNHIQ RHVNDMLGRVAIAWCELQNHELTLWNEARKLNPNAIASATVGRRVSARMLGDVMAVSTCVPVAADNVIVQNS MRISSRPGACYSRPLVSFRYEDQGPLVEGQLGENNELRLTRDAIEPCTVGHRRYFTFGGGYVYFEEYAYSHQLSR ADITTVSTFIDLNITMLEDHEFVPLEVYTRHEIKDSGLLDYTEVQRRNQLHDLRFADIDTVIHADANAAMFAGLGA FFEGMGDLGRAVGKVVMGIVGGVVSAVSGVSSFMSNPFGALAVGLLVLAGLAAAFFAFRYVMRLQSNPMKALYP LTTKELKNPTNPDASGEGEEGGDFDEAKLAEAREMIRYMALVSAMEHTEHKAKKKGTSALLSAKVTDMVMRKRR NTNYTQVPNKDGDADEDDL [SEQ ID NO: 125] (GenPept gbAAF04615.1).

[0363] This sequence comprises the following domains/moieties:

[0364] SP = 1-24

[0365] Ectodomain = 1-774

[0366] TM = 775-795

[0367] C = 796-904

[0368] Non-limiting examples of HSV1 Gb ectodomain polypeptides include:

Ectodomain 1-774:

[0369] MRQGAPARGCRWFVVWALLGLTLGVLVASAAPSSPGTPGVAAATQAANGGPATPAPPAL GAAPTGDPKPKKNKKPKNPTPPRPAGDNATVAAGHATLREHLRDIKAESTDANFYVCPPPTGATVVQFEQPRRCP TRPEGQNYTEGIAVVFKENIAPYKFKATMYYKDVTVSQVWFGHRYSQFMGIFEDRAPVPFEEVIDKINAKGVCRS TAKYVRNNLETTAFHRDDHETDMELKPANAATRTSRGWHTTDLKYNPSRVEAFHRYGTTVNCIVEEVDARSVYP YDEFVLATGDFVYMSPFYGYREGSHTEHTSYAADRFKQVDGFYARDLTTKARATAPTTRNLLTTPKFTVAWDWV PKRPSVCTMTKWQEVDEMLRSEYGGSFRFSSDAISTTFTTNLTEYPLSRVDLGDCIGKDARDAMDRIFARRYNA

THIKVGQPQYYLANGGFLIAYQPLLSNTLAELYVREHLREQSRKPPNPTPPPPGASANASVERIKTTSSIEFARLQF
TYNHIQRHVNDMLGRVAIAWCELQNHELTLWNEARKLNPNAIASATVGRRVSARMLGDVMAVSTCVPVAADNV
IVQNSMRISSRPGACYSRPLVSFRYEDQGPLVEGQLGENNELRLTRDAIEPCTVGHRRYFTFGGGYVYFEEYAYS
HQLSRADITTVSTFIDLNITMLEDHEFVPLEVYTRHEIKDSGLLDYTEVQRRNQLHDLRFADIDTVIHADANAAMF
AGLGAFFEGMGDLGRAVGKVVMGIVGGVVSAVSGVSSFMSNP [SEQ ID NO: 126] (GenPept gbAAF04615.1).

Ectodomain minus SP 25-775:

[0370] VLVASAAPSSPGTPGVAAATQAANGGPATPAPPALGAAPTGDPKPKKNKKPKNPTPPRPA
GDNATVAAGHATLREHLRDIKAESTDANFYVCPPPTGATVVQFEQPRRCPTRPEGQNYTEGIAVVFKENIAPYKF
KATMYYKDVTVSQVWFGHRYSQFMGIFEDRAPVPFEEVIDKINAKGVCRSTAKYVRNNLETTAFHRDDHETDME
LKPANAATRTSRGWHTTDLKYNPSRVEAFHRYGTTVNCIVEEVDARSVYPYDEFVLATGDFVYMSPFYGYREGS
HTEHTSYAADRFKQVDGFYARDLTTKARATAPTTRNLLTTPKFTVAWDWVPKRPSVCTMTKWQEVDEMLRSEY
GGSFRFSSDAISTTFTTNLTEYPLSRVDLGDCIGKDARDAMDRIFARRYNATHIKVGQPQYYLANGGFLIAYQPLL
SNTLAELYVREHLREQSRKPPNPTPPPPGASANASVERIKTTSSIEFARLQFTYNHIQRHVNDMLGRVAIAWCELQ
NHELTLWNEARKLNPNAIASATVGRRVSARMLGDVMAVSTCVPVAADNVIVQNSMRISSRPGACYSRPLVSFRY
EDQGPLVEGQLGENNELRLTRDAIEPCTVGHRRYFTFGGGYVYFEEYAYSHQLSRADITTVSTFIDLNITMLEDHE
FVPLEVYTRHEIKDSGLLDYTEVQRRNQLHDLRFADIDTVIHADANAAMFAGLGAFFEGMGDLGRAVGKVVMGI
VGGVVSAVSGVSSFMSNP [SEQ ID NO: 127] (GenPept gbAAF04615.1).

[0371] An ectodomain polypeptide sequence used to make the chimeric polypeptides of the invention may be found naturally within an enveloped virus fusion protein, and/or it may have one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30) single amino acid mutations (insertions, deletions or substitutions) relative to a natural fusion protein sequence. For instance, it is known to mutate F proteins to eliminate their furin cleavage sequences, thereby preventing intracellular processing. In particular embodiments, the ectodomain polypeptide lacks any one or more of SP, TM and C domains and optionally contains one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30) single amino acid mutations (insertions, deletions or substitutions) relative to a natural fusion protein sequence.

[0372] The invention may use any desired enveloped virus fusion protein amino acid sequence, such as the amino acid sequence of SEQ ID NO: 2 to 127, or a sequence having identity or similarity to SEQ ID NO: 2 to 127. Typically it will have at least 75% identity or or similarity to SEQ ID NO: 2 to 127, *e.g.*, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99%, identity or similarity to SEQ ID NO: 2 to 127.

2.3 Representative chimeric polypeptide constructs

[0373] Non-limiting examples of chimeric polypeptides of the present invention are set out below:

2.3.1 Inf A HA ectodomain – HIV GP160-based SSM

[0374] MKTIIAFSCILCLIFAQKLPGSDNSMATLCLGHHAVPNGTLVKTITDDQIEVTNATELVQSS
STGRICNSPHQILDGKNCTLIDALLGDPHCDDFQNKEWDLFVERSTAYSNCYPYYVPDYATLRSLVASSGNLEFT
QESFNWTGVAQDGSSYACRRGSVNSFFSRLNWLYNLNYKYPEQNVTMPNNDKFDKLYIWGVHHPGTDKDQTN
LYVQASGRVIVSTKRSQQTVIPNIGSRPWVRGVSSIISIYWTIVKPGDILLINSTGNLIAPRGYFKIQSGKSSIMRS
DAHIDECNSECITPNGSIPNDKPFQNVNKITYGACPRYVKQNTLKLATGMRNVPEKQTRGIFGAIAGFIENGWEG

MVDGWYGFRHQNSEGTGQAADLKSTQAAINQITGKLNRVIKKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKID
LWSYNAELLVALENQHTIDLTDSEMSKLFERTRRQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHDIYRN
EALNNRFQIKGVQLKSGYKD*GG*SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILA*GGSGG*HTTWMEWD
REINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 128].

2.3.2 Inf A HA stem domain – HIV GP160-based SSM

[0375] MKTIIALSYILCLVFAQKLPGNDNSTATLCLGHHAVPNGTIVKTITNDQIEVTNATELGFGQ
NTLKLATGMRNVPEKQTRGIFGAIAGFIENGWEGMLDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGMLNRL
IGSGGSGELLVALLNQHTIDLTDSEMNKLFEKTKKQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHDVYR
DEALNNRFQIKGVELKSGYKDGGRSGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILAGGSGGHTTWME
WDREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 129].

2.3.3 Inf A H5 ectodomain – HIV GP160-based SSM

[0376] MGWSCIILFLVATATGVHSEDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKL
CDLDGVKPLILRDCSVAGWLLGNPMCDEFINVPEWSYIVEKANPVNDLCYPGDFNDYEELKHLLSRINHFEKIQII
PKSSWSSHEASLGVSSACPYQGKSSFFRNVVWLIKKNSTYPTIKRSYNNTNQEDLLVLWGIHHPNDAAEQTKLY
QNPTTYISVGTSTLNQRLVPRIATRSKVNGQSGRMEFFWTILRPNDAINFESNGNFIAPEYAYKIVKKGDSTIMKS
ELEYGNCNAKCQTPMGAINSSMPFHNIHPLTIGECPKYVKSNRLVLATGLRNSPQRERRRKKRGLFGAIAGFIEG
GWQGMVDGWYGYHHSNEQGSGYAADKESTQKAIDGVTNKVNSIIDKMNTQFEAVGREFNNLERRIENLNKKM
EDGFLDVWTYNAELLVLMENERTLDFHDSNVKNLYDKVRLQLRDNAKELGNGCFEFYHKCDNECMESVRNGTY
DYPQYSEEARLKREEISGVKLESIGSGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILAGGSGGHTTWMEW
DREINNYTSLIHSLIEESQNQPAKDEQELLE [SEQ ID NO: 130].

2.3.4 Inf B HA ectodomain – HIV GP160-based SSM

[0377] MKAIIVLLMVVTSNADRICTGITSSNSPHVVKTATQGEVNVTGVIPLTTTPTKSHFANLKGT
QTRGKLCPNCFNCTDLDVALGRPKCMGNTPSAKVSILHEVKPATSGCFPIMHDRTKIRQLPNLLRGYENIRLSTS
NVINTETAPGGPYKVGTSGSCPNVANGNGFFNTMAWVIPKDNNKTAINPVTVEVPYICSEGEDQITVWGFHSDD
KTQMERLYGDSNPQKFTSSANGVTTHYVSQIGGFPNQTEDEGLKQSGRIVVDYMVQKPGKTGTIVYQRGILLPQ
KVWCASGRSKVIKGSLPLIGEADCLHEKYGGLNKSKPYYTGEHAKAIGNCPIWVKTPLKLANGTKYRPPAKLLKE
RGFFGAIAGFLEGGWEGMIAGWHGYTSHGAHGVAVAADLKSTQEAINKITKNLNYLSELEVKNLQRLSGAMNEL
HDEILELDEKVDDLRADTISSQIELAVLLSNEGIINSEDEHLLALERKLKKMLGPSAVEIGNGCFETKHKCNQTCL
DRIAAGTFNAGDFSLPTFDSLNITAASLNDDGLDNHTGGSGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARIL
AGGSGGHTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 131].

2.3.5 RSV F ectodomain – HIV GP160-based SSM

[0378] MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNI
KKNKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTQATNNRARRELPRFMNYTLNNAKKTNVTLSKKRKRRFL
GFLLGVGSAIASGVAVSKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSI
SNIETVIEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMS
IIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRV
FCDTMNSLTLPSEVNLCNVDIFNPKYDCEIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCDY
VSNKGVDTVSVGNTLYYVNKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVN
AGKSTTN*GG*SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILA*GGSGG*HTTWMEWDREINNYTSLIHSLIE
ESQNQQEKNEQELLE [SEQ ID NO: 132].

2.3.6 RSV F (1-520) – HIV GP160-based SSM:

[0379] MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNI KKNKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTQATNNRARRELPRFMNYTLNNAKKTNVTLSKKRKRRFL GFLLGVGSAIASGVAVSKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSI SNIETVIEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMS IIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRV FCDTMNSLTLPSEVNLCNVDIFNPKYDCEIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCDY VSNKGVDTVSVGNTLYYVNKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVN AGK*SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILA*GGSGG*HTTWMEWDREINNYTSLIHSLIEESQNQP AKDEQELLE* [SEQ ID NO: 147].

2.3.7 RSV F (1-520) – DScav mutations - HIV GP160-based SSM:

[0380] MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNI KKNKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTQATNNRARRELPRFMNYTLNNAKKTNVTLSKKRKRRFL GFLLGVGSAIASGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPILNKQSCSI SNIETVIEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMC IIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRV FCDTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCDY VSNKGVDTVSVGNTLYYVNKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVN AGK*SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILA*GGSGG*HTTWMEWDREINNYTSLIHSLIEESQNQP AKDEQELLE* [SEQ ID NO: 150].

2.3.8 hMPV F ectodomain – HIV GP160-based SSM

[0381] MSWKVVIIFSLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCAD GPSLIKTELDLTKSALRELRTVSADQLAREEQIENPRQSRFVLGAIALGVATAAAVTAGVAIAKTIRLESEVTAIKN ALKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGIT PAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMVQLPIFGVIDTPCWIVKAAP SCSEKKGNYACLLREDQGWYCQNAGSTVYYPNEKDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIK GRPVSSSFDPVKFPEDQFNVALDQVFESIENSQALVDQSNRILSSAEKGNTG*G*SGIVQQQNNLLRAIEAQQHLL QLTVWGIKQLQARILA*GGSGG*HTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 133].

2.3.9 PIV F ectodomain – HIV GP160-based SSM

[0382] MPTSILLIITTMIMASFCQIDITKLQHVGVLVNSPKGMKISQNFETRYLILSLIPKIEDSNSC GDQQIKQYKRLLDRLIIPLYDGLRLQKDVIVSNQESNENTDPRTKRFFGGVIGTIALGVATSAQITAAVALVEAKQ ARSDIEKLKEAIRDTNKAVQSVQSSIGNLIVAIKSVQDYVNKEIVPSIARLGCEAAGLQLGIALTQHYSELTNIFGD NIGSLQEKGIKLQGIASLYRTNITEIFTTSTVDKYDIYDLLFTESIKVRVIDVDLNDYSITLQVRLPLLTRLLNTQIYK VDSISYNIQNREWYIPLPSHIMTKGAFLGGADVKECIEAFSSYICPSDPGFVLNHEMESCLSGNISQCPRTVVTSD IVPRYAFVNGGVVANCITTTCTCNGIGNRINQPPDQGVKIITHKECNTIGINGMLFNTNKEGTLAFYTPNDITLNN SVALDPIDISIELNKAKSDLEESKEWIRRSNQKLDSIGNWHQSSTT*GG*SGIVQQQNNLLRAIEAQQHLLQLTVW GIKQLQARILA*GGSGG*HTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 134].

2.3.10 MeV F ectodomain – HIV GP160-based SSM

[0383] MGLKVNVSAIFMAVLLTLQTPTGQIHWGNLSKIGVVGIGSASYKVMTRSSHQSLVIKLMP NITLLNNCTRVEIAEYRRLLRTVLEPIRDALNAMTQNIRPVQSVASSRRHKRFAGVVLAGAALGVATAAQITAGIA LHQSMLNSQAIDNLRASLETTNQAIEAIRQAGQEMILAVQGVQDYINNELIPSMNQLSCDLIGQKLGLKLLRYYT

EILSLFGPSLRDPISAEISIQALSYALGGDINKVLEKLGYSGGDLLGILESRGIKARITHVDTESYLIVLSIAYPTLSE
IKGVIVHRLEGVSYNIGSQEWYTTVPKYVATQGYLISNFDESSCTFMPEGTVCSQNALYPMSPLLQECLRGSTKS
CARTLVSGSFGNRFILSQGNLIANCASILCKCYTTGTIINQDPDKILTYIAADHCPVVEVNGVTIQVGSRRYPDAV
YLHRIDLGPPILLERLDVGTNLGNAIAKLEDAKELLESSDQILRSMKGLSST*GG*SGIVQQQNNLLRAIEAQQHLLQ
LTVWGIKQLQARILA*GGSGG*HTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 135].

2.3.11 HeV F ectodomain – HIV GP160-based SSM

[0384] MATQEVRLKCLLCGIIVLVLSLEGLGILHYEKLSKIGLVKGITRKYKIKSNPLTKDIVIKMIPN
VSNVSKCTGTVMENYKSRLTGILSPIKGAIELYNNNTHDLVGDVKLAGVVMAGIAIGIATAAQITAGVALYEAMK
NADNINKLKSSIESTNEAVVKLQETAEKTVYVLTALQDYINTNLVPTIDQISCKQTELALDLALSKYLSDLLFVFGP
NLQDPVSNSMTIQAISQAFGGNYETLLRTLGYATEDFDDLLESDSIAGQIVYVDLSSYYIIVRVYFPILTEIQQAYV
QELLPVSFNNDNSEWISIVPNFVLIRNTLISNIEVKYCLITKKSVICNQDYATPMTASVRECLTGSTDKCPRELVVS
SHVPRFALSGGVLFANCISVTCQCQTTGRAISQSGEQTLLMIDNTTCTTVVLGNIIISLGKYLGSINYNSESIAVGP
PVYTDKVDISSQISSMNQSLQQSKDYIKEAQKILDTVNPS*GG*SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQ
ARILA*GGSGG*HTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 136].

2.3.12 NiV F ectodomain – HIV GP160-based SSM

[0385] MVVILDKRCYCNLLILILMISECSVGILHYEKLSKIGLVKGVTRKYKIKSNPLTKDIVIKMIPN
VSNMSQCTGSVMENYKTRLNGILTPIKGALEIYKNNTHDLVGDVRLAGVIMAGVAIGIATAAQITAGVALYEAMK
NADNINKLKSSIESTNEAVVKLQETAEKTVYVLTALQDYINTNLVPTIDKISCKQTELSLDLALSKYLSDLLFVFGP
NLQDPVSNSMTIQAISQAFGGNYETLLRTLGYATEDFDDLLESDSITGQIIYVDLSSYYIIVRVYFPILTEIQQAYIQ
ELLPVSFNNDNSEWISIVPNFILVRNTLISNIEIGFCLITKRSVICNQDYATPMTNNMRECLTGSTEKCPRELVVSS
HVPRFALSNGVLFANCISVTCQCQTTGRAISQSGEQTLLMIDNTTCPTAVLGNVIISLGKYLGSVNYNSEGIAIGP
PVFTDKVDISSQISSMNQSLQQSKDYIKEAQRLLDTVNPS*GG*SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQ
ARILA*GGSGG*HTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 137].

2.3.13 HIV GP160 ectodomain – RSV F-based SSM

[0386] MRVMGIERNYPCWWTWGIMILGMIIICNTAENLWVTVYYGVPIWKDANTTLFCASDAKAY
DTEVHNVWATHACVPTDPSPQELKMENVTEEFNMWKNNMVEQMHTDIISLWDQSLKPCVQLTPLCVTLDCSYN
ITNNITNSITNSSVNMREEIKNCSFNMTTELRDKNRKVYSLFYKLDVVQINNGNNSSNLYRLINCNTSALTQACPK
VTFEPIPIHYCAPAGYAILKCNDKEFNGTGLCKNVSTVQYTHGIRPVVSTQLLLNGSLAEGKVMIRSENITNNVKN
IIVQLNESVTINCTRPNNNTRRSVRIGPGQTFYATGDIIGDIRQAHCNVSGSQWNKTLHQVVEQLRKYWNNNTII
FNSSSGGDLEITTHSFNCAGEFFYCNTSGLFNSTWVNGTTSSMSNGTITLPCRIKQIINMWQRVGQAMYAPPIQ
GVIKCESNITGLILTRDGGVNSSDSETFRPGGGDMRDNWRSELYKYKVVKIEPLGVAPTKARRRVVEREKRAVTL
GAVFIGFLGTAGSTMGAVSITLTVQARKLLSGIVQQQSNLLRAIEAQQHLLKLTVWGIKQLQARVLAVERYLRDQ
QLLGIWGCSGKLICPTNVPWNSSWSNKSLDEIWENMTWLQWDKEISNYTIKIYELIEESQIQQERNEKDLLELD
KWASLWNWFDISKWLWYIK*GGG*VNKIKSALLSTNKAVVSLSNGVSVLTSKVLDLK*GGGGHHHHGGG*FDASIS
QVNEKINQSLAFIRKSDELLHNV [SEQ ID NO: 138].

2.3.14 EBOV GP ectodomain, minus mucin like domain (1-311,462-650)– HIV GP160-based SSM

[0387] MGVTGILQLPRDRFKRTSFFLWVIILFQRTFSIPLGVIHNSTLQVSDVDKLVCRDKLSSTNQ
LRSVGLNLEGNGVATDVPSATKRWGFRSGVPPKVVNYEAGEWAENCYNLEIKKPDGSECLPAAPDGIRGFPRCR
YVHKVSGTGPCAGDFAFHKEGAFFLYDRLASTVIYRGTTFAEGVVAFLILPQAKKDFFSSHPLREPVNATEDPSSG
YYSTTIRYQATGFGTNETEYLFEVDNLTYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKVNPEIDTTIGEWA

FWETKKNLTRKIRSEELSFTVV*GG*NNTHHQDTGEESASSGKLGLITNTIAGVAGLITGGRRTRREAIVNAQPKCN PNLHYWTTQDEGAAIGLAWIPYFGPAAEGIYIEGLMHNQDGLICGLRQLANETTQALQLFLRATTELRTFSILNRK AIDFLLQRWGGTCHILGPDCCIEPHDWTKNITDKIDQIIHDFVDKTLPDQGDNDNWWTGWRQ*GG*SGIVQQQN NLLRAIEAQQHLLQLTVWGIKQLQARILA*GGSGG*HTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 139].

2.3.15 MARV GP ectodomain, minus mucin like domain – HIV GP160-based SSM

[0388] MKTTCFLISLILIQGTKNLPILEIASNNQPQNVDSVCSGTLQKTEDVHLMGFTLSGQKVAD SPLEASKRWAFRTGVPPKNVEYTEGEEAKTCYNISVTDPSGKSLLLDPPTNIRDYPKCKTIHHIQGQNPHAQGIAL HLWGAFFLYDRIASTTMYRGKVFTEGNIAAMIVNKTVHKMIFSRQGQGYRHMNLTSTNKYWTSSNGTQTNDTG CFGALQEYNSTKNQTCAPSKIPPPLPTARPEIKL*GG*AQHLVYFRRKRSILWREGDMFPFLDGLINAPIDFDPVPNT KTIFDESSSSGASAEEDQHASPNISLTLSYFPNINENTAYSGENENDCDAELRIWSVQEDDLAAGLSWIPFFGPG IEGLYTAVLIKNQNNLVCRLRRLANQTAKSLELLLRVTTEERTFSLINRHAIDFLLTRWGGTCKVLGPDCCIGIEDL SKNISEQIDQIKKDEQKEGTGWGLGGKWWTSDW*GG*SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILA *GGSGG*HTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 140].

2.3.16 SARS-CoV S ectodomain – HIV GP160-based SSM

[0389] MFIFLLFLTLTSGSDLDRCTTFDDVQAPNYTQHTSSMRGVYYPDEIFRSDTLYLTQDLFLPF YSNVTGFHTINHTFGNPVIPFKDGIYFAATEKSNVVRGWVFGSTMNNKSQSVIIINNSTNVVIRACNFELCDNPFF AVSKPMGTQTHTMIFDNAFNCTFEYISDAFSLDVSEKSGNFKHLREFVFKNKDGFLYVYKGYQPIDVVRDLPSGF NTLKPIFKLPLGINITNFRAILTAFSPAQDIWGTSAAAYFVGYLKPTTFMLKYDENGTITDAVDCSQNPLAELKCSV KSFEIDKGIYQTSNFRVVPSGDVVRFPNITNLCPFGEVFNATKFPSVYAWERKKISNCVADYSVLYNSTFFSTFKC YGVSATKLNDLCFSNVYADSFVVKGDDVRQIAPGQTGVIADYNYKLPDDFMGCVLAWNTRNIDATSTGNYNYK YRYLRHGKLRPFERDISNVPFSPDGKPCTPPALNCYWPLNDYGFYTTTGIGYQPYRVVVLSFELLNAPATVCGPKL STDLIKNQCVNFNFNGLTGTGVLTPSSKRFQPFQQFGRDVSDFTDSVRDPKTSEILDISPCAFGGVSVITPGTNA SSEVAVLYQDVNCTNVSAAIHADQLTPAWRIYSTGNNVFQTQAGCLIGAEHVDTSYECDIPIGAGICASYHTVSL LRSTSQKSIVAYTMSLGADSSIAYSNNTIAIPTNFSISITTEVMPVSMAKTSVDCNMYICGDSTECANLLLQYGSF CTQLNRALSGIAAEQDRNTREVFAQVKQMYKTPTLKYFGGFNFSQILPDPLKPTKRSFIEDLLFNKVTLADAGFMK QYGECLGDINARDLICAQKFNGLTVLPPLLTDDMIAAYTAALVSGTATAGWTFGAGAALQIPFAMQMAYRFNGIG VTQNVLYENQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRLD KVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQAAPHGV VFLHVTYVPSQERNFTTAPAICHEGKAYFPREGVFVFNGTSWFITQRNFFSPQIITTDNTFVSGNCDVVIGIINNTV YDPLQPELDSFKGELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWP WYVW*GG*SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILA*GGSGG*HTTWMEWDREINNYTSLIHSLIEES QNQQEKNEQELLE [SEQ ID NO: 141].

2.3.17 MERS-CoV S ectodomain – HIV GP160-based SSM

[0390] MIHSVFLLMFLLTPTESYVDVGPDSVKSACIEVDIQQTFFDKTWPRPIDVSKADGIIYPQGR TYSNITITYQGLFPYQGDHGDMYVYSAGHATGTTPQKLFVANYSQDVKQFANGFVVRIGAAANSTGTVIISPSTS ATIRKIYPAFMLGSSVGNFSDGKMGRFFNHTLVLLPDGCGTLLRAFYCILEPRSGNHCPAGNSYTSFATYHTPATD CSDGNYNRNASLNSFKEYFNLRNCTFMYTYNITEDEILEWFGITQTAQGVHLFSSRYVDLYGGNMFQFATLPVYD TIKYYSIIPHSIRSIQSDRKAWAAFYVYKLQPLTFLLDFSVDGYIRRAIDCGFNDLSQLHCSYESFDVESGVYSVSS FEAKPSGSVVEQAEGVECDFSPLLSGTPPQVYNFKRLVFTNCNYNLTKLLSLFSVNDFTCSQISPAAIASNCYSSLI LDYFSYPLSMKSDLGVSSAGPISQFNYKQSFSNPTCLILATVPHNLTTITKPLKYSYINKCSRLLSDDRTEVPQLVN ANQYSPCVSIVPSTVWEDGDYYRKQLSPLEGGGWLVASGSTVAMTEQLQMGFGITVQYGTDTNSVCPKLEFAN DTKIASQLGNCVEYSLYGVSGRGVFQNCTAVGVRQQRFVYDAYQNLVGYYSDDGNYYCLRACVSVPVSVIYDKE

TKTHATLFGSVACEHISSTMSQYSRSTRSMLKRRDSTYGPLQTPVGCVLGLVNSSLFVEDCKLPLGQSLCALPDT
PSTLTPRSVRSVPGEMRLASIAFNHPIQVDQFNSSYFKLSIPTNFSFGVTQEYIQTTIQKVTVDCKQYICNGFQKC
EQLLREYGQFCSKINQALHGANLRQDDSVRNLFASVKSSQSSPIIPGFGGDFNLTLLEPVSISTGSRSARSAIEDL
LFDKVTIADPGYMQGYDDCMQQGPASARDLICAQYVAGYKVLPPLMDVNMEAAYTSSLLGSIAGVGWTAGLSS
FAAIPFAQSIFYRLNGVGITQQVLSENQKLIANKFNQALGAMQTGFTTTNEAFRKVQDAVNNNAQALSKLASELS
NTFGAISASIGDIIQRLDVLEQDAQIDRLINGRLTTLNAFVAQQLVRSESAALSAQLAKDKVNECVKAQSKRSGF
CGQGTHIVSFVVNAPNGLYFMHVGYYPSNHIEVVSAYGLCDAANPTNCIAPVNGYFIKTNNTRIVDEWSYTGSSF
YSPEPITSLNTKYVAPQVTYQNISTNLPPPLLGNSTGIDFQDELDEFFKNVSTSIPNFGSLTQINTTLLDLTYEMLSL
QQVVKALNESYIDLKELGNYTYYNKWPWYIWL*GG*SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILA*GGS*
*GG*HTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 142].

2.3.18 VSV G ectodomain – HIV GP160-based SSM

[0391] MKCLLYLAFLFIGVNCKFTIVFPHNQKGNWKNVPSNYHYCPSSSDLNWHNDLIGTAIQVK
MPKSHKAIQADGWMCHASKWVTTCDFRWYGPKYITQSIRSFTPSVEQCKESIEQTKQGTWLNPGFPPQSCGYA
TVTDAEAVIVQVTPHHVLVDEYTGEWVDSQFINGKCSNYICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSE
DGELSSLGKEGTGFRSNYFAYETGGKACKMQYCKHWGVRLPSGVWFEMADKDLFAAARFPECPEGSSISAPSQ
TSVDVSLIQDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIINGTLKYFETRYIRVDIAAPILSR
MVGMISGTTTERELWDDWAPYEDVEIGPNGVLRTSSGYKFPLYMIGHGMLDSDLHLSSKAQVFEHPHIQDAAS
QLPDDESLFFGDTGLSKNPIELVEGWFSSWK*GG*SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILA*GGSG*
*G*HTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 143].

2.3.19 RABV GP ectodomain – HIV GP160-based SSM

[0392] MIPQTLLFVPLLVFSLCFGKFPIYTIPDKLGPWSPIDIHHLSCPNNLVVEDEGCTNLSGFSYM
ELKVGYISAIKVNGFTCTGVVTEAETYTNFVGYVTTTFKRKHFRPTPDACRAAYNWKMAGDPRYEESLHNPYPDY
HWLRTVKTTKESLVIISPSVSDLDPYDKSLHSRVFPSGKCSGITVSSTYCPTNHDYTIWMPENPRLGTSCDIFTNS
RGKRASKGSKTCGFVDERGLYKSLKGACKLKLCGVLGLRLMDGTWAAIQTSDEAKWCPPDQLVNIHDFRSDEI
EHLVVEELVKKREECLDALESIMTTKSVSFRRLSHLRKLVPGFGKAYTIFNKTLMEADAHYKSVRTWNEIIPSKGC
LRVGGRCHPHVNGVFFNGIILGPDGHVLIPEMQSSLLQQHMELLESSVIPLMHPLADPSTVFKDGDEAEDFVEVH
LPDVHKQVSGVDLGLPSWGK*GG*SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILA*GGSGG*HTTWMEW
DREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 144].

2.3.20 HSV1 Gb ectodomain – HIV GP160-based SSM

[0393] MRQGAPARGRRWFVVWALLGLTLGVLVVSAAPSSPGTPGVAAATQAANGGPATPAPPAP
GAPPTGDPKPKKNKKPKPPKPPRPAGDNATVAAGHATLREHLRDIKAENTDANFYVCPPPTGATVVQFEQPRRCP
TRPEGQNYTEGIAVVFKENIAPYKFKATMYYKDVTVSQVWFGHRYSQFMGIFEDRAPVPFEEVIDKINAKGVCRS
TAKYVRNNLETTAFHRDDHETDMELKPANAATRTSRGWHTTDLKYNPSRVEAFHRYGTTVNCIVEEVDARSVYP
YDEFVLATGDFVYMSPFYGYREGSHTEHTSYAADRFKQVDGFYARDLTTKARATAPTTRNLLTTPKFTVAWDWV
PKRPSVCTMTKWQEVDEMLRSEYGGSFRFSSDAISTTFTTNLTEYPLSRVDLGDCIGKDARDAMDRIFARRYNA
THIKVGQPQYYLANGGFLIAYQPLLSNTLAELYVREHLREQSRKPPNPTPPPPGASANASVERIKTTSSIEFARLQF
TYNHIQRHVNDMLGRVAIAWCELQNHELTLWNEARKLNPNAIASATVGRRVSARMLGDVMAVSTCVPVAADNV
IVQNSMRISSRPGACYSRPLVSFRYEDQGPLVEGQLGENNELRLTRDAIEPCTVGHRRYFTFGGGYVYFEEYAYS
HQLSRADITTVSTFIDLNITMLEDHEFVPLEVYTRHEIKDSGLLDYTEVQRRNQLHDLRFADIDTVIHADANAAMF
AGLGAFFEGMGDLGRAVGKVVMGIVGGVVSAVSGVSSFMSNP*GG*SGIVQQQNNLLRAIEAQQHLLQLTVWGI
KQLQARILA*GGSGG*HTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 145].

2.4 Use of structure-stabilizing moiety as a universal oligomerization domain

[0394] In addition to its utility in stabilizing an ectodomain polypeptide of the invention against rearrangement to a post-fusion conformation, the structure-stabilizing moiety is useful as a universal oligomerization domain (UOD) for oligomerizing any heterologous molecules of interest into oligomers, particularly trimers. In specific embodiments, a UOD is fused upstream or downstream of a heterologous proteinaceous molecule to form a chimeric polypeptide. Typically, the UOD is fused downstream of the heterologous proteinaceous molecule. As with the ectodomain embodiments described herein, association of the complementary heptad repeats of the UOD to one another under conditions suitable for their association (*e.g.*, in aqueous solution) results in formation of an anti-parallel, two-helix bundle that trimerizes to form a highly stable six-helix bundle, thus permitting trimerization of the chimeric polypeptide to form a trimeric polypeptide complex.

[0395] The heterologous proteinaceous molecule may be a natural or non-natural polypeptide. In certain embodiments, the heterologous polypeptide is or comprises a therapeutic polypeptide. A vast variety of therapeutic polypeptides, including both ligands and receptors, are known in the art to be useful for treating a variety of diseases. Various examples of known targets and indications for therapeutic polypeptides are shown in Table 8.

TABLE 8

PEPTIDE TARGET	INDICATION	NAME/COMPANY
	Oncology	
GNRH receptor	Palliative prostate cancer treatment	Leuprorelin/Takeda Histrelin/Valera Goserelin/AstraZeneca
CXCR4 antagonist	Stem cell mobilizer, NHL, MM Hepatocellular Carcinoma	Mozobil/AnorMED Inc (now Genzyme) CTCE-9908/ Chemokine Therapeutics
Integrin alphaV-beta 3 antagonist	Head and Neck, glioblastoma	Cilengitide/Merck
Angiopoietin receptor kinase antagonist	Breast, ovarian, renal cell carcinoma	AMG-386 peptibody/Amgen,Takeda
IGF1-R antagonist	Hepatocellular Carcinoma	Allostera Pharmaceuticals
Gastrin-releasing peptide receptor bFGF	Inflammation, Cancer, Anti-angiogenesis various cancers	Academic
Gelatinase inhibitor	Cancer	CTT Technologies
GCSFR agonist	Neutropenia	Gematide/Affymax
Keratinocyte GFR	Mucositis	Keratide/Affymax
VEGF-R2/c-met receptor	Cancer	Dipeptide from Dyax
	Autoimmune	
TPO	ITP	Nplate peptibody/Amgen
GLP-2 analog	Crohn's Enterocolitis	Teduglutide/NPS Allelix
Treg	MS	Copaxone/Teva
GPCR agonists	Various	Compugen
	Diabetes	
GLP-1 analogues/R agonists		GLP-1 (7-37)/Biorexis Pfizer Exenatide/Amylin Liraglutide/Novo Nordisk

		ZP10/Zealand Pharma/Sanofi-Avanetis Pramlinitide/Amylin
Proislet peptide		CureDM
Glucagon antagonist		Glucagon
	Obesity	PYY/multiple companies Oxyntomodulin TKS-1225/Thiakis/Wyeth
Oxyntomodulin EPO	Anemic chronic kidney disease	Hematide/Affimax
Calcitonin PH receptor	Osteoporosis	Capsitonin/Bone Medical Teriparatide/Lilly
	Cardiovascular	
BNP	Congestive heart failure	Nesiritide/Scios
GIIb/IIIa antagonist	Myocardial infarction	Eptifibatide/COR Therapeutics/Schering Plough
Thrombin inhibitor	Thrombosis, Ischemic heart disease	Bivalirudin/TMC/Scherrer
Bradykinin B2 antagonist	Hereditary angioedema	Icatibant/Hoechst
GAP junction modulator	Heart Arrhythmia	Rotigaptide/Zealand/Wyeth
FPLRG1 agonist	Reperfusion injury	Compugen
BNP/ANP	Congestive heart failure	Bispecific/Academic
	Acromegaly	
Somatostatin receptor agonist	Acromegaly and neuroendocrine cancer	Octreotide/Valera Pharmaceuticals Lanreotide/Ibsen
	Enuresis	
Vasopressin V1 agonist		Desmopressin/Orphan Therapeutics/Lypressin Terlipressin/Orphan Therapeutics
	Labor	
Oxytocin antagonist	Halts Premature Labor	Retosiban/GSK/ Atosiban/Ferring
	Antiviral	
HIV fusion protein blocker	HIV	Enfuviritide/Roche
Immunostimulatory	HepC, Hep C	Thymalfasin/RegeneRx SCV-07/SciClone
CXCR4 antagonist	HIV	AnorMED Inc (now Genzyme)
CCR5 antagonist	HIV	
CXCR4/CCR5 bispecific	HIV	Genzyme
	Antibacterial	
	Staph. Aureus	Daptamycin Bacitracin
	Ophthalmic	Gramidicin/Bausch&Lomb Colistin Pexiganan Omiganan
	Staph. Aureus	Xoma-629
Glycophorin antagonist	Malaria	Academic
	CNS	
Norepinephrine transporter antagonist	Severe chronic pain	Conotoxin/Xenome
	Antidepressant	Nemifitide
Formyl peptide receptor-like 1	COPD	Various academics, Bayer 2003 patent

agonists/antagonists		
IL4/IL 13 antagonist Prokineticin receptor-1 and-2	Asthma	Synairgen Academic

[0396] The UOD of the invention can be used to create trimerized soluble receptors including for example TNF receptor superfamily members, Ig superfamily members, cytokine receptor superfamily members, chemokine receptor superfamily members, integrin family members, growth factor receptor family, hormone receptors, opioid receptors, other neuropeptide receptors, ion channels, among others, including CD1a-e, CD2 (LFA-2), CD2R, CD3γ, CD3δ, CD3ε, CD4-7, CD8a, CD8b, CD9, CD10 CD11a, CD11b, CD11c, CDwI2, CD13, CD14, CD15, CD15s, CD15u, CD16a (FcγRIIIA), CD16b (FcγRIIIB), CDw17, CD18 (Integrin β2), CD19-28, CD29 (Integrin β1), CD30, CD31 (PE-CAM-1), CD32 (FcγRII), CD33 (Siglec-3), CD34-41, CD42a-d, CD43, CD44, CD44R, CD45, CD45RA, CD45RB, CD45RO, DC47, CD47R, CD48, CD49a-f (VLA-1-6), CD50 (ICAM-3), CD51, CD52, CD53, CD54 (ICAM-1), CD55, CD56 (N-CAM), CD57, CD58 (LFA-3), CD59, CD60a-c, CD61, CD62E, CD62L, CD62P, CD63, CD64, CD65, CD65s, CD66a-f, CD68, CD69, CD70, CD71, CD72, CD73, CD74, CD75, CD75s, CD77, CD79a, CD79b, CD80, CD81, CD82, DC83, CDw84, CD85, CD86-CD91, CDw92, CD93, CD94-CD99, CD99R, CD100-CD106, CD107a, CD107b, CD108-CD112, CDw113, CD114 (G-CSFR), CD115 (M-CSFR), CD116, CD117, CD118. CDw119, CD120a, CD120b, CD121a (IL-1R type I), CDw121b (IL-1R, type II), CD122 (IL-2Rβ), CDw123 (IL-3R), CD124 (IL-4R), CDw125 (IL-5R), CD126 (IL-6R), CD127 (IL-7R), CDw128, CDw128b (IL-8Rβ, CD129 (IL-9R), CD130 (IL-6Rβ), CDw131, CD132, CD133, CD134 (Ox-40), CD135-CD139, CD140a (PDGFRα), CD140b (PDGFRβ), CD141-CD144, CDw145, CD146, CD147, CD148, CD15, CD151, CD152 (CTLA-4), CD153 (CD30L), CD154 (CD40L), CD155, CD156a-c, CD157, CD158a, CD158b, CD159a, CD159c, CD160, CD161, CD162, CD162R, CD163, CD164, CD165, CD166, CD167a, CD168, CD169, CD170, CD171, CD172a, CD172b, CD172g, CD173, CD174, CD175, CD175s, CD176, CD178 (FasL), CD179a, CD179b, CD180, CD181 (CXCR1), CD182 (CXCR2), CD183 (CXCR3), CD184 (CXCR4), CD185 (CXCR5), CDw186 (CXCR6), CD191 (CCR-1), CD192 (CCR2), CD193 (CCR3), CD194 (CCR4), CD195 (CCR5). CD196 (CCR6), CD197 (CCR7), CDw198 (CCR8), CDwI99 (CCR9), CD200 (Ox-2), CD201, CD202b, CD203c, CD204 (macrophage scavenger R), CD207 (Langerin), CD208 (DC-LAMP), CD209 (DC-SIGN), CDw210 (IL-10R), CD212 (IL-12-Rβ1), CD213a1 (IL-13-Rα1), CD213a2 (IL-13-Rα2), CDw217 (IL-17-R), CDw218a (IL-18Rα), CDw218b (IL-18Rβ), CD220 (Insulin-R), CD221 (IGF-1R), CD222 (IGF-II R), CD223-234, CD235a (glycophorin A), CD235ab (glycophorin A/B), CD235b (glycophorin B), CD236 (glycophorin C/D), CD236R (glycophorin C), CD238, CD239, CD240CE, CD240D, CD241-CD249, CD252 (Ox40L), CD254 (RANKL), CD256 (APRIL), CD257 (BAFF), CD258 (LIGHT), CD261 (TRAIL-R1), CD262 (TRAIL-R2), CD263 (DcR1), CD264 (DcR2), CD256 (RANK), CD266 (TWEAK-R), CD267 (TACI), CD268 (BAFFR), CD269 (BCMA), CD271 (NGFR), CD272 (BTLA), CD273 (PD-L2), CD274 (PD-L1), CD275 (B7-H2), CD276 (B7-H3), CD277, CD278 (ICOS), CD279 (PD1), CD280, CD281 (TLR1), CD282 (TLR2), CD283 (TLR3), CD284 (TLR4), CD289 (TLR9), CD292, CDw293, CD294, CD295 (LeptinR), CD296, CD297, CD298 (Na+/K+-ATPase β3 subunit), CD299 (L-SIGN), CD300a, CD300c, CD300e, CD301-CD307, CD309 (VEGF-R2), CD312, CD314-322, CD324, CDw325, CD326, CDw327, CDw328, CDw329, CD331-CD337, CDw338, CD339, B7-H4, Xedar, CCR10, CCR11, CX3CR1, chemokine-like receptor-1 (ChemR23), complement receptors, DARC, IL-11R, IL-12R, IL-13R, IL-15R, IL-20R, IL-21R, IL-22R, IL-23R, IL-27R, IL-28R, IL-31R, XCR1, CX3CR1, chemokine-binding protein 2 (D6), interferon receptors, leukocyte associated Ig-like receptor family, leukocyte

immunoglobulin-like receptor family including LILRC1 and LILRC2, leukotriene receptors, LAMP, nectin-like proteins 1-4, IgSF8, immunoglobulin-like transcript family LT1-6, EDAR, stromal derived factor (SDF), thymic stromal lymphopoietin receptor, erythropoietin receptor, thrombopoietin-receptor, epidermal growth factor receptor, fibroblast growth factor receptors FGF1-4, hepatocyte growth factor receptor (HGF-R), epaCAM, insulin-like growth factor receptors IGF1-R and IGF2-R, fibronectin, fibronectin leucine-rich transmembrane proteins FLRT1-3, Her2, 3 and 4, CRELD1 and 2, 8D6A, lipoprotein receptor (LDL-R), C-type lectin-like family members such as CLEC-1, CLEC-2, CLEC4D, 4F and Dectin 1 and 2, layilin, growth hormone receptor, prolactin-releasing hormone receptor (PRRP), corticotropin-releasing hormone receptors (CRHR), follicle stimulating hormone receptor (FSHR), gonadotropin-releasing hormone receptor (GNRHR), thyrotropin-releasing hormone receptor (TRHR), somatostatin receptors SSTR1-SSTR5, vasopressin receptors 1A, 1B, 2, Oxytocin receptor, luteinizing hormone/choriogonadotropin receptor (LHCGR), thyrotropin receptor, atrial natriuretic factor receptor NPR1-3, acetylcholine receptors (AChR), calcitonin receptor (CT), Cholecystokinin receptors CCKAR and CCKBR, vasoactive intestinal peptide receptors VPAC1 and 2, δ-opioid receptors, κ-opioid receptors, μ-opioid receptors, σ receptors σ 1 and σ, cannabinoid receptors R1 and 2, angiotensin receptors AT1-4, bradykinin receptors V1 and 2, tachykinin receptor 1 (TACR1), calcitonin receptor-like receptor (CRLR), galanin receptors R1-3, GPCR neuropeptide receptors neuropeptide B/W R1 and 2, neuropeptide FF receptors R1 and R2, neuropeptide S receptor R1, neuropeptide Y receptors Y1-5, neurotensin receptors, Type I and II activin receptors, activin receptor-like kinases (Alk-1 and Alk-7), betaglycan, BMP and Activin membrane bound inhibitor (BAMBI), cripto, Trk receptors TrkA, TrkB, TrkC, AXL receptor family, LTK receptor family, TIE-1, TIE-2, Ryk, Neuropilin 1, Eph receptors EPHA1, EPHA2, EPHA3, EPHA4, EPHA5, EPHA6, EPHA7, EPHA8, EPHA9, EPHA10, EPHB1, EPHB2, EPHB3, EPHB4, EPHB5, EPHB6, melanocortin receptors MC-3 and MC-4, AMICA, CXADR, corticotrophin-releasing hormone-binding protein, Class-I restricted T cell-associated molecule, MHCI, MHCII, ampoterin-induced gene and ORF (AMIGOs), APJ, asialoglycoprotein receptors 1 and 2 (ASGPR), brain-specific angiogenesis inhibitor 3 (BAI-3), basal cell adhesion molecule/Lutheran blood group glycoprotein (BCAM/Lu), cadherins, CDCP1, cystic fibrosis transmembrane conductance regulator MRP-7, chondrolectin, lung surfactin, claudins, ANTHXR2, collagens, complement receptors, contactins 1-6, cubulin, endoglycan, EpCAM (epithelial cellular adhesion molecule), Endothelial Protein C receptor (EPCR), Eph receptors, glucagon-like peptide receptors GLP-1R and 2R, glutamate receptors, glucose transporters, glycine receptor, glypicans, G-protein coupled bile acid receptor, G-protein coupled receptor 15, KLOTHO family members, leptin receptor, LIMPII, LINGO, NOGO, lymphatic bessel endothelial hyaluronan receptor 1 (LYVE-1), myeloid inhibitory C-type lectin-like receptor CLEC12A, neogenin, nephrin, NETO-1, NETO-2, NMDA receptor, opioid-binding cell adhesion molecule, osteoclast inhibitory lectin-related protein, oncostatin receptor, osteoclast associated receptor, osteoactivin, thrombin receptors, podoplanin, porimin, potassium channels, Pref-1, stem cell factor receptor, semaphorins, SPARC, scavenger receptor A1, stabilins, syndecans, T cell receptors, TCAM-1, T cell cytokine receptor TCCR, thrombospondins, TIM1-6, toll-like receptors, triggering receptors expressed on myeloid cells (TREM) and TREM-like proteins, TROP-2 or any mimetic or analog thereof.

[0397] Furthermore, the UOD of the invention can be used to trimerize ligands of any of the above receptors including for example TNF superfamily members, cytokine superfamily members, growth factors, chemokine superfamily members, pro-angiogenic factors, pro-apoptotic

factors, integrins, hormones and other soluble factors, among others, including RANK-L, Lymphotoxin (LT)-α, LT-β, LT-α1β2, zLIGHT, BTLA. TL1A, FasL, TWEAK, CD30L, 4-1BB-L (CD137L), CD27L, Ox40L (CD134L), GITRL, CD40L (CD154), APRIL (CD256), BAFF, EDA1, IL-1α, IL-1β, IL-1RA, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17A, IL-17F, IL-17A/F, IL-18, IL-1 g, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, IL-34, IL-35, IFN-gamma, IFN-alpha, IFN-beta, TNF-α, TNF-β, G-CMF, GM-CSF, TGF-β1, 2 and 3, TGF-α, cardiotrophin-1, leukemia inhibitory factor (LIF), betacellulin, amphiregulin, thymic stromal lymphopoietin (TSLP), flt-3, CXCL1-16, CCL1-3, CCL3L1, CCL4-CCL8, CCL9/10, CCL11-28, XCL1, XCL2, CX3CL1, HMG-B1, heat shock proteins, chemerin, defensins, macrophage migration inhibitory factor (MIF), oncostatin M, limitin, vascular endothelial growth factors VEGF A-D and PIGF, lens epithelium derived growth factor, erythropoietin, thrombopoietin, platelet derived growth factor, epidermal growth factor, fibroblast growth factors FGF1-14 and 16-23, hepatoma-derived growth factor, hepassocin, hepatocyte growth factor, platelet-derived endothelial growth factor (PD-ECGF), insulin-like growth factors IGF1 and IGF2, IGF binding proteins (IGFBP 1-6), GASPS (growth and differentiation-factor-associated serum proteins), connective tissue growth factor, epigen, epiregulin, developmental arteries and neural crest epidermal growth factor (DANCE), glial maturation factor-β, insulin, growth hormone, angiogenin, angiopoietin 1-4, angiopoietin-like proteins 1-4, integrins αVβ3, αVβ5 and α5β1, erythropoietin, thrombopoietin, prolactin releasing hormone, corticotropin-releasing hormone (CRH), gonadotropin releasing hormone, thyrotropin releasing hormone, somatostatin, vasopressin, oxytocin, demoxytocin, carbetocin, luteinizing hormone (LH) and chorionic gonadotropins, thyroid-stimulating hormone, ANP, BNP, CNP, calcitonin, CCK a, CCK B, vasoactive intestinal peptides 1 and 2, encephalin, dynorphin, β-endorphin, morphine, 4-PPBP, [1] SA 4503, Ditolylguanidine, siramesine angiotensin, kallidin, bradykinin, tachykinins, substance P, calcitonin, galanin, neurotensin, neuropeptides Y1-5, neuropeptide S, neuropeptide FF, neuropeptide B/W, brain-derived neurotrophic factors BDNF, NT-3, NT-4/5, activin A, AB, B and C, inhibin, Mullerian inhibiting hormone (MIH), bone morphogenetic proteins BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP10, BMP15, growth differentiating factors GDF1, GDF2, GDF3, GDF5, GDF6, GDF7, Myostatin/GDF8, GDF9, GDF10, GDF11, GDF15, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4), artemin, persephin, neurturin, GDNF, agrin, ephrin ligands EFNA1, EFNA2, EFNA3, EFNA4, EFNA5 EFNB1, EFNB2, EFNB3, adiponectin, α2-macroglobulin, agrecan, agouti-related protein (AgRP), α-melanocyte stimulating hormone, albumin, ameloblastin, plasminogen, angiostatin, apolipoproteins A1, AII, B, B100, E, amyloid, autophagin, TGF-beta induced protein Ig H3), biglycan, leukocyte cell-derived chemotaxin LECT2, C-reactive protein, complement components, chordin, chordin-like proteins, collectins, clusterin-like protein 1, cortisol, van willebrandt factor, cytostatins, endostatin, endoreppellin, ephrin ligands, fetuins, ficolins, glucagon, granulysin, gremlin, HGF activator inhibitors HAI-1 and 2, kallilcreins, laminins, leptins, lipocalins, mannan binding lectins (MBL), meteorin, MFG-E8, macrophage galactose N-acetyl-galactosamine-specific lectin (MGL), midkine, myocilin, nestin, osteoblast-specific factor 2, osteopontin, osteocrin, osteoadherin, pentraxin, persephin, placenta growth factor, relaxins, resistin and resistin-like molecules, stem cell factor, stanniocalcins, VE-statin, substance P, tenascins, vitronectin, tissue factor, tissue factor pathway inhibitors, as well as any other of the >7000 proteins identified in the human secretome as listed in

the secreted protein database (Chen Y *et al.*, 2005. *Nucleic Acids Res* 33 Database Issue:D169-173), or any mimetic or analog thereof.

[0398] Additionally, the UOD of the invention can be used to trimerize enzymes such as for example angiotensin converting enzymes (ACE), matrix metalloproteases, ADAM metalloproteases with thrombospondin type I motif (ADAMTS1, 4, 5, 13), aminopeptidases, beta-site APP-cleaving enzymes (BACE-1 and -2), chymase, kallilkreins, reelin, serpins, or any mimetic or analog thereof.

[0399] Also, the UOD of the invention can be used to trimerize chemotherapeutic agents, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof to increase potency of targeted compounds for therapeutic purposes, such as for example calicheamicin, pseudomonas exotoxin, diphtheria toxin, ricin, saporin, apoptosis-inducing peptides or any analog thereof.

[0400] In other embodiments, the UOD of the invention can also be used to fuse antigens for cancer vaccines such as for example the colorectal cancer antigen A33, α-fetoprotein, mucin 1 (MUC1), CDCP1, carcinoembryonic antigen cell adhesion molecules, Her-2, 3 and 4, mesothelin, CDCP1, NETO-1, NETO-2, syndecans, LewisY, CA-125, melanoma associated antigen (MAGE), tyrosinase, epithelial tumor antigen (ETA), among others, as well as for fusing viral envelope antigens or fungal antigens for treatment of infectious diseases.

2.5 Methods of preparing chimeric polypeptide constructs

[0401] The chimeric polypeptides of the present invention may be prepared by chemical synthesis or recombinant means. Usually, the chimeric polypeptides are prepared by expression of a recombinant construct that encodes the chimeric polypeptide in suitable host cells, although any suitable methods can be used. Suitable host cells include, for example, insect cells (*e.g.*, *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni*), mammalian cells (*e.g.*, human, non-human primate, horse, cow, sheep, dog, cat, and rodent (*e.g.*, hamster), avian cells (*e.g.*, chicken, duck, and geese), bacteria (*e.g.*, *Escherichia coli*, *Bacillus subtilis*, and *Streptococcus spp.*), yeast cells (*e.g.*, *Saccharomyces cerevisiae*, *Candida albicans*, *Candida maltosa*, *Hansenula* polymorphs, *Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Pichia guillerimondii*, *Pichia pastoris*, *Schizosaccharomyces pombe* and *Yarrowia lipolytica*), *Tetrahymena* cells (*e.g.*, *Tetrahymena thermophile*) or combinations thereof. Many suitable insect cells and mammalian cells are well-known in the art. Suitable insect cells include, for example, Sf9 cells, Sf21 cells, Tn5 cells, Schneider S2 cells, and High Five cells (a clonal isolate derived from the parental Trichoplusia ni BTI-TN-5B1-4 cell line (Invitrogen)). Suitable mammalian cells include, for example, Chinese hamster ovary (CHO) cells, human embryonic kidney cells (HEK293 cells, typically transformed by sheared adenovirus type 5 DNA), NIH-3T3 cells, 293-T cells, Vero cells, HeLa cells, PERC.6 cells (ECACC deposit number 96022940), Hep G2 cells, MRC-5 (ATCC CCL-171), WI-38 (ATCC CCL-75), fetal rhesus lung cells (ATCC CL-160), Madin-Darby bovine kidney ("MDBK") cells, Madin-Darby canine kidney ("MDCK") cells (*e.g.*, MDCK (NBL2), ATCC CCL34; or MDCK 33016, DSM ACC 2219), baby hamster kidney (BHK) cells, such as BHK21-F, HKCC cells, and the like. Suitable avian cells include, for example, chicken embryonic stem cells (*e.g.*, EBx® cells), chicken embryonic fibroblasts, chicken embryonic germ cells, duck cells (*e.g.*, AGE1.CR and AGE1.CR.pIX cell lines (ProBioGen) which are described, for example, in Vaccine 27:4975-4982 (2009) and WO2005/042728), EB66 cells, and the like.

[0402] Suitable insect cell expression systems, such as Baculovirus systems, are known to those of skill in the art and described in, *e.g.*, Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987). Materials and methods for Baculovirus/insert cell expression systems are commercially available in kit form from, inter alia, Invitrogen, San Diego Calif. Avian cell expression systems are also known to those of skill in the art and described in, *e.g.*, U.S. Pat. Nos. 5,340,740; 5,656,479; 5,830,510; 6,114,168; and 6,500,668; European Patent No. EP 0787180B; European Patent Application No. EP03291813.8; WO 03/043415; and WO 03/076601. Similarly, bacterial and mammalian cell expression systems are also known in the art and described in, *e.g.*, Yeast Genetic Engineering (Barr *et al.*, eds., 1989) Butterworths, London.

[0403] Recombinant constructs encoding the chimeric polypeptides of the present invention can be prepared in suitable vectors using conventional methods. A number of suitable vectors for expression of recombinant proteins in insect or mammalian cells are well-known and conventional in the art. Suitable vectors can contain a number of components, including, but not limited to one or more of the following: an origin of replication; a selectable marker gene; one or more expression control elements, such as a transcriptional control element (*e.g.*, a promoter, an enhancer, a terminator), and/or one or more translation signals; and a signal sequence or leader sequence for targeting to the secretory pathway in a selected host cell (*e.g.*, of mammalian origin or from a heterologous mammalian or non-mammalian species). For example, for expression in insect cells a suitable Baculovirus expression vector, such as pFastBac (Invitrogen), can be used to produce recombinant Baculovirus particles. The Baculovirus particles are amplified and used to infect insect cells to express recombinant protein. For expression in mammalian cells, a vector that will drive expression of the construct in the desired mammalian host cell (*e.g.*, Chinese hamster ovary cells) is used.

[0404] The chimeric polypeptides can be purified using any suitable method. Suitable methods for purifying desired proteins including precipitation and various types of chromatography, such as hydrophobic interaction, ion exchange, affinity, chelating and size exclusion are well-known in the art. Suitable purification schemes can be created using two or more of these or other suitable methods. If desired, the chimeric polypeptides can include a purification moiety or "tag" that facilitates purification, as described in Section 2.1.2. Such tagged polypeptides can conveniently be purified, for example from conditioned media, by chelating chromatography or affinity chromatography.

[0405] The chimeric polypeptides may include additional sequences. For example, for expression purposes, the natural leader peptide of a heterologous polypeptide of interest (*e.g.*, the natural leader peptide of an enveloped virus fusion protein) may be substituted for a different one.

3. Nucleic acid constructs for endogenous production of chimeric polypeptides

[0406] The present invention also contemplates nucleic acid constructs for endogenous production of chimeric polypeptides in a host organism, suitably a vertebrate animal, preferably a mammal such as a human). The nucleic acid constructs can be self-replicating extra-chromosomal vectors/replicons (*e.g.*, plasmids) or vectors that integrate into a host genome. In specific embodiments, the nucleic acid constructs are viral vectors. Exemplary viral vectors include retroviral vectors, lentiviral vectors, poxvirus vectors, vaccinia virus vectors, adenovirus vectors, adenovirus-associated virus vectors, herpes virus vectors, flavivirus vectors, and alphavirus

vectors. Viral vectors may be live, attenuated, replication conditional or replication deficient, and typically is a non-pathogenic (defective), replication competent viral vector.

[0407] By way of example, when the viral vector is a vaccinia virus vector, a polynucleotide encoding a chimeric polypeptide of the invention may be inserted into a non-essential site of a vaccinia viral vector genome. Such non-essential sites are described, for example, in Perkus *et al.* (1986. *Virology* 152:285); Hruby *et al.* (1983. *Proc. Natl. Acad. Sci. USA* 80:3411); Weir *et al.* (1983. *J. Virol.* 46:530). Suitable promoters for use with vaccinia viruses include but are not limited to P7.5 (*see*, *e.g.*, Cochran *et al.* 1985. *J. Virol.* 54:30); P11 (*see*, *e.g.*, Bertholet, *et al.*, 1985. *Proc. Natl. Acad. Sci. USA* 82:2096); and CAE-1 (*see*, *e.g.*, Patel *et al.*, 1988. *Proc. Natl. Acad. Sci. USA* 85:9431). Highly attenuated strains of vaccinia are more acceptable for use in humans and include Lister, NYVAC, which contains specific genome deletions (*see*, *e.g.*, Guerra *et al.*, 2006. *J. Virol.* 80:985-998); Tartaglia *et al.*, 1992. *AIDS Research and Human Retroviruses* 8:1445-1447), or MVA (*see*, *e.g.*, Gheradi *et al.*, 2005. *J. Gen. Virol.* 86:2925-2936); Mayr *et al.*, 1975. *Infection* 3:6-14). See also Hu *et al.* (2001. *J. Virol.* 75:10300-10308), describing use of a Yaba-Like disease virus as a vector for cancer therapy); U.S. Pat. Nos. 5,698,530 and 6,998,252. See also, e.g., U.S. Pat. No. 5,443,964. See also U.S. Pat. Nos. 7,247,615 and 7,368,116.

[0408] In certain embodiments, an adenovirus vector may be used for expressing a chimeric polypeptide of interest. The adenovirus on which a viral transfer vector may be based may be from any origin, any subgroup, any subtype, mixture of subtypes, or any serotype. For instance, an adenovirus can be of subgroup A (e.g., serotypes 12, 18, and 31), subgroup B (e.g., serotypes 3, 7, 11, 14, 16, 21, 34, 35, and 50), subgroup C (e.g., serotypes 1, 2, 5, and 6), subgroup D (e.g., serotypes 8, 9, 10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, and 42-48), subgroup E (e.g., serotype 4), subgroup F (e.g., serotypes 40 and 41), an unclassified serogroup (e.g., serotypes 49 and 51), or any other adenoviral serotype. Adenoviral serotypes 1 through 51 are available from the American Type Culture Collection (ATCC, Manassas, Va.). Non-group C adenoviruses, and even non-human adenoviruses, can be used to prepare replication-deficient adenoviral vectors. Non-group C adenoviral vectors, methods of producing non-group C adenoviral vectors, and methods of using non-group C adenoviral vectors are disclosed in, for example, U.S. Pat. Nos. 5,801,030, 5,837,511, and 5,849,561, and International Patent Applications WO 97/12986 and WO 98/53087. Any adenovirus, even a chimeric adenovirus, can be used as the source of the viral genome for an adenoviral vector. For example, a human adenovirus can be used as the source of the viral genome for a replication-deficient adenoviral vector. Further examples of adenoviral vectors can be found in Molin *et al.* (1998. *J. Virol.* 72:8358-8361), Narumi *et al.* (1998. *Am J. Respir. Cell Mol. Biol.* 19:936-941) Mercier *et al.* (2004. *Proc. Natl. Acad. Sci. USA* 101:6188-6193), U.S. Publication Nos. 20150093831, 20140248305, 20120283318, 20100008889, 20090175897 and 20090088398 and U.S. Pat. Nos. 6,143,290; 6,596,535; 6,855,317; 6,936,257; 7,125,717; 7,378,087; 7,550,296.

[0409] The viral vector can also be based on adeno-associated viruses (AAVs). For a description of AAV-based vectors, *see*, for example, U.S. Pat. Nos. 8,679,837, 8,637,255, 8,409,842, 7,803,622, and 7,790,449, and U.S. Publication Nos. 20150065562, 20140155469, 20140037585, 20130096182, 20120100606, and 20070036757. The AAV vectors may also be self-

complementary (sc) AAV vectors, which are described, for example, in U.S. Patent Publications 2007/01110724 and 2004/0029106, and U.S. Pat. Nos. 7,465,583 and 7,186,699.

[0410] Herpes simplex virus (HSV)-based viral vectors are also suitable for endogenous production of the chimeric polypeptides of the invention. Many replication-deficient HSV vectors contain a deletion to remove one or more intermediate-early genes to prevent replication. Advantages of the herpes vector are its ability to enter a latent stage that can result in long-term DNA expression, and its large viral DNA genome that can accommodate exogenous DNA up to 25 kb. For a description of HSV-based vectors, see, for example, U.S. Pat. Nos. 5,837,532, 5,846,782, 5,849,572, and 5,804,413, and International Patent Applications WO 91/02788, WO 96/04394, WO 98/15637, and WO 99/06583.

[0411] Retroviral vectors may include those based upon murine leukemia virus (MuLV), gibbon ape leukemia virus (GaLV), ecotropic retroviruses, simian immunodeficiency virus (SW), human immunodeficiency virus (HIV), and combinations (*see*, *e.g.*, Buchscher *et al.*, 1992. *J. Virol.* 66:2731-2739; Johann *et al.*, 1992. *J. Virol.* 66:1635-1640; Sommerfelt *et al.*, 1990. *Virology* 176:58-59; Wilson *et al.*, 1989. *J. Virol.* 63:2374-2378; Miller *et al.*, 1991. *J. Virol.* 65:2220-2224; Miller *et al.*, 1990. *Mol. Cell Biol.* 10:4239; Kolberg, 1992. *NIH Res.* 4:43; Cornetta *et al.*,1991. *Hum. Gene Ther.* 2:215).

[0412] In specific embodiments, the retroviral vector is a lentiviral vector. As would be understood by the skilled person, a viral vector, such as a lentiviral vector, generally refers to a viral vector particle that comprises the viral vector genome. For example, a lentiviral vector particle may comprise a lentiviral vector genome. With respect to lentiviral vectors, the vector genome can be derived from any of a large number of suitable, available lentiviral genome based vectors, including those identified for human gene therapy applications (*see*, *e.g.*, Pfeifer *et al.*, 2001. *Annu. Rev. Genomics Hum. Genet.* 2:177-211). Suitable lentiviral vector genomes include those based on Human Immunodeficiency Virus (HIV-1), HIV-2, feline immunodeficiency virus (FIV), equine infectious anemia virus, Simian Immunodeficiency Virus (SIV), and maedi/visna virus. A desirable characteristic of lentiviruses is that they are able to infect both dividing and non-dividing cells, although target cells need not be dividing cells or be stimulated to divide. Generally, the genome and envelope glycoproteins will be based on different viruses, such that the resulting viral vector particle is pseudotyped. Safety features of the viral vector are desirably incorporated. Safety features include self-inactivating LTR and integration deficiency as described in more detail herein. In certain embodiments integration deficiency may be conferred by elements of the vector genome but may also derive from elements of the packaging system (*e.g.*, a non functional integrase protein that may not be part of the vector genome but supplied in trans). Exemplary vectors contain a packaging signal (psi), a Rev-responsive element (RRE), splice donor, splice acceptor, optionally a central poly-purine tract (cPPT), and WPRE element. In certain exemplary embodiments, the viral vector genome comprises sequences from a lentivirus genome, such as the HIV-1 genome or the SIV genome. The viral genome construct may comprise sequences from the 5' and 3' LTRs of a lentivirus, and in particular may comprise the R and U5 sequences from the 5' LTR of a lentivirus and an inactivated or self-inactivating 3' LTR from a lentivirus. The LTR sequences may be LTR sequences from any lentivirus from any species. For example, they may be LTR sequences from HIV, SIV, FIV or BIV. Typically, the LTR sequences are HIV LTR sequences.

[0413] The vector genome may comprise an inactivated or self-inactivating 3' LTR (*see*, *e.g.*, Zufferey *et al.*, 1998. *J. Virol.* 72: 9873; Miyoshi *et al.*, 1998. *J. Virol.* 72:8150). A self-inactivating vector generally has a deletion of the enhancer and promoter sequences from the 3' long terminal repeat (LTR), which is copied over into the 5' LTR during vector integration. In one instance, the U3 element of the 3' LTR contains a deletion of its enhancer sequence, the TATA box, Spl and NF-kappa B sites. As a result of the self-inactivating 3' LTR, the provirus that is generated following entry and reverse transcription will comprise an inactivated 5' LTR. The rationale is to improve safety by reducing the risk of mobilization of the vector genome and the influence of the LTR on nearby cellular promoters. The self-inactivating 3' LTR may be constructed by any method known in the art.

[0414] Optionally, the U3 sequence from the lentiviral 5' LTR may be replaced with a promoter sequence in the viral construct, such as a heterologous promoter sequence. This can increase the titer of virus recovered from the packaging cell line. An enhancer sequence may also be included. Any enhancer/promoter combination that increases expression of the viral RNA genome in the packaging cell line may be used. In one example, the CMV enhancer/promoter sequence is used (*see*, *e.g.*, U.S. Pat. Nos. 5,385,839 and 5,168,062).

[0415] In certain embodiments, the risk of insertional mutagenesis is minimized by constructing the lentiviral vector to be integration defective. A variety of approaches can be pursued to produce a non-integrating vector genome. These approaches entail engineering a mutation(s) into the integrase enzyme component of the pol gene, such that it encodes a protein with an inactive integrase. The vector genome itself can be modified to prevent integration by, for example, mutating or deleting one or both attachment sites, or making the 3' LTR-proximal polypurine tract (PPT) non-functional through deletion or modification. In addition, non-genetic approaches are available; these include pharmacological agents that inhibit one or more functions of integrase. The approaches are not mutually exclusive, that is, more than one of them can be used at a time. For example, both the integrase and attachment sites can be non-functional, or the integrase and PPT site can be non-functional, or the attachment sites and PPT site can be non-functional, or all of them can be non-functional.

[0416] Exemplary lentivirus vectors are described for example in U.S. Publication Nos. 20150224209, 20150203870, 20140335607, 20140248306, 20090148936, and 20080254008.

[0417] The viral vectors may also be based on an alphavirus. Alphaviruses include *Sindbis virus* (and *Venezuelan equine encephalitis virus* (*VEEV*)), *Aura virus*, *Babanki virus*, *Barmah Forest virus*, *Bebaru virus*, *Cabassou virus*, *Chikungunya virus*, *Eastern equine encephalitis virus*, *Everglades virus*, *Fort Morgan virus*, *Getah virus*, *Highlands J virus*, *Kyzylagach virus*, *Mayaro virus*, *Me Tri virus*, *Middelburg virus*, *Mosso das Pedras virus*, *Mucambo virus*, *Ndumu virus*, *O'nyong-nyong virus*, *Pixuna virus*, *Rio Negro virus*, *Ross River virus*, *Salmon pancreas disease virus*, *Semliki Forest virus* (SFV), *Southern elephant seal virus*, *Tonate virus*, *Trocara virus*, *Una virus*, *Venezuelan equine encephalitis virus*, *Western equine encephalitis virus*, *and Whataroa virus*. Generally, the genome of such viruses encode nonstructural (*e.g.*, replicon) and structural proteins (*e.g.*, capsid and envelope) that can be translated in the cytoplasm of the host cell. *Ross River virus*, Sindbis virus, SFV, and VEEV have all been used to develop viral transfer vectors for transgene delivery. Pseudotyped viruses may be formed by combining alphaviral envelope

glycoproteins and retroviral capsids. Examples of alphaviral vectors can be found in U.S. Publication Nos. 20150050243, 20090305344, and 20060177819.

[0418] Alternatively, the viral vectors can be based on a flavivirus. Flaviviruses include *Japanese encephalitis virus*, *Dengue virus* (*e.g.*, *Dengue-1*, *Dengue-2*, *Dengue-3*, *Dengue-4*), *Yellow fever virus*, *Murray Valley encephalitis virus*, *St. Louis encephalitis virus*, *West Nile virus*, *Kunjin virus*, *Rocio encephalitis virus*, *Ilheus virus*, *Tick-borne encephalitis virus*, *Central European encephalitis virus*, *Siberian encephalitis virus*, *Russian Spring-Summer encephalitis virus*, *Kyasanur Forest Disease virus*, *Omsk Hemorrhagic fever virus*, *Louping ill virus*, *Powassan virus*, *Negishi virus*, *Absettarov virus*, *Hansalova virus*, *Apoi virus*, *and Hypr virus*. Examples of flavivirus vectors can be found in U.S. Publication Nos. 20150231226, 20150024003, 20140271708, 20140044684, 20130243812, 20120294889, 20120128713, 20110135686, 20110014229, 20110003884, 20100297167, 20100184832, 20060159704, 20060088937, 20030194801 and 20030044773.

4. Chimeric polypeptide complexes

[0419] The chimeric polypeptides of the invention can self-assemble under suitable conditions to form chimeric polypeptide complexes. Accordingly, the present invention further encompasses a method of producing a chimeric polypeptide complex, wherein the method comprises: combining chimeric polypeptides of the present invention under conditions (*e.g.*, in aqueous solution) suitable for the formation of a chimeric polypeptide complex, whereby a chimeric polypeptide complex is produced that comprises three chimeric polypeptides and is characterized by a six-helix bundle formed by the coiled coil structures of the respective structure-forming moieties of the chimeric polypeptides. The chimeric polypeptides that are combined may be identical or non-identical to thereby form homotrimers and heterotrimers, respectively.

[0420] Generally the chimeric polypeptides self-assemble in a buffered aqueous solution (*e.g.*, pH about 5 to about 9). If required, mild denaturing conditions can be used, such as, by including urea, small amounts of organic solvents or heat to mildly denature the chimeric polypeptides in order to facilitate refolding and self-assembly.

[0421] Any suitable preparation of chimeric polypeptides can be used in the method. For example, conditioned cell culture media that contains the desired chimeric polypeptide can be used in the method. However, it is preferable to use purified chimeric polypeptides in the method.

[0422] In particular embodiments in which the structure-stabilizing moiety / universal oligomerization domain is used to oligomerize ectodomain polypeptides to form chimeric polypeptide complexes, the ectodomain polypeptide subunits of the complexes are in the pre-fusion conformation. Without wishing to be bound by any particular theory or mode of operation, it is believed that the pre-fusion form of the ectodomain polypeptide trimer is stabilized in the complexes described herein because the heterologous structure-stabilizing moiety induces complex formation and prevents internal moieties or domains of the ectodomain polypeptide (*e.g.*, the HRA and HRB regions of a Class I ectodomain polypeptide, or the central α-helical coiled coil and fusion loop(s) at the C-terminal region of a Class III ectodomain) from interacting. The interaction of such internal moieties or domains leads to refolding into the post fusion form.

5. Screening methods

[0423] The present invention also encompasses methods of screening for agents that bind preferably specifically with a fusion protein of an enveloped virus, and/or complex of the

fusion protein. In specific embodiments, a compound library is screened for binding to an enveloped virus fusion ectodomain polypeptide-containing chimeric polypeptide, or complex thereof.

[0424] Candidate agents encompass numerous chemical classes including small molecules such as small organic compounds and macromolecules such as peptides, polypeptides and polysaccharides. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, desirably at least two of the functional chemical groups. The candidate compounds may comprise cyclical carbon or heterocyclic structures or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including, but not limited to: peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogues or combinations thereof. The compound library may comprise natural compounds in the form of bacterial, fungal, plant and animal extracts. Alternatively, or in addition, the compound library may include natural or synthetically produced compounds.

[0425] Methods for determining whether an agent binds to a target protein and/or the affinity for an agent for a target protein are known in the art. For example, the binding of an agent to a target protein can be detected and/or quantified using a variety of techniques such as, but not limited to, BioLayer Interferometry (BLI), Western blot, dot blot, surface plasmon resonance method (SPR), enzyme-linked immunosorbent assay (ELISA), AlphaScreen® or AlphaLISA® assays, or mass spectrometry based methods.

[0426] In some embodiments, agents can be assayed using any surface plasmon resonance (SPR)-based assays known in the art for characterizing the kinetic parameters of the interaction of the agent with ectodomain polypeptide-containing chimeric polypeptide, or complex of the invention. Any SPR instrument commercially available including, but not limited to, BIAcore Instruments (Biacore AB; Uppsala, Sweden); 1Asys instruments (Affinity Sensors; Franklin, Mass.); IBIS system (Windsor Scientific Limited; Berks, UK), SPR-CELLIA systems (Nippon Laser and Electronics Lab; Hokkaido, Japan), and SPR Detector Spreeta (Texas Instruments; Dallas, Tex.) can be used in the methods described herein. See, e.g., Mullett *et al.* (2000) Methods 22: 77-91; Dong *et al.* (2002) Reviews in Mol Biotech 82: 303-323; Fivash *et al.* (1998) Curr Opin Biotechnol 9: 97-101; and Rich *et al.* (2000) Curr Opin Biotechnol 11: 54-61.

[0427] In some embodiments, the biomolecular interactions between the agents and ectodomain polypeptide-containing chimeric polypeptide or complex of the invention can be assayed using BLI on an Octet (ForteBio Inc.). BLI is a label-free optical analytical technique that senses binding between a ligand (such as an ectodomain polypeptide-containing chimeric polypeptide or complex of the invention) that is immobilized on a biosensor tip and an analyte (such as a test compound) in solution by measuring the change in the thickness of the protein layer on the biosensor tip in real-time.

[0428] In some embodiments, AlphaScreen (PerkinElmer) assays can be used to characterize binding of test agents to the ectodomain polypeptide-containing chimeric polypeptide or complex of the invention. The acronym ALPHA stands for Amplified Luminescent Proximity Homogeneous Assay. AlphaScreen is a bead-based proximity assay that senses binding between

molecules (such as a subject chimeric polypeptide, or complex and a test compound) attached to donor and acceptor beads by measuring the signal produced by energy transfer between the donor and acceptor beads. (See e.g., Eglen *et al.* (2008) Curr Chem Genomics 1:2-10).

[0429] In some embodiments, AlphaLISA® (PerkinElmer) assays can be used to characterize binding of test agents to the chimeric polypeptide or complex of the invention. AlphaLISA is modified from the AlphaScreen assay described above to include europium-containing acceptor beads and functions as an alternative to traditional ELISA assays. (See, e.g., Eglen *et al.* (2008) Curr Chem Genomics 1:2-10.)

[0430] A variety of immunoassay techniques, including competitive and non-competitive immunoassays, can be used. The term "immunoassay" encompasses techniques including, without limitation, flow cytometry, FACS, enzyme immunoassays (EIA), such as enzyme multiplied immunoassay technique (EMIT), enzyme-linked immunosorbent assay (ELISA), IgM antibody capture ELISA (MAC ELISA) and microparticle enzyme immunoassay (MEIA), furthermore capillary electrophoresis immunoassays (CEIA), radio-immunoassays (RIA), immunoradiometric assays (IRMA), fluorescence polarization immunoassays (FPIA) and chemiluminescence assays (CL). If desired, such immunoassays can be automated. Immunoassays can also be used in conjunction with laser induced fluorescence. Liposome immunoassays, such as flow-injection liposome immunoassays and liposome immunosensors, are also suitable for use in the present invention. In addition, nephelometry assays, in which, for example, the formation of protein/antibody complexes results in increased light scatter that is converted to a peak rate signal as a function of the marker concentration, are suitable for use in the methods of the present invention.

[0431] In some embodiments, binding of test agents to the subject chimeric polypeptide, or complex can be assayed using thermodenaturation methods involving differential scanning fluorimetry (DSF) and differential static light scattering (DSLS).

[0432] In some embodiments, binding of test agents to the chimeric polypeptide or complex of the invention can be assayed using a mass spectrometry based method such as, but not limited to, an affinity selection coupled to mass spectrometry (AS-MS) platform. This is a label-free method where the protein and test compound are incubated, unbound molecules are washed away and protein-ligand complexes are analyzed by MS for ligand identification following a decomplexation step.

[0433] In some embodiments, binding of test agents to the subject chimeric polypeptide or complex can be quantitated using, for example, detectably labeled proteins such as radiolabeled (*e.g.*, ^{32}P, ^{35}S, ^{14}C or ^{3}H), fluorescently labeled (*e.g.*, FITC), or enzymatically labeled chimeric polypeptide or complex or test compound, by immunoassay, or by chromatographic detection.

[0434] In some embodiments, the present invention contemplates the use of fluorescence polarization assays and fluorescence resonance energy transfer (FRET) assays in measuring, either directly or indirectly, the degree of interaction between a chimeric polypeptide or complex and a test compound.

[0435] All of the above embodiments are suitable for development into high-throughput platforms.

[0436] Compounds may be further tested in the animal models to identify those compounds having the most potent *in vivo* effects, *e.g.*, those that bind specifically to a fusion protein of an enveloped virus, or complex of the fusion protein and preferably stimulate or enhance a therapeutically useful effect, *e.g.*, reduced viral load, reduced infection or symptoms associated therewith. These molecules may serve as "lead compounds" for the further development of pharmaceuticals by, for example, subjecting the compounds to sequential modifications, molecular modeling, and other routine procedures employed in rational drug design.

6. Antigen-binding molecules

[0437] The ectodomain-containing chimeric polypeptides and complexes of the present invention are useful for producing antigen-binding molecules, which are preferably proteins (*i.e.*, "antigen-binding protein") that are immuno-interactive with an envelope virus fusion protein. In specific embodiments, the ectodomain-containing chimeric polypeptides and complexes include at least one pre-fusion epitope that is not present in the post-fusion form of the envelope virus fusion protein, and therefore useful for preparation of antigen-binding molecules that are immuno-interactive with a metastable or pre-fusion form of an enveloped virus fusion protein.

[0438] Those of ordinary skill in the art will appreciate the well developed knowledge base on antigen-binding proteins such as set forth, for example, in Abbas *et al.*, Cellular and Molecular Immunology, 6th ed., W.B. Saunders Company (2010) or Murphey *et al.*, Janeway's Immunobiology, 8th ed., Garland Science (2011), each of which is incorporated herein by reference in its entirety.

[0439] In some embodiments, antigen binding proteins that are immuno-interactive with the chimeric polypeptides and complexes of the present invention are antibodies. Antibodies include intact antibodies and antigen binding fragments thereof, as described in the definition section. An antibody may comprise a complete antibody molecule (including polyclonal, monoclonal, chimeric, humanized, or human versions having full length heavy and/or light chains), or comprise an antigen binding fragment thereof. Antibody fragments include F(ab')$_2$, Fab, Fab', Fv, Fc, and Fd fragments, and can be incorporated into single domain antibodies, single-chain antibodies, maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see *e.g.*, Hollinger and Hudson, 2005, Nature Biotechnology, 23, 9, 1126-1136). Also included are antibody polypeptides such as those disclosed in U.S. Pat. No. 6,703,199, including fibronectin polypeptide monobodies. Other antibody polypeptides are disclosed in U.S. Patent Publication 2005/0238646, which are single-chain polypeptides.

[0440] Numerous methods of preparing antibodies to antigens of interest are known in the art. For example, monoclonal antibodies to the chimeric polypeptides and complexes of the present invention can be made using conventional hybridoma methods that are often based on the seminal method of Kohler, G. *et al.* (1975, "Continuous Cultures Of Fused Cells Secreting Antibody Of Predefined Specificity," Nature 256:495-497) or a modification thereof. Typically, monoclonal antibodies are developed in non-human species, such as mice. In general, a mouse or rat is used for immunization but other animals may also be used. The antibodies may be produced by immunizing mice with an immunogenic amount of an immunogen, in this case a chimeric polypeptide or complex of the present invention. The immunogen may be administered multiple times at periodic intervals such as, bi weekly, or weekly, or may be administered in such a way as to maintain viability in the animal.

[0441] To monitor the antibody response, a small biological sample (*e.g.*, blood) may be obtained from the animal and tested for antibody titer against the immunogen. The spleen and/or several large lymph nodes can be removed and dissociated into single cells. If desired, the spleen cells may be screened (after removal of non-specifically adherent cells) by applying a cell suspension to a plate or to a well coated with the antigen. B-cells, expressing membrane-bound immunoglobulin specific for the antigen, will bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, can then be fused with myeloma cells (*e.g.*, X63-Ag8.653 and those from the Salk Institute, Cell Distribution Center, San Diego, Calif.). Polyethylene glycol (PEG) may be used to fuse spleen or lymphocytes with myeloma cells to form a hybridoma. The hybridoma is then cultured in a selective medium (*e.g.*, hypoxanthine, aminopterin, thymidine medium, otherwise known as "HAT medium"). The resulting hybridomas are then plated by limiting dilution, and are assayed for the production of antibodies that bind specifically to the immunogen, using, for example, FACS (fluorescence activated cell sorting) or immunohistochemistry (IHC) screening. The selected monoclonal antibody-secreting hybridomas are then cultured either in vitro (*e.g.*, in tissue culture bottles or hollow fiber reactors), or in vivo (*e.g.*, as ascites in mice).

[0442] As another alternative to the cell fusion technique, Epstein-Barr Virus (EBV)-immortalized B cells may be used to produce monoclonal antibodies that are immuno-interactive with a subject chimeric polypeptide or complex. The hybridomas are expanded and subcloned, if desired, and supernatants are assayed for anti-immunogen activity by conventional assay procedures (*e.g.*, FACS, IHC, radioimmunoassay, enzyme immunoassay, fluorescence immunoassay, *etc.*).

[0443] Thus, the present invention further contemplates methods of producing an antigen-binding molecule that is immuno-interactive with a fusion protein of an enveloped virus, or complex of the fusion protein, wherein the method comprises: (1) immunizing an animal with a chimeric polypeptide complex or composition, as broadly described above and elsewhere herein, wherein an ectodomain polypeptide of the chimeric polypeptide complex corresponds to the fusion protein of the enveloped virus; (2) isolating a B cell from the animal, which is immuno-interactive with the fusion protein or complex thereof; and (3) producing the antigen-binding molecule expressed by that B cell. The present invention also encompasses antigen-binding molecule that are produced by such methods as well as derivatives thereof. Also encompassed are cells including hybridomas that are capable of producing the antigen-binding molecules of the invention, and methods of producing antigen-binding molecules from those cells. In specific embodiments, the antigen-binding molecules produced by the methods and cells of the invention are preferably neutralizing antigen-binding molecules.

[0444] Also contemplated are chimeric antibodies and humanized antibodies. In some embodiments, a humanized monoclonal antibody comprises the variable domain of a murine antibody (or all or part of the antigen binding site thereof) and a constant domain derived from a human antibody. Alternatively, a humanized antibody fragment may comprise the antigen binding site of a murine monoclonal antibody and a variable domain fragment (lacking the antigen-binding site) derived from a human antibody. Procedures for the production of engineered monoclonal antibodies include those described in Riechmann *et al.*, 1988, *Nature* 332:323, Liu *et al.*, 1987, *Proc. Nat. Acad. Sci. USA* 84:3439, Larrick *et al.*, 1989, *Bio/Technology* 7:934, and Winter *et al.*,

1993, *TIPS* 14:139. In one embodiment, the chimeric antibody is a CDR grafted antibody. Techniques for humanizing antibodies are discussed in, *e.g.*, U.S. Pat. Nos. 5,869,619; 5,225,539; 5,821,337; 5,859,205; 6,881,557, Padlan *et al.*, 1995, *FASEB J.* 9:133-39, Tamura *et al.*, 2000, J. Immunol. 164:1432-41, Zhang, W., *et al.*, *Molecular Immunology* 42(12):1445-1451, 2005; Hwang W. *et al.*, *Methods* 36(1):35-42, 2005; Dall'Acqua W F, *et al.*, *Methods* 36(1):43-60, 2005; and Clark, M., *Immunology Today* 21(8):397-402, 2000.

[0445] An antibody of the present invention may also be a fully human monoclonal antibody. Fully human monoclonal antibodies may be generated by any number of techniques with which those having ordinary skill in the art will be familiar. Such methods include, but are not limited to, Epstein Barr Virus (EBV) transformation of human peripheral blood cells (*e.g.*, containing B lymphocytes), *in vitro* immunization of human B-cells, fusion of spleen cells from immunized transgenic mice carrying inserted human immunoglobulin genes, isolation from human immunoglobulin V region phage libraries, or other procedures as known in the art and based on the disclosure herein.

[0446] Procedures have been developed for generating human monoclonal antibodies in non-human animals. For example, mice in which one or more endogenous immunoglobulin genes have been inactivated by various means have been prepared. Human immunoglobulin genes have been introduced into the mice to replace the inactivated mouse genes. In this technique, elements of the human heavy and light chain locus are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy chain and light chain loci (see also Bruggemann *et al.*, *Curr. Opin. Biotechnol.* 8:455-58 (1997)). For example, human immunoglobulin transgenes may be mini-gene constructs, or transloci on yeast artificial chromosomes, which undergo B-cell-specific DNA rearrangement and hypermutation in the mouse lymphoid tissue.

[0447] Antibodies produced in the animal incorporate human immunoglobulin polypeptide chains encoded by the human genetic material introduced into the animal. In one embodiment, a non-human animal, such as a transgenic mouse, is immunized with a subject chimeric polypeptide or complex immunogen.

[0448] Examples of techniques for production and use of transgenic animals for the production of human or partially human antibodies are described in U.S. Pat. Nos. 5,814,318, 5,569,825, and 5,545,806, Davis *et al.*, Production of human antibodies from transgenic mice in Lo, ed. Antibody Engineering: Methods and Protocols, Humana Press, NJ:191-200 (2003), Kellermann *et al.*, 2002, *Curr Opin Biotechnol.* 13:593-97, Russel *et al.*, 2000, *Infect Immun.* 68:1820-26, Gallo *et al.*, 2000, *Eur J. Immun.* 30:534-40, Davis *et al.*, 1999, *Cancer Metastasis Rev.* 18:421-25, Green, 1999, *J Immunol Methods* 231:11-23, Jakobovits, 1998, *Advanced Drug Delivery Reviews* 31:33-42, Green *et al.*, 1998, *J Exp Med.* 188:483-95, Jakobovits A, 1998, *Exp. Opin. Invest. Drugs* 7:607-14, Tsuda *et al.*, 1997, *Genomics* 42:413-21, Mendez *et al.*, 1997, *Nat. Genet.* 15:146-56, Jakobovits, 1994, *Curr Biol.* 4:761-63, Arbones *et al.*, 1994, *Immunity* 1:247-60, Green *et al.*, 1994, *Nat. Genet.* 7:13-21, Jakobovits *et al.*, 1993, *Nature* 362:255-58, Jakobovits *et al.*, 1993, *Proc Natl Acad Sci USA* 90:2551-55. Chen, J., M. *et al. Int. Immunol.* 5 (1993): 647-656, Choi *et al.*, 1993, *Nature Genetics* 4: 117-23, Fishwild *et al.*, 1996, *Nature Biotech.* 14: 845-51, Harding *et al.*, 1995, *Annals of the New York Academy of Sciences*, Lonberg *et al.*, 1994, *Nature* 368: 856-59, Lonberg, 1994, Transgenic Approaches to Human Monoclonal

Antibodies in Handbook of Experimental Pharmacology 113: 49-101, Lonberg *et al.*, 1995, *Int. Rev. Immunol.* 13: 65-93, Neuberger, 1996, *Nature Biotech.* 14: 826, Taylor *et al.*, 1992, *Nucleic Acids Research* 20: 6287-95, Taylor *et al.*, 1994, *Int. Immunol.* 6: 579-91, Tomizuka *et al.*, 1997, *Nature Genetics* 16: 133-43, Tomizuka *et al.*, 2000, *Proc Natl Acad Sci USA* 97: 722-27, Tuaillon *et al.*, 1993, *Proc Natl Acad Sci USA* 90: 3720-24, and Tuaillon *et al.*, 1994, *J. Immunol.* 152: 2912-20.; Lonberg *et al.*, *Nature* 368:856, 1994; Taylor *et al.*, *Int. Immunol.* 6:579, 1994; U.S. Pat. No. 5,877,397; Bruggemann *et al.*, 1997 Curr. Opin. Biotechnol. 8:455-58; Jakobovits *et al.*, 1995. *Ann. N.Y. Acad. Sci.* 764:525-35. In addition, protocols involving the XenoMouse®. (Abgenix, now Amgen, Inc.) are described, for example in U.S. 05/0118643 and WO 05/694879, WO 98/24838, WO 00/76310, and U.S. Pat. No. 7,064,244.

[0449] The present invention further encompasses fragments of an anti-chimeric polypeptide/complex antibody of the invention. Such fragments can consist entirely of antibody-derived sequences or can comprise additional sequences. Examples of antigen-binding fragments include Fab, $F(ab')_2$, single chain antibodies, diabodies, triabodies, tetrabodies, and domain antibodies. Other examples are provided in Lunde *et al.*, 2002, *Biochem. Soc. Trans.* 30:500-06.

[0450] Single chain antibodies may be formed by linking heavy and light chain variable domain (Fv region) fragments *via* an amino acid bridge (short peptide linker), resulting in a single polypeptide chain. Such single-chain Fvs (scFvs) have been prepared by fusing DNA encoding a peptide linker between DNAs encoding the two variable domain polypeptides (V_L and V_H). The resulting polypeptides can fold back on themselves to form antigen-binding monomers, or they can form multimers (e.g., dimers, trimers, or tetramers), depending on the length of a flexible linker between the two variable domains (Kortt *et al.*, 1997, *Prot. Eng.* 10:423; Kortt *et al.*, 2001, *Biomol. Eng.* 18:95-108). By combining different V_L and V_H-comprising polypeptides, one can form multimeric scFvs that bind to different epitopes (Kriangkum *et al.*, 2001, *Biomol. Eng.* 18:31-40). Techniques developed for the production of single chain antibodies include those described in U.S. Pat. No. 4,946,778; Bird, 1988, *Science* 242:423; Huston *et al.*, 1988, *Proc. Natl. Acad. Sci. USA* 85:5879; Ward *et al.*, 1989, *Nature* 334:544, de Graaf *et al.*, 2002, *Methods Mol. Biol.* 178:379-87.

[0451] Antigen binding fragments derived from an antibody can also be obtained, for example, by proteolytic hydrolysis of the antibody, for example, pepsin or papain digestion of whole antibodies according to conventional methods. By way of example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment termed $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent to produce 3.5S Fab' monovalent fragments. Optionally, the cleavage reaction can be performed using a blocking group for the sulfhydryl groups that result from cleavage of disulfide linkages. As an alternative, an enzymatic cleavage using papain produces two monovalent Fab fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. Pat. No. 4,331,647, Nisonoff *et al.*, *Arch. Biochem. Biophys.* 89:230, 1960; Porter, *Biochem. J.* 73:119, 1959; Edelman *et al.*, in Methods in Enzymology 1:422 (Academic Press 1967); and by Andrews, S. M. and Titus, J. A. in Current Protocols in Immunology (Coligan J. E., *et al.*, eds), John Wiley & Sons, New York (2003), pages 2.8.1-2.8.10 and 2.10A.1-2.10A.5. Other methods for cleaving antibodies, such as separating heavy chains to form monovalent light-heavy chain fragments (Fd), further cleaving of

fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

[0452] Another form of an antibody fragment is a peptide comprising one or more complementarity determining regions (CDRs) of an antibody. CDRs can be obtained by constructing polynucleotides that encode the CDR of interest. Such polynucleotides are prepared, for example, by using the polymerase chain reaction to synthesize the variable region using mRNA of antibody-producing cells as a template (see, for example, Larrick *et al.*, Methods: A Companion to Methods in Enzymology 2:106, 1991; Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in Monoclonal Antibodies: Production, Engineering and Clinical Application, Ritter *et al.* (eds.), page 166 (Cambridge University Press 1995); and Ward *et al.*, "Genetic Manipulation and Expression of Antibodies," in Monoclonal Antibodies: Principles and Applications, Birch *et al.*, (eds.), page 137 (Wiley-Liss, Inc. 1995)). The antibody fragment further may comprise at least one variable region domain of an antibody described herein. Thus, for example, the V region domain may be monomeric and be a V_L and V_H domain, which is capable of independently binding a subject ectodomain polypeptide or complex with an affinity at least equal to 10^{-7} M or less.

[0453] The variable region domain may be any naturally occurring variable domain or an engineered version thereof. By engineered version is meant a variable region domain that has been created using recombinant DNA engineering techniques. Such engineered versions include those created, for example, from a specific antibody variable region by insertions, deletions, or changes in or to the amino acid sequences of the specific antibody. Particular examples include engineered variable region domains containing at least one CDR and optionally one or more framework amino acids from a first antibody and the remainder of the variable region domain from a second antibody.

[0454] The variable region domain may be covalently attached at a C-terminal amino acid to at least one other antibody domain or a fragment thereof. Thus, for example, a V_H domain that is present in the variable region domain may be linked to an immunoglobulin CH1 domain, or a fragment thereof. Similarly a V_L domain may be linked to a C_K domain or a fragment thereof. In this way, for example, the antibody may be a Fab fragment wherein the antigen binding domain contains associated V_H and V_L domains covalently linked at their C-termini to a CH1 and C_K domain, respectively. The CH1 domain may be extended with further amino acids, for example to provide a hinge region or a portion of a hinge region domain as found in a Fab' fragment, or to provide further domains, such as antibody CH2 and CH3 domains.

7. Compositions

[0455] The present invention further provides compositions, including pharmaceutical compositions, comprising a chimeric polypeptide or complex, or a nucleic acid construct from which the chimeric polypeptide or complex is expressible, as broadly described above and elsewhere herein. Representative compositions may include a buffer, which is selected according to the desired use of the chimeric polypeptide or complex, and may also include other substances appropriate to the intended use. Where the intended use is to induce an immune response, the composition is referred to as an "immunogenic" or "immunomodulating" composition. Such compositions include preventative compositions (*i.e.*, compositions administered for the purpose of preventing a condition such as an infection) and therapeutic compositions (*i.e.*, compositions administered for the purpose of treating conditions such as an infection). An immunomodulating

composition of the present invention may therefore be administered to a recipient for prophylactic, ameliorative, palliative, or therapeutic purposes.

[0456] Those skilled in the art can readily select an appropriate buffer, a wide variety of which are known in the art, suitable for an intended use. In some instances, the composition can comprise a pharmaceutically acceptable excipient, a variety of which are known in the art and need not be discussed in detail herein. Pharmaceutically acceptable excipients have been amply described in a variety of publications, including, for example, A. Gennaro (2000) "Remington: The Science and Practice of Pharmacy", 20th edition, Lippincott, Williams, & Wilkins; Pharmaceutical Dosage Forms and Drug Delivery Systems (1999) H. C. Ansel *et al.*, eds 7.sup.th ed., Lippincott, Williams, & Wilkins; and Handbook of Pharmaceutical Excipients (2000) A. H. Kibbe *et al.*, eds., 3.sup.rd ed. Amer. Pharmaceutical Assoc.

[0457] In some embodiments, the compositions comprise more than one (*i.e.*, different) chimeric polypeptide or complex of the invention (*e.g.*, chimeric polypeptides the respective ectodomain polypeptides of which correspond to different enveloped virus fusion proteins), or one or more nucleic acid constructs from which the chimeric polypeptide(s) or complex(es) is/are expressible.

[0458] Pharmaceutical compositions of the present invention may be in a form suitable for administration by injection, in a formulation suitable for oral ingestion (such as, for example, capsules, tablets, caplets, elixirs), in the form of an ointment, cream or lotion suitable for topical administration, in a form suitable for delivery as an eye drop, in an aerosol form suitable for administration by inhalation, such as by intranasal inhalation or oral inhalation, or in a form suitable for parenteral administration, that is, subcutaneous, intramuscular or intravenous injection.

[0459] Supplementary active ingredients such as adjuvants or biological response modifiers can also be incorporated into pharmaceutical compositions of the present invention. Although adjuvant(s) may be included in pharmaceutical compositions of the present invention they need not necessarily comprise an adjuvant. In such cases, reactogenicity problems arising from the use of adjuvants may be avoided.

[0460] In general, adjuvant activity in the context of a pharmaceutical composition of the present invention includes, but is not limited to, an ability to enhance the immune response (quantitatively or qualitatively) induced by immunogenic components in the composition (*e.g.*, a chimeric polypeptide or complex of the present invention). This may reduce the dose or level of the immunogenic components required to produce an immune response and/or reduce the number or the frequency of immunizations required to produce the desired immune response.

[0461] Any suitable adjuvant may be included in a pharmaceutical composition of the present invention. For example, an aluminum-based adjuvant may be utilized. Suitable aluminum-based adjuvants include, but are not limited to, aluminum hydroxide, aluminum phosphate and combinations thereof. Other specific examples of aluminum-based adjuvants that may be utilized are described in European Patent No. 1216053 and United States Patent No. 6,372,223. Other suitable adjuvants include Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, Mich.); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, N.J.); AS-2 (SmithKline Beecham, Philadelphia, Pa.); aluminum salts such as aluminum hydroxide gel (alum)

or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A; oil in water emulsions including those described in European Patent No. 0399843, United States Patent No. 7,029,678 and PCT Publication No. WO 2007/006939; and/or additional cytokines, such as GM-CSF or interleukin-2, -7, or -12, granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF) monophosphoryl lipid A (MPL), cholera toxin (CT) or its constituent subunit, heat labile enterotoxin (LT) or its constituent subunit, toll-like receptor ligand adjuvants such as lipopolysaccharide (LPS) and derivatives thereof (*e.g.*, monophosphoryl lipid A and 3-Deacylated monophosphoryl lipid A), *Flavivirus* NS1 and muramyl dipeptide (MDP).

[0462] Pharmaceutical compositions of the present invention may be provided in a kit. The kit may comprise additional components to assist in performing the methods of the present invention such as, for example, administration device(s), buffer(s), and/or diluent(s). The kits may include containers for housing the various components and instructions for using the kit components in the methods of the present invention.

8. Dosages and routes of administration

[0463] The composition is administered in an "effective amount" that is, an amount effective to achieve an intended purpose in a subject. The dose of active compound(s) administered to a patient should be sufficient to achieve a beneficial response in the subject over time such as a reduction in at least one symptom associated with an infections. The quantity or dose frequency of the pharmaceutically active compounds(s) to be administered may depend on the subject to be treated inclusive of the age, sex, weight and general health condition thereof. In this regard, precise amounts of the active compound(s) for administration will depend on the judgment of the practitioner. One skilled in the art would be able, by routine experimentation, to determine an effective, non-toxic amount of a chimeric polypeptide or complex described herein to include in a pharmaceutical composition of the present invention for the desired therapeutic outcome.

[0464] In general, a pharmaceutical composition of the present invention can be administered in a manner compatible with the route of administration and physical characteristics of the recipient (including health status) and in such a way that it elicits the desired effect(s) (*i.e.* therapeutically effective, immunogenic and/or protective). For example, the appropriate dosage of a pharmaceutical composition of the present invention may depend on a variety of factors including, but not limited to, a subject's physical characteristics (*e.g.*, age, weight, sex), whether the compound is being used as single agent or adjuvant therapy, the type of MHC restriction of the patient, the progression (*i.e.*, pathological state) of a virus infection, and other factors that may be recognized by one skilled in the art. Various general considerations that may be considered when determining an appropriate dosage of a pharmaceutical composition of the present invention are described, for example, in Gennaro (2000) "Remington: The Science and Practice of Pharmacy", 20th edition, Lippincott, Williams, & Wilkins; and Gilman *et al.*, (Eds), (1990), "Goodman And Gilman's: The Pharmacological Bases of Therapeutics", Pergamon Press.

[0465] In some embodiments, an "effective amount" of a subject chimeric polypeptide or complex, or a nucleic acid construct from which the chimeric polypeptide or complex is expressible, is an amount sufficient to achieve a desired prophylactic or therapeutic effect, *e.g.*, to

reduce a symptom associated with infection, and/or to reduce the number of infectious agents in the individual. In these embodiments, an effective amount reduces a symptom associated with infection and/or reduces the number of infectious agents in an individual by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, or more, when compared to the symptom or number of infectious agents in an individual not treated with the chimeric polypeptide or complex. Symptoms of infection by a pathogenic organism, as well as methods for measuring such symptoms, are known in the art. Methods for measuring the number of pathogenic organisms in an individual are standard in the art.

[0466] In some embodiments, an "effective amount" of a subject chimeric polypeptide or complex, or a nucleic acid construct from which the chimeric polypeptide or complex is expressible, is an amount that is effective in a selected route of administration to elicit an immune response to an enveloped virus fusion protein.

[0467] In some embodiments, *e.g.*, where the chimeric polypeptide comprises a heterologous antigen, an "effective amount" is an amount that is effective to facilitate elicitation of an immune response against that antigen. For example, where the heterologous antigen is an antigen from a different pathogenic organism than the one from which the ectodomain polypeptide is derived), an "effective amount" of a subject chimeric polypeptide or complex, or a nucleic acid construct from which the chimeric polypeptide or complex is expressible, is an amount that is effective for elicitation of an immune response against that antigen and preferably protection of the host against infection, or symptoms associated with infection, by that pathogenic organism. In these embodiments, an effective amount reduces a symptom associated with infection by the pathogenic organism and/or reduces the number of infectious agents corresponding to the pathogenic organism in an individual by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, or more, when compared to the symptom or number of infectious agents in an individual not treated with the chimeric polypeptide or complex. Symptoms of infection by a pathogenic organism, as well as methods for measuring such symptoms, are known in the art.

[0468] Alternatively, where a heterologous antigen is a cancer- or tumor-associated antigen, an "effective amount" of a chimeric polypeptide or complex, or a nucleic acid construct from which the chimeric polypeptide or complex is expressible, is an amount that is effective in a route of administration to elicit an immune response effective to reduce or inhibit cancer or tumor cell growth, to reduce cancer or tumor cell mass or cancer or tumor cell numbers, or to reduce the likelihood that a cancer or tumor will form. In these embodiments, an effective amount reduces tumor growth and/or the number of tumor cells in an individual by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, or more, when compared to the tumor growth and/or number of tumor cells in an individual not treated with the chimeric polypeptide or complex. Methods of measuring tumor growth and numbers of tumor cells are known in the art.

[0469] The amount of chimeric polypeptide or complex in each dose is selected as an amount that induces an immune response to the encoded ectodomain polypeptide, and/or that

induces an immunoprotective or other immunotherapeutic response without significant, adverse side effects generally associated with typical vaccines. Such amount will vary depending upon which specific ectodomain polypeptide is employed, whether or not the vaccine formulation comprises an adjuvant, and a variety of host-dependent factors.

[0470] A pharmaceutical composition of the present invention can be administered to a recipient by standard routes, including, but not limited to, parenteral (*e.g.*, intravenous).

[0471] A pharmaceutical composition of the present invention may be administered to a recipient in isolation or in conjunction with additional therapeutic agent(s). In embodiments where a pharmaceutical composition is concurrently administered with therapeutic agent(s), the administration may be simultaneous or sequential (*i.e.*, pharmaceutical composition administration followed by administration of the agent(s) or vice versa).

[0472] Typically, in treatment applications, the treatment may be for the duration of the disease state or condition. Further, it will be apparent to one of ordinary skill in the art that the optimal quantity and spacing of individual dosages will be determined by the nature and extent of the disease state or condition being treated, the form, route and site of administration, and the nature of the particular individual being treated. Optimum conditions can be determined using conventional techniques.

[0473] In many instances (*e.g.*, preventative applications), it may be desirable to have several or multiple administrations of a pharmaceutical composition of the present invention. For example, a pharmaceutical composition may be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more times. The administrations may be from about one to about twelve week intervals, and in certain embodiments from about one to about four week intervals. Periodic re-administration may be desirable in the case of recurrent exposure to a particular pathogen or other disease-associated component targeted by a pharmaceutical composition of the present invention.

[0474] It will also be apparent to one of ordinary skill in the art that the optimal course of administration can be ascertained using conventional course of treatment determination tests.

[0475] Where two or more entities are administered to a subject "in conjunction" or "concurrently" they may be administered in a single composition at the same time, or in separate compositions at the same time, or in separate compositions separated in time.

[0476] Certain embodiments of the present invention involve the administration of pharmaceutical compositions in multiple separate doses. Accordingly, the methods for the prevention (*i.e.* vaccination) and treatment of infection described herein encompass the administration of multiple separated doses to a subject, for example, over a defined period of time. Accordingly, the methods for the prevention (*i.e.*, vaccination) and treatment of infection disclosed herein include administering a priming dose of a pharmaceutical composition of the present invention. The priming dose may be followed by a booster dose. The booster may be for the purpose of re-vaccination. In various embodiments, the pharmaceutical composition or vaccine is administered at least once, twice, three times or more.

[0477] Methods for measuring the immune response are known to persons of ordinary skill in the art. Exemplary methods include solid-phase heterogeneous assays (*e.g.*, enzyme-linked immunosorbent assay), solution phase assays (*e.g.*, electrochemiluminescence assay), amplified

luminescent proximity homogeneous assays, flow cytometry, intracellular cytokine staining, functional T-cell assays, functional B-cell assays, functional monocyte-macrophage assays, dendritic and reticular endothelial cell assays, measurement of NK cell responses, IFN-γ production by immune cells, quantification of virus RNA/DNA in tissues or biological fluids (*e.g.*, quantification of viral RNA or DNA in serum or other fluid or tissue/organ), oxidative burst assays, cytotoxic-specific cell lysis assays, pentamer binding assays, and phagocytosis and apoptosis evaluation.

[0478] It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the present invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

[0479] In order that the invention may be readily understood and put into practical effect, particular preferred embodiments will now be described by way of the following non-limiting examples.

EXAMPLES

EXAMPLE 1

RSV F

[0480] As an illustration of the ability of the present invention to produce chimeric polypeptides that comprise an ectodomain of a viral fusion protein constrained in a pre-fusion conformation, representative evidence is provided for Respiratory Syncytial virus Fusion protein.

Materials and Methods

CHIMERIC POLYPEPTIDE DESIGN:

[0481] The ectodomain of RSV F as well as a RSV-F ectodomain mutant (RSV F ds cav) comprising mutations at 4 sites (S155C, S290C, S190F and V207L), as per McLellan *et al.*, (Science, 2013. 342(6158):592-8), were each operably connected to a downstream heterologous structure stabilizing moiety (SSM) that comprises a pair of complementary heptad repeat regions derived from HIV-1 GP160. A control ectodomain construct lacking this SSM and a positive control construct comprising the RSV F ds cav operably connected to the foldon SSM (RSV F ds cav foldon) were also produced. The amino acid sequences of the relevant proteins are presented below.

Ectodomain of RSV F (1-520):

[0482] MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNI KKNKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTQATNNRARRELPRFMNYTLNNAKKTNVTLSKKRKRRFL GFLLGVGSAIASGVAVSKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSI SNIETVIEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMS IIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRV FCDTMNSLTLPSEVNLCNVDIFNPKYDCEIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCDY VSNKGVDTVSVGNTLYYVNKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVN AGK [SEQ ID NO: 146].

Ectodomain of RSV F (1-520) – HIV GP160-based SSM:

[0483] MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNI KKNKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTQATNNRARRELPRFMNYTLNNAKKTNVTLSKKRKRRFL

GFLLGVGSAIASGVAVSKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSI
SNIETVIEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMS
IIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRV
FCDTMNSLTLPSEVNLCNVDIFNPKYDCEIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCDY
VSNKGVDTVSVGNTLYYVNKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVN
AGK*SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILA*GGSGG*HTTWMEWDREINNYTSLIHSLIEESQNQP
AKDEQELLE [SEQ ID NO: 147].

Ectodomain of RSV F (1-520) – DScav mutations - HIV GP160-based SSM:

[0484] MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNI
KKNKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTQATNNRARRELPRFMNYTLNNAKKTNVTLSKKRKRRFL
GFLLGVGSAIASGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPILNKQSCSI
SNIETVIEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMC
IIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRV
FCDTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCDY
VSNKGVDTVSVGNTLYYVNKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVN
AGK*SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILA*GGSGG*HTTWMEWDREINNYTSLIHSLIEESQNQP
AKDEQELLE [SEQ ID NO: 150].

Ectodomain of RSV F (1-513) – DScav mutations -Foldon SSM (control as per McLellan et al., (Science, 2013. 342(6158):592-8)):

[0485] MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNI
KKNKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTQATNNRARRELPRFMNYTLNNAKKTNVTLSKKRKRRFL
GFLLGVGSAIASGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPILNKQSCSI
SNIETVIEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMC
IIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRV
FCDTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCDY
VSNKGVDTVSVGNTLYYVNKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELL*SAIG
GYIPEAPRDGQAYVRKDGEWVLLSTFLGGLVPRGSGSAWSHPQFEK* [SEQ ID NO: 151]

PROTEIN EXPRESSION AND PURIFICATION:

[0486] Codon optimized DNA sequences encoding the chimeric fusion proteins RSV F clamp, RSV F ds cav clamp, RSV F ds cav foldon, and a control RSV F ectodomain lacking HIV-1 HRA and HRB sequences were each incorporated into the pIRES-2 eukaryotic expression vector downstream of the CMV promoter. The resulting plasmids were transfected into Chinese hamster ovary (CHO) cells grown in chemically defined CHO (CD-CHO) media (Gibco) containing 600 µg plasmid and 2.4 mg of linear polyethylenimine in 300 mL of CHO cells at a density of $1x10^6$ cells per mL. After 4 h of incubation with the transfection reagent cells were pelleted and resuspended in 300 mL of CD-CHO containing 8 mM Glutamax (Gibco), 100 units/mL of penicillin (Gibco), 100 µg/mL of streptomycin (Gibco), 7.5% CHO CD Efficient Feed A (Gibco), and 7.5% CHO CD Efficient Feed B (Gibco). Cells were then incubated for 7 days at 37° C, 5% CO2, shaking at ~120 rpm. After 7 days cells were removed by centrifugation for 10 min at 6,000 x g and supernatant was filtered.

[0487] Recombinant proteins were purified by affinity chromatography with specific monoclonal antibodies covalently coupled to HiTrap NHS-activated HP columns (GE). Chimeric

clamp stabilized RSV F was purified by mAb 1281 (Frey, *et al.*, Nat Struct Mol Biol. 2010. 17(12):1486-91) which binds to the 6-helix bundle formed by HIV-1 HRA and HRB. The RSV F ds cav clamp, RSV F ds cav foldon chimeric proteins and control RSV F were purified using mAb 101F (McLellan, *et al. J Virol.* 2010. 84(23):12236-44). Purification of the desired protein was confirmed by SDS-PAGE.

Results

PROTEIN CONFORMATION:

[0488] Protein conformation was assessed with conformation specific monoclonal antibodies. Incorporation of the SSM based on HIV-1 GP160 HR1 and HR2 sequences downstream of the ectodomain acts as a kind of 'molecular clamp' that inhibits the fusion ectodomain polypeptide from rearranging to a post-fusion conformation. Evidence that such chimeric fusion protein is stabilized in the pre-fusion conformation while the corresponding naked ectodomain (*i.e.*, expressed alone) forms the post fusion form is shown in Figures 1A-C.

EXAMPLE 2

INFA HA

[0489] As another illustration of the ability of the present invention to produce chimeric polypeptides that comprise an ectodomain of a viral fusion protein constrained in a pre-fusion conformation, representative evidence is provided for Influenza A (INFA) Hemagglutinin (HA) protein. Furthermore, representative evidence is provided for INFA HA protein that the chimeric protein constrained in the pre-fusion conformation *via* the aforementioned method is able to induce an improved neutralizing immune response upon administration to mice.

Materials and Methods

CHIMERIC POLYPEPTIDE DESIGN:

[0490] The ectodomain of INFA HA was operably connected to a downstream heterologous structure stabilizing moiety that comprises a pair of complementary heptad repeat regions derived from HIV-1 GP160. The amino acid sequences of the resulting chimeric protein and its control are presented below.

Ectodomain of INFA HA (1-529):

[0491] MKTIIALSYILCLVFAQKLPGNDNSTATLCLGHHAVPNGTIVKTITNDQIEVTNATELVQSS STGEICDSPHQILDGKNCTLIDALLGDPQCDGFQNKKWDLFVERSKAYSNCYPYDVPDYASLRSLVASSGTLEF NNESFNWTGVTQNGTSSACIRRSKNSFFSRLNWLTHLNFKYPALNVTMPNNEQFDKLYIWGVHHPGTDKDQIF LYAQASGRITVSTKRSQQTAIPNIGSRPRVRNIPSRISIYWTIVKPGDILLINSTGNLIAPRGYFKIRSGESSIMRS DAPIGKCNSECITPNGSIPNDKPFQNVNRITYGACPRYVKQNTLKLATGMRNVPEKQTRGIFGAIAGFIENGWEG MVDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGKLNRLIGKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKID LWSYNAELLVALENQHTIDLTDSEMNKLFEKTKKQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHDVYRD EALNNRFQIKGVELKSGYKD [SEQ ID NO: 148].

Ectodomain of INFA HA (1-529) – HIV GP160-based SSM:

[0492] MKTIIALSYILCLVFAQKLPGNDNSTATLCLGHHAVPNGTIVKTITNDQIEVTNATELVQSS STGEICDSPHQILDGKNCTLIDALLGDPQCDGFQNKKWDLFVERSKAYSNCYPYDVPDYASLRSLVASSGTLEF NNESFNWTGVTQNGTSSACIRRSKNSFFSRLNWLTHLNFKYPALNVTMPNNEQFDKLYIWGVHHPGTDKDQIF

LYAQASGRITVSTKRSQQTAIPNIGSRPRVRNIPSRISIYWTIVKPGDILLINSTGNLIAPRGYFKIRSGESSIMRS
DAPIGKCNSECITPNGSIPNDKPFQNVNRITYGACPRYVKQNTLKLATGMRNVPEKQTRGIFGAIAGFIENGWEG
MVDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGKLNRLIGKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKID
LWSYNAELLVALENQHTIDLTDSEMNKLFEKTKKQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHDVYRD
EALNNRFQIKGVELKSGYKDG*SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILA*GGSGGH*TTWMEWDRE*
INNYTSLIHSLIEESQNQPAKDEQELLE [SEQ ID NO: 149].

PROTEIN EXPRESSION AND PURIFICATION:

[0493] Codon optimized DNA sequences encoding the chimeric fusion protein Influenza H3 clamp or a control ectodomain lacking HIV-1 HRA and HRB sequences were incorporated into the pIRES-2 eukaryotic expression vector following the CMV promoter. The resulting plasmids were transfected into CHO cells and the transfected cells were grown and collected according to Example 1.

[0494] Recombinant proteins were purified by affinity chromatography with specific monoclonal antibodies covalently coupled to HiTrap NHS-activated HP columns (GE). Chimeric clamp stabilized Influenza HA was purified by mAb 1281 (Frey, *et al.*, Nat Struct Mol Biol. 2010. 17(12):1486-91) which binds to the 6-helix bundle formed by HIV-1 HRA and HRB. The ectodomain of Influenza HA was purified with mAb C05 (Ekiert, *et al.*, Nature, 2012. 489(7417): 526-32). Purification of the desired protein was confirmed by SDS-PAGE. Commercial quadrivalent influenza vaccine (QIV) for the 2015 season was purchased from Sanofi Pasteur, Fluquadri™.

Results

PROTEIN CONFORMATION:

[0495] Protein conformation was assessed with conformation specific monoclonal antibodies and size-exclusion chromatography. Incorporation of the SSM based on HIV-1 GP160 HR1 and HR2 sequences downstream of the respective ectodomains acts as a kind of 'molecular clamp' that inhibits the fusion ectodomain polypeptide from rearranging to a post-fusion conformation. Evidence that such chimeric fusion protein is stabilized in the pre-fusion conformation while the naked ectodomain (*i.e.*, expressed alone) forms the post fusion form is shown in Figures 2 and 3.

ANIMAL IMMUNIZATION:

[0496] To test the utility of the viral fusion proteins stabilized in their pre-fusion form *via* the incorporation of the 6-helix bundle forming moiety as immunogens, BALB/c mice were immunized with the chimeric clamp stabilized influenza HA, the corresponding non-stabilized ectodomain or the commercial QIV. Five BALB/c mice per group were immunized with 5 µg of purified protein (or PBS) with 3 µg of saponin adjuvant Quil-A. Immunization was *via* intradermal delivery and mice were immunized twice, three weeks apart. Three weeks following the second immunization mice were sacrificed and sera collected. Neutralization effect of pooled sera from each group was assessed against Influenza A/Hebei Baoding Anguo/51/2010 (H3N2) in a plaque reduction neutralization test (PRNT). Sera from mice vaccinated chimeric clamp stabilized Influenza HA showed strong neutralizing activity with an IC50 value of 1:14,000 (95%CI 11,000-17,000), while sera from mice vaccinated with the corresponding HA ectodomain showed no neutralizing activity even at the highest dose tested of 1:20 and sera from mice vaccinated with commercial QIV show neutralization with an IC50 value of ~1:180. Therefore, stabilization of the pre-fusion

form of the influenza HA *via* the incorporation of a structure-stabilizing moiety comprised of HIV-1 HR1 and HR2 is critical for a strong neutralizing immune response and is able to increase the neutralizing immune response by approximately 80-fold compared to a current commercial inactivated vaccine.

[0497] To confirm that clamp stabilized HA induced a novel population of antibodies that did not interact with post-fusion HA sol or the commercial QIV H3clamp vaccinated mouse sera were pre-incubated with H3sol or QIV to remove any antibodies capable of binding these forms (Figure 4, white bars). Neither incubation with H3sol or QIV resulted in a decrease in virus neutralization, however pre-incubation with H3clamp resulted in the complete removal of virus neutralization activity. To test if induced immune response was specific for the head or stem subdomain of HA, ELISA reactivity was compared against the whole H3clamp and a H3 stem only domain (as outlined above in Section 2.3.2). Prior to ELISA mouse sera were pre-incubated with EBOV GP clamp (as outlined above in Section 2.3.12) to reabsorb antibodies specific for the clamp domain itself. Sera were then added to an ELISA plate coated with H3clamp or H3stem and humoral antibody reactivity measured via ELISA. Dilution factors producing half maximal absorbance were compared to estimate the percentage of immunity specific for the stem domain. Immunity to the head domain was estimated by subtracting stem domain specific immunity from the total (see, Figure 5). H3 clamp immunization resulted in approximately 25% of humoral immunity reactive with the stem domain and 75% with the head domain. In contrast, QIV immunization resulted in only 4% of humoral immunity reactive with the stem domain and 96% with the head and H3sol immunization resulted in only 1% of humoral immunity reactive with the stem domain and 99% with the head.

[0498] Reactivity of the immune response with H5 from avian influenza H5N1 was compared using the H5clamp construct (as outlined above in Section 2.3.3). Sera from mice vaccinated with H5clamp or H1clamp was also included in the analysis. Prior to ELISA mouse sera was pre-incubated with EBOV GP clamp (as outlined above in Section 2.3.12) to reabsorb antibodies specific for the clamp domain itself. Sera was then added to an ELISA plate coated with H5clamp and humoral antibody reactivity measured via ELISA. Endpoint tires were calculated and are presented in Figure 6. H3clamp and H1clamp both showed substantial cross reactivity with H5, which was significantly greater than that of QIV (27-fold increase and 81-fold increase, respectively).

[0499] The present inventors next set out to determine the subdomain responsible for H5 cross-reactivity. Prior to measuring ELISA reactivity with H5 ELISA, mouse sera was pre-incubated with EBOV GP clamp (as outlined above in Section 2.3.12) and/or H3stem H3 stem only domain (as outlined above in Section 2.3.2) to pre-absorb antibodies specific for the clamp domain itself and/or the H3 stem domain or human monoclonal antibody FI6v3 (Corti *et al.*, PNAS 2011) was added to outcompete stem specific antibodies. Sera was then added to an ELISA plate coated with H5clamp and endpoint titres we calculated via ELISA (Figure 7). QIV immunized mice showed low reactivity with H5 that was only minimally affected by stem absorption/Fi6V3 competition. Sera from mice immunized with H5 clamp showed high reactivity that was not affected by stem absorption/Fi6V3 competition indicating a strong head specific immune response. However, sera from mice immunized with H1 clamp or H3 clamp showed strong reactivity with H5 and this was

significantly reduced by stem absorption/Fi6V3 competition indicating a stem specific response is responsible for H5 cross-reactivity.

EXAMPLE 3

MERS SPIKE

[0500] As yet another illustration of the ability of the present invention to produce chimeric polypeptides that comprise an ectodomain of a viral fusion protein constrained in a pre-fusion conformation, representative evidence is provided for Middle East Respiratory Syndrome (MERS) virus Spike protein.

Materials and Methods

CHIMERIC POLYPEPTIDE DESIGN:

[0501] The ectodomain of MERS Spike protein was operably connected to a downstream heterologous structure stabilizing moiety that comprises a pair of complementary heptad repeat regions derived from HIV-1 GP160. The amino acid sequence of the resulting chimeric protein is presented below.

Ectodomain of MERS Spike (1-1296) – HIV GP160-based SSM:

[0502] MIHSVFLLMFLLTPTESYVDVGPDSVKSACIEVDIQQTFFDKTWPRPIDVSKADGIIYPQGR TYSNITITYQGLFPYQGDHGDMYVYSAGHATGTTPQKLFVANYSQDVKQFANGFVVRIGAAANSTGTVIISPSTS ATIRKIYPAFMLGSSVGNFSDGKMGRFFNHTLVLLPDGCGTLLRAFYCILEPRSGNHCPAGNSYTSFATYHTPATD CSDGNYNRNASLNSFKEYFNLRNCTFMYTYNITEDEILEWFGITQTAQGVHLFSSRYVDLYGGNMFQFATLPVYD TIKYYSIIPHSIRSIQSDRKAWAAFYVYKLQPLTFLLDFSVDGYIRRAIDCGFNDLSQLHCSYESFDVESGVYSVSS FEAKPSGSVVEQAEGVECDFSPLLSGTPPQVYNFKRLVFTNCNYNLTKLLSLFSVNDFTCSQISPAAIASNCYSSLI LDYFSYPLSMKSDLGVSSAGPISQFNYKQSFSNPTCLILATVPHNLTTITKPLKYSYINKCSRLLSDDRTEVPQLVN ANQYSPCVSIVPSTVWEDGDYYRKQLSPLEGGGWLVASGSTVAMTEQLQMGFGITVQYGTDTNSVCPKLEFAN DTKIASQLGNCVEYSLYGVSGRGVFQNCTAVGVRQQRFVYDAYQNLVGYYSDDGNYYCLRACVSVPVSVIYDKE TKTHATLFGSVACEHISSTMSQYSRSTRSMLKRRDSTYGPLQTPVGCVLGLVNSSLFVEDCKLPLGQSLCALPDT PSTLTPRSVRSVPGEMRLASIAFNHPIQVDQFNSSYFKLSIPTNFSFGVTQEYIQTTIQKVTVDCKQYICNGFQKC EQLLREYGQFCSKINQALHGANLRQDDSVRNLFASVKSSQSSPIIPGFGGDFNLTLLEPVSISTGSRSARSAIEDL LFDKVTIADPGYMQGYDDCMQQGPASARDLICAQYVAGYKVLPPLMDVNMEAAYTSSLLGSIAGVGWTAGLSS FAAIPFAQSIFYRLNGVGITQQVLSENQKLIANKFNQALGAMQTGFTTTNEAFRKVQDAVNNNAQALSKLASELS NTFGAISASIGDIIQRLDVLEQDAQIDRLINGRLTTLNAFVAQQLVRSESAALSAQLAKDKVNECVKAQSKRSGF CGQGTHIVSFVVNAPNGLYFMHVGYYPSNHIEVVSAYGLCDAANPTNCIAPVNGYFIKTNNTRIVDEWSYTGSSF YSPEPITSLNTKYVAPQVTYQNISTNLPPPLLGNSTGIDFQDELDEFFKNVSTSIPNFGSLTQINTTLLDLTYEMLSL QQVVKALNESYIDLKELGNYTYYNKWP*GGSGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILAGGSGGHTT WMEWDREINNYTSLIHSLIEESQNQQEKNEQELLE* [SEQ ID NO: 152].

PROTEIN EXPRESSION AND PURIFICATION:

[0503] A codon optimized DNA sequence encoding the chimeric fusion protein MERS S clamp was incorporated into the pIRES-2 eukaryotic expression vector following the CMV promoter. The resulting plasmid was transfected into CHO cells and transfected cells were grown and collected according to Example 1. The recombinant protein was purified by affinity chromatography with mAb 1281 (Frey, *et al.*, Nat Struct Mol Biol. 2010. 17(12):1486-91) as described in Example 1.

Results

PROTEIN CONFORMATION:

[0504] Protein conformation was assessed using SDS-PAGE and size exclusion chromatography. The results presented in Figure 8 indicate that the MERS S clamp chimeric fusion protein is stabilized in a pre-fusion conformation.

EXAMPLE 4

EBOV GP

[0505] Still another illustration of the ability of the present invention to produce chimeric polypeptides that comprise an ectodomain of a viral fusion protein constrained in a pre-fusion conformation, representative evidence is provided for EBOV Glycoprotein (GP). Further evidence is provided as illustration of the stability of this chimeric polypeptide at high temperatures for extended periods. Furthermore, representative evidence is provided for EBOV GP that the chimeric protein constrained in the pre-fusion conformation *via* the aforementioned method is able to induce a neutralizing immune response upon administration to mice.

Materials and Methods

CHIMERIC POLYPEPTIDE DESIGN:

[0506] An EBOV GP lacking the mucin-like domain was operably connected to a downstream heterologous structure stabilizing moiety that comprises a pair of complementary heptad repeat regions derived from HIV-1 GP160. The amino acid sequence of the resulting chimeric protein is presented below.

EBOV GP ectodomain, minus mucin like domain (1-311,462-650)– HIV GP160-based SSM

[0507] MGVTGILQLPRDRFKRTSFFLWVIILFQRTFSIPLGVIHNSTLQVSDVDKLVCRDKLSSTNQ LRSVGLNLEGNGVATDVPSATKRWGFRSGVPPKVVNYEAGEWAENCYNLEIKKPDGSECLPAAPDGIRGFPRCR YVHKVSGTGPCAGDFAFHKEGAFFLYDRLASTVIYRGTTFAEGVVAFLILPQAKKDFFSSHPLREPVNATEDPSSG YYSTTIRYQATGFGTNETEYLFEVDNLTYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKVNPEIDTTIGEWA FWETKKNLTRKIRSEELSFTVV*GG*NNTHHQDTGEESASSGKLGLITNTIAGVAGLITGGRRTRREAIVNAQPKCN PNLHYWTTQDEGAAIGLAWIPYFGPAAEGIYIEGLMHNQDGLICGLRQLANETTQALQLFLRATTELRTFSILNRK AIDFLLQRWGGTCHILGPDCCIEPHDWTKNITDKIDQIIHDFVDKTLPDQGDNDNWWTGWRQ*GG*SGIVQQQN NLLRAIEAQQHLLQLTVWGIKQLQARILA*GGSGG*HTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 139].

PROTEIN EXPRESSION AND PURIFICATION:

[0508] A codon optimized DNA sequence encoding the EBOV GP delta mucin clamp was incorporated into the pIRES-2 eukaryotic expression vector downstream of the CMV promoter. The resulting plasmid was transfected into CHO cells and transfected cells were grown and collected according to Example 1. The recombinant protein was purified by affinity chromatography using mAb Kz52 (Murin *et al.*, PNAS. 2014 11(48):17182-7).

Results

PROTEIN CONFORMATION:

[0509] Protein conformation was assessed by SDS-PAGE in the presence and absence of reducing conditions, and with conformation specific monoclonal antibodies. The results presented in

Figures 9 to 11 indicate that the EBOV GP delta mucin clamp chimeric fusion protein is stabilized in a pre-fusion conformation, and that this conformation is stable even at relatively high temperatures for extended periods (*see*, Figure 11).

ANIMAL IMMUNIZATION:

[0510] To further test the utility of the viral fusion proteins stabilized in their pre-fusion form *via* the incorporation of the 6-helix bundle forming moiety as immunogens, BALB/c mice were immunized with the chimeric clamp stabilized EBOV GP delta mucin construct. Five BALB/c mice per group were immunized with 1 µg of purified protein (or PBS) with or without 3 µg of saponin adjuvant Quil-A. Immunization was *via* intradermal delivery and mice were immunized three times, three weeks apart. Three weeks following the third immunization mice were sacrificed and sera collected. EBOV GP specific response was assessed after each immunization (Figure 12) Neutralization effect of sera from each mouse was assessed against live ZEBOV under PC4 conditions at the Australian Animal Health Laboratory (AAHL) in a plaque reduction neutralization test (PRNT) (Figure 13). Sera from mice vaccinated chimeric clamp stabilized EBOV GP delta mucin construct showed strong neutralizing activity with a geometric mean titer producing 50% reduction in plaque forming units calculated to be 52.8 (95%CI 24.5-114.0).

EXAMPLE 5

CLAMP IMMUNOSILENCING

[0511] This example illustrates the ability of the present invention to produce chimeric polypeptides, in which solvent exposed regions of the clamp sequence are modified to incorporate *N*-linked glycosylations. Representative evidence is provided for EBOV GP and these modifications are shown to facilitate shielding of the clamp domain from recognition by the adaptive immune system.

Materials and Methods

CHIMERIC POLYPEPTIDE DESIGN:

[0512] An EBOV GP lacking the mucin-like domain was operably connected to four different downstream heterologous SSMs, each comprising a pair of complementary HRA and HRB regions derived from HIV-1 GP160 in which individual HRB regions carried different mutations facilitating the incorporation of N-linked glycosylations. The amino acid sequence of these chimeric proteins are presented below.

EBOV GP ectodomain, minus mucin like domain (1-311,462-650)– HIV GP160-based SSM + G1:

[0513] MGVTGILQLPRDRFKRTSFFLWVIILFQRTFSIPLGVIHNSTLQVSDVDKLVCRDKLSSTNQ LRSVGLNLEGNGVATDVPSATKRWGFRSGVPPKVVNYEAGEWAENCYNLEIKKPDGSECLPAAPDGIRGFPRCR YVHKVSGTGPCAGDFAFHKEGAFFLYDRLASTVIYRGTTFAEGVVAFLILPQAKKDFFSSHPLREPVNATEDPSSG YYSTTIRYQATGFGTNETEYLFEVDNLTYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKVNPEIDTTIGEWA FWETKKNLTRKIRSEELSFTVV*GG*NNTHHQDTGEESASSGKLGLITNTIAGVAGLITGGRRTRREAIVNAQPKCN PNLHYWTTQDEGAAIGLAWIPYFGPAAEGIYIEGLMHNQDGLICGLRQLANETTQALQLFLRATTELRTFSILNRK AIDFLLQRWGGTCHILGPDCCIEPHDWTKNITDKIDQIIHDFVDKTLPDQGDNDNWWTGWRQ*GGSGIVQQQN NLLRAIEAQQHLLQLTVWGIKQLQARILAGGSGGTTWM**N**W**T***REINNYTSLIHSLIEESQNQQEKNEQELLE* [SEQ ID NO: 153]

EBOV GP ectodomain, minus mucin like domain (1-311,462-650)– HIV GP160-based SSM + G2:

[0514] MGVTGILQLPRDRFKRTSFFLWVIILFQRTFSIPLGVIHNSTLQVSDVDKLVCRDKLSSTNQ LRSVGLNLEGNGVATDVPSATKRWGFRSGVPPKVVNYEAGEWAENCYNLEIKKPDGSECLPAAPDGIRGFPRCR YVHKVSGTGPCAGDFAFHKEGAFFLYDRLASTVIYRGTTFAEGVVAFLILPQAKKDFFSSHPLREPVNATEDPSSG YYSTTIRYQATGFGTNETEYLFEVDNLTYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKVNPEIDTTIGEWA FWETKKNLTRKIRSEELSFTVV*GG*NNTHHQDTGEESASSGKLGLITNTIAGVAGLITGGRRTRREAIVNAQPKCN PNLHYWTTQDEGAAIGLAWIPYFGPAAEGIYIEGLMHNQDGLICGLRQLANETTQALQLFLRATTELRTFSILNRK AIDFLLQRWGGTCHILGPDCCIEPHDWTKNITDKIDQIIHDFVDKTLPDQGDNDNWWTGWRQ*GGSGIVQQQN NLLRAIEAQQHLLQLTVWGIKQLQARILAGGSGGTTWMEWDREINNYTSLIH**N**L**T***EESQNQQEKNEQELL*E [SEQ ID NO: 154]

EBOV GP ectodomain, minus mucin like domain (1-311,462-650)– HIV GP160-based SSM + G3:

[0515] MGVTGILQLPRDRFKRTSFFLWVIILFQRTFSIPLGVIHNSTLQVSDVDKLVCRDKLSSTNQ LRSVGLNLEGNGVATDVPSATKRWGFRSGVPPKVVNYEAGEWAENCYNLEIKKPDGSECLPAAPDGIRGFPRCR YVHKVSGTGPCAGDFAFHKEGAFFLYDRLASTVIYRGTTFAEGVVAFLILPQAKKDFFSSHPLREPVNATEDPSSG YYSTTIRYQATGFGTNETEYLFEVDNLTYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKVNPEIDTTIGEWA FWETKKNLTRKIRSEELSFTVV*GG*NNTHHQDTGEESASSGKLGLITNTIAGVAGLITGGRRTRREAIVNAQPKCN PNLHYWTTQDEGAAIGLAWIPYFGPAAEGIYIEGLMHNQDGLICGLRQLANETTQALQLFLRATTELRTFSILNRK AIDFLLQRWGGTCHILGPDCCIEPHDWTKNITDKIDQIIHDFVDKTLPDQGDNDNWWTGWRQ*GGSGIVQQQN NLLRAIEAQQHLLQLTVWGIKQLQARILAGGSGGTTWMEWDREINNYTSLIHSLIEESQNQ**T***EKNEQELLE* [SEQ ID NO: 155]

EBOV GP ectodomain, minus mucin like domain (1-311,462-650)– HIV GP160-based SSM + G4:

[0516] MGVTGILQLPRDRFKRTSFFLWVIILFQRTFSIPLGVIHNSTLQVSDVDKLVCRDKLSSTNQ LRSVGLNLEGNGVATDVPSATKRWGFRSGVPPKVVNYEAGEWAENCYNLEIKKPDGSECLPAAPDGIRGFPRCR YVHKVSGTGPCAGDFAFHKEGAFFLYDRLASTVIYRGTTFAEGVVAFLILPQAKKDFFSSHPLREPVNATEDPSSG YYSTTIRYQATGFGTNETEYLFEVDNLTYVQLESRFTPQFLLQLNETIYTSGKRSNTTGKLIWKVNPEIDTTIGEWA FWETKKNLTRKIRSEELSFTVV*GG*NNTHHQDTGEESASSGKLGLITNTIAGVAGLITGGRRTRREAIVNAQPKCN PNLHYWTTQDEGAAIGLAWIPYFGPAAEGIYIEGLMHNQDGLICGLRQLANETTQALQLFLRATTELRTFSILNRK AIDFLLQRWGGTCHILGPDCCIEPHDWTKNITDKIDQIIHDFVDKTLPDQGDNDNWWTGWRQ*GGSGIVQQQN NLLRAIEAQQHLLQLTVWGIKQLQARILAGGSGGTTWMEWDREINNYTSLIHSLIEESQNQQEKNE**N**E**T***LE* [SEQ ID NO: 156]

PROTEIN EXPRESSION AND PURIFICATION:

[0517] Codon optimized DNA sequences encoding the above constructs were each incorporated into the pIRES-2 eukaryotic expression vector downstream of the CMV promoter. The resulting plasmids were transfected into CHO cells and transfected cells were grown and collected according to Example 1. Recombinant proteins were purified by affinity chromatography using mAb Kz52 (Murin *et al.*, PNAS. 2014 11(48):17182-7).

Results

[0518] To test the potential to decrease recognition of the clamp by the adaptive immune system (immunosilencing) four separate mutations were introduced within the HRB of the

clamp sequence based on the HIV GP160-based SSM which could facilitate the incorporation of *N*-linked glycosylations.

[0519] Chimeric EBOV GP (lacking the mucin-like domain) incorporating the modified GP160-based SSM were purified and the reactivity with Kz52 was assessed to confirm correct conformation of the purified proteins. The reactivity of sera from mice immunized with the chimeric clamp stabilized influenza HA was tested against Chimeric EBOV proteins incorporating the modified clamp sequences (Figure 14). Reactivity was significantly reduced by glycosylation at each individual site supporting the hypothesis that this method can be used to reduce reactivity to the clamp domain.

EXAMPLE 6

PURIFICATION OF CLAMP STABILIZED ANTIGEN FROM 8 VIRUSES

[0520] This example demonstrates the generic ability of the present invention to produce chimeric polypeptides that comprise an ectodomain of a viral fusion protein from a wide range of enveloped viruses and for these polypeptides to be purified by a monoclonal antibody specific to the clamp domain.

Materials and Methods

CHIMERIC POLYPEPTIDE DESIGN:

[0521] The ectodomain of INFA HA, RSV F, Nipah F and HSV2 gB is operably connected downstream to the heterologous structure stabilizing moiety that comprises a pair of complementary heptad repeat regions derived from HIV-1 GP160. The amino acid sequences of the resulting chimeric protein and their respective controls are presented below. For influenza the soluble ectodomain lacking a SSM was generated, as were controls incorporating the foldon SSM. For RSV-F, a non-essential region of the ectodomain (aa106-144) was removed from the design. The soluble ectodomain lacking a SSM was generated, as was a positive control produced described by McLellan *et al.* (*Science*, 2013. 342(6158): 592-598) was also produced that included mutations at 4 sites as (S155C, S290C, S190F and V207L and the foldon SSM. For Nipah and HSV non-stabilized controls were not produced.

Ectodomain of Influenza HA (A Switzerland 2013, H3N2) (1-533):

[0522] MGWSCIILFLVATATGVHSEQKLPGNDNSTATLCLGHHAVPNGTIVKTITNDRIEVTNATE LVQNSSIGEICDSPHQILDGENCTLIDALLGDPQCDGFQNKKWDLFVERSKAYSNCYPYDVPDYASLRSLVASS GTLEFNNESFNWAGVTQNGTSSSCRRGSNSSFFSRLNWLTHLNSKYPALNVTMPNNEQFDKLYIWGVHHPVTD KDQIFLYAQSSGRITVSTKRSQQAVIPNIGYRPRIRDIPSRISIYWTIVKPGDILLINSTGNLIAPRGYFKIRSGKSS IMRSDAPIGKCKSECITPNGSIPNDKPFQNVNRITYGACPRYVKQSTLKLATGMRNVPERQTRGIFGAIAGFIENG WEGMVDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGKLNRLIGKTNEKFHQIEKEFSEVEGRIQDLEKYVED TKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEKTKKQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHD VYRDEALNNRFQIKGVELKSGYKD [SEQ ID NO: 157].

Ectodomain of Influenza HA (A Switzerland 2013, H3N2) (1-533) – Foldon SSM:

[0523] MGWSCIILFLVATATGVHSEQKLPGNDNSTATLCLGHHAVPNGTIVKTITNDRIEVTNATE LVQNSSIGEICDSPHQILDGENCTLIDALLGDPQCDGFQNKKWDLFVERSKAYSNCYPYDVPDYASLRSLVASS GTLEFNNESFNWAGVTQNGTSSSCRRGSNSSFFSRLNWLTHLNSKYPALNVTMPNNEQFDKLYIWGVHHPVTD KDQIFLYAQSSGRITVSTKRSQQAVIPNIGYRPRIRDIPSRISIYWTIVKPGDILLINSTGNLIAPRGYFKIRSGKSS

IMRSDAPIGKCKSECITPNGSIPNDKPFQNVNRITYGACPRYVKQSTLKLATGMRNVPERQTRGIFGAIAGFIENG
WEGMVDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGKLNRLIGKTNEKFHQIEKEFSEVEGRIQDLEKYVED
TKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEKTKKQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHD
VYRDEALNNRFQIKGVELKSGYKDGGSAIGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGLVPRGSGSAWSHPQ
FEK [SEQ ID NO: 158].

Ectodomain of Influenza HA (A Switzerland 2013 H3N2) (1-533) - HIV GP160-based SSM:

[0524] MGWSCIILFLVATATGVHSEQKLPGNDNSTATLCLGHHAVPNGTIVKTITNDRIEVTNATE
LVQNSSIGEICDSPHQILDGENCTLIDALLGDPQCDGFQNKKWDLFVERSKAYSNCYPYDVPDYASLRSLVASS
GTLEFNNESFNWAGVTQNGTSSSCRRGSNSSFFSRLNWLTHLNSKYPALNVTMPNNEQFDKLYIWGVHHPVTD
KDQIFLYAQSSGRITVSTKRSQQAVIPNIGYRPRIRDIPSRISIYWTIVKPGDILLINSTGNLIAPRGYFKIRSGKSS
IMRSDAPIGKCKSECITPNGSIPNDKPFQNVNRITYGACPRYVKQSTLKLATGMRNVPERQTRGIFGAIAGFIENG
WEGMVDGWYGFRHQNSEGRGQAADLKSTQAAIDQINGKLNRLIGKTNEKFHQIEKEFSEVEGRIQDLEKYVED
TKIDLWSYNAELLVALENQHTIDLTDSEMNKLFEKTKKQLRENAEDMGNGCFKIYHKCDNACIGSIRNGTYDHD
VYRDEALNNRFQIKGVELKSGYKDGSGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILAGGSGGHTTWME
WDREINNYTSLIHSLIEESQNQPAKDEQELLE [SEQ ID NO: 159].

Ectodomain of Influenza HA (A California 2009, H1N1pdm) (1-526):

[0525] MKVKLLVLLCTFATANADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLEDKHNGKLCKL
RGVAPLHLGKCNIAGWILGNPECESLSTASSWSYIVETPSSDNGTCYPGDFIDYEELREQLSSVSSFERFEIFPKT
SSWPNHDSNKGVTAACPHAGAKSFYKNLIWLVKKGNSYPKLSKSYINDKGKEVLVLWGIHHPSTSADQQSLYQ
NADTYVFVGSSRYSKKFKPEIAIRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPRYAFAMERNAGSGIIIS
DTPVHDCNTTCQTPKGAINTSLPFQNIHPITIGKCPKYVKSTKLRLATGLRNIPSIQSRGLFGAIAGFIEGGWTGM
VDGWYGYHHQNEQGSGYAADLKSTQNAIDEITNKVNSVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDGFLD
IWTYNAELLVLLENERTLDYHDSNVKNLYEKVRSQLKNNAKEIGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEE
AKLNREEIDGVKLESTR [SEQ ID NO: 160].

Ectodomain of Influenza HA (A California 2009, H1N1pdm) (1-526) – Foldon SSM:

[0526] MKVKLLVLLCTFATANADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLEDKHNGKLCKL
RGVAPLHLGKCNIAGWILGNPECESLSTASSWSYIVETPSSDNGTCYPGDFIDYEELREQLSSVSSFERFEIFPKT
SSWPNHDSNKGVTAACPHAGAKSFYKNLIWLVKKGNSYPKLSKSYINDKGKEVLVLWGIHHPSTSADQQSLYQ
NADTYVFVGSSRYSKKFKPEIAIRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPRYAFAMERNAGSGIIIS
DTPVHDCNTTCQTPKGAINTSLPFQNIHPITIGKCPKYVKSTKLRLATGLRNIPSIQSRGLFGAIAGFIEGGWTGM
VDGWYGYHHQNEQGSGYAADLKSTQNAIDEITNKVNSVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDGFLD
IWTYNAELLVLLENERTLDYHDSNVKNLYEKVRSQLKNNAKEIGNGCFEFYHKCDNTCMESVKNGTYDYPKYSEE
AKLNREEIDGVKLESTRGGSAIGGYIPEAPRDGQAYVRKDGEWVLLSTFLGGLVPRGSGSAWSHPQFEK [SEQ
ID NO: 161].

Ectodomain of Influenza HA (A California 2009, H1N1pdm) (1-526) - HIV GP160-based SSM:

[0527] MMKVKLLVLLCTFATANADTLCIGYHANNSTDTVDTVLEKNVTVTHSVNLLEDKHNGKLC
KLRGVAPLHLGKCNIAGWILGNPECESLSTASSWSYIVETPSSDNGTCYPGDFIDYEELREQLSSVSSFERFEIFP
KTSSWPNHDSNKGVTAACPHAGAKSFYKNLIWLVKKGNSYPKLSKSYINDKGKEVLVLWGIHHPSTSADQQSL
YQNADTYVFVGSSRYSKKFKPEIAIRPKVRDQEGRMNYYWTLVEPGDKITFEATGNLVVPRYAFAMERNAGSGII
ISDTPVHDCNTTCQTPKGAINTSLPFQNIHPITIGKCPKYVKSTKLRLATGLRNIPSIQSRGLFGAIAGFIEGGWTG
MVDGWYGYHHQNEQGSGYAADLKSTQNAIDEITNKVNSVIEKMNTQFTAVGKEFNHLEKRIENLNKKVDDGFL

DIWTYNAELLVLLENERTLDYHDSNVKNLYEKVRSQLKNNAKEIGNGCFEFYHKCDNTCMESVKNGTYDYPKYS EEAKLNREEIDGVKLESTRGSGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILAGGSGGHTTWMEWDREI NNYTSLIHSLIEESQNQPAKDEQELLE [SEQ ID NO: 162].

Ectodomain of RSV F (1-516) – His tag:

[0528] MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNI KKNKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTQATNNRARRELPRFMNYTLNNAKKTNVTLSKKRKRRFL GFLLGVGSAIASGVAVSKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSI SNIETVIEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMS IIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRV FCDTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCDY VSNKGVDTVSVGNTLYYVNKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLHNVG GGGHHHHHH [SEQ ID NO: 163].

Ectodomain of RSV F (1-513) – DScav mutations -Foldon SSM:

[0529] MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNI KKNKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTQATNNRARRELPRFMNYTLNNAKKTNVTLSKKRKRRFL GFLLGVGSAIASGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPILNKQSCSI SNIETVIEFQQKNNRLLEITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMC IIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRV FCDTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCDY VSNKGVDTVSVGNTLYYVNKQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKINQSLAFIRKSDELLSAI*G GYIPEAPRDGQAYVRKDGEWVLLSTFLGGLVPRGSGSAWSHPQFEK* [SEQ ID NO: 164]

Ectodomain of RSV F (1-105,145-511) – HIV GP160-based SSM

[0530] MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAVSKGYLSALRTGWYTSVITIELSNI KKNKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTQATNNGSGSAIASGVAVSKVLHLEGEVNKIKSALLSTN KAVVSLSNGVSVLTSKVLDLKNYIDKQLLPIVNKQSCSISNIETVIEFQQKNNRLLEITREFSVNAGVTTPVSTYML TNSELLSLINDMPITNDQKKLMSNNVQIVRQQSYSIMSIIKEEVLAYVVQLPLYGVIDTPCWKLHTSPLCTTNTKE GSNICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDV SSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVNKQEGKSLYVKGEPIINFY DPLVFPSDEFDASISQVNEKINQSLAFIRKSDEGSGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILAGGSG GHTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 165].

Ectodomain of Nipah F (1-483) – HIV GP160-based SSM

[0531] MVVILDKRCYCNLLILILMISECSVGILHYEKLSKIGLVKGVTRKYKIKSNPLTKDIVIKMIPN VSNMSQCTGSVMENYKTRLNGILTPIKGALEIYKNNTHDLVGDVRLAGVIMAGVAIGIATAAQITAGVALYEAMK NADNINKLKSSIESTNEAVVKLQETAEKTVYVLTALQDYINTNLVPTIDKISCKQTELSLDLALSKYLSDLLFVFGP NLQDPVSNSMTIQAISQAFGGNYETLLRTLGYATEDFDDLLESDSITGQIIYVDLSSYYIIVRVYFPILTEIQQAYIQ ELLPVSFNNDNSEWISIVPNFILVRNTLISNIEIGFCLITKRSVICNQDYATPMTNNMRECLTGSTEKCPRELVVSS HVPRFALSNGVLFANCISVTCQCQTTGRAISQSGEQTLLMIDNTTCPTAVLGNVIISLGKYLGSVNYNSEGIAIGP PVFTDKVDISSQISSMNQSLQQSKDYIKEAQRLLDTGSGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILA GGSGGHTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 166].

Ectodomain of HSV1 gB (28-741) – IgK signal peptide – HIV GP160-based SSM:

[0532] MGWSCIILFLVATATGVHSERASGDVAATVAANGGPASQPPPVPSPATTKARKRKTKKPP KRPEATPPPDANATVAAGHATLRAHLREIKVENADAQFYVCPPPTGATVVQFEQPRRCPTRPEGQNYTEGIAVVF KENIAPYKFKATMYYKDVTVSQVWFGHRYSQFMGIFEDRAPVPFEEVIDKINAKGVCRSTAKYVRNNMETTAFH RDDHETDMELKPAKVATRTSRGWHTTDLKYNPSRVEAFHRYGTTVNCIVEEVDARSVYPYDEFVLATGDFVYMS PFYGYREGSHTEHTSYAADRFKQVDGFYARDLTTKARATSPTTRNLLTTPKFTVAWDWVPKRPAVCTMTKWQEV DEMLRAEYGGSFRFSSDAISTTFTTNLTQYSLSRVDLGDCIGRDAREAIDRMFARKYNATHIKVGQPQYYLATGG FLIAYQPLLSNTLAELYVREYMREQDRKPRNATPAPLREAPSANASVERIKTTSSIEFARLQFTYNHIQRHVNDML GRIAVAWCELQNHELTLWNEARKLNPNAIASATVGRRVSARMLGDVMAVSTCVPVAPDNVIVQNSMRVSSRPG TCYSRPLVSFRYEDQGPLIEGQLGENNELRLTRDALEPCTVGHRRYFIFGGGYVYFEEYAYSHQLSRADVTTVSTF IDLNITMLEDHEFVPLEVYTRHEIKDSGLLDYTEVQRRNQLHDLRFADIDTVIRADANAAMFAGLCAFFEGMGDL GRGGGGSGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARILAGGSGGHTTWMEWDREINNYTSLIHSLIEES QNQQEKNEQELLE [SEQ ID NO: 167].

Ectodomain of Measles F (1-488) – HIV GP160-based SSM:

[0533] MGLKVNVSAIFMAVLLTLQTPTGQIHWGNLSKIGVVGIGSASYKVMTRSSHQSLVIKLMP NITLLNNCTRVEIAEYRRLLRTVLEPIRDALNAMTQNIRPVQSVASSRRHKRFAGVVLAGAALGVATAAQITAGIA LHQSMLNSQAIDNLRASLETTNQAIEAIRQAGQEMILAVQGVQDYINNELIPSMNQLSCDLIGQKLGLKLLRYYT EILSLFGPSLRDPISAEISIQALSYALGGDINKVLEKLGYSGGDLLGILESRGIKARITHVDTESYFIVLSIAYPTLSE IKGVIVHRLEGVSYNIGSQEWYTTVPKYVATQGYLISNFDESSCTFMPEGTVCSQNALYPMSPLLQECLRGSTKS CARTLVSGSFGNRFILSQGNLIANCASILCKCYTTGTIINQDPDKILTYIAADHCPVVEVNGVTIQVGSRRYPDAV YLHRIDLGPPISLERLDVGTNLGNAIAKLEDAKELLESSDQILRSMKGGRSGIVQQQNNLLRAIEAQQHLLQLTV WGIKQLQARILAGGSGGHTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 168].

Ectodomain of Lassa virus GPC (1-423) – HIV GP160-based SSM:

[0534] MGQIVTFFQEVPHVIEEVMNIVLIALSVLAVLKGLYNFATCGLVGLVTFLLLCGRSCTTSLYK GVYELQTLELNMETLNMTMPLSCTKNNSHHYIMVGNETGLELTLTNTSIINHKFCNLSDAHKKNLYDHALMSIIST FHLSIPNFNQYEAMSCDFNGGKISVQYNLSHSYAGDAANHCGTVANGVLQTFMRMAWGGSYIALDSGRGNWD CIMTSYQYLIIQNTTWEDHCQFSRPSPIGYLGLLSQRTRDIYISRRLLGTFTWTLSDSEGKDTPGGYCLTRWMLIE AELKCFGNTAVAKCNEKHDEEFCDMLRLFDFNKQAIQRLKAEAQMSIQLINKAVNALINDQLIMKNHLRDIMGIP YCNYSKYWYLNHTTTGRTSLPKCWLVSNGSYLNETHFSDDIEQQADNMITEMLQKEYMERQGSGIVQQQNNLL RAIEAQQHLLQLTVWGIKQLQARILAGGSGGHTTWMEWDREINNYTSLIHSLIEESQNQQEKNEQELLE [SEQ ID NO: 169].

PROTEIN EXPRESSION AND PURIFICATION:

[0535] Codon optimized DNA sequences encoding the chimeric fusion proteins, Influenza H1 foldon, Influenza H1 clamp, Influenza H3 foldon, Influenza H3 clamp, RSV F clamp, RSV F ds cav Foldon, MERS S clamp and Ebola GP clamp delta mucin, Nipah virus F clamp, Measles virus F clamp, Lassa virus GPC clamp, Herpes Simplex 2 virus gB clamp (as well as the corresponding ectodomains of RSV F and Influenza H3 lacking HIV-1 HRA and HRB sequences) were incorporated into the pIRES-2 or pNBF eukaryotic expression vector following the CMV promoter. The resulting plasmids were transfected into Chinese hamster ovary (CHO) cells grown in chemically defined CHO (CD-CHO) media (Gibco) or in ExpiCHO expression media (Gibco). CHO cells were transfected by either combining 600 µg plasmid and 2.4 mg of linear polyethylenimine in 300 mL of CHO cells at a density of $1x10^6$ cells per mL or 16 µg plasmid with 640 µl OPTI-pro

serum free media (SFM), and 51.2 µl of expifectamine (Gibco). After 4 h of incubation CHO cells transfected with linear polyethylenimine were pelleted, media containing transfection reagent was removed and cells were resuspended in 300 mL of CD-CHO containing 8mM Glutamax (Gibco), 100 units/mL of penicillin (Gibco), 100 µg/mL of streptomycin (Gibco), 7.5% CHO CD Efficient Feed A (Gibco), and 7.5% CHO CD Efficient Feed B (Gibco). For cells transfected with Expifectamine, after 24 h incubation 96 µl of ExpiCHO enhancer and 3.84 ml of ExpiCHO feed was added. Both sets of cells were then incubated for 7 days at 37° C, 7.5% CO2, shaking at ~120 rpm. After 7 days cells were removed by centrifugation for 10 min at 6,000 x g and supernatant was filtered.

[0536] Recombinant proteins were purified by affinity chromatography with specific monoclonal antibodies covalently coupled to HiTrap NHS-activated HP columns (GE). Chimeric clamp stabilized Influenza HA, RSV F, MERS S, Ebola GP, Lassa GPC, Nipah F, Measles F and HSV2 gB were purified by mAb 1281 (Frey, *et al.*, Nat Struct Mol Biol. 2010. 17(12):1486-91) which binds to the 6-helix bundle formed by HIV-1 HRA and HRB. Chimeric clamp stabilized and foldon stabilized Influenza H1 and the corresponding ectodomain for H1 were purified with mAb 5J8 (Hong, *et al.*, J Virol, 2013. 87(22): p. 12471-80). Chimeric clamp stabilized and foldon stabilized Influenza H3 and the corresponding ectodomain for H3 were purified with mAb C05 (Ekiert, *et al.*, Nature, 2012. 489(7417): 526-32). Chimeric clamp stabilized and foldon stabilized RSV F incorporating ds cav mutations as well as the corresponding ectodomain of RSV F were purified with mAb 101F (McLellan, *et al. J Virol.* 2010. 84(23):12236-44). Purification of the desired protein was confirmed by SDS-PAGE.

Results

Molecular Clamp as a Generic Method for Protein Expression and Purification:

[0537] Expression and purification of chimeric clamp stabilized viral fusion proteins from eight viruses was confirmed by SDS-PAGE (Figure 15). Incorporation of the SSM based on HIV-1 GP160 HR1 and HR2 sequences downstream of ectodomains allows recovery of diverse chimeric proteins using the by mAb 1281 (Frey, *et al.*, Nat Struct Mol Biol. 2010. 17(12):1486-91) which binds to the 6-helix bundle formed by HIV-1 HRA and HRB.

Mouse Protection Study Following Challenge with Influenza Virus H1N1pdm:

[0538] To extend results showing inclusion of the 6-helix bundle forming moiety into the influenza HA ectodomain can elicit a broadly cross-reactive immune response, we conducted an influenza challenge experiment within C57b mice. This study also set out to directly compare the molecular clamp the 6-helix bundle forming moiety with the foldon SSM with regard to the induction of broad-spectrum cross-protection from divergent influenza subtypes. C57b mice were vaccinated with either PBS, H1sol, H3sol, H1foldon, H3foldon, H1clamp or H3clamp. Vaccines were dose matched, each containing 5 µg of HA and 3 µg of QuilA with mice receiving three doses of vaccine given two weeks apart. Mice were then challenged with Influenza Cal/09 (H1N1pdm) six weeks after the initial dose (n= 5 mice per group). This study was designed to assess both protection against an identical strain and protection against a highly divergent subtype. Mice were challenged via the intranasal route with influenza A virus H1N1pdm at a 'low dose' of $1x10^2$ plaque-forming units (PFU) and a 'high dose' of $5.5x10^3$ PFU. Weight loss was measured daily over a 14-day period with mice culled if they lost >20% of their original body weight.

[0539] This study confirmed that H1clamp is able to provide complete protection against a matched strain of influenza, as did H1sol and H1foldon (Figures 16A and B). Of particular interest, immunization with H3clamp also showed partial protection against influenza H1N1pdm. For H3clamp 3/5 mice survived when challenged with the low dose influenza H1N1pdm and 2/5 mice survived when challenged with the high dose influenza H1N1pdm (Figures 16C and D). In comparison none of the mice immunized with H3sol nor H3 foldon survived when challenged with either the low or high dose of influenza H1N1pdm, and only 1/5 mice mock immunized with PBS survived when challenged with the low dose of influenza H1N1pdm. A comparison between mice surviving H1N1pdm after H3clamp vaccination (5/10) compared to mice surviving after mock vaccination or vaccination with H3sol or H3 foldon (1/30), demonstrates the statistical significance of broad spectrum influenza protection being mediated by H3clamp (p=0.0003; chi square calculator). As H3 and H1 subtypes belong to separate groups within the influenza phylogenetic tree this result indicates a broad level of protection and it can be reasonably expected that broad spectrum all influenza strains and subtypes which are equally or less divergent.

INCREASED THERMAL STABILITY UPON INCLUSION OF THE CLAMP SSM:

[0540] To directly compare stability afforded by the clamp SSM and the foldon SSM, purified antigens Influenza H1 foldon, Influenza H1 clamp, RSV F ds cav foldon, and RSV F clamp were incubated at 43°C for 72hrs and the reactivity with mAbs used as a measure of thermal stability. For the RSV F comparison three pre-fusion specific mAbs were used, D25 (McLellan *et al.*, Science, 2013. 340(6136): p. 1113-7), MPE8 (Corti *et al.*, Nature, 2013. 501(7467): p. 439-43) and AM22 (McLellan *et al.*, Science, 2013. 340(6136): p. 1113-7). For the influenza HA comparison two stem specific mAbs were used, CR6261 (Ekiert *et al.*, Science, 2009. 324(5924): p. 246-51) and Fi6V3 (Corti *et al.*, Science, 2011. 333(6044): p. 850-6), and one head specific mAb was used, 5J8 (Hong, *et al.*, J Virol, 2013. 87(22): p. 12471-80). Direct comparison revealed that RSV F clamp retained significantly higher reactivity with prefusion specific mAbs than RSV F ds cav foldon following incubation at elevated temperature (Figure 17A). Similarly, direct comparison revealed that influenza H1clamp retained significantly higher reactivity with HA stem specific mAbs than influenza H1 foldon (Figure 17B). Retention of reactivity with the head specific mAb was comparable between influenza H1 clamp and influenza H1 foldon. Together these results demonstrates the superior stability of the clamp SSM compared to the foldon SSM.

NEUTRALIZING IMMUNE RESPONSE INDUCED BY RSV F CLAMP:

[0541] To test the utility of the viral fusion proteins stabilized in their pre-fusion form via the incorporation of the 6-helix bundle forming moiety as immunogens, BALB/c mice were immunized with the RSV F clamp, RSV F ds cav foldon, the corresponding RSV ectodomain (RSV F sol) or mock immunized with PBS. Five BALB/c mice per group were immunized with 5 µg of purified protein (or PBS) with 3 µg of saponin adjuvant Quil-A. Immunization was via intradermal delivery and mice were immunized three times each three weeks apart. Three weeks following the third immunization mice were sacrificed and sera collected. Neutralization effect of sera from individual mice was assessed against RSV strain A2 in a plaque reduction neutralization test (PRNT) (Figure 18). Sera from mice vaccinated chimeric clamp stabilized RSV F clamp showed strong neutralizing activity with a geometric mean IC50 value of 8,124 (95%CI = 1,968-33,543), while sera from mice vaccinated with the RSV F ds cav foldon showed neutralizing activity with a geometric mean IC50 value of 2,859 (95%CI = 794-10,290) and sera from mice vaccinated with

the RSV F sol showed neutralizing activity with an a geometric mean IC50 value of 562 (95%CI = 242-1,410). Therefore, stabilization of the pre-fusion form of the RSV F via the incorporation of a structure-stabilizing moiety comprised of HIV-1 HR1 and HR2 is critical for a strong neutralizing immune response, which is approximately 3-fold higher than that induced by the alternate stabilization approach 'ds cav foldon' (McLellan *et al.*, Science, 2013. 342(6158): p. 592-8).

STABILIZATION OF THE PREFUSION CONFORMATION OF NIPAH VIRUS F:

[0542] To further validate the utility of the clamp SSM to stabilize the prefusion conformation of viral fusion proteins the clamp SSM was incorporated into the ectodomain of Nipah virus F and expressed in CHO cells. Protein purified by immunoaffinity chromatography was then analyzed by size exclusion chromatography using a superdex 200 column. The major portion of the Nipah virus F clamp eluted at approximately 11.5 mL which is equivalent to roughly the expected size of the trimeric protein, ~180kDa (Figure 19). As has been well demonstrated for Paramyxovirus F (Connolly *et al.*, PNAS, 2006. 103(47): P. 17903-8), transition of the Nipah virus F into the post-fusion conformation would be expected to result in the exposure of the hydrophobic fusion peptide thereby driving protein aggregation. The presence of the soluble trimeric protein is therefore evidence supporting the presence of the pre-fusion conformation. Purified Nipah virus F clamp was also analyzed by negative stain transmission electron microscopy (TEM) (Figure 19, inset). Within the TEM image presented, it is clearly visible that particles of Nipah virus F clamp have a homogenous size and a topology consistent with the expected prefusion conformation. The data presented further supports the ability of the clamp SSM to stabilize the prefusion conformation of viral fusion proteins.

NEUTRALIZING IMMUNE RESPONSE INDUCED BY NIPAH VIRUS F CLAMP:

[0543] To further test the utility of the viral fusion proteins stabilized in their pre-fusion form via the incorporation of the 6-helix bundle forming moiety as immunogens, BALB/c mice were immunized with the Nipah virus F clamp or mock immunized with PBS. Four BALB/c mice per group were immunized with 5 µg of purified protein (or PBS) with 3 µg of saponin adjuvant Quil-A. Immunization was via intradermal delivery and mice were immunized twice, three weeks apart. Three weeks following the second immunization mice were sacrificed and sera collected. Neutralization effect of sera from individual mice was assessed against live Nipah virus (Malaysian strain) in a plaque reduction neutralization test (PRNT) under BSL4 containment (Figure 20). Sera from mice vaccinated chimeric clamp stabilized Nipah virus F clamp all showed strong neutralizing activity with a geometric mean IC50 value of 48 (95%CI= 6-384), while sera from mice mock immunized with PBS showed no neutralizing activity at the highest serum concentration tested. This result therefore provides further evidence that chimeric viral fusion proteins incorporating the clamp SSM are able to elicit a neutralizing immune response upon vaccination.

INCORPORATION OF THE CLAMP SSM INTO A CLASS III VIRAL FUSION PROTEIN:

[0544] To further validate the utility of the clamp SSM to the stabilization of viral fusion proteins, the clamp SSM was incorporated into the ectodomain of HSV2 gB and expressed in CHO cells. Protein purified by immunoaffinity chromatography was then analyzed by size exclusion chromatography on a superose 6 column. The major portion of the HSV2 gB clamp eluted at approximately 15 mL which is equivalent to roughly the expected size of the trimeric protein, ~300kDa (Figure 21). Purified HSV2 gB clamp was also analyzed by negative stain transmission electron microscopy (TEM) (Figure 21, inset). Within the TEM image presented, it is clearly visible

that particles of HSV2 gB clamp have a homogenous size and a topology consistent with the structure previously resolved by x-ray crystallography (Heldwein *et al.*, Science, 2006. 313(5784): 217-20). This conformation is hypothesized to be the HSV2 gB post-fusion conformation. Notably, HSV2 gB clamp is also able to bind most neutralizing antibodies for HSV2 and may be useful as a subunit vaccine candidate (Cairns *et al.*, JVi, 2014. 88(5): P. 2677-89). The data presented supports the ability of the clamp SSM to stabilize and purify viral Class III fusion proteins, in addition to viral Class I fusion proteins.

[0545] The results presented herein demonstrate that chimeric polypeptides that comprise an ectodomain of a viral fusion protein constrained in a pre-fusion conformation can:

- induce a more broadly cross-protective immune response;
- are stable at elevated temperatures;
- induce a superior neutralizing immune response; and
- form using Class I or Class III ectodomains.

[0546] The disclosure of every patent, patent application, and publication cited herein is hereby incorporated herein by reference in its entirety.

[0547] The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the instant application.

[0548] Throughout the specification the aim has been to describe the preferred embodiments of the invention without limiting the invention to any one embodiment or specific collection of features. Those of skill in the art will therefore appreciate that, in light of the instant disclosure, various modifications and changes can be made in the particular embodiments exemplified without departing from the scope of the present invention. All such modifications and changes are intended to be included within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A chimeric polypeptide comprising an enveloped virus fusion ectodomain polypeptide operably connected downstream to a heterologous, structure-stabilizing moiety comprising complementary first heptad repeat (HR1) and second heptad repeat (HR2) regions that associate with each other under conditions suitable for their association (*e.g.*, in aqueous solution) to form an anti-parallel, two-helix bundle.

2. The chimeric polypeptide of claim 1, wherein the HR1 and HR2 regions lack complementarity to the ectodomain polypeptide, so that they preferentially form with each other an anti-parallel, two-helix bundle, rather than with structural elements of the ectodomain polypeptide.

3. The chimeric polypeptide of claim 1 or claim 2, wherein each of the HR1 and HR2 regions is independently characterized by a n-times repeated 7-residue pattern of amino acid types, represented as $(a\text{-}b\text{-}c\text{-}d\text{-}e\text{-}f\text{-}g\text{-})_n$ or $(d\text{-}e\text{-}f\text{-}g\text{-}a\text{-}b\text{-}c\text{-})_n$, wherein the pattern elements 'a' to 'g' denote conventional heptad positions at which the amino acid types are located and n is a number equal to or greater than 2, and at least 50% (or at least 51% to at least 99% and all integer percentages in between) of the conventional heptad positions 'a' and 'd' are occupied by hydrophobic amino acid types and at least 50% (or at least 51% to at least 99% and all integer percentages in between) of the conventional heptad positions 'b', 'c', 'e', 'f' and 'g' are occupied by hydrophilic amino acid types, the resulting distribution between hydrophobic and hydrophilic amino acid types enabling the identification of the heptad repeat regions.

4. The chimeric polypeptide of any one of claims 1 to 3, wherein one or both of the HR1 and HR2 regions comprises, consists or consists essentially of an endogenous Class I enveloped virus fusion protein heptad repeat region amino acid sequence.

5. The chimeric polypeptide of claim 4, wherein the HR1 and HR2 regions comprise, consist or consist essentially of complementary endogenous heptad repeat A (HRA) and heptad repeat B (HRB) regions, respectively, of one or more Class I enveloped virus fusion proteins.

6. The chimeric polypeptide of claim 5, wherein the HRA region amino acid sequence and the HRB region amino acid sequence are derived from the same Class I enveloped virus fusion protein.

7. The chimeric polypeptide of claim 5, wherein the HRA region amino acid sequence and the HRB region amino acid sequence are derived from the different Class I enveloped virus fusion proteins.

8. The chimeric polypeptide of any one of claims 1 to 7, wherein the HR1 and HR2 regions are independently selected from HRA and HRB regions of fusion proteins expressed by orthomyxoviruses, paramyxoviruses, retroviruses, coronaviruses, filoviruses and arenaviruses.

9. The chimeric polypeptide of any one of claims 1 to 8, wherein the structure-stabilizing moiety (*e.g.*, including one or both of HR1 and HR2 regions) comprises an immune-silencing moiety that inhibits elicitation of an immune response to the structure-stabilizing moiety.

10. The chimeric polypeptide of claim 9, wherein immune-silencing moiety is a glycosylation site that is recognized and glycosylated by a glycosylation enzyme (*e.g.*, a glycosyltransferase).

11. The chimeric polypeptide of any one of claims 1 to 10, wherein the structure-stabilizing moiety (*e.g.*, including one or both of HR1 and HR2 regions) comprises one or more unnatural amino acids.

12. The chimeric polypeptide of claim 11, wherein the one or more unnatural amino acids permit coupling of polyethylene glycol.

13. The chimeric polypeptide of claim 11, wherein the one or more unnatural amino acids permit coupling of an immune stimulating moiety.

14. The chimeric polypeptide of claim 11, wherein the one or more unnatural amino acids permit coupling of a lipid (*e.g.*, to facilitate formation of lipid vesicles, or virus-like particles, which display the ectodomain of the chimeric polypeptide, *inter alia* for stimulating a host immune response).

15. The chimeric polypeptide of any one of claims 1 to 14, wherein the ectodomain polypeptide corresponds to a Class I enveloped virus fusion protein ectodomain.

16. The chimeric polypeptide of claim 15, wherein the ectodomain polypeptide comprises one or both of an endogenous HRA region and an endogenous HRB region.

17. The chimeric polypeptide of claim 15 or claim 16, wherein the Class I fusion protein is one from a Class I enveloped fusion protein virus selected from orthomyxoviruses, paramyxoviruses, retroviruses, coronaviruses, filoviruses and arenaviruses.

18. The chimeric polypeptide of any one of claims 1 to 14, wherein the ectodomain polypeptide corresponds to a Class III enveloped virus fusion protein ectodomain.

19. The chimeric polypeptide of claim 18, wherein the Class III fusion protein is one from a Class III enveloped fusion protein virus selected from rhabdoviruses and herpesviruses.

20. The chimeric polypeptide of any one of claims 1 to 19, wherein the ectodomain polypeptide (*e.g.*, Class I or Class III) comprises or consists of a whole precursor ectodomain polypeptide or a portion thereof.

21. The chimeric polypeptide of claim 20, wherein the ectodomain polypeptide or portion lacks any one or more of an endogenous signal peptide, a protease cleavage site, an endogenous head portion of an ectodomain, an endogenous stem portion of an ectodomain, an endogenous mucin-like domain, an endogenous membrane proximal external region and an endogenous fusion peptide.

22. The chimeric polypeptide of claim 20 or claim 21, wherein one or more endogenous proteolytic cleavage sites (*e.g.*, one or more furin cleavage sites) of a wild-type or reference fusion protein are altered or deleted to render the ectodomain polypeptide less susceptible to proteolytic cleavage by a protease.

23. The chimeric polypeptide of any one of claims 1 to 22, wherein the ectodomain polypeptide comprises at least one pre-fusion epitope that is not present in the post-fusion form of an enveloped virus fusion protein to which the ectodomain polypeptide corresponds.

24. The chimeric polypeptide of any one of claims 1 to 23, wherein the HR1 and HR2 regions of the structure-stabilizing moiety are connected by a linker.

25. The chimeric polypeptide of claim 24, wherein the linker consists of about 1 to about 100 amino acid residues (and all integer amino acid residues therebetween).

26. The chimeric polypeptide of claim 24, wherein the linker consists of about 1 to about 50 amino acid residues (and all integer amino acid residues therebetween).

27. The chimeric polypeptide of claim 24, wherein the linker consists of about 50 to about 100 amino acid residues (and all integer amino acid residues therebetween).

28. The chimeric polypeptide of any one of claims 24 to 27, wherein the linker comprises at least one moiety selected from a purification moiety that facilitates purification of the chimeric polypeptide, an immune-modulating moiety that modulates an immune response to the chimeric polypeptide, a cell-specific moiety and a structural flexibility-conferring moiety.

29. A chimeric polypeptide comprising a proteinaceous molecule operably connected downstream to a heterologous, structure-stabilizing moiety comprising complementary first heptad repeat (HR1) and second heptad repeat (HR2) regions that associate with each other under conditions suitable for their association (*e.g.*, in aqueous solution) to form an anti-parallel, two-helix bundle.

30. The chimeric polypeptide of claim 29, wherein the proteinaceous molecule is a therapeutic polypeptide.

31. A nucleic acid construct that comprises a coding sequence for a chimeric polypeptide according to any one of claims 1 to 30, operably linked to a regulatory element that is operable in the host cell.

32. A host cell that contains the nucleic acid construct of claim 31.

33. The host cell of claim 32, wherein the host cell is a prokaryotic host cell.

34. The host cell of claim 33, wherein the host cell is a eukaryotic host cell.

35. A method of producing a chimeric polypeptide complex, wherein the method comprises: combining chimeric polypeptides according to any one of claims 1 to 30 under conditions (*e.g.*, in aqueous solution) suitable for the formation of a chimeric polypeptide complex, whereby a chimeric polypeptide complex is produced that comprises three chimeric polypeptide subunits and is characterized by a six-helix bundle formed by oligomerization of the two-helix bundles of the respective structure-forming moieties of the chimeric polypeptides.

36. A chimeric polypeptide complex that comprises three chimeric polypeptide subunits according to any one of claims 1 to 30 and is characterized by a six-helix bundle formed by oligomerization of the two-helix bundles of the respective structure-forming moieties of the chimeric polypeptides.

37. The complex of claim 36, wherein the chimeric polypeptide subunits each comprise an enveloped virus fusion ectodomain polypeptide, and wherein the complex comprises at least one pre-fusion epitope of an enveloped virus fusion protein of interest (*e.g.*, a wild-type enveloped virus fusion protein), or complex thereof, which is not present on a post-fusion form of the enveloped virus fusion protein, or complex thereof.

38. A composition comprising a chimeric polypeptide according to any one of claims 1 to 30, or a chimeric polypeptide complex according to claim 36 or claim 37, and a pharmaceutically acceptable carrier, diluent or adjuvant.

39. A method of identifying an agent (*e.g.*, a small molecule or macromolecule) that binds with a fusion protein of an enveloped virus, or complex of the fusion protein, wherein the method comprises: contacting the candidate agent with an ectodomain polypeptide-containing chimeric polypeptide or chimeric polypeptide complex, as broadly described above and elsewhere herein, wherein the ectodomain polypeptide corresponds to the fusion protein of the enveloped virus, and detecting binding of the candidate agent to the chimeric polypeptide or chimeric polypeptide complex.

40. The method of claim 39, further comprising contacting the candidate agent with the fusion protein or complex of the fusion protein and detecting binding of the candidate agent to the fusion protein or the complex.

41. The method of claim 39 or claim 40, wherein the candidate agent is part of a compound library (*e.g.*, small molecule or macromolecule library).

42. The method of any one of claims 39 to 41, further comprising isolating the candidate agent from the library.

43. The method of any one of claims 39 to 42, wherein the candidate agent binds specifically to the chimeric polypeptide or chimeric polypeptide complex.

44. The method of any one of claims 39 to 43, wherein the candidate agent binds specifically to the fusion protein or complex of the fusion protein.

45. A method of producing an antigen-binding molecule (*e.g.*, an antibody such as a neutralizing antibody) that is immuno-interactive with a fusion protein of an enveloped virus, or complex of the fusion protein, wherein the method comprises: (1) immunizing an animal with an ectodomain polypeptide-containing chimeric polypeptide according to any one of claims 1 to 28, or an ectodomain polypeptide-containing chimeric polypeptide complex according to claim 36 or claim 37, or a composition thereof according to claim 38, wherein the ectodomain polypeptide corresponds to the fusion protein of the enveloped virus; (2) identifying and/or isolating a B cell from the animal, which is immuno-interactive with the fusion protein or complex thereof; and (3) producing the antigen-binding molecule expressed by that B cell.

46. An antigen-binding molecule that is produced by the method of claim 45, or a derivative antigen-binding molecule with the same epitope-binding specificity as the antigen-binding molecule.

47. The derivative antigen-binding molecule of claim 46, which is selected from antibody fragments (such as Fab, Fab', $F(ab')_2$, Fv), single chain (scFv) and domain antibodies (including, for example, shark and camelid antibodies), and fusion proteins comprising an antibody, and any other modified configuration of the immunoglobulin molecule that comprises an antigen binding/recognition site.

48. An immune-modulating composition comprising an antigen binding molecule according to claim 46 or claim 47, and a pharmaceutically acceptable carrier, diluent or adjuvant.

49. A method of eliciting an immune response to a fusion protein of an enveloped virus, or complex of the fusion protein, in a subject, wherein the method comprises administering to the subject an enveloped virus fusion ectodomain-containing chimeric polypeptide complex or composition according to any preceding claim, wherein an

ectodomain polypeptide subunit of the chimeric polypeptide complex corresponds to the fusion protein of the enveloped virus.

50. A method of eliciting an immune response to a fusion protein of an enveloped virus, or complex of the fusion protein, in a subject, wherein the method comprises administering to the subject a DNA vaccine or viral vector/replicon capable of expressing an enveloped virus fusion ectodomain-containing chimeric polypeptide complex or composition according to any preceding claim, wherein an ectodomain polypeptide subunit of the chimeric polypeptide complex corresponds to the fusion protein of the enveloped virus.

51. A method for treating or preventing an enveloped virus infection in a subject, wherein the method comprises administering to the subject an effective amount of an enveloped virus fusion ectodomain-containing chimeric polypeptide complex according to any preceding claim, and/or an antigen binding molecule according to claim 46 or claim 47, and/or composition according to claim 48.

52. A chimeric polypeptide comprising a proteinaceous molecule operably connected downstream to a heterologous, structure-stabilizing moiety comprising complementary first heptad repeat (HR1) and second heptad repeat (HR2) regions that associate with each other under conditions suitable for their association (*e.g.*, in aqueous solution) to form an anti-parallel, two-helix bundle.

53. A method of producing a chimeric polypeptide complex, wherein the method comprises: combining chimeric polypeptides according to claim 52 under conditions (*e.g.*, in aqueous solution) suitable for the formation of a chimeric polypeptide complex, whereby a chimeric polypeptide complex is produced that comprises three chimeric polypeptide subunits and is characterized by a six-helix bundle formed by oligomerization of the two-helix bundles of the respective structure-forming moieties of the chimeric polypeptides.

54. A chimeric polypeptide complex that comprises three chimeric polypeptide subunits according to claim 52 and is characterized by a six-helix bundle formed by oligomerization of the two-helix bundles of the respective structure-forming moieties of the chimeric polypeptides.

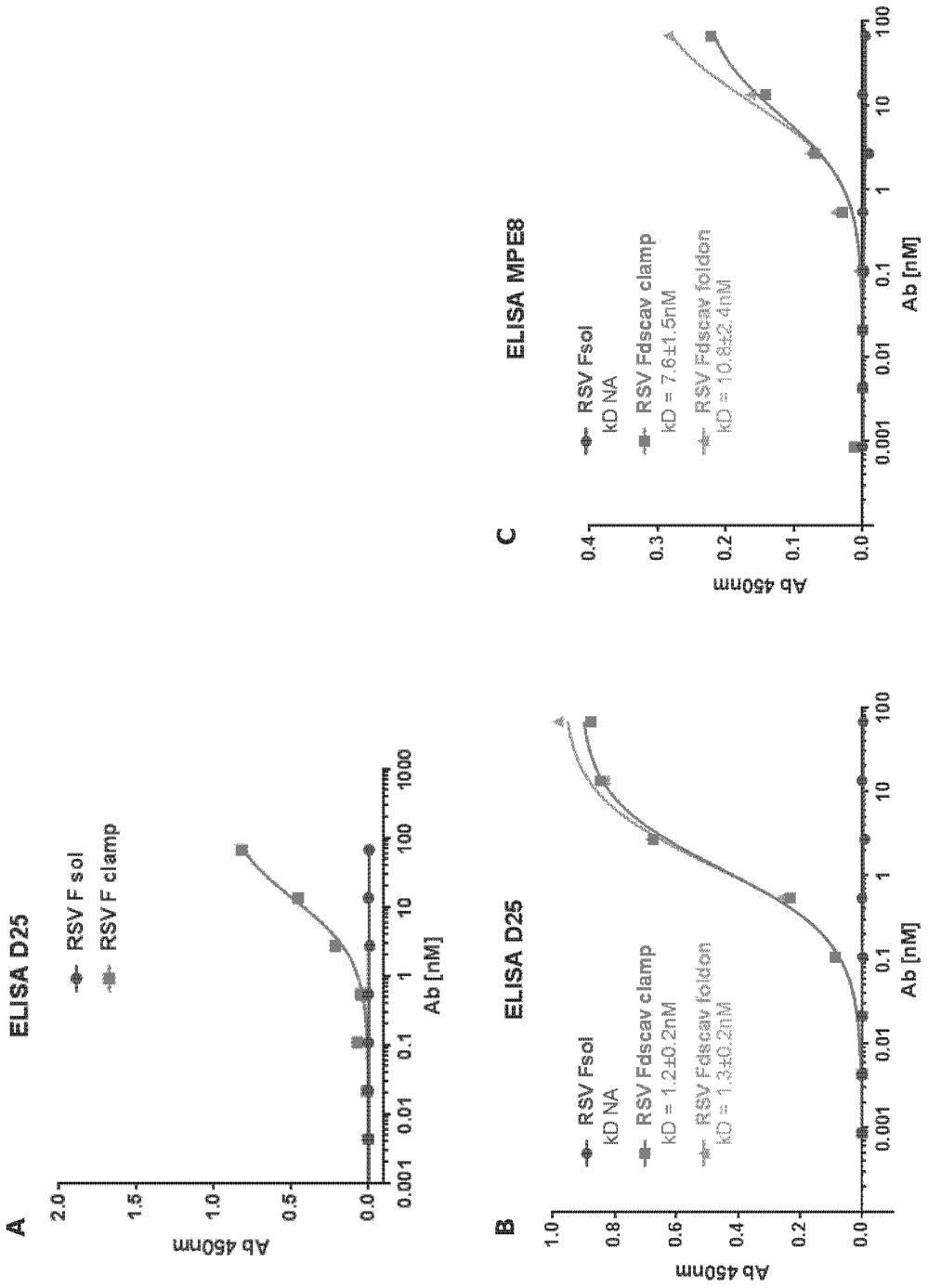
A
ELISA D25
RSV F sol
RSV F clamp
Ab 450nm
Ab [nM]
B
ELISA D25
RSV Fsol
kD NA
RSV Fdscav clamp
kD = 1.2±0.2nM
RSV Fdscav foldon
kD = 1.3±0.2nM
Ab 450nm
Ab [nM]
C
ELISA MPE8
RSV Fsol
kD NA
RSV Fdscav clamp
kD = 7.6±1.5nM
RSV Fdscav foldon
kD = 10.8±2.4nM
Ab 450nm
Ab [nM]

FIGURE 1

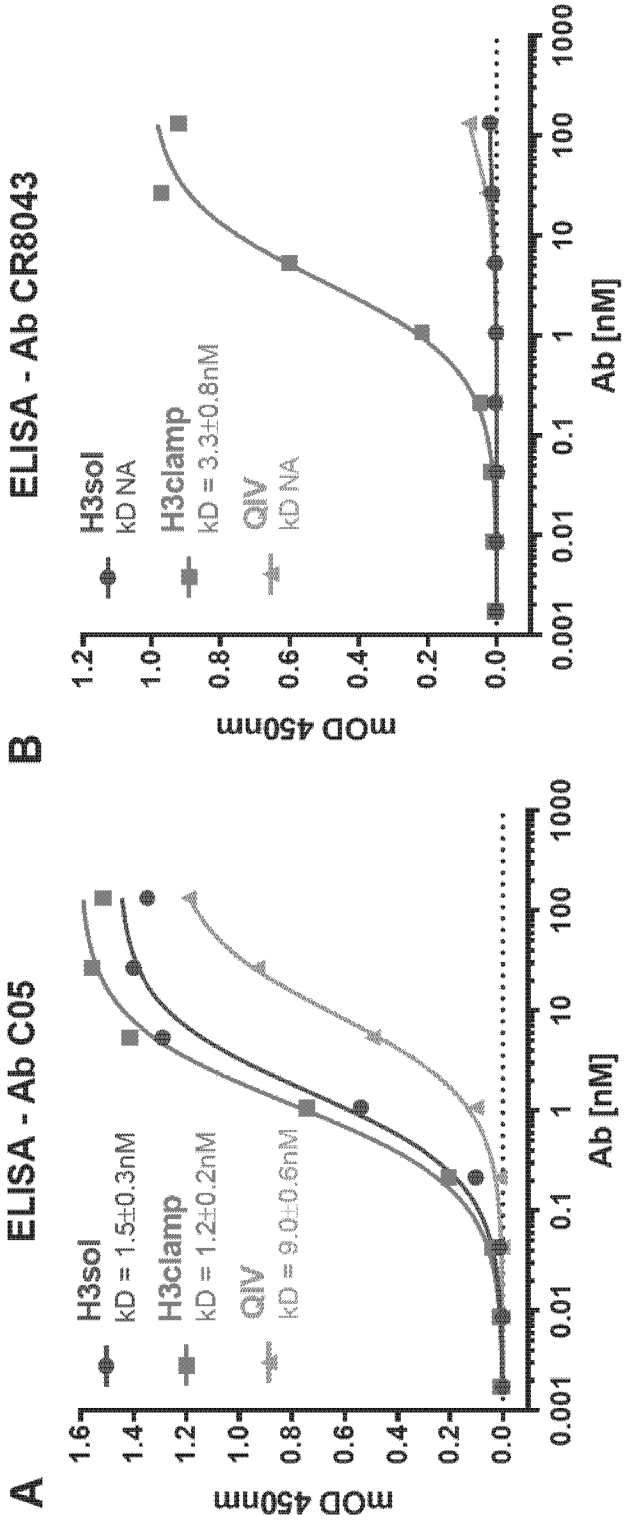
A
ELISA - Ab C05
mOD 450nm
Ab [nM]
H3sol
kD = 1.5±0.3nM
H3clamp
kD = 1.2±0.2nM
QIV
kD = 9.0±0.6nM
B
ELISA - Ab CR8043
mOD 450nm
Ab [nM]
H3sol
kD NA
H3clamp
kD = 3.3±0.8nM
QIV
kD NA

FIGURE 2

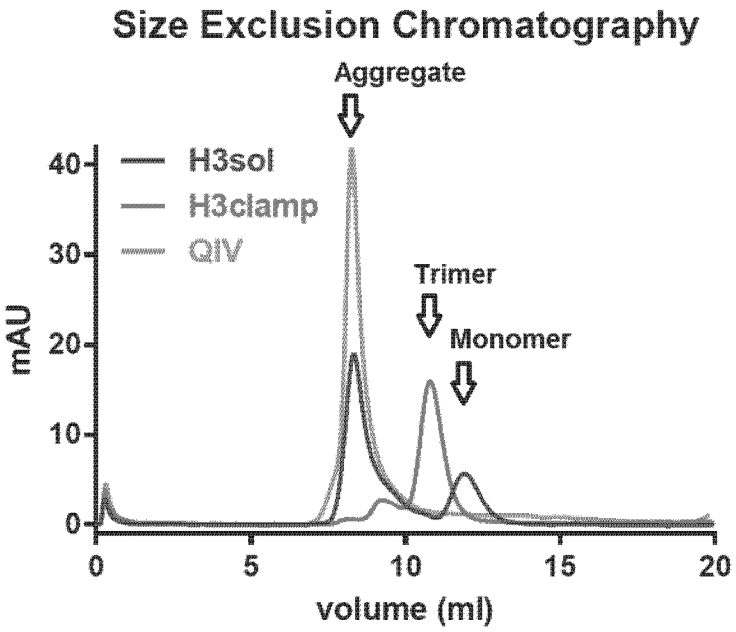

FIGURE 3

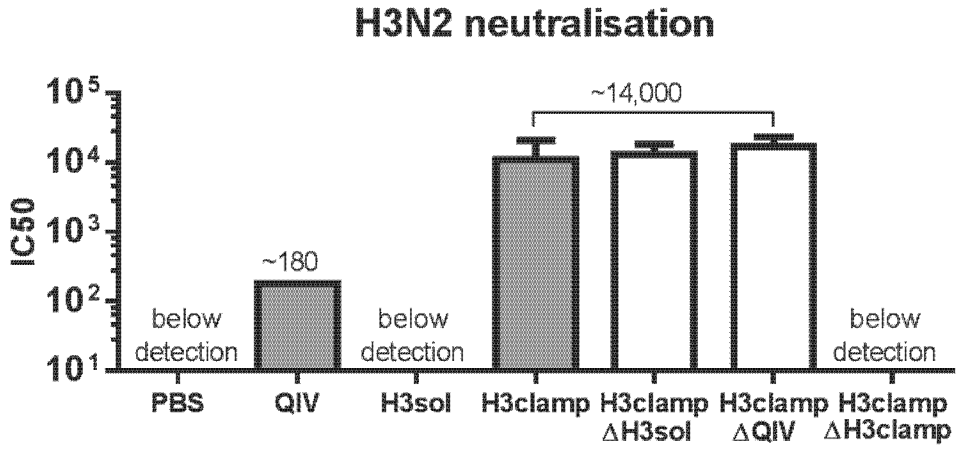

FIGURE 4

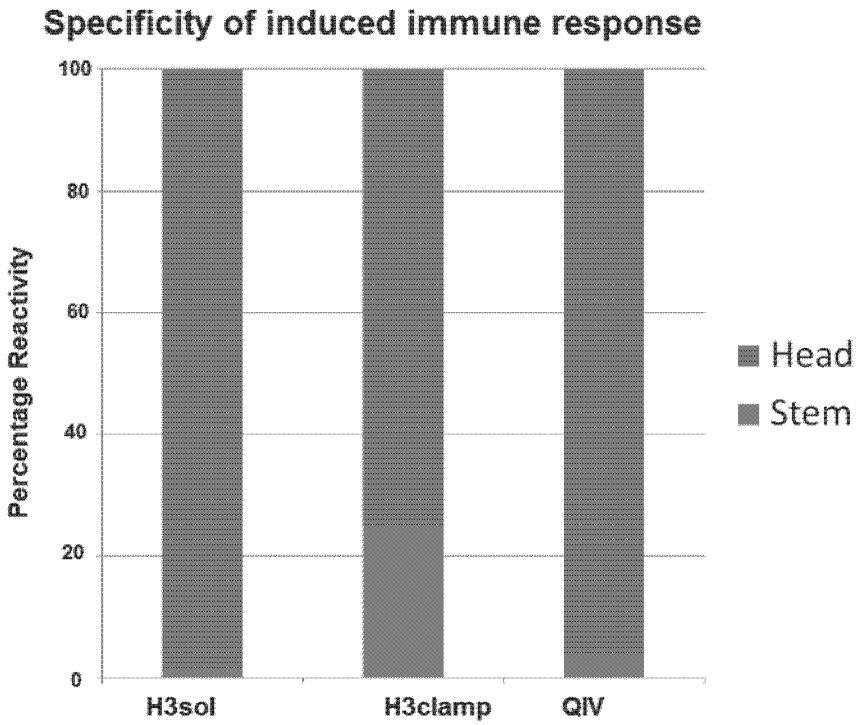

FIGURE 5

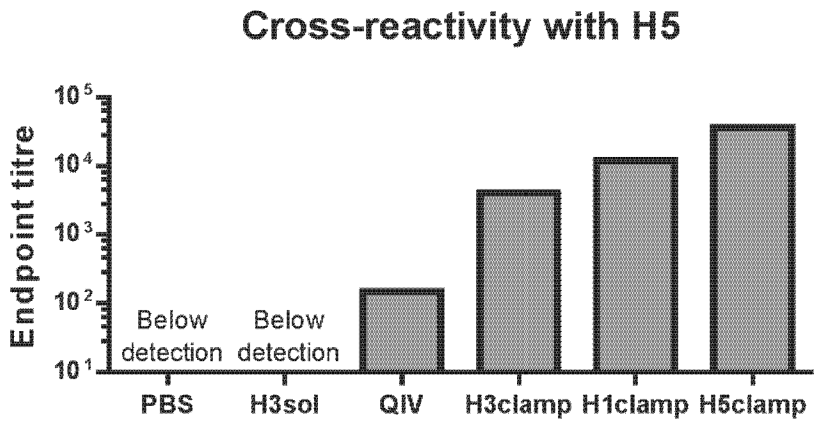

FIGURE 6

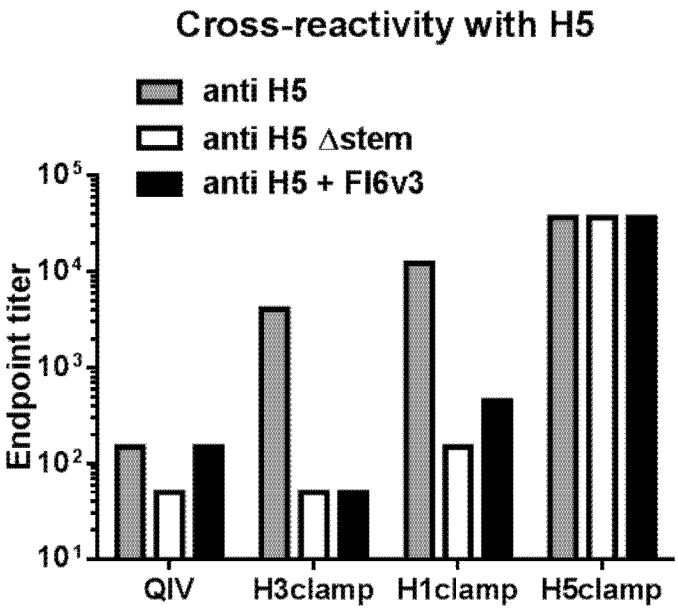
Cross-reactivity with H5
anti H5
anti H5 Δstem
anti H5 + FI6v3
Endpoint titer
10^5
10^4
10^3
10^2
10^1
QIV
H3clamp
H1clamp
H5clamp

FIGURE 7

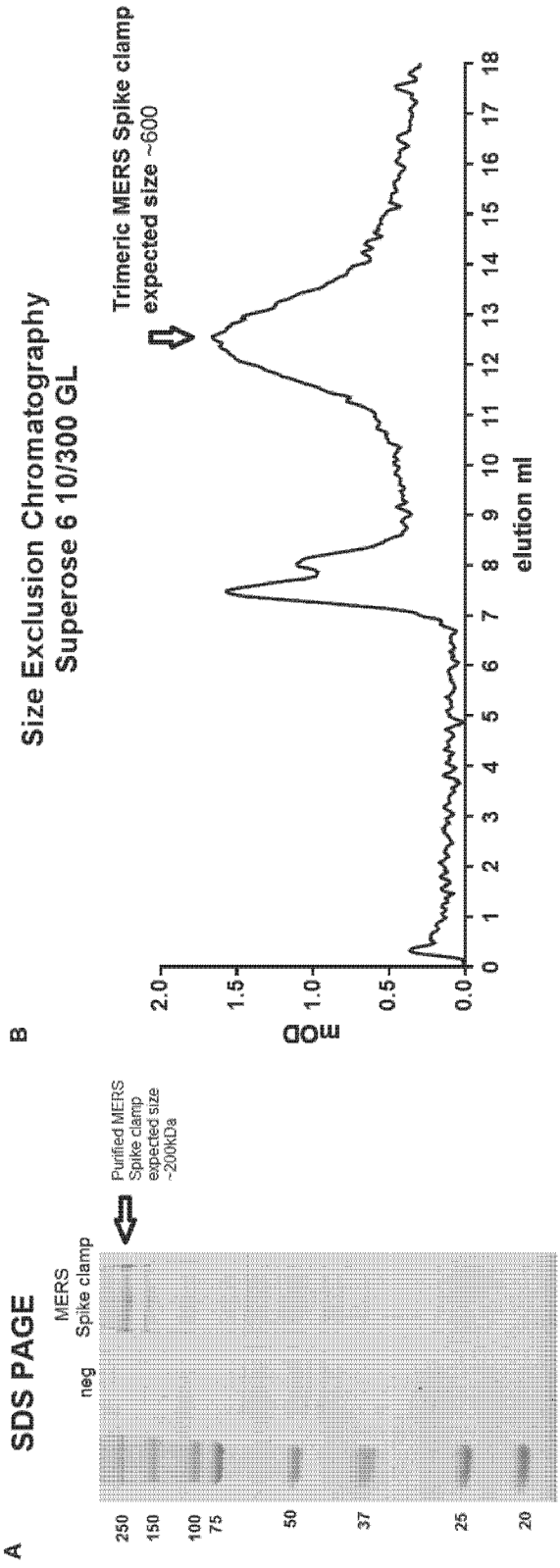
A
SDS PAGE
neg
MERS Spike clamp
Purified MERS Spike clamp expected size ~200kDa
250
150
100
75
50
37
25
20
B
Size Exclusion Chromatography
Superose 6 10/300 GL
Trimeric MERS Spike clamp expected size ~600
mOD
2.0
1.5
1.0
0.5
0.0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
elution ml

FIGURE 8

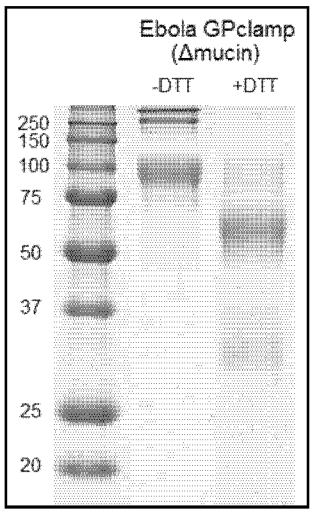

FIGURE 9

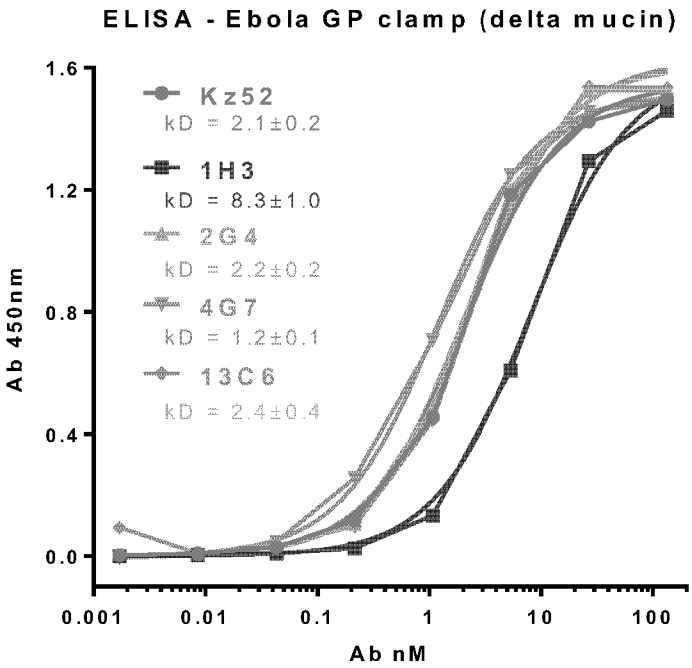

FIGURE 10

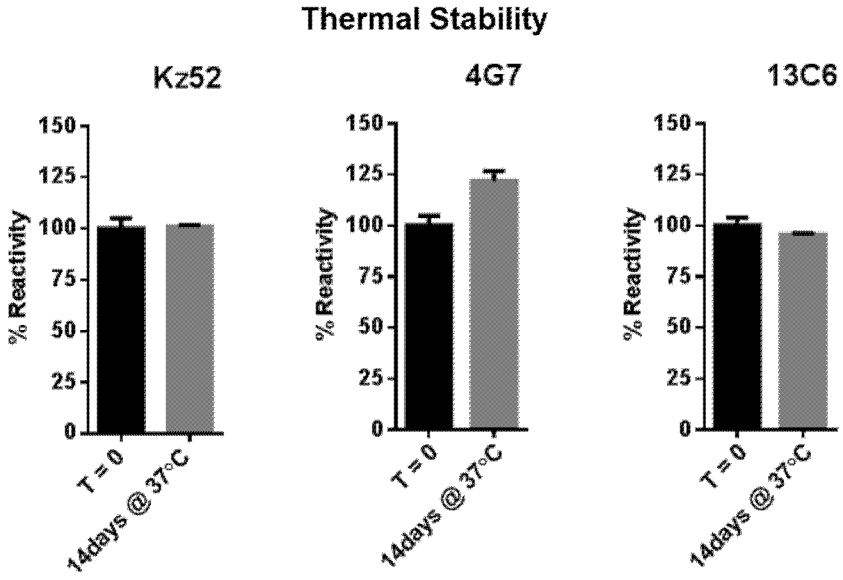
Thermal Stability
Kz52
4G7
13C6
% Reactivity
150
125
100
75
50
25
0
T = 0
14days @ 37°C

FIGURE 11

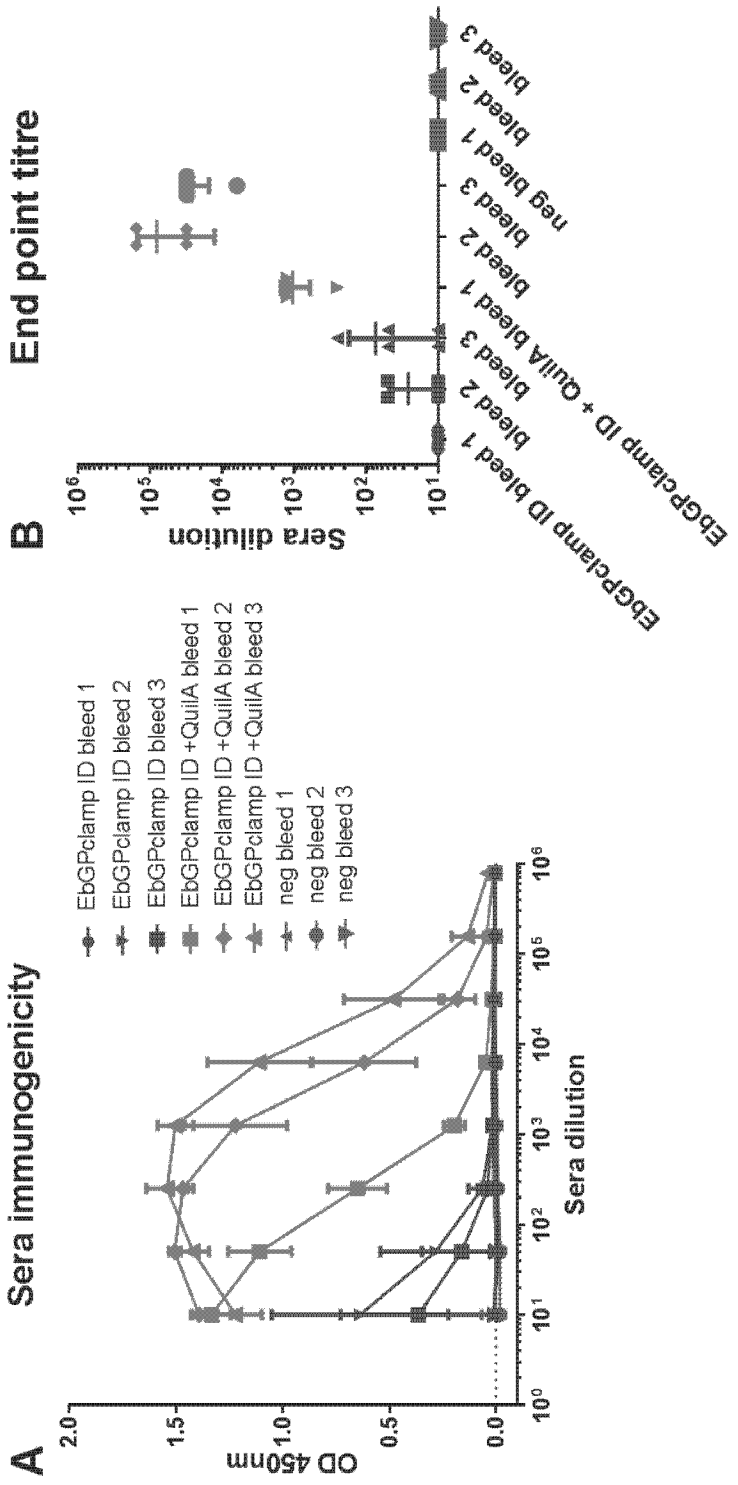
A
Sera immunogenicity
OD 450nm
Sera dilution
EbGPclamp ID bleed 1
EbGPclamp ID bleed 2
EbGPclamp ID bleed 3
EbGPclamp ID +QuilA bleed 1
EbGPclamp ID +QuilA bleed 2
EbGPclamp ID +QuilA bleed 3
neg bleed 1
neg bleed 2
neg bleed 3
B
End point titre
Sera dilution
EbGPclamp ID bleed 1
bleed 2
bleed 3
EbGPclamp ID + QuilA bleed 1
bleed 2
bleed 3
neg bleed 1
bleed 2
bleed 3

FIGURE 12

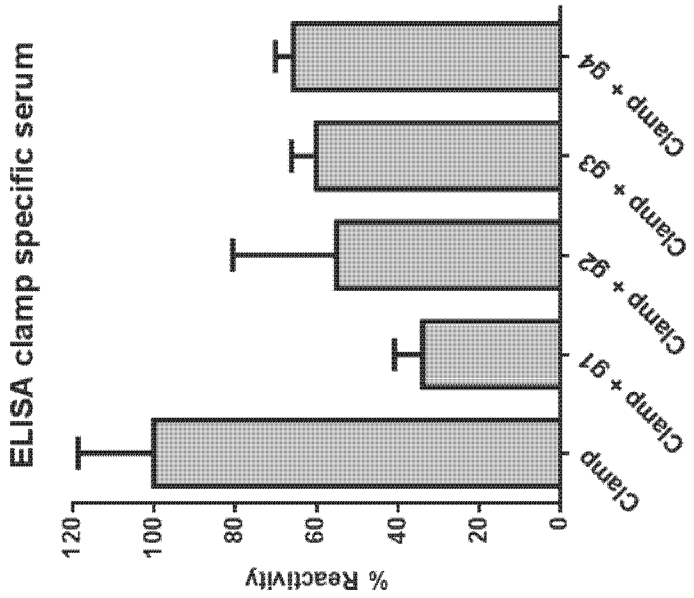

FIGURE 14

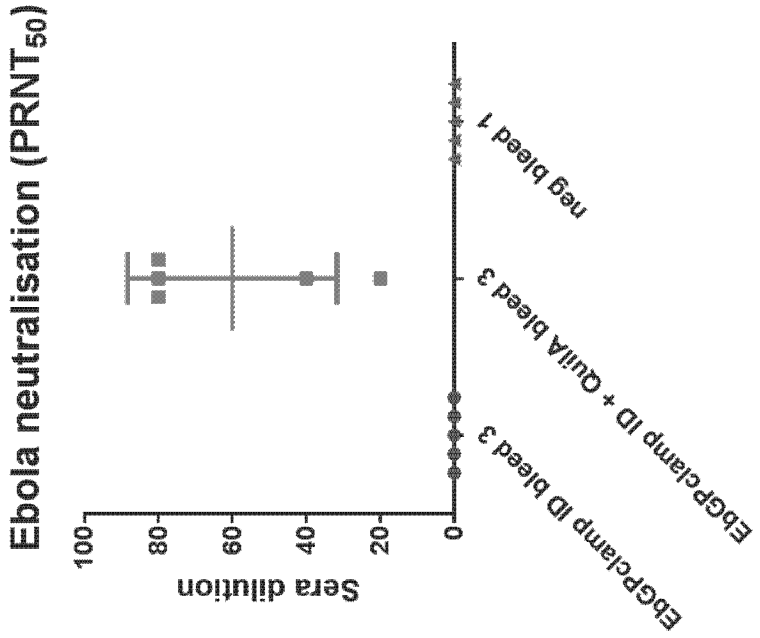

FIGURE 13

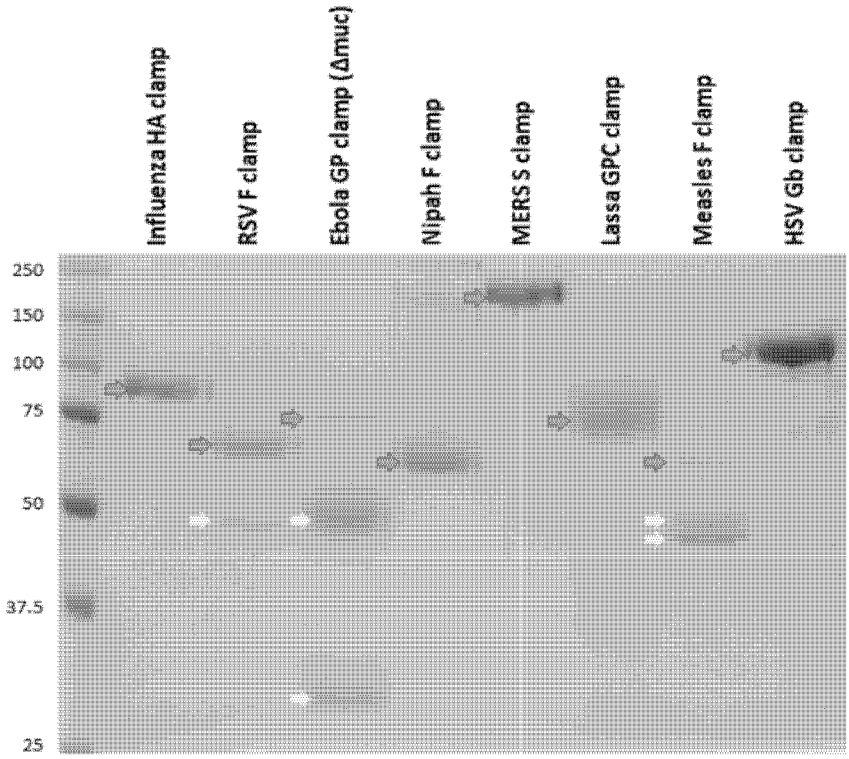

FIGURE 15

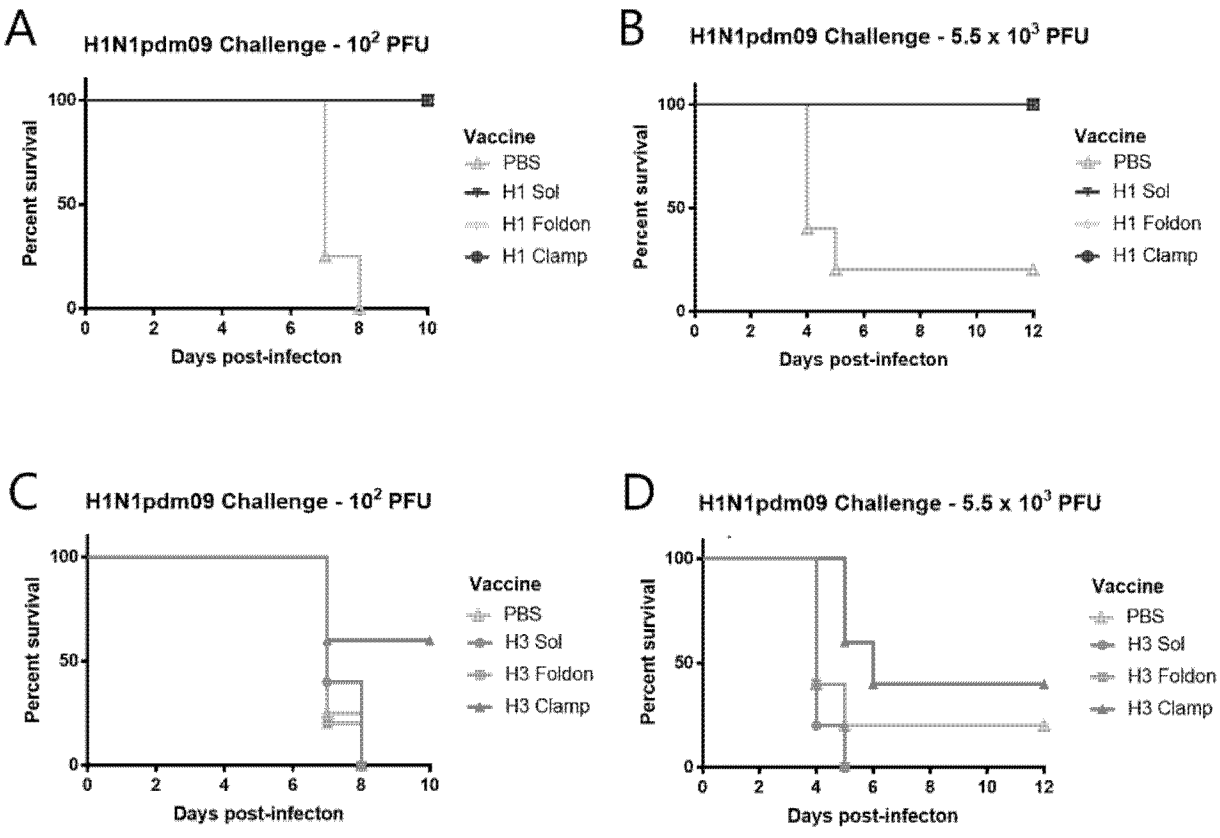

FIGURE 16

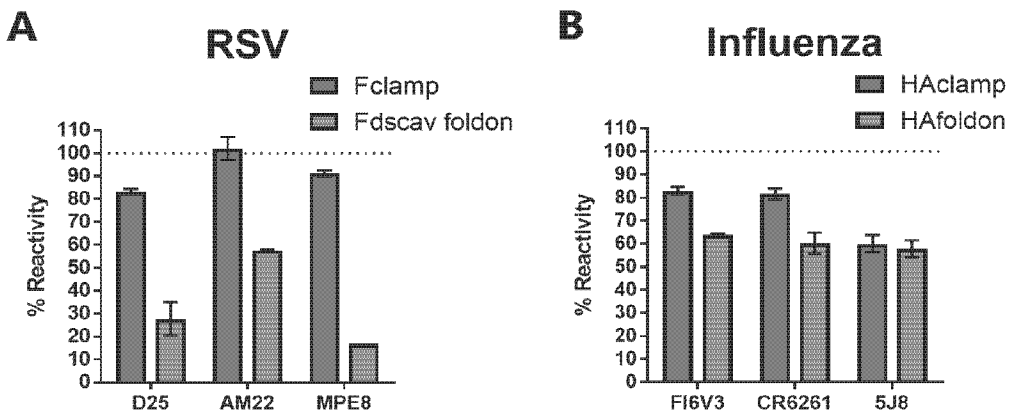

FIGURE 17

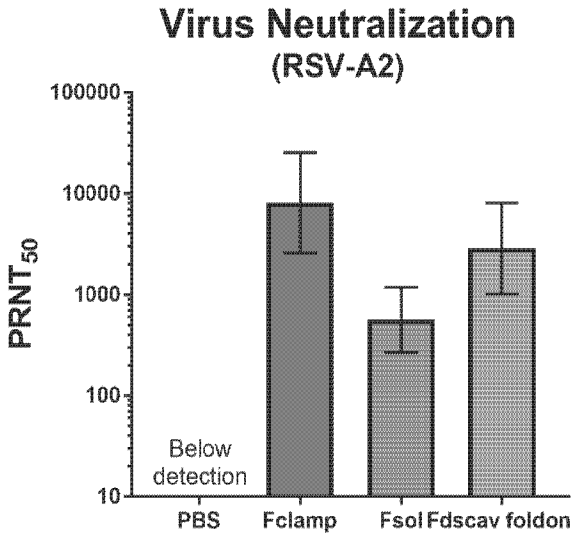

FIGURE 18

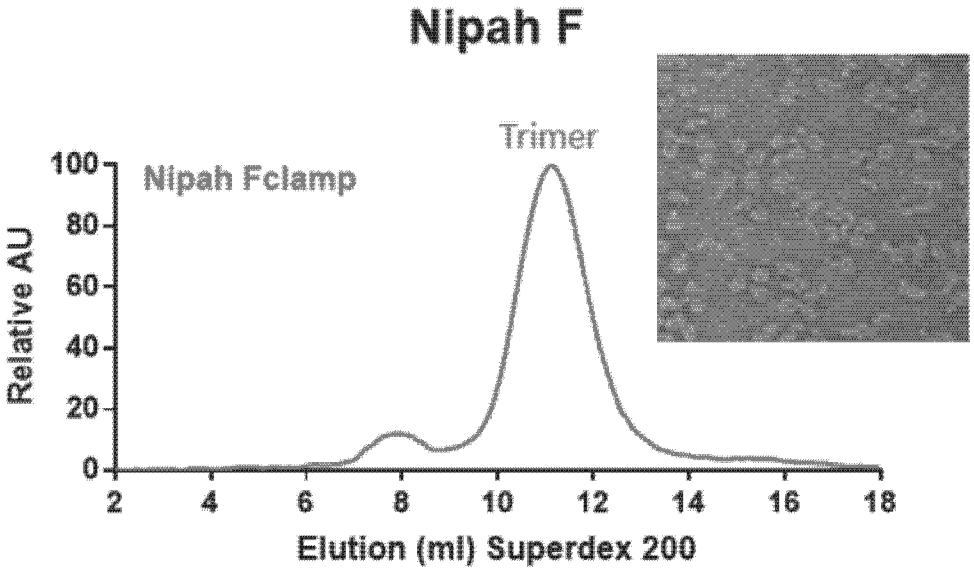

FIGURE 19

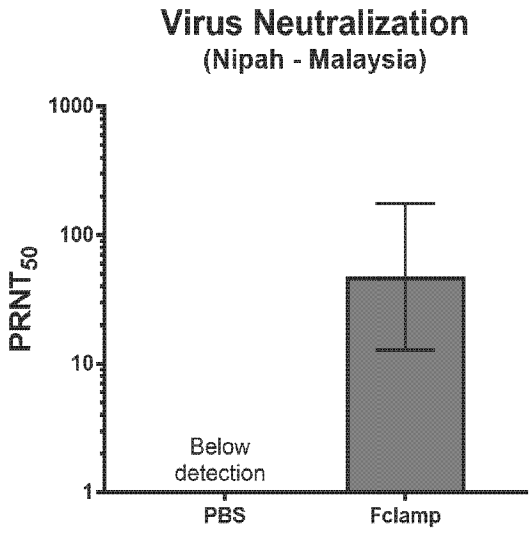

FIGURE 20

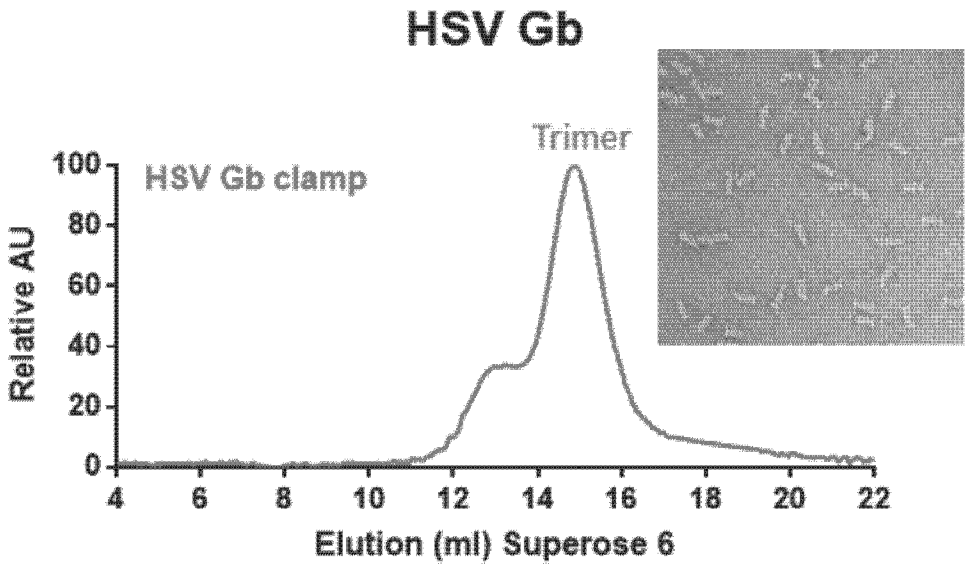
HSV Gb
Trimer
HSV Gb clamp
Relative AU
100
80
60
40
20
0
4
6
8
10
12
14
16
18
20
22
Elution (ml) Superose 6

FIGURE 21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2018/050299

A. CLASSIFICATION OF SUBJECT MATTER

C07K 19/00 (2006.01) ***C07K 14/11 (2006.01)*** ***C07K 14/115 (2006.01)*** ***C07K 14/15 (2006.01)*** ***C07K 14/165 (2006.01)*** ***C07K 14/08 (2006.01)*** ***A61P 31/14 (2006.01)***

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)

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

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

PATENW, MEDLINE, CAPLUS, BIOSIS, EMBASE: Chimeric, ectodomain, exposed domain, heptad, pre-fusion, two-helix, anti-parallel, respiratory syncytial virus, orthopneumovirus, influenza, orthomyxovirus, middle east respiratory syndrome, coronavirus, ebola, nipah, henipavirus, herpes, HIV, GP-160 and similar terms. **Internal databases provided by IP Australia, PubMed, Espacenet:** Applicant/Inventors and keywords search.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] 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

"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	Date of mailing of the international search report
25 May 2018	25 May 2018

Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Lauren Howitt AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262256130

INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/AU2018/050299**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2015/177312 A1 (GLAXOSMITHKLINE BIOLOGICALS SA) 26 November 2015 See Abstract, page 2, line 28 – page 3, line 30, Example 4, Figure 3	1-38 and 45-54
A	WO 2014/079842 A1 (NOVARTIS AG) 30 May 2014 See Abstract, page 3, lines 2-12	1-38 and 45-54
A	WO 2001/004335 A2 (THE GOVERNMENT OF THE UNITED STATES OF AMERICA, as represented by THE DEPARTMENT OF HEALTH AND HUMAN SERVICES) 18 January 2001 See Abstract, page 7, line 28 – page 8, line 14	1-38 and 45-54
A	SCHMIDT, M.R. et al., 'Modification of the respiratory syncytial virus F protein in virus-like particles impacts generation of B cell memory', Journal of Virology. 2014, Vol. 88, No. 17, pages 10165-10176 See Abstract, Materials and Methods	1-38 and 45-54
P,X	WO 2018/027252 A1 (THE COUNCIL OF THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH) 15 February 2018 See [0019]-[0023], [0065], [0094], [0142], [0164], [0229]	29-36, 38 and 52-54

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2018/050299

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:

the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including

2. ☒ Claims Nos.: **39-44**

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:

See Supplemental Box

3. ☐ Claims Nos:

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:

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.:

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.

Supplemental Box

Continuation of **Box II**

Claims 39-44 do not comply with Rule 6.2(a) because they rely on references to the description and/or drawings.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2018/050299**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2018/050299**

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End of Annex

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