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(54) **Title:** ANTIGENIC EPSTEIN BARR VIRUS POLYPEPTIDES

(57) **Abstract:** This disclosure relates to antigenic EBV polypeptides and their use in eliciting antibodies against EBV. Also disclosed are antigenic polypeptides comprising an EBV polypeptide and a ferritin protein.

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ANTIGENIC EPSTEIN BARR VIRUS POLYPEPTIDES

[0001] This application claims the benefit of priority of U.S. Provisional Patent Application No. 62/652,201, filed April 3, 2018, which is incorporated herein by reference in its entirety.

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 27, 2019, is named 2019-03-27_01121-0032-00PCT_SL_ST25.txt and is 377,803 bytes in size.

[0003] Even with many successes in the field of vaccinology, new breakthroughs are needed to protect humans against many life-threatening infectious diseases. Many currently licensed vaccines rely on decade-old technologies to produce live-attenuated or inactivated killed pathogens, which carry inherent safety concerns and in many cases, stimulate only short-lived, weak immune responses that require the administration of multiple doses. While advances in genetic and biochemical engineering have made it possible to develop therapeutic agents to challenging disease targets, these applications to the field of vaccinology have not been fully realized. Recombinant protein technologies now allow the design of optimal antigens. Additionally, nanoparticles have increasingly demonstrated the potential for optimal antigen presentation and targeted drug delivery. Nanoparticles with multiple attached antigens have been shown to have increased binding avidity afforded by the multivalent display of their molecular cargos, and an ability to cross biological barriers more efficiently due to their nanoscopic size. *Helicobacter pylori* (*H. pylori*) ferritin nanoparticles fused to influenza virus haemagglutinin (HA) protein has allowed improved antigen stability and increased immunogenicity in mouse influenza models (*see* Kanekiyo et al., Nature 499:102-106 (2013)). This fusion protein self-assembled into an octahedrally-symmetric nanoparticle and presented 8 trimeric HA spikes to give a robust immune response in various pre-clinical models when used with an adjuvant.

[0004] Epstein Barr virus (EBV) infects about 95% of the adult population worldwide and has been known to be associated with two B-cell lymphomas, Burkitt's and Hodgkin's lymphomas. The virus can also infect epithelial cells and is associated with nasopharyngeal cancer. Furthermore, EBV causes most cases of infectious mononucleosis in developed countries, affecting mainly children and young adults. Infectious mononucleosis can result in

a long recovery period of up to one month. There are currently no approved vaccines on the market, so there is a strong need for a preventive vaccine.

[0005] Here, a set of new polypeptides, nanoparticles, compositions, methods, and uses involving EBV polypeptides is presented. Novel EBV single-chain gL and gH (sometimes depicted as gL/gH or gH/gL) polypeptides were generated, as were antigenic polypeptides comprising these novel EBV polypeptides and ferritin. Antigenic polypeptides and nanoparticles comprising the single-chain gL and gH polypeptides can comprise a relatively long linker between the gL and gH sequences, which was observed to provide an increase in immunogenicity. Antigenic ferritin polypeptides and nanoparticles comprising EBV gp220 polypeptides were also generated. Furthermore, self-adjuvanting antigenic polypeptides comprising the described EBV polypeptides and ferritin were developed wherein immune-stimulatory moieties, such as adjuvants, were directly, chemically attached to the antigenic polypeptide. The direct conjugation of an immune-stimulatory moiety to the antigenic polypeptide allows for targeted co-delivery of the immune-stimulatory moiety and EBV polypeptide in a single macromolecular entity, which can greatly decrease the potential for systemic toxicity that is feared with traditional vaccines that comprise antigens and immune-stimulatory molecules such as adjuvants as separate molecules. The co-delivery of immune-stimulatory moieties together with EBV polypeptides in a macromolecular entity and their multivalent presentation may also reduce the overall dose needed to elicit protection, reducing manufacturing burdens and costs.

SUMMARY

[0006] It is an object of this disclosure to provide compositions, kits, methods, and uses that can provide one or more of the advantages discussed above, or at least provide the public with a useful choice. Accordingly, the following embodiments are disclosed herein.

[0007] Embodiment 1 is an antigenic EBV polypeptide comprising an Epstein Barr Virus (EBV) gL polypeptide and an EBV gH polypeptide, wherein a linker having a length of at least 15 amino acids separates the EBV gL polypeptide and the EBV gH polypeptide.

[0008] Embodiment 2 is an antigenic EBV polypeptide comprising an Epstein Barr Virus (EBV) gL polypeptide, an EBV gH polypeptide, and an EBV gp42 polypeptide, wherein a linker having a length of at least 15 amino acids separates the EBV gL polypeptide and the EBV gH polypeptide.

[0009] Embodiment 3 is the antigenic EBV polypeptide of claim 1 or claim 2, further comprising a ferritin.

[0010] Embodiment 4 is an antigenic EBV polypeptide comprising an EBV polypeptide and a ferritin protein, wherein the ferritin protein comprises a mutation replacing a surface-exposed amino acid with a cysteine.

[0011] Embodiment 5 is the antigenic EBV polypeptide of claim 4, wherein the EBV polypeptide comprises an EBV gL polypeptide, an EBV gH polypeptide, or an EBV gp220 polypeptide.

[0012] Embodiment 6 is the antigenic EBV polypeptide of claim 5, wherein the EBV polypeptide comprises a gL polypeptide and the polypeptide further comprises an EBV gH polypeptide.

[0013] Embodiment 7 is the antigenic EBV polypeptide of any one of claims 1 or 3-6, wherein the polypeptide further comprises an EBV gp42 polypeptide.

[0014] Embodiment 8 is a composition comprising a first antigenic EBV polypeptide and a second antigenic EBV polypeptide, wherein the first antigenic EBV polypeptide comprises a ferritin heavy chain and a first EBV polypeptide, the second antigenic EBV polypeptide comprises a ferritin light chain and a second EBV polypeptide, and the first and second EBV polypeptides are different.

[0015] Embodiment 9 is the composition of claim 8, wherein the first EBV polypeptide or the second EBV polypeptide comprises a gp220 polypeptide.

[0016] Embodiment 10 is the composition of any one of claims 8 to 9, wherein (i) the first antigenic EBV polypeptide comprises one or both of a gL polypeptide and a gH polypeptide and the second antigenic EBV polypeptide comprises a gp220 polypeptide, or (ii) the first antigenic EBV polypeptide comprises a gp220 polypeptide and the second antigenic EBV polypeptide comprises one or both of a gL polypeptide and a gH polypeptide.

[0017] Embodiment 11 is the composition of any one of claims 8 to 10, wherein the first antigenic EBV polypeptide comprises a gL polypeptide and a gH polypeptide; or the second antigenic EBV polypeptide comprises a gL polypeptide and an EBV gH polypeptide.

[0018] Embodiment 12 is the composition of claim 10 or 11, wherein the antigenic EBV polypeptide comprising a gL polypeptide and/or a gH polypeptide further comprises a gp42 polypeptide.

[0019] Embodiment 13 is the antigenic EBV polypeptide or composition of any one of claims 1-12, comprising a gH and gL polypeptide, wherein the gH polypeptide is C-terminal to the gL polypeptide, optionally comprising a gp42 polypeptide, wherein the gp42 polypeptide is C-terminal to the gH polypeptide.

[0020] Embodiment 14 is the antigenic EBV polypeptide or composition of any one of claims 1-13, comprising a gp42 polypeptide, wherein the gp42 polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 239 or 240.

[0021] Embodiment 15 is the antigenic EBV polypeptide or composition of any one of claims 1-14, comprising an EBV gH polypeptide and an EBV gp42 polypeptide, wherein a linker having a length of at least 15 amino acids separates the EBV gH polypeptide and the EBV gp42 polypeptide, optionally wherein the linker has a length of 15 to 60 amino acids, 20 to 60 amino acids, 30 to 60 amino acids, 40 to 60 amino acids, 30 to 50 amino acids, or 40 to 50 amino acids, further optionally wherein the linker comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 234.

[0022] Embodiment 16 is the antigenic EBV polypeptide or composition of any one of the preceding claims, comprising a linker, wherein the linker has a length of at least 15 amino acids, optionally wherein the linker separates a first EBV polypeptide and a second EBV polypeptide.

[0023] Embodiment 17 is the antigenic EBV polypeptide or composition of claim 16, wherein the linker has a length of 15 to 60 amino acids, 20 to 60 amino acids, 30 to 60 amino acids, 40 to 60 amino acids, 30 to 50 amino acids, or 40 to 50 amino acids.

[0024] Embodiment 18 is the antigenic EBV polypeptide or composition of any one of the preceding claims, wherein the EBV polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 36.

[0025] Embodiment 19 is the antigenic EBV polypeptide or composition of any one of the preceding claims, wherein the EBV polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 37.

[0026] Embodiment 20 is the antigenic EBV polypeptide or composition of any one of the preceding claims, wherein the polypeptide comprises a linker comprising an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 30, optionally wherein the linker separates a first EBV polypeptide and a second EBV polypeptide.

[0027] Embodiment 21 is the antigenic EBV polypeptide or composition of any one of the preceding claims, wherein the EBV polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 38.

[0028] Embodiment 22 is the antigenic EBV polypeptide or composition of any one of claims 3-21, further comprising a further linker that separates the EBV polypeptide and the ferritin.

[0029] Embodiment 23 is the antigenic EBV polypeptide or composition of any one of claims 3-22, comprising an EBV gp42 polypeptide located N-terminal to the ferritin and C-terminal to the gH polypeptide, wherein a linker separates the EBV gp42 polypeptide and the ferritin, optionally wherein the linker has a length of at least 15 amino acids or has a length of 15 to 60 amino acids, 20 to 60 amino acids, 30 to 60 amino acids, 40 to 60 amino acids, 30 to 50 amino acids, or 40 to 50 amino acids, further optionally wherein the linker comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to any one of SEQ ID NOs: 233, 234, 235, 236, 237, or 238.

[0030] Embodiment 24 is the antigenic EBV polypeptide or composition of claim 22 or 23, wherein the linker comprises a cysteine.

[0031] Embodiment 25 is the antigenic EBV polypeptide or composition of any one of claims 22-24, wherein the linker comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 33.

[0032] Embodiment 26 is the antigenic EBV polypeptide or composition of claim 24-25, wherein the cysteine is conjugated to an immune-stimulatory moiety, optionally wherein the immune-stimulatory moiety is an agonist of TLR2, TLR7/8, TLR9, or STING.

[0033] Embodiment 27 is the antigenic EBV polypeptide or composition of any one of claims 3-26, wherein the ferritin comprises one or more of E12C, S26C, S72C, A75C, K79C, S100C, and S111C mutations of *H. pylori* ferritin or one or more corresponding mutations in a non-*H. pylori* ferritin as determined by pairwise or structural alignment.

[0034] Embodiment 28 is the antigenic EBV polypeptide or composition of any one of claims 3-27, wherein the ferritin comprises a mutation replacing a surface-exposed asparagine with a non-asparagine amino acid, optionally wherein the asparagine is at position 19 of *H. pylori* ferritin, or an analogous position in a non- *H. pylori* ferritin as determined by pairwise or structural alignment.

[0035] Embodiment 29 is the antigenic EBV polypeptide or composition of any one of claims 3-28, wherein the ferritin comprises a mutation replacing an internal cysteine with a non-cysteine amino acid, optionally wherein the internal cysteine is at position 31 of *H. pylori* ferritin, or a position that corresponds to position 31 of *H. pylori* ferritin as determined by pair-wise or structural alignment.

[0036] Embodiment 30 is the antigenic EBV polypeptide or composition of any one of claims 3-29, wherein the ferritin comprises an amino acid sequence with 80%, 85%, 90%, 95%, 98%, or 99% identity to any one of SEQ ID NOs: 201-207 or 211-215.

[0037] Embodiment 31 is the antigenic EBV polypeptide or composition of any one of claims 1-31, wherein the antigenic EBV polypeptide comprises a sequence with at least 80%, 85%, 90%, 95%, 98%, or 99% identity to amino acids 23-1078 of SEQ ID NO: 226.

[0038] Embodiment 32 is the antigenic EBV polypeptide or composition of any one of claims 1-31, wherein the antigenic EBV polypeptide comprises a sequence with at least 80%, 85%, 90%, 95%, 98%, or 99% identity to any one of SEQ ID NOs: 226-231 or 241-242, optionally lacking the leader sequence.

[0039] Embodiment 33 is a ferritin particle comprising the antigenic EBV polypeptide or the first and second polypeptides of any one of claims 3-32.

[0040] Embodiment 34 is a composition comprising the antigenic EBV polypeptide(s) or ferritin particle of any one of the preceding claims and a pharmaceutically acceptable carrier.

[0041] Embodiment 35 is the composition of claim 34, wherein the ferritin particle comprises an EBV gL polypeptide and an EBV gH polypeptide, and the composition further comprises a second ferritin particle comprising a gp220 polypeptide.

[0042] Embodiment 36 is the antigenic EBV polypeptide, ferritin particle, or composition of any one of the preceding claims for use in a method of eliciting an immune response to influenza or in protecting a subject against infection with EBV.

[0043] Embodiment 37 is a method of eliciting an immune response to EBV or protecting a subject against infection with EBV comprising administering any one or more antigenic EBV polypeptide, ferritin particle, or composition of any one of the preceding claims to a subject.

[0044] Embodiment 38 is the antigenic EBV polypeptide, ferritin particle, composition, or method of any one of claims 36-37, wherein the subject is human.

[0045] Embodiment 39 is a nucleic acid encoding the antigenic EBV polypeptide of any one of claims 1-32, optionally wherein the nucleic acid is an mRNA.

[0046] Additional objects and advantages will be set forth in the description which follows, and/or will be obvious from the description, or may be learned by practice. The objects and advantages will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

[0047] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the claims.

[0048] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate certain embodiments and together with the description, serve to explain the principles described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0049] **FIGs 1A-1B** show purified single-chain gL and gH monomer **(FIG 1A)** (SEQ ID NO: 6) and trimer **(FIG 1B)** (SEQ ID NO: 11) with and without removal of the His-tag by Coomassie and Western blot analysis. **FIG 1B** also presents the UV absorbance trace of fractions from a size exclusion column (Superose® 6) purification.

[0050] **FIGs 2A-2E** shows purification and characterization of single-chain gL/gH-ferritin nanoparticles (SEQ ID NO: 14). A UV absorbance trace of Superose® 6 purification fractions is shown **(FIG 2A)**, as well as Coomassie **(FIG 2B)** and Western blot **(FIG 2C)** analysis of selected fractions from the purification (L indicates molecular weight ladder; the positions of the 150 and 250 kDa bands are indicated at right in FIG 2B). Dynamic light scattering **(FIG 2D)** and electron microscopy **(FIG 2E)** analyses of the single-chain nanoparticles are also presented.

[0051] **FIG 3** shows different representative single-chain gL/gH-ferritin constructs.

[0052] **FIG 4** shows antibody titers following immunization of mice with single-chain gL/gH trimers or nanoparticles (NP) admixed with AF03 adjuvant, which is a squalene emulsion-based adjuvant. *p-value = <0.05 when comparing the NP construct with its corresponding trimer construct. From left to right, constructs were SEQ ID NOs: 16, 10, 11, 13, 12, and 14.

[0053] **FIGs 5A-5B** show anti-gL/gH antibody response in mice to a bivalent composition comprising both gp220 nanoparticles (SEQ ID NO: 1) and a single-chain gL/gH nanoparticle (“gL_gH_C5 NP,” SEQ ID NO: 19) compared to the single-chain gL/gH nanoparticle and negative control naked ferritin (i.e., ferritin not conjugated to any non-ferritin polypeptide or immune-stimulatory moiety). The results indicate that using the bivalent composition did not result in interference with the anti-gL/gH antibody response relative to the results with single-chain gL/gH with negative control naked ferritin. Both compositions included AF03 adjuvant. ELISA results at individual dilutions **(FIG 5A)** and binding titer **(FIG 5B)** are shown.

[0054] **FIGs 6A-6B** show anti-gp220 antibody response to a bivalent composition comprising both gp220 nanoparticles and single-chain gL/gH nanoparticles as described for FIGs 5A-B. The results indicate that using the bivalent composition did not result in interference with the anti-gp220 antibody response relative to the results with gp220 nanoparticles with negative control naked ferritin. Both compositions included AF03 adjuvant. ELISA results at individual dilutions **(FIG 6A)** and binding titer **(FIG 6B)** are shown.

[0055] **FIG 7A** shows the design of a nanoparticle comprising an EBV polypeptide and ferritin comprising a mutation replacing a surface-exposed amino acid with a cysteine for conjugation to an immune-stimulatory moiety such as a toll-like receptor (TLR) agonist. For an exemplary sequence corresponding to this design, *see* SEQ ID NO: 14, in which a single-chain gL/gH antigen is linked to ferritin by a flexible 46 amino acid linker. **FIG 7B** shows a representative toll-like receptor (TLR) agonist (SM7/8a with a PEG4-maleimide linker) suitable for conjugation to a construct according to FIG 7A. **FIG 7C** shows an electron micrograph (EM) image of a gL/gH nanoparticle with SM7/8a conjugated thereto via the cysteine on the ferritin surface and a PEG4-maleimide linker.

[0056] **FIG 8A** shows a structure of part of a ferritin comprising a mutation replacing a surface-exposed amino acid with a cysteine, in which the location of the cysteine is indicated. **FIG 8B** illustrates conjugation of a CpG adjuvant (SEQ ID NO: 247) to ferritin by juxtaposing the ferritin, linker, and CpG adjuvant, oriented to show the parts of each moiety that become attached to each other in proximity.

[0057] **FIGs 9A-9B** show mass spectrometry (MS) spectra of the unconjugated **(FIG 9A)** and SM7/8a-conjugated **(FIG 9B)** forms of a gL/gH-ferritin. The difference in mass of the main peaks was 711 Da, which approximately corresponds to the predicted difference from conjugating the SM7/8a with linker.

[0058] **FIGs 10A-10B** show mass spectrometry (MS) spectra of the unconjugated **(FIG 10A)** and SM7/8a-conjugated **(FIG 10B)** forms of a gp220-ferritin. The difference in mass of the main peaks was 714.7 Da, which approximately corresponds to the predicted difference from conjugating the SM7/8a with linker.

[0059] **FIGs 11A-11D** show electron microscopy (EM) images of unconjugated **(FIGs 11A, C)** and conjugated **(FIGs 11B, D)** single-chain gL/gH **(FIGs 11A, B)** and gp220 **(FIGs 11C, D)** ferritin nanoparticles, indicating that conjugation of SM7/8a to these nanoparticles did not disrupt nanoparticle structure.

[0060] **FIGs 12A-12B** show antibody responses in mice after treatment with ferritin nanoparticles comprising single-chain gL/gH either without conjugated SM7/8a or other adjuvant, with AF03 adjuvant as a separate molecule, or with conjugated SM7/8a. ELISA results are shown as individual dilutions **(FIG 12A)** and binding titers **(FIG 12B)**.

[0061] **FIGs 13A-13B** show antibody responses in mice after treatment with nanoparticles comprising gp220 either alone, with AF03 adjuvant as a separate molecule, or with conjugated SM7/8a. ELISA results are shown as individual dilutions **(FIG 13A)** and binding titers **(FIG 13B)**.

[0062] **FIGs 14A-14B** show anti-gL/gH antibody responses in mice after treatment with gp220 nanoparticles conjugated to SM7/8a and single-chain gL/gH ferritin nanoparticles conjugated to SM7/8a compared to treatment with single-chain gL/gH ferritin nanoparticles conjugated to SM7/8a and naked ferritin, as measured by ELISA. Results are shown for experiments without **(FIG 14A)** or with **(FIG 14B)** admixed AF03.

[0063] **FIGs 15A-15B** show anti-gp220 antibody responses in mice after treatment with gp220 nanoparticles conjugated to SM7/8a and single-chain gL/gH nanoparticles conjugated to SM7/8a compared to treatment with gp220 nanoparticles conjugated to SM7/8a and naked ferritin, as measured by ELISA. Results are shown for experiments without **(FIG 15A)** or with **(FIG 15B)** admixed AF03.

[0064] **FIG 16** shows anti-gL/gH antibody responses in mice after treatment with single-chain gL/gH nanoparticles (gL/gH_C5; SEQ ID NO: 19) and naked ferritin, with or without either or both of admixed AF03 adjuvant and/or SM7/8a conjugated to the single-chain gL/gH nanoparticles, as measured by ELISA endpoint titer.

[0065] **FIG 17** shows anti-gp220 antibody responses in mice after treatment with gp220 nanoparticles and naked ferritin, with or without either or both of admixed AF03 adjuvant and/or SM7/8a conjugated to the gp220 nanoparticles.

[0066] **FIG 18** shows anti-gL/gH antibody responses in mice after treatment with bivalent compositions comprising gp220 nanoparticles and single-chain gL/gH nanoparticles. As indicated in the legend, some nanoparticles were conjugated to SM7/8a, and some nanoparticles were admixed with AF03. The order of symbols from top to bottom in the key matches the order of symbols from left to right in the graph.

[0067] **FIG 19** shows anti-gp220 antibody responses in mice after treatment with bivalent compositions comprising single-chain gL/gH nanoparticles and gp220 nanoparticles and/or naked ferritin. As indicated in the legend, some nanoparticles were conjugated to

SM7/8a, and some nanoparticles were admixed with AF03. The order of symbols from top to bottom in the key matches the order of symbols from left to right in the graph.

[0068] **FIGs 20A-20D** show antibody responses in mice after treatment with gL_gH_C7 nanoparticle (SEQ ID NO: 20) with or without admixed AF03 adjuvant and/or conjugation to SM7/8a (**FIGs 20A, 20C, or 20D**) or CpG oligodeoxynucleotide (**FIG 20B**). Shown are endpoint titer from prime bleed measured by ELISA (**FIG 20A**), ELISA results at individual dilutions from booster bleed (**FIG 20B**), and endpoint titers measured by ELISA from booster bleed (**FIG 20C**) and terminal bleed (**FIG 20D**).

[0069] **FIG 21** shows antibody responses in mice after treatment with the gL_gH_C5 nanoparticle (SEQ ID NO: 19) with or without admixed AF03 adjuvant and/or conjugation to SM7/8a as endpoint titers measured by ELISA from the prime, boost, and terminal bleeds.

[0070] **FIG 22** shows antibody responses in mice after treatment with a gp220 nanoparticle with or without admixed AF03 adjuvant and/or conjugation to SM7/8a as endpoint titers measured by ELISA from the prime, boost, and terminal bleeds.

[0071] **FIG 23A** shows the light and heavy chains of *T. ni* ferritin with or without fusion to either gp220 or gL/gH, visualized by Coomassie staining (**FIG 23A**). The 20, 25, 75, 100, and 150 kDa markers in the rightmost lane of **FIG 23A** are labeled. **FIG 23B** provides an illustration of the constructs comprising light and heavy chains of *T. ni* ferritin with or without fusion to either gp220 or gL/gH.

[0072] **FIGs 24A-24D** demonstrate ion exchange (Q column) chromatographic and size-exclusion chromatographic (SEC) purification of gp220-*T. ni* ferritin. Shown are absorbance traces from the Q column at pH 7 (**FIG 24A**) and SEC Superose® 6, 16/600 (**FIG 24B**); Coomassie staining of fractions from Q column at pH 7 (**FIG 24C**) (lanes from left are input ("In"), flow-through ("FT"), mol. wt. ladder (sizes labeled at left in kD), and selected fractions); and Coomassie staining of fractions from SEC Superose® 6, 16/600 (**FIG 24D**) (lanes from left are mol. wt. ladder (sizes labeled at left in kD) and selected fractions). **FIG 24E** shows an illustration of the constructs.

[0073] **FIGs 25A-25D** demonstrate ion exchange (Q column) chromatographic and size-exclusion chromatographic (SEC) purification of gL/gH (Light)/gp220 (Heavy)- *T. ni* ferritin. Shown are absorbance traces from the Q column at pH 7 (**FIG 25A**) and SEC Superose® 6, 16/600 (**FIG 25B**); Coomassie staining of fractions from Q column at pH 7 (**FIG 25C**) (lanes from left are input ("In"), flow-through ("FT"), mol. wt. ladder (sizes labeled at left in kD), and selected fractions); and Coomassie staining of fractions from SEC

Superose® 6, 16/600 (**FIG 25D**) (lanes from left are mol. wt. ladder (sizes labeled at left in kD) and selected fractions). **FIG 25E** shows an illustration of the constructs.

[0074] **FIGs 26A-26H** show gp220-*T. ni* ferritin or gL/gH (light chain)/gp220(heavy chain)-*T. ni* ferritin constructs visualized by Coomassie staining Coomassie (**FIG 26A** and **FIG 26E**), illustrated diagrammatically (**FIG 26B** and **Fig 26F**), characterized by dynamic light scattering (DLS) (**FIG 26D** and **FIG 26H**), and visualized in electron micrographs (**FIG 26C** and **FIG 26G**). **FIGs 26A-26D** present data with gp220 fused to both the light and the heavy chain (diagram in **FIG 26B**). **FIGs 26E-26H** present data with gp220 fused to the heavy chain and gL/gH fused to the light chain (diagram in **FIG 26F**).

[0075] **FIGs 27A-FIG 27C** show naked *T. ni* ferritin particles (i.e., not fused to a non-ferritin polypeptide) visualized by Coomassie staining (**FIG 27A**), visualized in electron micrographs (**FIG 27B**), and characterized by DLS (**FIG 27C**).

[0076] **FIG 28A** shows a SDS denaturing Coomassie-stained gel (at left) with a purified gH/gL/gp42 NP construct (SEQ ID NO: 227), which was expressed in 293 expi cells, and (at right) the size exclusion chromatography (SEC) peak of the gH/gL/gp42 NP. Units of the horizontal axis of the SEC chromatogram are mL. **FIG 28B** shows the gH/gL/gp42 NP purified from the CHO pools to have a dynamic light scattering radius of around 26.2nm.

[0077] **FIG 29A-B** show an assessment of the immune response elicited by a monovalent gH/gL/gp42 nanoparticle composition in combination with a naked ferritin nanoparticle or a bivalent composition (gH/gL/gp42 nanoparticle in combination with a gp220). **FIG 29A** shows B cell neutralization. **FIG 29B** shows epithelial cell neutralization.

[0078] **FIGs 30A-E** show endpoint binding titers against the indicated antigens. **FIGs 30F-G** show an EBV viral neutralizing assay (in B cells and epithelial cells, respectively) of sera from ferrets vaccinated as indicated. Prime = Inj. 1 and Boost = Inj. 2.

[0079] **FIGs 31A-B**: FIG 31A shows purification of gH/gL/gp42_NP_ C12 (SEQ ID NO: 228) using Superose 6 size exclusion chromatography. The arrow depicts the fractions collected from the peak with a denaturing coomassie gel analysis and a western blot analysis using anti-ferritin antibodies. FIG 31B is a dynamic light scattering analysis of the sample in FIG 31A, which shows the particle size radius of 20.6 nm.

[0080] **FIGs 32A-B**: FIG 32A shows purification of gH/gL/gp42_NP_ C13 (SEQ ID NO: 229) using the Superose 6 size exclusion chromatography. The arrow depicts the fractions collected from the peak with a denaturing coomassie gel analysis and a western blot

analysis using anti-ferritin antibodies. FIG 32B is a dynamic light scattering analysis of the sample in FIG 32A, which shows the particle size radius of 17.1 nm.

[0081] **FIGs 33A-B**: FIG 33A shows purification of gH/gL/gp42_NP_ C14 (SEQ ID NO: 230) using the Superose 6 size exclusion chromatography. The arrow depicts the fractions collected from the peak with a denaturing coomassie gel analysis and a western blot analysis using anti-ferritin antibodies. FIG 33B is a dynamic light scattering analysis of the sample in FIG 33A, which shows the particle size radius of 16.9 nm.

[0082] **FIG 34**: An SDS reducing coomassie gel on the left shows the purified single-chain gH/gL/gp42-His product (SEQ ID NO: 226). The protein was purified using Nickel affinity chromatography. On the right is a 2.9 Angstrom crystal structure of the single-chain gH/gL/gp42-His product (SEQ ID NO: 226). Gp42 (in dark gray and indicated with arrows) interacts with the gH/gL heterodimer.

[0083] **FIG 35A-E**: A cartoon of a single-chain construct of gH/gL/gp42 fused to ferritin (as in each of SEQ ID NOs: 227-231) is shown in FIG 35A. The fusion between each protein is via a flexible amino acid linker or a rigid amino acid linker specified above. The single-chain gH/gL/gp42 molecule will assure a 1:1:1 ratio of heterotrimer formation on the nanoparticle. The crystal structure of this heterotrimer has been solved to show that the single-chain gH/gL/gp42 can adopt a heterotrimer formation similar to wild-type gH, gL, and gp42 proteins found in nature (FIG 35B; see also FIG 34). FIG 35C is a model of how this single-chain gH/gL/gp42 heterotrimer is displayed on the nanoparticle through the fusion with ferritin. There are twenty-four copies of the single-chain gH/gL/gp42 displayed on a single nanoparticle. FIG 35D shows the purification after expression of SEQ ID NOs: 227 in 293Expi cells. A denaturing SDS Coomassie gel shows the gH/gL/gp42 fused to ferritin to be above 150kD with glycosylation. FIG 35E shows negative stain electron microscopy analysis of the purified product, indicating that the single-chain gH/gL/gp42 fused to ferritin can successfully form nanoparticles displaying the gH/gL/gp42 antigens on the surface.

DETAILED DESCRIPTION

[0084] EBV polypeptides are provided, which can be antigenic when administered alone, with adjuvant as a separate molecule, and/or as part of a nanoparticle (e.g., ferritin particle or lumazine synthase particle), which can be self-adjuvanting. Such polypeptides and compositions comprising such polypeptides can be used to elicit antibody responses against Epstein Barr virus (EBV). The EBV polypeptide can comprise a gL, gH, gL/gH, gp220, or gp42 polypeptide, or combinations thereof, and a multimerization domain such as a ferritin.

The ferritin may comprise a mutation replacing a surface-exposed amino acid with a cysteine, which can facilitate conjugating immune-stimulatory moieties to the ferritin via the cysteine. Such conjugation may eliminate or reduce the need for separately administered adjuvant, and may also potentially reduce the amount of adjuvant/immune-stimulatory moiety needed to elicit an immune response to the EBV polypeptide. In some embodiments, an antigenic EBV polypeptide comprising (i) an EBV polypeptide, and (ii-a) a ferritin comprising a surface-exposed cysteine, or (ii-b) a ferritin and an N- or C- terminal linker comprising a cysteine is provided. Any of the EBV polypeptides described herein can be combined with any of the ferritins described below. Nucleic acids that encode the polypeptides described herein are also provided.

A. Definitions

[0085] As used herein, an “EBV polypeptide” refers to a polypeptide comprising all or part of an amino acid sequence encoded by EBV. Similarly, gL, gH, gp42, and gp220 polypeptides refer to polypeptides comprising all or part of a gL, gH, gp42, or gp220 amino acid sequence, respectively, encoded by EBV. Polypeptides with, e.g., at least 80% identity to an EBV-encoded polypeptide will necessarily comprise part of the EBV-encoded polypeptide. The terms “gL polypeptide,” “gH polypeptide,” “gp42 polypeptide,” and “gp220 polypeptide” are used interchangeably with “EBV gL polypeptide,” “EBV gH polypeptide,” “EBV gp42 polypeptide,” and “EBV gp220 polypeptide,” respectively. Immunization with an EBV polypeptide as part or all of an antigenic polypeptide may confer protection from infection with EBV. Unless the context dictates otherwise, any polypeptide disclosed herein comprising an EBV polypeptide can comprise all or part of multiple sequences encoded by EBV (for example, all or part of gL and gH of EBV, or all or part of gL, gH, and gp42 of EBV).

[0086] As used herein, a “monomer,” or “monomer construct” refers to a construct expressed as a single-chain protein. A monomer may comprise gL and gH of EBV expressed in a single chain, or gL, gH, and gp42 of EBV expressed in a single chain.

[0087] As used herein, a “trimer,” or “trimer construct” refers to a construct comprising gL and/or gH of EBV together with a trimerization domain, such as a foldon trimerization domain derived from T4 phage fibritin. Other trimerization domains, such as the human collagen XVIII trimerization domain (*see, e.g.*, Alvarez-Cienfuegos et al., Scientific Reports 2016; 6:28643) and the L1ORF1p trimerization domain (*see, e.g.*, Khazina et al.,

Proc Natl Acad Sci U S A 2009 Jan 12; 106(3):731-36) are also known in the art and can be used in trimeric constructs.

[0088] "Ferritin" or "ferritin protein," as used herein, refers to a protein with detectable sequence identity to *H. pylori* ferritin (SEQ ID NO: 208 or 209) or another ferritin discussed herein, such as *P. furiosus* ferritin, *Trichoplusia ni* ferritin, or human ferritin, that serves to store iron, e.g., intracellularly or in tissues or to carry iron in the bloodstream. Such exemplary ferritins, including those that occur as two polypeptide chains, known as the heavy and light chains (e.g., *T. ni* and human ferritin), are discussed in detail below. In some embodiments, a ferritin comprises a sequence with at least 15%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 99.5% identity to a ferritin sequence disclosed herein, e.g., in Table 2 (Sequence Table). A ferritin may be a fragment of a full-length naturally-occurring sequence.

[0089] "Wild-type ferritin," as used herein, refers to a ferritin whose sequence consists of a naturally-occurring sequence. Ferritins also include full-length ferritin or a fragment of ferritin with one or more differences in its amino acid sequence from a wild-type ferritin.

[0090] As used herein, a "ferritin monomer" refers to a single ferritin molecule (or, where applicable, a single ferritin heavy or light chain) that has not assembled with other ferritin molecules. A "ferritin multimer" comprises multiple associated ferritin monomers. A "ferritin protein" includes monomeric ferritin and multimeric ferritin.

[0091] As used herein, "ferritin particle," refers to ferritin that has self-assembled into a globular form. Ferritin particles are sometimes referred to as "ferritin nanoparticles" or simply "nanoparticles". In some embodiments, a ferritin particle comprises 24 ferritin monomers (or, where applicable, 24 total heavy and light chains).

[0092] "Hybrid ferritin," as used herein, refers to ferritin comprising *H. pylori* ferritin with an amino terminal extension of bullfrog ferritin. An exemplary sequence used as an amino terminal extension of bullfrog ferritin appears as SEQ ID NO: 217. In hybrid ferritin, the amino terminal extension of bullfrog ferritin can be fused to *H. pylori* ferritin such that immune-stimulatory moiety attachment sites are distributed evenly on the ferritin particle surface. "Bullfrog linker" as used herein is a linker comprising the sequence of SEQ ID NO: 217. Hybrid ferritin is also sometimes referred to as "bfpFerr" or "bfp ferritin." Any of the constructs comprising a bullfrog sequence can be provided without the bullfrog sequence, such as, for example, without a linker or with an alternative linker. Exemplary bullfrog linker

sequences are provided in Table 2. Where Table 2 shows a bullfrog linker, the same construct may be made without a linker or with an alternative linker.

[0093] "N-glycan," as used herein, refers to a saccharide chain attached to a protein at the amide nitrogen of an N (asparagine) residue of the protein. As such, an N-glycan is formed by the process of N-glycosylation. This glycan may be a polysaccharide.

[0094] "Glycosylation," as used herein, refers to the addition of a saccharide unit to a protein.

[0095] "Immune response," as used herein, refers to a response of a cell of the immune system, such as a B cell, T cell, dendritic cell, macrophage or polymorphonucleocyte, to a stimulus such as an antigen or vaccine. An immune response can include any cell of the body involved in a host defense response, including for example, an epithelial cell that secretes an interferon or a cytokine. An immune response includes, but is not limited to, an innate and/or adaptive immune response. As used herein, a "protective immune response" refers to an immune response that protects a subject from infection (e.g., prevents infection or prevents the development of disease associated with infection). Methods of measuring immune responses are well known in the art and include, for example, by measuring proliferation and/or activity of lymphocytes (such as B or T cells), secretion of cytokines or chemokines, inflammation, antibody production and the like. An "antibody response" is an immune response in which antibodies are produced.

[0096] As used herein, an "antigen" refers to an agent that elicits an immune response, and/or an agent that is bound by a T cell receptor (e.g., when presented by an MHC molecule) or to an antibody (e.g., produced by a B cell) when exposed or administered to an organism. In some embodiments, an antigen elicits a humoral response (e.g., including production of antigen-specific antibodies) in an organism. Alternatively, or additionally, in some embodiments, an antigen elicits a cellular response (e.g., involving T-cells whose receptors specifically interact with the antigen) in an organism. A particular antigen may elicit an immune response in one or several members of a target organism (e.g., mice, rabbits, primates, humans), but not in all members of the target organism species. In some embodiments, an antigen elicits an immune response in at least about 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% of the members of a target organism species. In some embodiments, an antigen binds to an antibody and/or T cell receptor, and may or may not induce a particular physiological response in an organism. In some embodiments, for example, an antigen may

bind to an antibody and/or to a T cell receptor in vitro, whether or not such an interaction occurs in vivo. In some embodiments, an antigen reacts with the products of specific humoral or cellular immunity, including those induced by heterologous immunogens. Antigens include antigenic ferritin proteins comprising ferritin (e.g., comprising one or more mutations) and a non-ferritin polypeptide as described herein.

[0097] An "immune-stimulatory moiety," as used herein, refers to a moiety that is covalently attached to a ferritin or antigenic ferritin polypeptide and that can activate a component of the immune system (either alone or when attached to ferritin or antigenic ferritin polypeptide). Exemplary immune-stimulatory moieties include agonists of toll-like receptors (TLRs), e.g., TLR 4, 7, 8, or 9. In some embodiments, an immune-stimulatory moiety is an adjuvant.

[0098] "Adjuvant," as used herein, refers to a substance or vehicle that non-specifically enhances the immune response to an antigen. Adjuvants can include, without limitation, a suspension of minerals (e.g., alum, aluminum hydroxide, or phosphate) on which antigen is adsorbed; a water-in-oil or oil-in-water emulsion in which antigen solution is emulsified in mineral oil or in water (e.g., Freund's incomplete adjuvant). Sometimes killed mycobacteria is included (e.g., Freund's complete adjuvant) to further enhance antigenicity. Immuno-stimulatory oligonucleotides (e.g., a CpG motif) can also be used as adjuvants (for example, *see* U.S. Patent Nos. 6,194,388; 6,207,646; 6,214,806; 6,218,371; 6,239,116; 6,339,068; 6,406,705; and 6,429,199). Adjuvants can also include biological molecules, such as Toll-Like Receptor (TLR) agonists and costimulatory molecules. An adjuvant may be administered as a separate molecule in a composition or covalently bound (conjugated) to ferritin or an antigenic ferritin polypeptide.

[0099] An "antigenic EBV polypeptide" is used herein to refer to a polypeptide comprising all or part of an EBV amino acid sequence of sufficient length that the molecule is antigenic with respect to EBV. Antigenicity may be a feature of the EBV sequence as part of a construct further comprising a heterologous sequence, such as a ferritin or lumazine synthase protein and/or immune-stimulatory moiety. That is, if an EBV sequence is part of a construct further comprising a heterologous sequence, then it is sufficient that the construct can serve as an antigen that generates anti-EBV antibodies, regardless of whether the EBV sequence without the heterologous sequence could do so.

[00100] "Antigenic ferritin polypeptide" and "antigenic ferritin protein" are used interchangeably herein to refer to a polypeptide comprising a ferritin and a non-ferritin

polypeptide (e.g., an EBV polypeptide) of sufficient length that the molecule is antigenic with respect to the non-ferritin polypeptide. The antigenic ferritin polypeptide may further comprise an immune-stimulatory moiety. Antigenicity may be a feature of the non-ferritin sequence as part of the larger construct. That is, it is sufficient that the construct can serve as an antigen against the non-ferritin polypeptide, regardless of whether the non-ferritin polypeptide without the ferritin (and immune-stimulatory moiety if applicable) could do so. In some embodiments, the non-ferritin polypeptide is an EBV polypeptide, in which case the antigenic ferritin polypeptide is also an "antigenic EBV polypeptide." To be clear, however, an antigenic EBV polypeptide does not need to comprise ferritin. "Antigenic polypeptide" is used herein to refer to a polypeptide which is either or both of an antigenic ferritin polypeptide and an antigenic EBV polypeptide.

[00101] "Self-adjuvanting," as used herein, refers to a composition or polypeptide comprising a ferritin and an immune-stimulatory moiety directly conjugated to the ferritin so that the ferritin and immune-stimulatory moiety are in the same molecular entity. An antigenic ferritin polypeptide comprising a non-ferritin polypeptide may be conjugated to an immune-stimulatory moiety to generate a self-adjuvanting polypeptide.

[00102] A "surface-exposed" amino acid, as used herein, refers to an amino acid residue in a protein (e.g., a ferritin) with a side chain that can be contacted by solvent molecules when the protein is in its native three-dimensional conformation after multimerization, if applicable. Thus, for example, in the case of ferritin that forms a 24-mer, a surface-exposed amino acid residue is one whose side chain can be contacted by solvent when the ferritin is assembled as a 24-mer, e.g., as a ferritin multimer or ferritin particle.

[00103] As used herein, a "subject" refers to any member of the animal kingdom. In some embodiments, "subject" refers to humans. In some embodiments, "subject" refers to non-human animals. In some embodiments, subjects include, but are not limited to, mammals, birds, reptiles, amphibians, fish, insects, and/or worms. In certain embodiments, the non-human subject is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, and/or a pig). In some embodiments, a subject may be a transgenic animal, genetically-engineered animal, and/or a clone. In certain embodiments of the present invention the subject is an adult, an adolescent or an infant. In some embodiments, terms "individual" or "patient" are used and are intended to be interchangeable with "subject".

[00104] As used herein, the term “vaccination” or “vaccinate” refers to the administration of a composition intended to generate an immune response, for example to a disease-causing agent. Vaccination can be administered before, during, and/or after exposure to a disease-causing agent, and/or to the development of one or more symptoms, and in some embodiments, before, during, and/or shortly after exposure to the agent. In some embodiments, vaccination includes multiple administrations, appropriately spaced in time, of a vaccinating composition.

[00105] The disclosure describes nucleic acid sequences and amino acid sequences having a certain degree of identity to a given nucleic acid sequence or amino acid sequence, respectively (a references sequence).

[00106] “Sequence identity” between two nucleic acid sequences indicates the percentage of nucleotides that are identical between the sequences. “Sequence identity” between two amino acid sequences indicates the percentage of amino acids that are identical between the sequences.

[00107] The terms “% identical”, “% identity” or similar terms are intended to refer, in particular, to the percentage of nucleotides or amino acids which are identical in an optimal alignment between the sequences to be compared. Said percentage is purely statistical, and the differences between the two sequences may be but are not necessarily randomly distributed over the entire length of the sequences to be compared. Comparisons of two sequences are usually carried out by comparing said sequences, after optimal alignment, with respect to a segment or “window of comparison”, in order to identify local regions of corresponding sequences. The optimal alignment for a comparison may be carried out manually or with the aid of the local homology algorithm by Smith and Waterman, 1981, Ads App. Math. 2, 482, with the aid of the local homology algorithm by Needleman and Wunsch, 1970, J. Mol. Biol. 48, 443, with the aid of the similarity search algorithm by Pearson and Lipman, 1988, Proc. Natl Acad. Sci. USA 88, 2444, or with the aid of computer programs using said algorithms (GAP, BESTFIT, FASTA, BLAST P, BLAST N and TFASTA in Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, Wis.).

[00108] Percentage identity is obtained by determining the number of identical positions at which the sequences to be compared correspond, dividing this number by the number of positions compared (e.g., the number of positions in the reference sequence) and multiplying this result by 100.

[00109] In some embodiments, the degree of identity is given for a region which is at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90% or about 100% of the entire length of the reference sequence. For example, if the reference nucleic acid sequence consists of 200 nucleotides, the degree of identity is given for at least about 100, at least about 120, at least about 140, at least about 160, at least about 180, or about 200 nucleotides, in some embodiments in continuous nucleotides. In some embodiments, the degree of identity is given for the entire length of the reference sequence.

[00110] Nucleic acid sequences or amino acid sequences having a particular degree of identity to a given nucleic acid sequence or amino acid sequence, respectively, may have at least one functional property of said given sequence, e.g., and in some instances, are functionally equivalent to said given sequence. One important property includes the ability to act as a cytokine, in particular when administered to a subject. In some embodiments, a nucleic acid sequence or amino acid sequence having a particular degree of identity to a given nucleic acid sequence or amino acid sequence is functionally equivalent to said given sequence.

[00111] As used herein, the term "kit" refers to a packaged set of related components, such as one or more compounds or compositions and one or more related materials such as solvents, solutions, buffers, instructions, or desiccants.

B. Antigenic EBV polypeptides comprising gL and gH polypeptides

[00112] EBV has three glycoproteins, glycoprotein B (gB), gH, and gL, that form the core membrane fusion machinery to allow viral penetration into a cell. gL and gH have been previously described, for example, in Matsuura et al., Proc Natl Acad Sci U S A. 2010 Dec 28;107(52):22641-6. Monomers and trimers of gL and gH for use as vaccines have been described, for example, in Cui et al., Vaccine. 2016 Jul 25;34(34):4050-5. The gH and gL proteins associate to form a heterodimeric complex considered necessary for efficient membrane fusion and binding to epithelial cell receptors required for viral entry.

[00113] Disclosed herein are antigenic polypeptides comprising EBV gL and EBV gH. In some embodiments, the polypeptide exists as a single-chain. In some embodiments, the polypeptide forms a trimer, e.g., through trimerization of a trimerization domain, such as a T4 phage fibritin trimerization domain. In some embodiments, the polypeptide forms a nanoparticle (e.g., ferritin or lumazine synthase particle), e.g., through multimerization of a ferritin or lumazine synthase. In some embodiments, an antigenic EBV polypeptide according

to this disclosure comprises an EBV gL polypeptide and an EBV gH polypeptide, and a linker having a length of at least 15 amino acids separating the EBV gL polypeptide and the EBV gH polypeptide. It has been found that a relatively long linker can provide benefits such as improved expression and/or immunogenicity.

[00114] In some embodiments, the EBV gH and/or gL polypeptides are full-length gH and/or gL (for exemplary full-length sequences, *see* GenBank Accession Nos. CEQ35765.1 and YP_001129472.1, respectively). In some embodiments, the EBV gH and/or gL polypeptides are fragments of gH and/or gL. In some embodiments, the gL polypeptide is a gL(D7) construct with a 7-amino acid deletion at the end of the gL C terminus. In some embodiments, the gH polypeptide comprises a mutation at C137, such as a C137A mutation. In some embodiments, the C137 mutation removes a native, unpaired cysteine to avoid non-specific conjugation. In some embodiments, the gH polypeptide comprises a mutation to remove a cysteine corresponding to cysteine 137 of SEQ ID NO: 37, such as a C137A mutation. In some embodiments, the C137 mutation removes a native, unpaired cysteine to avoid non-specific conjugation.

[00115] In some embodiments, the EBV gL polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%,98%, 99%, or 100% identity to SEQ ID NO: 36. In some embodiments, the EBV gH polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 37.

[00116] In some embodiments, a mammalian leader sequence (also known as a signal sequence) is appended N-terminally to an EBV polypeptide such as a gH or gL polypeptide, e.g., at the N-terminus of the polypeptide. In some embodiments, a mammalian leader sequence results in secretion of a protein when expressed in mammalian cells.

[00117] Native EBV gH and/or gL sequences are shown in GenBank Accession No. NC_009334.1 (Human herpesvirus 4, complete genome, dated 26-Mar-2010). For some of the constructs disclosed herein, amino acids 23-137 of the gL amino acid sequence in NC_009334.1 was used as the gL polypeptide, and the native signal peptide (amino acids 1-22 of the NCBI sequence) was replaced with an IgGκ leader sequence. For some of the constructs, amino acids 19-678 of the gH amino acid sequence in NC_009334.1 was used as the gH polypeptide. In some embodiments, the gL and gH were linked via a linker as shown in the table of sequences herein.

[00118] In some embodiments, gL and gH polypeptides are expressed as a single-chain monomer. In some embodiments, the monomer composition comprises or consists of a

sequence shown in the Sequence Table and denoted in the description as “monomer”. A single-chain comprising gL and gH polypeptides may be referred to as “gL/gH,” which can be used interchangeably with “gH_gL,” “gL_gH,” or “gL/gH.”

[00119] In some embodiments, gL and gH are provided as a trimer. In some embodiments, a trimerization domain is placed after (C-terminal to) the gH sequence and in some embodiments, this is followed by a His_6 (SEQ ID NO: 243) sequence. The foldon trimerization domain is exemplary, as any trimerization domain known in the art can be used, such as collagen or L1ORF1p trimerization domains referenced herein. A gL and gH trimer has been shown to induce higher serum neutralization titers relative to a gL and gH monomer using peripheral blood human naïve B cells (*see*, for example, Cui et al., Vaccine. 2016 Jul 25;34(34):4050-5).

[00120] In some embodiments, a gL/gH trimer has an amino acid sequence comprising or consisting of a sequence shown in the Sequence Table and denoted in the description as “trimer.”

[00121] The gL/gH polypeptide can be combined with any of the ferritins or lumazine synthases discussed herein. For example, in some embodiments, an antigenic EBV polypeptide comprises a monomer or trimer gL/gH polypeptide (+/- gp42 and/or gp220) and i) a heavy or light chain ferritin (e.g., *T. ni* heavy or light chain ferritin); or ii) a ferritin, optionally comprising a surface-exposed cysteine.

[00122] Additionally, in some embodiments, any antigenic EBV polypeptide comprising an EBV gL/gH polypeptide and a ferritin can be present in a composition comprising another polypeptide disclosed herein.

C. Antigenic EBV polypeptides comprising a gp220 polypeptide

[00123] In some embodiments, an antigenic EBV polypeptide comprises a gp220 polypeptide. A gp220-hybrid bullfrog/*H. pylori* ferritin nanoparticle has been previously described in Kanekiyo Cell. 2015 Aug 27;162(5):1090-100. This nanoparticle did not comprise a mutation providing a surface-exposed cysteine or a linker comprising a cysteine, among other differences from certain ferritins described herein.

[00124] In some embodiments, the gp220 polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 38.

[00125] In some embodiments, a mammalian leader sequence (also known as a signal sequence) is N-terminally appended to a gp220 polypeptide. In some embodiments, a mammalian leader sequence results in secretion of a protein when expressed in mammalian cells.

[00126] The gp220 polypeptide can be combined with any of the ferritins or lumazine synthases discussed herein. For example, in some embodiments, an antigenic EBV polypeptide comprises a gp220 polypeptide (+/- gL/gH and/or gp42) and i) a heavy or light chain ferritin (e.g., *T. ni* heavy or light chain ferritin); or ii) a ferritin, optionally comprising a surface-exposed cysteine as described herein.

[00127] Additionally, in some embodiments, any antigenic EBV polypeptide comprising a gp220 polypeptide and a ferritin can be present in a composition comprising another polypeptide disclosed herein.

D. Antigenic EBV polypeptides comprising a gp42 polypeptide

[00128] In some embodiments, an antigenic EBV polypeptide comprises a gp42 polypeptide. An exemplary gp42 sequence is provided as SEQ ID NO: 34. A further exemplary gp42 sequence, suitable for inclusion in fusions e.g. with gL and gH polypeptides, is provided as SEQ ID NO: 239. Another exemplary gp42 sequence, suitable for inclusion in fusions e.g. with gL and gH polypeptides, is provided as SEQ ID NO: 240.

[00129] In some embodiments, the gp42 polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 34. In some embodiments, the gp42 polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 239. In some embodiments, the gp42 polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 240.

[00130] In some embodiments, a mammalian leader sequence (also known as a signal sequence) is N-terminally appended to a gp42 polypeptide. In some embodiments, a mammalian leader sequence results in secretion of a protein when expressed in mammalian cells. An exemplary leader sequence is amino acids 1-22 of SEQ ID NO: 226.

[00131] In some embodiments, an antigenic EBV polypeptide comprising a gH and/or gL polypeptide further comprises a gp42 polypeptide. Any of the EBV polypeptides comprising a gH and/or gL polypeptide described above can further comprise a gp42 polypeptide. In some embodiments, the gp42 polypeptide is located C-terminal to the gH

and/or gL polypeptide(s), as exemplified in SEQ ID NOs: 21 and 226-231. In some embodiments, the gp42 polypeptide is located N-terminal to a ferritin, also as exemplified in SEQ ID NOs: 21 and 227-231. Thus, for example, an antigenic EBV polypeptide may comprise, in N- to C-terminal orientation, a gL polypeptide, a gH polypeptide, a gp42 polypeptide, and optionally a ferritin. Linkers such as those described herein can separate the gp42 polypeptide from EBV polypeptides and/or ferritins located N-terminal and/or C-terminal thereto. In some embodiments, a linker separates each EBV polypeptide in an antigenic ferritin polypeptide (e.g., a gL polypeptide, a gH polypeptide, and a gp42 polypeptide), and a further linker may be present between the ferritin if present and the EBV polypeptide proximal thereto (e.g., a gp42 polypeptide).

[00132] In some embodiments, a linker having a length of at least 15 amino acids separates the EBV gH polypeptide and the EBV gp42 polypeptide. Such a linker may have a length of 15 to 60 amino acids, 20 to 60 amino acids, 30 to 60 amino acids, 40 to 60 amino acids, 30 to 50 amino acids, or 40 to 50 amino acids. In some embodiments, the linker comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 234.

[00133] In some embodiments, where gp42 and ferritin are present in a polypeptide, a linker separates the EBV gp42 polypeptide and the ferritin. Such a linker may have a length of at least 15 amino acids or has a length of 15 to 60 amino acids, 20 to 60 amino acids, 30 to 60 amino acids, 40 to 60 amino acids, 30 to 50 amino acids, or 40 to 50 amino acids. In some embodiments, such a linker comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to any one of SEQ ID NOs: 233, 234, 235, 236, 237, or 238.

[00134] The gp42 polypeptide can be combined with any of the ferritins or lumazine synthases discussed herein. For example, in some embodiments, a polypeptide comprises a gp42 polypeptide (+/- gL/gH and/or gp220) and a heavy or light chain ferritin (e.g., *T. ni* heavy or light chain ferritin); or ii) ferritin, optionally comprising a surface-exposed cysteine as described herein.

[00135] In some embodiments, the antigenic EBV polypeptide comprises a sequence with at least 80%, 85%, 90%, 95%, 98%, or 99% identity to amino acids 23-1078 of SEQ ID NO: 226. In some embodiments, the antigenic EBV polypeptide comprises a sequence with 80%, 85%, 90%, 95%, 98%, or 99% identity to amino acids 1-1078 of SEQ ID NO: 226. In some embodiments, the antigenic EBV polypeptide comprises a sequence with at least 80%,

85%, 90%, 95%, 98%, or 99% identity to any one of SEQ ID NOs: 226, 227, 228, 229, 230, or 231, optionally lacking the leader sequence (e.g., lacking any or all of amino acids 1-22 of these sequences).

[00136] Additionally, in some embodiments, any antigenic EBV polypeptide comprising a gp42 polypeptide and a ferritin can be present in a composition comprising another polypeptide disclosed herein.

E. Linkers

[00137] In some embodiments, an antigenic EBV polypeptide comprises a linker between gL and gH polypeptides. In some embodiments, an antigenic EBV polypeptide comprises a linker between an EBV polypeptide and a ferritin or lumazine synthase. The following features are described with respect to either of such linkers, although the present invention provides that a relatively long linker between the gL and gH sequences may provide an increase in immunogenicity. Any linker may be used; for example, in some embodiments, the linker is 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids in length. In some embodiments, the linker is about 2-4, 2-6, 2-8, 2-10, 2-12, or 2-14 amino acids in length. In some embodiments, the linker is a peptide linker, which can facilitate expression of the antigenic ferritin polypeptide as a fusion protein (e.g., from a single open reading frame). In some embodiments, the linker is a glycine-serine linker. In some embodiments, the glycine-serine linker is GS, GGGS (SEQ ID NO: 244), 2XGGGS (i.e., GGGSGGGS) (SEQ ID NO: 245), or 5XGGGS (SEQ ID NO: 246). In some embodiments, the linker between the EBV polypeptide and ferritin is GS, GGGS (SEQ ID NO: 244), 2XGGGS (i.e., GGGSGGGS) (SEQ ID NO: 245), or 5XGGGS (SEQ ID NO: 246).

[00138] In some embodiments, the linker is at least 15 amino acids in length. In some embodiments, the linker is at least 25 amino acids in length. In some embodiments, the linker is at least 30 amino acids in length. In some embodiments, the linker is at least 35 amino acids in length. In some embodiments, the linker is at least 40 amino acids in length. In some embodiments, the linker is less than or equal to 60 amino acids in length. In some embodiments, the linker is less than or equal to 50 amino acids in length. In some embodiments, the linker is about 16, 28, 40, 46, or 47 amino acids in length. In some embodiments, the linker is flexible. In some embodiments, the linker comprises a cysteine, e.g., for use as a site for conjugation of an immune-stimulatory moiety (e.g., adjuvant); an exemplary linker comprising a cysteine is provided as SEQ ID NO: 225. In some

embodiments, the linker comprises a sequence with at least 75%, 80%, 85%, 90%, or 95% identity to SEQ ID NO: 225, and further comprises a cysteine corresponding to the cysteine in SEQ ID NO: 225. In some embodiments, the linker comprises at least 25 amino acids (e.g., 25 to 60 amino acids), wherein a cysteine is located at a position ranging from the 8th amino acid from the N-terminus to the 8th amino acid from the C-terminus, or within 10 amino acids of the central residue or bond of the linker.

[00139] In some embodiments, the linker comprises glycine (G) and/or serine (S) amino acids. In some embodiments, the linker comprises or consists of glycine (G), serine (S), asparagine (N), and/or alanine (A) amino acids, and optionally a cysteine as discussed above. In some embodiments, the linker comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 222. In some embodiments, the linker comprises GGGGSGGGGSGGGGSG (SEQ ID NO: 28), GGSGSGSNSSASSGASSGGASGGSGGSG (SEQ ID NO: 29), GGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGASGGSGGSG (SEQ ID NO: 30), or GS. In some embodiments, the linker comprises FR1 (SEQ ID NO: 31) or FR2 (SEQ ID NO: 32). In some embodiments, the linker comprises SEQ ID NO: 233-238.

[00140]

[00141] In some embodiments, a linker comprising a cysteine as a conjugation site for an immune-stimulatory moiety such as an adjuvant is used in a construct comprising a ferritin molecule lacking an unpaired, surface-exposed cysteine, or in a construct comprising a ferritin molecule comprising an unpaired, surface-exposed cysteine.

[00142] In some embodiments, the linker is a cysteine-thrombin-histidine linker. In some embodiments, this linker is used to directly conjugate an EBV polypeptide to ferritin via click chemistry. An exemplary sequence comprising a cysteine-thrombin-histidine linker is SEQ ID NO: 39. Click chemistry suitable for conjugation reactions involving the cysteine-thrombin-histidine linker is discussed herein.

[00143] In some embodiments, a construct does not comprise a linker. In some embodiments, a construct comprises one linker. In some embodiments, a construct comprises two or more than two linkers.

[00144] In some embodiments, the construct comprises a linker between gH and gL but not between the polypeptide and ferritin or vice versa. In some embodiments, the construct only comprises a linker between the polypeptide and ferritin.

F. Antigenic EBV polypeptides comprising an EBV polypeptide and ferritin or lumazine synthase

[00145] In some embodiments, an antigenic EBV polypeptide is provided, comprising an EBV polypeptide and ferritin. The EBV polypeptide can be any of the EBV polypeptides described herein, such as a gL, gH, gL/gH, gp220, gp42 polypeptide, or combinations thereof. The ferritin component of the polypeptide may be a ferritin from any species, and may or may not have mutations, such as a mutation replacing a surface-exposed amino acid with a cysteine as described herein. In some embodiments, the polypeptide comprises the amino acids of any one of SEQ ID NOS: 1-27.

[00146] In some embodiments, the ferritin in the polypeptide is a wild-type ferritin. In some embodiments, the ferritin is bacterial, insect, fungal, bird, or mammalian. In some embodiments, the ferritin is human. In some embodiments, the ferritin is bacterial.

[00147] In some embodiments, the ferritin is a light chain and/or heavy chain ferritin. In some embodiments, the ferritin is an insect ferritin, such as *Trichoplusia ni* heavy chain ferritin (SEQ ID NO: 211) or *Trichoplusia ni* light chain ferritin (SEQ ID NO: 212). In some embodiments, the ferritin is a human ferritin, such as human heavy chain ferritin (SEQ ID NO: 214 or FTH1, GENE ID No: 2495) or human light chain ferritin (SEQ ID NO: 215 or FTL, GENE ID No: 2512). In some embodiments, a ferritin nanoparticle comprises 24 total subunits of heavy chain ferritin and light chain ferritin, such as in human or *Trichoplusia ni* ferritin nanoparticles. *T. ni* ferritin nanoparticles can comprise 12 subunits of heavy chain ferritin and 12 subunits of light chain ferritin.

[00148] In some embodiments, an antigenic EBV polypeptide comprises a light chain ferritin and an EBV polypeptide. In some embodiments, an antigenic EBV polypeptide comprises a heavy chain ferritin and an EBV polypeptide. In some embodiments, an antigenic EBV polypeptide comprising a light chain ferritin and an EBV polypeptide can assemble with a heavy chain ferritin that is not linked to an EBV polypeptide. In some embodiments, an antigenic EBV polypeptide comprising a heavy chain ferritin and an EBV polypeptide can assemble with a light chain ferritin that is not linked to an EBV polypeptide. A ferritin not linked to an EBV polypeptide (or, more generally, a non-ferritin polypeptide) may be referred as a "naked ferritin."

[00149] In some embodiments, an antigenic polypeptide comprising a heavy chain ferritin and a polypeptide can assemble with an antigenic polypeptide comprising a light chain ferritin and an EBV polypeptide to allow presentation of two of the same or different

non-ferritin polypeptides on a single ferritin nanoparticle. In some embodiments, the two different non-ferritin polypeptides are EBV polypeptides. In some embodiments, the two different non-ferritin polypeptides are encoded by EBV and a different infectious agent. In some embodiments, the different non-ferritin polypeptide from a different infectious agent is from a virus or bacterium.

[00150] In some embodiments, an antigenic polypeptide comprising a heavy chain ferritin and a non-ferritin polypeptide can assemble with a polypeptide comprising a light chain ferritin and a non-ferritin polypeptide to produce a bivalent composition.

[00151] In some embodiments, an antigenic polypeptide comprises a light chain ferritin and a gp220 and/or gp42 polypeptide. In some embodiments, an antigenic polypeptide comprises a heavy chain ferritin and a gp220 and/or gp42 polypeptide.

[00152] In some embodiments, an antigenic polypeptide comprises a light chain ferritin and a single-chain gL and gH polypeptide. In some embodiments, an antigenic polypeptide comprises a heavy chain ferritin and a single-chain gL and gH polypeptide.

[00153] In some embodiments, an antigenic polypeptide comprising a light chain ferritin and a gp220 and/or gp42 polypeptide assembles with an antigenic polypeptide comprising a heavy chain ferritin and a single-chain gL and gH polypeptide.

[00154] In some embodiments, an antigenic polypeptide comprising a heavy chain ferritin and a gp220 and/or gp42 polypeptide assembles with an antigenic polypeptide comprising a light chain ferritin and a single-chain gL and gH polypeptide. In some embodiments, twelve (12) gp220 and/or gp42 polypeptides and twelve (12) single-chain gL and gH polypeptides are comprised in an assembled ferritin nanoparticle, as in the case of an assembled *T. ni* ferritin nanoparticle.

[00155] Any type of ferritin nanoparticle(s) that comprises both gp220 and/or gp42 and single-chain gL and gH polypeptides may be referred to as a "bivalent" or "bivalent EBV" particle or construct. A composition comprising a gL and gH trimer together with a ferritin that comprises gp220 and/or gp42 would also be a bivalent EBV composition.

[00156] In some embodiments, the ferritin is *H. pylori* ferritin (*see* SEQ ID NO: 208 or 209 for an exemplary *H. pylori* ferritin sequence), optionally with one or more mutations such as those described herein. In some embodiments, the lower sequence homology between *H. pylori* ferritin (or other bacterial ferritins) and human ferritin may decrease the potential for autoimmunity when used as a vaccine platform (*see* Kanekiyo et al., Cell 162, 1090–1100 (2015)).

[00157] In some embodiments, a nanoparticle is provided comprising an antigenic EBV polypeptide as disclosed herein comprising an EBV polypeptide and a ferritin.

1. Ferritin mutations

[00158] In some embodiments, the ferritin comprises one or more mutations are disclosed herein. In some embodiments, the one or more mutations comprise changes to the amino acid sequence of a wild-type ferritin and/or an insertion, e.g., at the N- or C-terminus. In some embodiments, one, two, three, four, five, or more different amino acids are mutated in the ferritin as compared to wild-type ferritin (in some embodiments, in addition to any N-terminal insertion). The one or more mutations can change functional properties of the ferritin, e.g., as discussed in detail below. In general, a mutation simply refers to a difference in the sequence (such as a substituted, added, or deleted amino acid residue or residues) relative to the corresponding wild-type ferritin.

2. Cysteine for conjugation

[00159] In some embodiments, ferritin is mutated to provide a chemical handle for conjugation of an immune-stimulatory moiety and/or EBV polypeptide. This can be achieved with a mutation replacing a surface-exposed non-cysteine amino acid with a cysteine. For the avoidance of doubt, language such as "replacing a surface-exposed amino acid with a cysteine" necessarily implies that the surface-exposed amino acid in the wild-type or pre-mutation sequence is not cysteine. Another approach for providing a chemical handle for conjugation of an immune-stimulatory moiety or EBV polypeptide is to include a segment of amino acids, such as a linker, N- or C-terminal to the ferritin, wherein the segment of amino acids comprises a cysteine. In some embodiments, this cysteine (whether replacing a surface-exposed amino acid or in an N- or C-terminal linker) is unpaired, which means that it does not have an appropriate partner cysteine to form a disulfide bond. In some embodiments, this cysteine does not change the secondary structure of ferritin. In some embodiments, this cysteine does not change the tertiary structure of ferritin.

[00160] In some embodiments, this cysteine can be used to conjugate agents, such as immune-stimulatory moieties, to ferritin. In some embodiments, this cysteine provides a free thiol group that is reactive. In some embodiments, agents conjugated to this cysteine on ferritin are exposed on the surface of an assembled ferritin particle. In some embodiments, this cysteine can interact with molecules and cells of the subject after administration while the ferritin particle is assembled.

[00161] In some embodiments, the presence of this cysteine allows conjugation of one or more immune-stimulatory moieties, e.g., adjuvants. In some embodiments, conjugation of the immune-stimulatory moiety would not occur in the absence of this cysteine.

[00162] In some embodiments, the non-cysteine amino acid that is replaced with a cysteine is selected from E12, S72, A75, K79, S100, and S111 of *H. pylori* ferritin. Thus, in some embodiments, the surface-exposed amino acid that is replaced in favor of cysteine is an amino acid residue that corresponds to E12, S26, S72, A75, K79, S100, or S111 of *H. pylori* ferritin. Analogous amino acids can be found in non-*H. pylori* ferritin by pair-wise or structural alignment. In some embodiments, the non-cysteine amino acid that is replaced with a cysteine can be selected from an amino acid that corresponds to S3, S19, S33, I82, A86, A102, and A120 of human light chain ferritin. In some embodiments, the surface-exposed amino acid to be replaced with a cysteine is selected based on the understanding that if the native amino acid were replaced with cysteine, it would be reactive in an assembled ferritin multimer or particle and/or that this cysteine does not disrupt the stability of the ferritin multimer or particle and/or that this cysteine does not lead to reduction in expression levels of ferritin.

[00163] In some embodiments, the ferritin comprises an E12C mutation. In some embodiments, the E12C residue can be used to conjugate agents (e.g., immune-stimulatory moieties and/or EBV polypeptides) to ferritin. In some embodiments, the E12C residue provides a free thiol group that is reactive. In some embodiments, agents conjugated to the E12C residue on ferritin monomers are expressed on the surface on an assembled ferritin multimer or particle. In some embodiments, twenty-four E12C residues (one from each monomer) are present on the surface of a ferritin multimer or particle.

[00164] In some embodiments, the ferritin comprises an S26C mutation. In some embodiments, the S26C residue can be used to conjugate agents (e.g., immune-stimulatory moieties and/or EBV polypeptides) to ferritin. In some embodiments, the S26C residue provides a free thiol group that is reactive. In some embodiments, agents conjugated to the S26C residue on ferritin monomers are expressed on the surface on an assembled ferritin multimer or particle. In some embodiments, twenty-four S26C residues (one from each monomer) are present on the surface of a ferritin multimer or particle.

[00165] In some embodiments, the ferritin comprises an S72C mutation. In some embodiments, the S72C residue can be used to conjugate agents (e.g., immune-stimulatory moieties and/or EBV polypeptides) to ferritin. In some embodiments, the S72C residue

provides a free thiol group that is reactive. In some embodiments, agents conjugated to the S72C residue on ferritin monomers are expressed on the surface on an assembled ferritin multimer or particle. In some embodiments, twenty-four S72C residues (one from each monomer) are present on the surface of a ferritin multimer or particle.

[00166] In some embodiments, the ferritin comprises an A75C mutation. In some embodiments, the A75C residue can be used to conjugate agents (e.g., immune-stimulatory moieties and/or EBV polypeptides) to ferritin. In some embodiments, the A75C residue provides a free thiol group that is reactive. In some embodiments, agents conjugated to the A75C residue on ferritin monomers are expressed on the surface on an assembled ferritin multimer or particle. In some embodiments, twenty-four A75C residues (one from each monomer) are present on the surface of a ferritin multimer or particle.

[00167] In some embodiments, the ferritin comprises an K79C mutation. In some embodiments, the K79C residue can be used to conjugate agents (e.g., immune-stimulatory moieties and/or EBV polypeptides) to ferritin. In some embodiments, the K79C residue provides a free thiol group that is reactive. In some embodiments, agents conjugated to the K79C residue on ferritin monomers are expressed on the surface on an assembled ferritin multimer or particle. In some embodiments, twenty-four K79C residues (one from each monomer) are present on the surface of a ferritin multimer or particle.

[00168] In some embodiments, the ferritin comprises an S100C mutation. In some embodiments, the S100C residue can be used to conjugate agents (e.g., immune-stimulatory moieties and/or EBV polypeptides) to ferritin. In some embodiments, the S100C residue provides a free thiol group that is reactive. In some embodiments, agents conjugated to the S100C residue on ferritin monomers are expressed on the surface on an assembled ferritin multimer or particle. In some embodiments, twenty-four S100C residues (one from each monomer) are present on the surface of a ferritin multimer or particle.

[00169] In some embodiments, the ferritin comprises an S111C mutation. In some embodiments, the S111C residue can be used to conjugate agents (e.g., immune-stimulatory moieties and/or EBV polypeptides) to ferritin. In some embodiments, the S111C residue provides a free thiol group that is reactive. In some embodiments, agents conjugated to the S111C residue on ferritin monomers are expressed on the surface on an assembled ferritin multimer or particle. In some embodiments, twenty-four S111C residues (one from each monomer) are present on the surface of a ferritin multimer or particle.

3. Removal of internal cysteine

[00170] In some embodiments, the ferritin comprises a mutation replacing an internal cysteine with a non-cysteine amino acid. Removal of a native internal cysteine residue can ensure that there is only one unpaired cysteine per ferritin monomer and avoid undesired reactions such as disulfide formation and may result in a more stable and efficient result (e.g., adjuvant presentation). In some embodiments, C31 of *H. pylori* ferritin is replaced with a non-cysteine amino acid. In some embodiments, C31 of *H. pylori* ferritin is replaced with a serine (C31S), although any non-cysteine residue may be used, e.g., alanine, glycine, threonine, or asparagine. Analogous amino acids can be found in non-*H. pylori* ferritin by pair-wise or structural alignment. Thus, in some embodiments, the internal cysteine that is replaced in favor of non-cysteine is an amino acid residue that aligns with C31 of *H. pylori* ferritin. Exemplary ferritin sequences showing a C31S mutation are shown in SEQ ID NOS: 201-207. In some embodiments, when more than one internal cysteine is present in ferritin, two or more (e.g., each) internal cysteine is replaced with a non-cysteine amino acid, such as serine or an amino acid selected from serine, alanine, glycine, threonine, or asparagine.

4. Glycosylation

[00171] Human-compatible glycosylation can contribute to safety and efficacy in recombinant drug products. Regulatory approval may be contingent on demonstrating appropriate glycosylation as a critical quality attribute (*see* Zhang et al., Drug Discovery Today 21(5):740-765 (2016)). N-glycans can result from glycosylation of asparagine side chains and can differ in structure between humans and other organisms such as bacteria and yeast. Thus, it may be desirable to reduce or eliminate non-human glycosylation and/or N-glycan formation in ferritin according to the disclosure. In some embodiments, controlling glycosylation of ferritin improves the efficacy and/or safety of the composition, especially when used for human vaccination.

[00172] In some embodiments, ferritin is mutated to inhibit formation of an N-glycan. In some embodiments, a mutated ferritin has reduced glycosylation as compared to its corresponding wild type ferritin.

[00173] In some embodiments, the ferritin comprises a mutation replacing a surface-exposed asparagine with a non-asparagine amino acid. In some embodiments, the surface-exposed asparagine is N19 of *H. pylori* ferritin or a position that corresponds to position 31 of *H. pylori* ferritin as determined by pair-wise or structural alignment In some embodiments, mutating such an asparagine, e.g., N19 of *H. pylori* ferritin, decreases glycosylation of

ferritin. In some embodiments, the mutation replaces the asparagine with a glutamine. In some embodiments, the ferritin is an *H. pylori* ferritin comprising an N19Q mutation. SEQ ID NOS: 201-207 are exemplary ferritin sequences comprising N19Q mutations.

[00174] A mammal exposed to a glycosylated protein produced in bacteria or yeast may generate an immune response to the glycosylated protein, because the pattern of glycosylation of a given protein in bacterial or yeast could be different from the pattern of glycosylation of the same protein in a mammal. Thus, some glycosylated therapeutic proteins may not be appropriate for production in bacteria or yeast.

[00175] In some embodiments, decreased glycosylation of ferritin by amino acid mutation facilitates protein production in bacteria or yeast. In some embodiments, decreased glycosylation of ferritin reduces the potential for adverse effects in mammals upon administration of mutated ferritin that is expressed in bacteria or yeast. In some embodiments, the reactogenicity in a human subject of a mutated ferritin produced in bacteria or yeast is lower because glycosylation is decreased. In some embodiments, the incidence of hypersensitivity responses in human subjects is lower following treatment with a mutated ferritin with reduced glycosylation compared to wildtype ferritin.

[00176] In some embodiments, degradation in a subject of a composition comprising a mutated ferritin with reduced glycosylation is slower compared with a composition comprising a wild-type ferritin, or a composition comprising a corresponding ferritin with wild-type glycosylation. In some embodiments, a composition comprising a mutated ferritin with reduced glycosylation has reduced clearance in a subject compared with a composition comprising a wild-type ferritin, or a composition comprising a corresponding ferritin with wild-type glycosylation. In some embodiments, a composition comprising a mutated ferritin with reduced glycosylation has a longer-serum half-life compared to wild-type ferritin, or a composition comprising a corresponding ferritin with wild-type glycosylation.

5. Combinations of mutations

[00177] In some embodiments, a ferritin comprises more than one type of mutation described herein. In some embodiments, the ferritin comprises one or more mutations independently selected from: a mutation to decrease glycosylation, a mutation to remove an internal cysteine, and a mutation to generate a surface-exposed cysteine. In some embodiments, the ferritin comprises a mutation to decrease glycosylation, a mutation to remove an internal cysteine, and a mutation to generate a surface-exposed cysteine.

[00178] In some embodiments, the ferritin comprises an N19Q mutation, a C31S mutation, and a mutation to generate a surface-exposed cysteine. In some embodiments, the ferritin comprises an N19Q mutation, a C31S mutation, and an E12C mutation. In some embodiments, the ferritin comprises an N19Q mutation, a C31S mutation, and an S72C mutation. In some embodiments, the ferritin comprises an N19Q mutation, a C31S mutation, and an A75C mutation. In some embodiments, the ferritin comprises an N19Q mutation, a C31S mutation, and an K79C mutation. In some embodiments, the ferritin comprises an N19Q mutation, a C31S mutation, and an S100C mutation. In some embodiments, the ferritin comprises an N19Q mutation, a C31S mutation, and an S111C mutation. In some embodiments, the ferritin comprises mutations corresponding to any of the foregoing sets of mutations, wherein the corresponding mutations change an N to a Q, a C to an S, and a non-cysteine surface-exposed amino acid to a cysteine at positions determined by pair-wise alignment of the ferritin amino acid sequence to an *H. pylori* ferritin amino acid sequence (SEQ ID NO: 208 OR 209).

[00179] Exemplary ferritins comprising more than one type of mutation are provided in SEQ ID NOS: 201-207.

6. Structural alignment

[00180] As discussed herein, positions of mutations corresponding to those described with respect to a given polypeptide (e.g, *H. pylori* ferritin) can be identified by pairwise or structural alignment. Structural alignment is relevant to large protein families such as ferritin where the proteins share similar structures despite considerable sequence variation and many members of the family have been structurally characterized, and can also be used to identify corresponding positions in different versions of other polypeptides described herein, such as EBV polypeptides (e.g., gL, gH, gp220, or gp42). The protein databank (PDB) comprises 3D structures for many ferritins, including those listed below with their accession numbers.

[00181] 2jd6, 2jd7 – PfFR - Pyrococcus furiosus. 2jd8 – PfFR+Zn. 3a68 – soFR from gene SferH4 – soybean. 3a9q - soFR from gene SferH4 (mutant). 3egm, 3bvf, 3bvi, 3bvk, 3bvl – HpFR – Heliobacter pylori. 5c6f – HpFR (mutant) + Fe. 1z4a, 1vlg – FR – Thermotoga maritime. 1s3q, 1sq3, 3kx9 – FR – Archaeoglubus fulgidus. 1krq – FR – Campylobacter jejuni. 1eum - EcFR – Escherichia coli. 4reu – EcFR + Fe. 4xgs – EcFR (mutant) + Fe2O2. 4ztt – EcFR (mutant) + Fe2O + Fe2 + Fe + O2. 1qgh – LiFR - Listeria innocua. 3qz3 - VcFR – Vibrio cholerae. 3vnx – FR – Ulva pertusa. 4ism, 4isp, 4itt, 4itw, 4iwj, 4iwk, 4ixk, 3e6s – PnmFR – Pseudo-nitschia multiseries. 4zkh, 4zkw, 4zkx, 4zl5, 4zl6,

4zlw, 4zmc – PnmFR (mutant) + Fe. 1z6o – FR – Trichoplusia ni. 4cmy – FR + Fe – Chlorobaculum tepidum. Ferritin light chain (FTL). 1lb3, 1h96 – mFTL – mouse. 1rcc, 1rcd, 1rci – bFTL+tartrate+Mg. 1rce, 1rcg - bFTL+tartrate+Mn. 3noz, 3np0, 3np2, 3o7r – hoFTL (mutant) - horse. 3o7s, 3u90 - hoFTL. 4v1w – hoFTL – cryo EM. 3rav, 3rd0 – hoFTL + barbiturate. Ferritin light+heavy chains: 5gn8 – hFTH + Ca.

[00182] Structural alignment involves identifying corresponding residues across two (or more) polypeptide sequences by (i) modeling the structure of a first sequence using the known structure of the second sequence or (ii) comparing the structures of the first and second sequences where both are known, and identifying the residue in the first sequence most similarly positioned to a residue of interest in the second sequence. Corresponding residues are identified in some algorithms based on alpha-carbon distance minimization in the overlaid structures (e.g., what set of paired alpha carbons provides a minimized root-mean-square deviation for the alignment). When identifying positions in a non-*H. pylori* ferritin corresponding to positions described with respect to *H. pylori* ferritin, *H. pylori* ferritin can be the “second” sequence. Where a non-*H. pylori* ferritin of interest does not have an available known structure, but is more closely related to another non-*H. pylori* ferritin that does have a known structure than to *H. pylori* ferritin, it may be most effective to model the non-*H. pylori* ferritin of interest using the known structure of the closely related non-*H. pylori* ferritin, and then compare that model to the *H. pylori* ferritin structure to identify the desired corresponding residue in the ferritin of interest. There is an extensive literature on structural modeling and alignment; representative disclosures include US 6859736; US 8738343; and those cited in Aslam et al., Electronic Journal of Biotechnology 20 (2016) 9–13. For discussion of modeling a structure based on a known related structure or structures, *see, e.g.*, Bordoli et al., Nature Protocols 4 (2009) 1–13, and references cited therein.

7. Lumazine synthase

[00183] In some embodiments, the antigenic polypeptide comprises a lumazine synthase protein. Lumazine synthases can form higher-order structures, e.g., a 60-subunit lumazine synthase particle. Exemplary lumazine synthases are *Aquifex aeolicus* lumazine synthase (SEQ ID NO: 40) and *E. coli* lumazine synthase (SEQ ID NO: 41). In some embodiments, the lumazine synthase has at least 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NOS: 40 or 41. The lumazine synthase can be located C-terminal to the EBV polypeptide and can be separated from the EBV polypeptide by a linker as discussed herein.

G. Mutations in gL, gH, gp42, linker, and/or ferritin sequences to eliminate potential oxidation, deamidation, or Isoaspartate formation sites

[00184] In some embodiments, an antigenic EBV polypeptide comprises one or more mutations to eliminate potential oxidation, deamidation, or Isoaspartate formation sites, such as the exemplary mutations set forth in Table 1 below.

[00185] For example, in some embodiments, a gL sequence comprises one or more mutations to eliminate a potential succinimide/isoaspartate or deamidation site. For example, a gL sequence can comprise a G to A mutation at a position corresponding to position 36 of SEQ ID NO: 227; an N to Q mutation at a position corresponding to position 47 of SEQ ID NO: 227; or an N to Q mutation at a position corresponding to position 105 of SEQ ID NO: 227. A position in an amino acid sequence "corresponds" to a given position in SEQ ID NO: 227 if it aligns to that position according to a standard sequence alignment algorithm such as the Smith-Waterman algorithm using default parameters.

[00186] In some embodiments, a linker comprises one or more mutations to eliminate a potential deamidation site. For example, a linker sequence can comprise an N to G mutation at a position corresponding to position 132 or 141 of SEQ ID NO: 227.

[00187] In some embodiments, a gH sequence comprises one or more mutations to eliminate a potential succinimide/isoaspartate or oxidation site. For example, a gH sequence can comprise an M to L mutation at a position corresponding to position 189, 401, or 729 of SEQ ID NO: 227; a D to E mutation at a position corresponding to position 368 of SEQ ID NO: 227; an M to I mutation at a position corresponding to position 499 or 639 of SEQ ID NO: 227; or an N to Q mutation at a position corresponding to position 653 of SEQ ID NO: 227.

[00188] In some embodiments, a gp42 sequence comprises one or more mutations to eliminate a potential deamidation site. For example, a gp42 sequence can comprise an N to Q mutation at a position corresponding to position 959 or 990 of SEQ ID NO: 227; or an N to S mutation at a position corresponding to position 988 of SEQ ID NO: 227.

[00189] In some embodiments, a ferritin sequence comprises one or more mutations to eliminate a potential deamidation, oxidation, or isoaspartate formation site. For example, a ferritin sequence can comprise a Q to S mutation at a position corresponding to position 1150 of SEQ ID NO: 227; an M to I mutation at a position corresponding to position 1168 of SEQ ID NO: 227; an M to L mutation at a position corresponding to position 1177 of SEQ ID NO:

227; a G to A mutation at a position corresponding to position 1188 of SEQ ID NO: 227; or an N to Q mutation at a position corresponding to position 1253 or 1296 of SEQ ID NO: 227.

[00190] Exemplary mutations are shown below in Table 1. The position numbering corresponds to SEQ ID NO: 227.

[00191] Table 1. Exemplary mutations.

Location	Modification	START	END	MOTIF	solvent expsoure	Mutation
gL	Succinimide/IsoAsp	35	36	DG	Exposed	G36A
gL	deamidation	47	47	N	likely exposed	N47Q
gL	deamidation	105	105	N	exposed	N105Q
linker	deamidation	132	132	N	exposed	N132G
linker	deamidation	141	141	N	exposed	N141G
gH	oxidation	189	189	M	exposed	M189L
gH	Succinimide/IsoAsp	368	369	DY	exposed	D368E
gH	oxidation	401	401	M	buried	M401L
gH	Succinimide/IsoAsp	429	430	DT	exposed	D429E
gH	oxidation	499	499	M	exposed	M499I
gH	oxidation	639	639	M	exposed	M639I
gH	oxidation	653	653	N	exposed	N653Q
gH	oxidation	729	729	M	exposed	M729L
gp42	deamidation	959	959	N	exposed	N959Q
gp42	deamidation	988	988	N	exposed	N988S
gp42	deamidation	990	990	N	exposed	N990Q
ferritin	deamidation	1150	1150	Q	exposed	Q1150S
ferritin	oxidation	1168	1168	M	buried	M1168I
ferritin	deamidation	1177	1177	M	buried	M1177L
ferritin	IsoAsp	1187	1188	DG	buried	G1188A
ferritin	deamidation	1253	1253	N	exposed	N1253Q
ferritin	deamidation	1296	1296	N	exposed	N1296Q

H. Immune-stimulatory moieties; Adjuvants; Conjugated EBV polypeptides

[00192] In some embodiments, an EBV polypeptide and/or an immune-stimulatory moiety, such as an adjuvant, is attached to a surface-exposed amino acid. In some embodiments, the surface-exposed amino acid is a cysteine, e.g., resulting from a mutation discussed above. In some embodiments, the surface-exposed amino acid is a lysine, aspartate, or glutamate. Conjugation procedures using glutaraldehyde (for conjugation of a lysine with an amino-bearing linker or moiety) or a carbodiimide (e.g., 1-Cyclohexyl-3-(2-morpholin-4-yl-ethyl) carbodiimide or 1-Ethyl-3-(3-dimethyl-aminopropyl) carbodiimide (EDC; EDAC) for conjugating an aspartate or glutamate to an amino-bearing linker or moiety, or a lysine to a carboxyl-bearing linker or moiety) are described in, e.g., Chapter 4 of Holtzhauer, M., Basic Methods for the Biochemical Lab, Springer 2006, ISBN 978-3-540-32785-1, available from www.springer.com.

[00193] In some embodiments, an immune-stimulatory moiety, such as an adjuvant, is attached to a surface-exposed amino acid of ferritin. In some embodiments, more than one immune-stimulatory moiety, such as an adjuvant, is attached to a surface-exposed amino acid of ferritin. In some embodiments, twenty-four immune-stimulatory moieties are attached to a ferritin multimer or particle (e.g., one moiety for each monomer in the *H. pylori* ferritin particle). In some embodiments with multiple immune-stimulatory moieties attached to a ferritin nanoparticle, all of the immune-stimulatory moieties are identical. In some embodiments with multiple immune-stimulatory moieties attached to a ferritin nanoparticle, all of the immune-stimulatory moieties are not identical.

1. Types of immune-stimulatory moieties; Adjuvants

[00194] Any immune-stimulatory moiety that can be attached to a surface-exposed amino acid (e.g., cysteine) can be used in ferritins according to this disclosure. In some embodiments, the immune-stimulatory moiety is a B cell agonist.

[00195] In some embodiments, the immune-stimulatory moiety is not hydrophobic. In some embodiments, the immune-stimulatory moiety is hydrophilic. In some embodiments, the immune-stimulatory moiety is polar. In some embodiments, the immune-stimulatory moiety is capable of hydrogen bonding or ionic bonding, e.g., comprises a hydrogen bond donor, hydrogen bond acceptor, cationic moiety, or anionic moiety. A moiety is considered

cationic or anionic if it would be ionized in aqueous solution at a physiologically relevant pH, such as pH 6, 7, 7.4, or 8.

[00196] In some embodiments, the immune-stimulatory moiety is an adjuvant. In some embodiments, the adjuvant comprises a pathogen associated molecular pattern (PAMP). In some embodiments, the adjuvant is a toll-like receptor (TLR) agonist or stimulator of interferon genes (STING) agonist. In some embodiments, the adjuvant activates TLR signaling in B and/or T cells. In some embodiments, the adjuvant regulates the adaptive immune response.

a) **TLR2 agonists**

[00197] In some embodiments, the immune-stimulatory moiety is a TLR2 agonist. In some embodiments, the immune-stimulatory moiety stimulates TLR2 signaling. In some embodiments, the immune-stimulatory moiety is a synthetic small molecule ligand of TLR2. In some embodiments, the immune-stimulatory moiety is a synthetic small molecule agonist of TLR2 signaling.

[00198] In some embodiments, the TLR2 agonist is PAM2CSK4, FSL-1, or PAM3CSK4.

b) **TLR7/8 agonists**

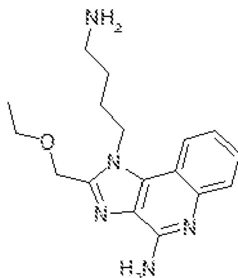
[00199] In some embodiments, the immune-stimulatory moiety is a TLR7 and/or TLR8 agonist (i.e., an agonist of at least one of TLR7 and TLR8). In some embodiments, the immune-stimulatory moiety stimulates TLR7 and/or TLR8 signaling. In some embodiments, the immune-stimulatory moiety is a synthetic small molecule ligand of TLR7 and/or TLR8. In some embodiments, the immune-stimulatory moiety is a synthetic small molecule agonist of TLR7 and/or TLR8 signaling.

[00200] In some embodiments, the TLR7 and/or TLR8 agonist is single-stranded (ssRNA). In some embodiments, the TLR7 and/or TLR8 agonist is an imidazoquinoline. In some embodiments, the TLR7 and/or TLR8 agonist is a nucleoside analog.

[00201] In some embodiments, the TLR7 and/or TLR8 agonist is an imidazoquinolinamine Toll-like receptor (TLR) agonist, such as 3M-012 (3M Pharmaceuticals). The structure of free 3M-012 is:

. It is understood that an immune-stimulatory moiety such as 3M-012 or any moiety discussed herein can be conjugated to a ferritin by substituting an appropriate peripheral atom of the moiety (e.g., a hydrogen) with a bond to a ferritin described herein, e.g., at the sulfur of a surface-exposed cysteine or a linker attached to such a sulfur. Thus, when conjugated to a ferritin, the structure of the immune-stimulatory moiety will differ slightly from the structure of the free molecule.

[00202] In some embodiments the TLR7 and/or TLR8 agonist is SM 7/8a. The structure of free SM 7/8a is:



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[00203] *See, e.g.*, Nat Biotechnol. 2015 Nov;33(11):1201-10. doi: 10.1038/nbt.3371.

c) **TLR9 agonists**

[00204] In some embodiments, the immune-stimulatory moiety is a TLR9 agonist. In some embodiments, the immune-stimulatory moiety stimulates TLR9 signaling. In some embodiments, the immune-stimulatory moiety is a synthetic small molecule ligand of TLR9. In some embodiments, the immune-stimulatory moiety is a synthetic small molecule agonist of TLR9 signaling.

[00205] In some embodiments, the TLR9 agonist is a CpG oligodeoxynucleotide (ODN). In some embodiments, the TLR9 agonist is an unmethylated CpG ODN. In some embodiments, the CpG ODN comprises a partial or complete phosphorothioate (PS) backbone instead of the natural phosphodiester (PO) backbone found in ordinary DNA.

[00206] In some embodiments, the CpG ODN is a Class B ODN, which comprises one or more 6mer CpG motif comprising 5' Purine (Pu)-Pyrimidine (Py)-C-G-Py-Pu 3'; has a fully phosphorothioated (i.e., PS-modified) backbone; and has a length of 18-28 nucleotides.

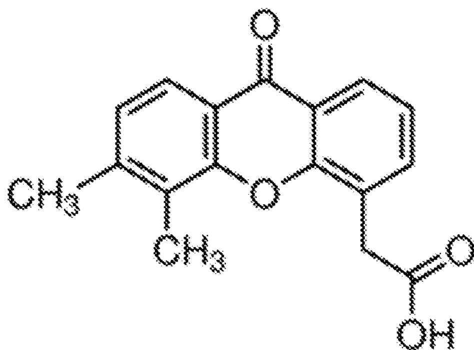
In some embodiments, the CpG ODN comprises the sequence of SEQ ID NO: 210, optionally comprising phosphorothioate linkages in the backbone.

[00207] In some embodiments, the TLR9 agonist comprises an immune-stimulatory sequence (ISS). In some embodiments the TLR9 agonist is ISS-1018 (Dynavax) (SEQ ID NO: 210).

d) STING agonists

[00208] In some embodiments, the immune-stimulatory moiety is a STING (Stimulator of Interferon Genes Protein, also known as Endoplasmic Reticulum IFN Stimulator) agonist. In some embodiments, the immune-stimulatory moiety stimulates STING signaling. In some embodiments, the immune-stimulatory moiety is a synthetic small molecule ligand of STING. In some embodiments, the immune-stimulatory moiety is a synthetic small molecule agonist of STING signaling.

[00209] In some embodiments the STING agonist is a cyclic dinucleotide (CDN). *See, e.g.*, Danilchanka et al., Cell 154:962-970 (2013). Exemplary CDNs include cdA, cdG, cAMP-cGMP, and 2'-5',3'-5' cGAMP (*see* Danilchanka et al. for structures). STING agonists also include synthetic agonists such as DMXAA



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2. Conjugated EBV polypeptides

[00210] In some embodiments, an EBV polypeptide is conjugated to a surface-exposed amino acid of ferritin. In some embodiments, the EBV polypeptide renders the ferritin protein antigenic. In some embodiments, the EBV polypeptide is antigenic alone, whereas in some embodiments, the EBV polypeptide is antigenic because of its association with ferritin. In some embodiments, the EBV polypeptide is any one of the EBV polypeptides described herein.

3. Conjugation

[00211] In some embodiments, a surface-exposed cysteine (e.g., resulting from a mutation described herein) or a cysteine in a peptide linker attached to ferritin (e.g., N-terminally to ferritin) is used to conjugate an immune-stimulatory moiety, such as an adjuvant, or an EBV polypeptide to a ferritin. In some embodiments, a linker is conjugated to such a cysteine, which linker can be subsequently conjugated to an immune-stimulatory moiety, such as an adjuvant, or an EBV polypeptide. In some embodiments, such a cysteine creates a chemical handle for conjugation reactions to attach an adjuvant, linker, or an EBV polypeptide. In some embodiments, bioconjugates are produced, wherein an immune-stimulatory moiety, such as an adjuvant, or an EBV polypeptide is linked to a ferritin after reduction of such a cysteine. In some embodiments, the cysteine is an unpaired surface-exposed cysteine, i.e., that lacks a partner cysteine in an appropriate position to form a disulfide bond. In some embodiments, the cysteine is an unpaired cysteine that comprises a free thiol side chain.

a) Types of conjugation chemistries

[00212] Any type chemistry can be used to conjugate the immune-stimulatory moiety, such as an adjuvant, or an EBV polypeptide to the ferritin, e.g., via reaction a surface-exposed amino acid such as cysteine or another amino acid such as Lys, Glu, or Asp.

[00213] In some embodiments, the conjugation is performed using click chemistry. As used herein, “click chemistry” refers to a reaction between a pair of functional groups that rapidly and selective react (i.e., “click”) with each other. In some embodiments, the click chemistry can be performed under mild, aqueous conditions. In some embodiments, a click chemistry reaction takes advantage of a cysteine on the surface of the ferritin, such as a cysteine resulting from mutation of a surface-exposed amino acid, to perform click chemistry using a functional group that can react with the cysteine.

[00214] A variety of reactions that fulfill the criteria for click chemistry are known in the field, and one skilled in the art could use any one of a number of published methodologies (*see, e.g.*, Hein et al., Pharm Res 25(10):2216-2230 (2008)). A wide range of commercially available reagents for click chemistry could be used, such as those from Sigma Aldrich, Jena Bioscience, or Lumiprobe. In some embodiments, conjugation is performed using click chemistry as described in the Examples below.

[00215] In some embodiments, the click chemistry reaction occurs after reduction of the ferritin.

[00216] In some embodiments, the click chemistry may be a 1-step click reaction. In some embodiments, the click chemistry may be a 2-step click reaction.

[00217] In some embodiments, the reaction(s) comprises metal-free click chemistry. In some embodiments, the reaction(s) comprise thiol-maleimide and/or disulfide exchange.

Metal-free click chemistry

[00218] Metal-free click chemistry can be used for conjugation reactions to avoid potential oxidation of proteins. Metal-free click chemistry has been used to form antibody conjugates (*see* van Geel et al., Bioconjugate Chem. 2015, 26, 2233−2242).

[00219] In some embodiments, metal-free click chemistry is used in reactions to attach adjuvant to ferritin. In some embodiments, copper-free conjugation is used in reactions to attach adjuvant to ferritin. In some embodiments, the metal-free click chemistry uses bicyclo[6.1.0]nonyne (BCN). In some embodiments, the metal-free click chemistry uses dibenzoazacyclooctyne (DBCO). In some embodiments BCN or DBCO reacts with an azide group.

[00220] DBCO has high specificity for azide groups via a strain-promoted click reaction in the absence of a catalyst, resulting in high yield of a stable triazole. In some embodiments, DBCO reacts with azide in the absence of copper catalyst.

[00221] In some embodiments, metal-free click chemistry is used in a 1-step click reaction. In some embodiments, metal-free click chemistry is used in a 2-step click reaction.

Thiol-maleimide and disulfide exchange

[00222] Ferritins described herein can comprise a cysteine comprising a thiol, also known as a sulfhydryl, which is available for reaction with sulfhydryl-reactive chemical groups (or which can be made available through reduction). Thus, the cysteine allows chemoselective modification to add an immune-stimulatory moiety, such as an adjuvant, to the ferritin. Under basic conditions, the cysteine will be deprotonated to generate a thiolate nucleophile, which can react with soft electrophiles, such as maleimides and iodoacetamides. The reaction of the cysteine with a maleimide or iodoacetamide results in a carbon-sulfur bond.

[00223] In some embodiments, a sulfhydryl-reactive chemical group reacts with the surface-exposed cysteine or cysteine in the linker of the ferritin. In some embodiments, the sulfhydryl-reactive chemical group is a haloacetyl, maleimide, aziridine, acryloyl, arylating agent, vinylsulfone, pyridyl disulfide, or TNB-thiol.

[00224] In some embodiments, the sulfhydryl-reactive chemical group conjugates to the sulfhydryl of the cysteine by alkylation (i.e., formation of a thioether bond)). In some embodiments, the sulfhydryl-reactive chemical group conjugates to the sulfhydryl of the cysteine by disulfide exchange (i.e., formation of a disulfide bond).

[00225] In some embodiments, the reaction to conjugate an immune-stimulatory moiety, such as an adjuvant, to the ferritin is a thiol-maleimide reaction.

[00226] In some embodiments, the sulfhydryl-reactive chemical group is a maleimide. In some embodiments, reaction of a maleimide with the cysteine results in formation of a stable thioester linkage, e.g., that is not reversible. In some embodiments, the maleimide does not react with tyrosines, histidines, or methionines in the ferritin. In some embodiments, unreacted maleimides are quenched at the end of the reaction by adding a free thiol, e.g., in excess.

[00227] In some embodiments, the reaction to conjugate an immune-stimulatory moiety, such as an adjuvant, to the ferritin is a thiol-disulfide exchange, also known as a disulfide interchange. In some embodiments, the reaction involves formation of a mixed disulfide comprising a portion of the original disulfide. In some embodiments, the original disulfide is the cysteine introduced in the ferritin by mutation of a surface-exposed amino acid or addition of an N-terminal linker.

[00228] In some embodiments, the sulfhydryl-reactive chemical group is a pyridyl dithiol. In some embodiments, the sulfhydryl-reactive chemical group is a TNB-thiol group.

b) Linkers

[00229] In some embodiments, an immune-stimulatory moiety, such as an adjuvant, or an EBV polypeptide is attached to the ferritin via a linker that is covalently bound to a surface-exposed amino acid such as a cysteine. In some embodiments, the linker comprises a polyethylene glycol, e.g., a PEG linker. In some embodiments, the polyethylene glycol (e.g., PEG) linker increases water solubility and ligation efficiency of the ferritin linked to the immune-stimulatory moiety, such as an adjuvant. The PEG linker is between 2 and 18 PEGs long, e.g., PEG4, PEG5, PEG6, PEG7, PEG8, PEG9, PEG10, PEG11, PEG12, PEG13, PEG14, PEG15, PEG16, PEG17, and PEG18.

[00230] In some embodiments, the linker comprises a maleimide. In some embodiments, the linker comprises the components of immune-stimulatory moiety (ISM)-linker-maleimide. In some embodiments, the ISM-linker-maleimide is conjugated to ferritin in a 1-step click chemistry reaction by reaction of the maleimide with a cysteine of the

ferritin. In some embodiments, the ISM of the adjuvant-linker-maleimide is SM7/8a. In some embodiments, the linker of the ISM-linker-maleimide is PEG4. In some embodiments, the ISM-linker-maleimide is SM7/8a-PEG4-maleimide.

[00231] In some embodiments, a 2-step click chemistry protocol is used with a linker comprising a sulfhydryl-reactive chemical group at one end and an amine-reactive group at the other end. In such a 2-step click chemistry protocol, a sulfhydryl-reactive chemical group reacts with a cysteine of the ferritin, while the amine-reactive group reacts with a reagent attached to the ISM. In this way, the ISM is conjugated to the ferritin via a set of 2 click chemistry reagents.

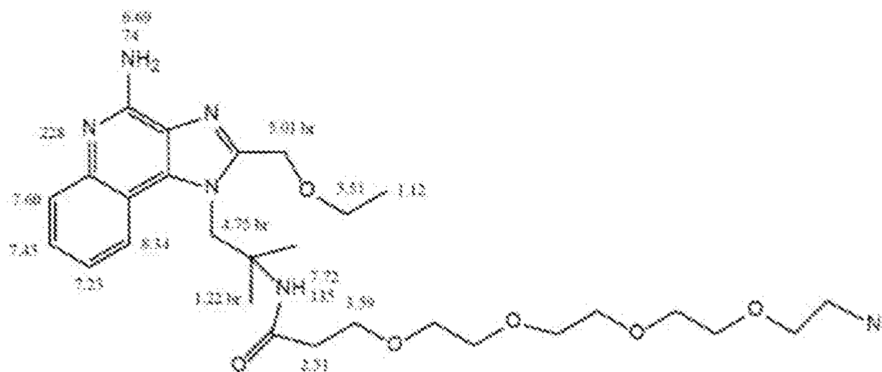
[00232] In some embodiments of the 2-step click chemistry protocol, the sulfhydryl-reactive chemical group is maleimide. In some embodiments of the 2-step click chemistry protocol, the maleimide reacts with the cysteine introduced in the ferritin by mutation of a surface-exposed amino acid or addition of an N-terminal linker.

[00233] In some embodiments of the 2-step click chemistry protocol, the amine-reactive group is DBCO. In some embodiments of the 2-step click chemistry protocol, the DBCO reacts with an azide group attached to an ISM.

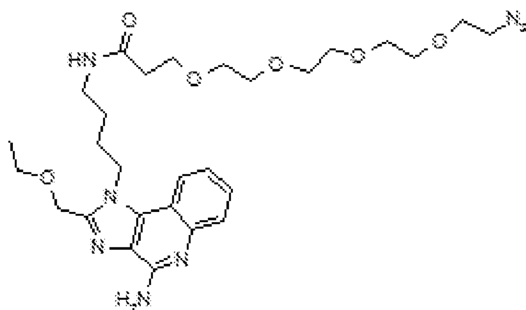
[00234] In some embodiments, a maleimide-linker-DBCO is used. In some embodiments, the maleimide-linker-DBCO is conjugated to ferritin after the ferritin is reduced. In some embodiments, the maleimide-linker-reagent is conjugated to ferritin by reaction of the maleimide with the cysteine of the ferritin in a first step. In some embodiments, the DBCO is used to link to an ISM attached to azide. In some embodiments, the ISM coupled to azide is ISS-1018. In some embodiments, the adjuvant coupled to azide is 3M-012 or CpG.

[00235] In some embodiments, a linker with a reactive group is added to the ISM. In some embodiments, the linker is a PEG4-azide linker or a PEG4-maleimide linker.

[00236] In some embodiments, a PEG4-azide linker is conjugated to 3M-012. An exemplary structure of 3M-012 conjugated to a PEG4-azide linker is:

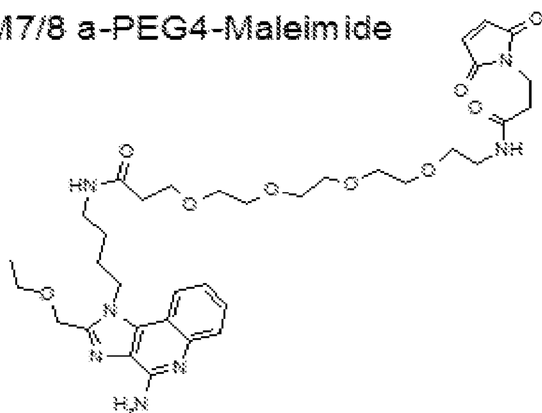


[00237] In some embodiments, a PEG4-azide linker is conjugated to SM7/8a. An exemplary structure of SM7/8a conjugated to a PEG4-azide linker is:



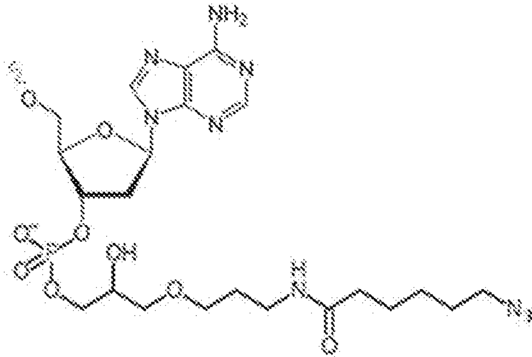
SM7/8a -PEG4-Azide

[00238] In some embodiments, a PEG4-maleimide linker is conjugated to SM7/8a. An exemplary structure of SM7/8a conjugated to a PEG4-maleimide linker is:



SM7/8 a-PEG4-Maleimide

[00239] In some embodiments, an azide group is conjugated to ISS-1018. An exemplary structure of ISS-1018 conjugated to an NHS ester-azide linker is:



I. Exemplary compositions, kits, nucleic acids, uses, and methods

[00240] In some embodiments, the present invention provides methods of immunizing a subject against infection with EBV. The present invention further provides methods of eliciting an immune response against EBV in a subject. In some embodiments, the present methods comprise administering to the subject an effective amount of a pharmaceutical composition described herein to a subject. In some embodiments, the present methods comprises administering to the subject an effective amount of an antigenic EBV polypeptide or nanoparticle described herein to a subject.

[00241] In some embodiments, a composition comprising any one or more of the antigenic EBV polypeptides described herein and a pharmaceutically acceptable vehicle, adjuvant, or excipient is provided.

[00242] In some embodiments, an antigenic EBV polypeptide, nanoparticle, or composition described herein is administered to a subject, such as a human or any of the subjects discussed below, to immunize against infection caused by EBV. In some embodiments, an antigenic EBV polypeptide or nanoparticle described herein is administered to a subject, such as a human, to produce a protective immune response to future infection with EBV. In some embodiments, an antigenic EBV polypeptide is administered. In some embodiments, an antigenic EBV polypeptide comprising an EBV polypeptide and ferritin is administered, wherein the ferritin can have one or more mutations described herein. In some embodiments, an antigenic EBV polypeptide or nanoparticle comprising any one of SEQ ID NOS: 1-27 is administered.

[00243] In some embodiments, the protective immune response decreases the incidence of hospitalization. In some embodiments, the protective immune response decreases the incidence of EBV infection, mononucleosis, complications caused by

mononucleosis (e.g. hepatitis, encephalitis, severe hemolytic anemia, or splenomegaly), nasopharyngeal cancer, gastric cancer, or B lymphoma (e.g., Burkitt's or Hodgkin's lymphoma).

[00244] In some embodiments, a composition comprises one antigenic EBV polypeptide (e.g., a monovalent composition). In some embodiments, a composition comprises an antigenic EBV polypeptide comprising a gH polypeptide. In some embodiments, a composition comprises an antigenic EBV polypeptide comprising a gL polypeptide. In some embodiments, a composition comprises an antigenic EBV polypeptide comprising a gp220 polypeptide.

[00245] In some embodiments, a composition comprises more than one antigenic EBV polypeptide. In some embodiments, a composition comprises one or more antigenic EBV polypeptides comprising more than one polypeptide encoded by EBV (i.e., a multivalent composition). In some embodiments, an EBV vaccine comprises nanoparticles comprising a gp220 polypeptide and, separately, nanoparticles comprising gH and gL polypeptides.

[00246] In some embodiments, any one or more of the antigenic EBV polypeptides, nanoparticles, or compositions described herein are provided for use in immunizing against infection caused by EBV. In some embodiments, any one or more of the polypeptides, nanoparticles, or compositions described herein are provided for use in producing a protective immune response to future infection with EBV.

1. Subjects

[00247] In some embodiments, the subject is a mammal. In some embodiments, the subject is a human.

[00248] In some embodiments, the subject is an adult (greater than or equal to 18 years of age). In some embodiments, the subject is a child or adolescent (less than 18 years of age). In some embodiments, the subject is elderly (greater than 60 years of age). In some embodiments, the subject is a non-elderly adult (greater than or equal to 18 years of age and less than or equal to 60 years of age).

[00249] In some embodiments, the composition is suitably formulated for an intended route of administration. Examples of suitable routes of administration include intramuscular, transcutaneous, subcutaneous, intranasal, oral, or transdermal.

[00250] In some embodiments, more than one administration of the composition is administered to the subject. In some embodiments, a booster administration improves the immune response.

[00251] In some embodiments, any one or more of the antigenic polypeptides, or compositions described herein are for use in a mammal, such as a primate (e.g., non-human primate, such as a monkey (e.g., a macaque, such as rhesus or cynomolgus) or ape), rodent (e.g., mouse or rat), or domesticated mammal (e.g., dog, rabbit, cat, horse, sheep, cow, goat, camel, or donkey).

2. Adjuvants

[00252] An adjuvant may be administered together with the antigenic EBV polypeptides and/or nanoparticles described herein to a subject, wherein administration of such a combination may produce a higher titer of antibodies against the EBV polypeptide(s) in the subject as compared to administration of the EBV polypeptide(s) without the adjuvant. An adjuvant may promote earlier, more potent, or more persistent immune response to the EBV polypeptide(s).

[00253] In some embodiments, a composition comprises one adjuvant. In some embodiments, a composition comprises more than one adjuvant. In some embodiments, a composition does not comprise an adjuvant.

[00254] In some embodiments, an adjuvant comprises aluminum. In some embodiments, an adjuvant is aluminum phosphate. In some embodiments, an adjuvant is Alum (Alyhydrogel '85 2%; Brenntag – Cat# 21645-51-2).

[00255] In some embodiments, an adjuvant is an organic adjuvant. In some embodiments, an adjuvant is an oil-based adjuvant. In some embodiments, an adjuvant comprises an oil-in-water nanoemulsion.

[00256] In some embodiments, an adjuvant comprises squalene. In some embodiments, the adjuvant comprising squalene is Ribi (Sigma adjuvant system Cat #S6322-1vl), Addavax™ MF59, AS03, or AF03 (*see* US9703095). In some embodiments, the adjuvant comprising squalene is a nanoemulsion.

[00257] In some embodiments, an adjuvant comprises a polyacrylic acid polymer (PAA). In some embodiments, the adjuvant comprising PAA is SPA09 (*see* WO 2017218819).

[00258] In some embodiments, an adjuvant comprises non-metabolizable oils. In some embodiments, the adjuvant is Incomplete Freund's Adjuvant (IFA).

[00259] In some embodiments, an adjuvant comprises non-metabolizable oils and killed Mycobacterium tuberculosis. In some embodiments, the adjuvant is Complete Freund's Adjuvant (CFA).

[00260] In some embodiments, an adjuvant is a lipopolysaccharide. In some embodiments, an adjuvant is monophosphoryl A (MPL or MPLA).

[00261]

3. Pharmaceutical compositions

[00262] In various embodiments, a pharmaceutical composition comprising an antigenic EBV polypeptide described herein and/or related entities is provided. In some embodiments, the pharmaceutical composition is an immunogenic composition (e.g., a vaccine) capable of eliciting an immune response such as a protective immune response against a pathogen.

[00263] For example, in some embodiments, the pharmaceutical compositions may comprise one or more of the following: (1) an antigenic EBV polypeptide comprising an EBV polypeptide and a ferritin comprising a mutation replacing a surface-exposed amino acid with a cysteine; (2) an antigenic EBV polypeptide comprising an EBV polypeptide and a ferritin comprising a mutation replacing a surface exposed amino acid with a cysteine and an immune-stimulatory moiety linked to the cysteine; (3) an antigenic EBV polypeptide comprising an EBV polypeptide and a ferritin comprising (i) a surface-exposed cysteine, (ii) a peptide linker N- terminal to the ferritin protein, wherein the EBV polypeptide is N-terminal to the peptide linker; (4) an antigenic EBV polypeptide comprising an EBV polypeptide and a ferritin comprising (i) a mutation replacing a surface exposed amino acid with a cysteine and an immune-stimulatory moiety linked to the cysteine, (ii) a mutation replacing the internal cysteine at position 31 of *H. pylori* ferritin, or a mutation of an internal cysteine at a position that is analogous to position 31 of a non-*H. pylori* ferritin as determined by pair-wise or structural alignment, with a non-cysteine amino acid, and (iii) a mutation replacing a surface-exposed asparagine with a non-asparagine amino acid; or (5) a ferritin particle comprising any of the foregoing polypeptides. In some embodiments, the pharmaceutical compositions may comprise an antigenic EBV gL/gH polypeptide, e.g., wherein the polypeptide comprises a linker of at least 15 amino acids between the gL and gH polypeptide sequences.

[00264] In some embodiments, the present invention provides pharmaceutical compositions comprising antibodies or other agents related to the antigenic polypeptides described herein. In an embodiment, the pharmaceutical composition comprises antibodies that bind to and/or compete with an antigenic polypeptide described herein. Alternatively,

the antibodies may recognize viral particles or bacteria comprising the non-ferritin polypeptide component of an antigenic polypeptide described herein.

[00265] In some embodiments, the pharmaceutical compositions as described herein are administered alone or in combination with one or more agents to enhance an immune response, e.g., an adjuvant described above. In some embodiments, a pharmaceutical composition further comprises an adjuvant described above.

[00266] In some embodiments, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier or excipient. As used herein, the term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which a pharmaceutical composition is administered. In exemplary embodiments, carriers can include sterile liquids, such as, for example, water and oils, including oils of petroleum, animal, vegetable, or synthetic origin, such as, for example, peanut oil, soybean oil, mineral oil, sesame oil and the like. In some embodiments, carriers are or include one or more solid components. Pharmaceutically acceptable carriers can also include, but are not limited to, saline, buffered saline, dextrose, glycerol, ethanol, and combinations thereof. As used herein, an excipient is any non-therapeutic agent that may be included in a pharmaceutical composition, for example to provide or contribute to a desired consistency or stabilizing effect. Suitable pharmaceutical excipients include, but are not limited to, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. In various embodiments, the pharmaceutical composition is sterile.

[00267] In some embodiments, the pharmaceutical composition contains minor amounts of wetting or emulsifying agents, or pH buffering agents. In some embodiments, the pharmaceutical compositions of may include any of a variety of additives, such as stabilizers, buffers, or preservatives. In addition, auxiliary, stabilizing, thickening, lubricating, and coloring agents can be included.

[00268] In various embodiments, the pharmaceutical composition may be formulated to suit any desired mode of administration. For example, the pharmaceutical composition can take the form of solutions, suspensions, emulsion, drops, tablets, pills, pellets, capsules, capsules containing liquids, gelatin capsules, powders, sustained-release formulations, suppositories, emulsions, aerosols, sprays, suspensions, lyophilized powder, frozen suspension, desiccated powder, or any other form suitable for use. General considerations in the formulation and manufacture of pharmaceutical agents may be found, for example, in

Remington's Pharmaceutical Sciences, 19th ed., Mack Publishing Co., Easton, PA, 1995; incorporated herein by reference.

[00269] The pharmaceutical composition can be administered via any route of administration. Routes of administration include, for example, oral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, mucosal, epidural, sublingual, intranasal, intracerebral, intravaginal, transdermal, rectally, by intratracheal instillation, bronchial instillation, inhalation, or topically. Administration can be local or systemic. In some embodiments, administration is carried out orally. In another embodiment, the administration is by parenteral injection. In some instances, administration results in the release of the antigenic ferritin polypeptide described herein into the bloodstream. The mode of administration can be left to the discretion of the practitioner.

[00270] In some embodiments, the pharmaceutical composition is suitable for parenteral administration (e.g. intravenous, intramuscular, intraperitoneal, and subcutaneous). Such compositions can be formulated as, for example, solutions, suspensions, dispersions, emulsions, and the like. They may also be manufactured in the form of sterile solid compositions (e.g. lyophilized composition), which can be dissolved or suspended in sterile injectable medium immediately before use. For example, parenteral administration can be achieved by injection. In such embodiments, injectables are prepared in conventional forms, i.e., either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. In some embodiments, injection solutions and suspensions are prepared from sterile powders, lyophilized powders, or granules.

[00271] In a further embodiment, the pharmaceutical composition is formulated for delivery by inhalation (e.g., for direct delivery to the lungs and the respiratory system). For example, the composition may take the form of a nasal spray or any other known aerosol formulation. In some embodiments, preparations for inhaled or aerosol delivery comprise a plurality of particles. In some embodiments, such preparations can have a mean particle size of about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, or about 13 microns. In some embodiments, preparations for inhaled or aerosol delivery are formulated as a dry powder. In some embodiments, preparations for inhaled or aerosol delivery are formulated as a wet powder, for example through inclusion of a wetting agent. In some embodiments, the wetting agent is selected from the group consisting of water, saline, or other liquid of physiological pH.

[00272] In some embodiments, the pharmaceutical composition in accordance with the invention are administered as drops to the nasal or buccal cavity. In some embodiments, a dose may comprise a plurality of drops (e.g., 1-100, 1-50, 1-20, 1-10, 1-5, etc.).

[00273] The present pharmaceutical composition may be administered in any dose appropriate to achieve a desired outcome. In some embodiments, the desired outcome is the induction of a long-lasting adaptive immune response against a pathogen, such as the source of a non-ferritin polypeptide present in an antigenic ferritin polypeptide present in the composition. In some embodiments, the desired outcome is a reduction in the intensity, severity, frequency, and/or delay of onset of one or more symptoms of infection. In some embodiments, the desired outcome is the inhibition or prevention of infection. The dose required will vary from subject to subject depending on the species, age, weight, and general condition of the subject, the severity of the infection being prevented or treated, the particular composition being used, and its mode of administration.

[00274] In some embodiments, pharmaceutical compositions in accordance with the invention are administered in single or multiple doses. In some embodiments, the pharmaceutical compositions are administered in multiple doses administered on different days (e.g., prime-boost vaccination strategies). In some embodiments, the pharmaceutical composition is administered as part of a booster regimen.

[00275] In various embodiments, the pharmaceutical composition is co-administered with one or more additional therapeutic agents. Co-administration does not require the therapeutic agents to be administered simultaneously, if the timing of their administration is such that the pharmacological activities of the additional therapeutic agent and the active ingredient(s) in the pharmaceutical composition overlap in time, thereby exerting a combined therapeutic effect. In general, each agent will be administered at a dose and on a time schedule determined for that agent.

4. Nucleic acid/mRNA

[00276] Also provided is a nucleic acid encoding an antigenic EBV polypeptide described herein. In some embodiments, the nucleic acid is an mRNA. Any nucleic acid capable of undergoing translation resulting in a polypeptide is considered an mRNA for purposes of this disclosure.

5. Kits

[00277] Also provided herein are kits comprising one or more antigenic EBV polypeptides, nucleic acids, antigenic ferritin particles, antigenic lumazine synthase particles, compositions, or pharmaceutical compositions described herein. In some embodiments, a kit further comprises one or more of a solvent, solution, buffer, instructions, or desiccant.

[00278] * * *

[00279] This description and exemplary embodiments should not be taken as limiting. For the purposes of this specification and appended claims, unless otherwise indicated, all numbers expressing quantities, percentages, or proportions, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term “about,” to the extent they are not already so modified. “About” indicates a degree of variation that does not substantially affect the properties of the described subject matter, e.g., within 10%, 5%, 2%, or 1%. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed considering the number of reported significant digits and by applying ordinary rounding techniques.

[00280] It is noted that, as used in this specification and the appended claims, the singular forms “a,” “an,” and “the,” and any singular use of any word, include plural referents unless expressly and unequivocally limited to one referent. As used herein, the term “include” and its grammatical variants are intended to be non-limiting, such that recitation of items in a list is not to the exclusion of other like items that can be substituted or added to the listed items. The term “or” is used in the inclusive sense, i.e., equivalent to “and/or,” unless the context dictates otherwise.

TABLE 2 (SEQUENCE TABLE): DESCRIPTION OF THE SEQUENCES

Description	Sequences	SEQ ID NO
	Key for SEQ ID NOs: 1-41 **Leader Sequence – underlined** **gL – *Italicized*** **Linker – double underlined** **gH – Bold** **bfpFerr (ferritin) – wavy underline** **FR – *Italicized and double underline*** **gp220 – *Italicized and bold*** **gp42 – *Italicized and underlined*** ***T. ni* ferritin heavy chain – double wavy underline** **Foldon sequence: *Italicized and wavy underline*** **Thrombin cleavage site: *Italicized and dashed underline*** **6X His Tag (SEQ ID NO: 243): *Bold, italicized and curvy underline***	
SIB 7187 leader sequence gp220 bfpFerr Nanoparticle N19Q/C31S/S111C	MDSKGSSQKGSRLLLLLVVSNLLLPQGVLAEAALLVCQYTIQSLIHLTGEDPGFFNVEIPEFPFYPTCNVCTADVNVTINFDVGGKKHQLDLDFGQLTPHTKAVYQPRGAFGGSENATNLFLLELLGAGELALTMRSKKLPINVTTGEEQQVSLESVDVYFQDVFGTMWCHHAEMQNPVYLIPETVPYIKWDNCNSTNITAVVRAQGLDVTLPLSLPTSAQDSNFSVKTEMLGNEIDIECIMEDGEISQVLPGDNKFNITCSGYESHVPSGGILTSTSPVATPIPGTGYAYSLRLTPRPVSRFLGNNSILYVFYSGNGPKASGGDYCIQSNIVFSDEIPASQDMPTNTTDITYVGDNATYSVPMVTSEDANSPNVTVTAFWAWPNNTETDFKCKWTLTSGTPSGCENISGAFASNRTFDITVSGLGTAPKTLIITRTATNATTTTHKVIFSKAPEGSESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKCKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	1
SIB 7340 leader sequence gL(D7)_linker_gH bfpFerr Nanoparticle N19Q/C31S/S111C	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSFFISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRGGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGASGGSGGSGAASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKMEELGHLT	2

	TEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSA LEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMI IPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTT YITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGGGSGGGSGGGSGGGSGGGSESQVRQQFSKDIEKLLNEQVN KEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINN IVDHAIKCKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	
SIB 7342 leader sequence gL(D7)_ linker _gH bfpFerr Nanoparticle N19Q/C31S/S111C	METDTLLLWVLLLWVPGSTGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSFFI SILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRGGGSGGGSGGGSGGGSGAASLSEVKLHLDIEGHASHYTIPWTELMAKV PGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIG TMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGD YSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNG CVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKMEELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSV LLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLT RDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSS SLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTT NGTVMEIAGLYEERASGGGSGGGSGGGSGGGSGGGSESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDH AAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKCKDHATFNFLQWYVAEQHEEEVLF KDILDKIELIGNENHGLYLADQYVKGIAKSRKS	3
SIB 7379 leader sequence gL(D7)_linker_gH Trimer	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSF FISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRGGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGASGGSGGSG AASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDA SKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEH VLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCRE PELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKMEELGHLT TEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSA LEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMI IPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTT YITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGSGYIPEAPRDGQAYVRKDGEWVLLSTFLGSGSGSGLVPRG SGAGGGHHHHHH	4

SIB 7380 leader sequence gL(D7)_linker_gH Trimer	METDTLLLWVLLLWVPGSTGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSFFISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRGGGSGGGSGGGSGGGSGAASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKMEELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGSGYIPEAPRDGQAYVRKDGEWVLLSTFLGSGSGSGLVPRGSGAGGGHHHHHH	5
SIB 7381 leader sequence gL(D7)_linker_gH Monomer	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSFFISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRGGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGASGGSGGSGAASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKMEELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGSGSGSGLVPRGSGAGGGHHHHHH	6
SIB 7382 leader sequence gL(D7)_linker_gH Monomer	METDTLLLWVLLLWVPGSTGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSFFISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRGGGSGGGSGGGSGGGSGAASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKMEELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGSGSGSGLVPRGSGAGGGHHHHHH	7

SIB 7392 leader sequence gL_linker_gH Monomer	MRAVGVFLAICLVTIFVLPTWG*NWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSF* *FISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGG*GGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGAS GGSGGSG**AASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSE** **GSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRV** **TEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLE** **MKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKM** **EELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLL** **STALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDR** **LDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEK** **EGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERA**SGSGSGSG*LVPRGS*GAGGG***HHHHHH***	8
SIB 7397 leader sequence gL_linker_gH Monomer	MRAVGVFLAICLVTIFVLPTWG*NWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSF* *FISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGG*GGSGSGSNSSASSGASSGGASGGSGGSG**AASLSEVKLHL** **DIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDASKVHPGVISGL** **NSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEHVLSLVFGKTKD** **LPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCREPELDTETLTTM** **FEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKMEELGHLTTEKQEYALRLA** **TVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSALEIGEVLRGLA** **LGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMIIPLINVTFIIS** **SDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTTYITSQEVQNSI** **LSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERA**SGSGSGSG*LVPRGS*GAGGG***HHHHHH***	9
SIB 7400 leader sequence gL_linker_gH bfpFerr Nanoparticle N19Q/C31S/S111C	METDTLLLWVLLLWVPGSTG*NWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSFFI* *SILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGG*GGGGSGGGGSGGGGSG**AASLSEVKLHLDIEGHASHYTIPWT** **ELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDASKVHPGVISGLNSPACMLSAPLEKQ** **LFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLT** **SAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCREPELDTETLTTMFEVSVAFFKVGHAV** **GETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKMEELGHLTTEKQEYALRLATVGYPKAGVYSGLI** **GGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSALEIGEVLRGLALGTESGLFSPCYLS** **LRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMIIPLINVTFIISSDREVRGSALYEAS** **TTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTTYITSQEVQNSILSSNYFDFDNLHVH** **YLLLTTNGTVMEIAGLYEERA**SGGGSGGGSGGGSGGGSGGGSESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAG	10

	LFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKCKDHATFNFLQWYVAEQH EEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	
SIB 7402 **leader sequence gL_linker_gH_trimer**	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSF FISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGGGGSGSGSNSSASSGASSGGASGGSGGSGAASLSEVKLHL DIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDASKVHPGVISGL NSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEHVLSLVFGKTKD LPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCREPELDTETLTTM FEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKMEELGHLTTEKQEYALRLA TVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSALEIGEVLRGLA LGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMIIPLINVTFIIS SDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTTYITSQEVQNSI LSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGSGYIPEAPRDGQAYVRKDGEWVLLSTFLGSGSGSGLVPRGSGAGGGHHHHH H	11
SIB 7403 **leader sequence gL_linker_gH_trimer**	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSF FISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGGGGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGAS GGSGGSGAASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSE GSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRV TEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLE MKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKM EELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLL STALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDR LDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEK EGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGSGYIPEAPRDGQAYVRKDGEWVLLSTFLGSGSG SGLVPRGSGAGGGHHHHHH	12
SIB 7404 **leader sequence gL_linker_gH bfpFerr Nanoparticle N19Q/C31S/S111C**	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSF FISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGGGGSGSGSNSSASSGASSGGASGGSGGSGAASLSEVKLHL DIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDASKVHPGVISGL NSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEHVLSLVFGKTKD LPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCREPELDTETLTTM FEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKMEELGHLTTEKQEYALRLA TVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSALEIGEVLRGLA	13

	LGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMIIPLINVTFIIS SDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTTYITSQEVQNSI LSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGGGSGGGSGGGSGGGSGGGSESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMS MSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKCKDH ATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	
SIB 7406 leader sequence gL_linker_gH bfpFerr Nanoparticle N19Q/C31S/S111C	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSF FISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGGGGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGAS GGSGGSGAASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSE GSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRV TEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLE MKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKM EELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLL STALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDR LDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEK EGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGGGSGGGSGGGSGGGSGGGSESQVRQQFSKDIEK LLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQH ISESINNIVDHAIKCKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	14
SIB 7414 leader sequence gL_linker_gH Monomer	METDTLLLWVLLLWVPGSTGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSFFI SILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGGGGGSGGGGSGGGGSGAASLSEVKLHLDIEGHASHYTIPWT ELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDASKVHPGVISGLNSPACMLSAPLEKQ LFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLT SAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCREPELDTETLTTMFEVSVAFFKVGHAV GETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKMEELGHLTTEKQEYALRLATVGYPKAGVYSGLI GGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSALEIGEVLRGLALGTESGLFSPCYLS LRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMIIPLINVTFIISSDREVRGSALYEAS TTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTTYITSQEVQNSILSSNYFDFDNLHVH YLLLTTNGTVMEIAGLYEERASGSGSGSGLVPRGSGAGGGHHHHHH	15
SIB 7429	METDTLLLWVLLLWVPGSTGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSFFI SILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGGGGGSGGGGSGGGGSGAASLSEVKLHLDIEGHASHYTIPWT ELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDASKVHPGVISGLNSPACMLSAPLEKQ LFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLT	16

leader sequence gL_linker_gH_trimer	SAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCREPELDTETLTTMFEVSVAFFKVGHAV GETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKMEELGHLTTEKQEYALRLATVGYPKAGVYSGLI GGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSALEIGEVLRGLALGTESGLFSPCYLS LRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMIIPLINVTFIISSDREVRGSALYEAS TTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTTYITSQEVQNSILSSNYFDFDNLHVH YLLLTTNGTVMEIAGLYEERASGSGYIPEAPRDGQAYVRKDGEWVLLSTFLGSGSGSGLVPRGSGAGGGHHHHHH	
SIB 15000 leader sequence gL_FR1_gH bfpFerr Nanoparticle N19Q/C31S/S111C	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSF FISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGGGGSGSASAEAAAKEAAAKAGGSGGSGAASLSEVKLHLDI EGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDASKVHPGVISGLNS PACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEHVLSLVFGKTKDLP DLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCREPELDTETLTTMFE VSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKMEELGHLTTEKQEYALRLATV GYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSALEIGEVLRGLALG TESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMIIPLINVTFIISSD REVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTTYITSQEVQNSILS SNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGGGSGGGSGGGSGGGSGGGSESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMSMS SWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKCKDHAT FNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	17
SIB 15001 leader sequence gL_FR2_gH bfpFerr Nanoparticle N19Q/C31S/S111C	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSF FISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGGGGSGSASAEAAAKEAAAKEAAAKASGGSGGSGAASLSEV KLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDASKVHPGV ISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEHVLSLVFG KTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCREPELDTET LTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKMEELGHLTTEKQEYA LRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSALEIGEVL RGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMIIPLINVT FIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTTYITSQEV QNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGGGSGGGSGGGSGGGSGGGSESQVRQQFSKDIEKLLNEQVNKEMQSSN LYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIK CKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	18
SIB 15002 leader sequence Construct 5 gL_linker_gH_lin	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSF FISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGGGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGAS GGSGGSGAASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSE GSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRV TEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLE	19

ker_bfpFerr Nanoparticle N19Q/C31S/S111C	MKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKM EELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLL STALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDR LDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEK EGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGGGSGSASSGASASGSSGSGSGSGSSSASSGASS GGASGGGSGGSGESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQL TSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKCKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYV KGIAKSRKS	
SIB 15003 leader sequence Construct 7 gL_linker_gH_lin ker_bfpFerr Nanoparticle N19Q/C31S	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSF FISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGGGGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGAS GGSGGSGAASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSE GSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRV TEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLE MKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKM EELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLL STALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDR LDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEK EGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGGGSGSASSGASASGSSCSGSGSGSSSASSGASS GGASGGGSGGSGESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQL TSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKSKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYV KGIAKSRKS	20
SIB 15004 leader sequence gL_gH_gp42_bfpFe rr Nanoparticle N19Q/C31S/S111C	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSF FISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGGGGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGAS GGSGGSGAASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSE GSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRV TEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLE MKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKM EELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLL STALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDR LDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEK EGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGGGSGSASSGASASGSSGSGSGSGSSSASSGDSK GSSQKGSRLLLLLVVSNLLLPQGVLAYFLPPRVRGGGRVAAAAITWVPKPNVEVWPVDPPPPVNFNKTAEQEYGDKEVKLPHWTPTLH TFQVPQNYTKANCTYCNTREYTFSYKGCCFYFTKKKHTWNGCFQACAELYPCTYFYGPTPDILPVVTRNLNAIESLWVGVYRVGEGNW	21

	TSLDGGTFKVYQIFGSHCTYVSKFSTVPVSHHECSFLKPCLCVSQRSNSGGSGSASSGASASGSSGSGSGSGSSSASSGASSGGASGGSGGSGGGSGSASSGASASGSSGSGSGSGSSSASSGASSGGASGGSGGSGESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKCKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	
SIB 15005 leader sequence Construct 5 gL_gH_C137A_bfpFerr Nanoparticle N19Q/C31S/S111C	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSFFISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGGGGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGASGGSGGSGAASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRAHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKMEELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGGGSGSASSGASASGSSGSGSGSGSSSASSGASSGGASGGGSGGSGESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKCKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	22
SIB 15006 leader sequence Construct 7 gL_gH_C137A_bfpFerr Nanoparticle N19Q/C31S	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSFFISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGGGGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGASGGSGGSGAASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRAHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKMEELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGGGSGSASSGASASGSSCSGSGSGSSSASSGASSGGASGGGSGGSGESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKSKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	23
SIB 17395	MDSKGSSQKGSRLLLLLVVSNLLLPQGVLAEAALLVCQYTIQSLIHLTGEDPGFFNVEIPEFPFYPTCNVCTADVNVTINFDVGGKKHQLDLDFGQLTPHTKAVYQPRGAFGGSENATNLFLLELLGAGELALTMRSKKLPINVTTGEEQQVSLESVDVYFQDVFGTMWCHHAEMQNPVYLIPETVPYIKWDNCNSTNITAVVRAQGLDVTLPLSLPTSAQDSNFSVKTEMLGNEIDIECIMEDGEISQVLPGDNKFNITCSGY	24

leader sequence gp220-T.ni ferritin heavy chain	ESHVPSGGILTSTSPVATPIPGTGYAYSLRLTPRPVSRFLGNNSILYVFYSGNGPKASGGDYCIQSNIVFSDEIPASQDMPTNTTDIT YVGDNATYSVPMVTSEDANSPNVTVTAFWAWPNNTETDFKCKWTLTSGTPSGCENISGAFASNRTFDITVSGLGTAPKTLIITRTATN ATTTTHKVIFSKAPEGSTQCNVNPVQIPKDWITMHRSCRNSMRQQIQMEVGASLQYLAMGAHFSKDVVNRPGFAQLFFDAASEEREHA MKLIEYLLMRGELTNDVSSLLQVRPPTRSSWKGGVEALEHALSMESDVTKSIRNVIKACEDDSEFNDYHLVDYLTGDFLEEQYKGQRD LAGKASTLKKLMDRHEALGEFIFDKKLLGIDV	
SIB 17396 leader sequence gL_linker_gH-*T. ni* ferritin heavy chain	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSF FISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGGGGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGAS GGSGGSGAASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSE GSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRV TEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLE MKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKM EELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLL STALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDR LDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEK EGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGSTQCNVNPVQIPKDWITMHRSCRNSMRQQIQME VGASLQYLAMGAHFSKDVVNRPGFAQLFFDAASEEREHAMKLIEYLLMRGELTNDVSSLLQVRPPTRSSWKGGVEALEHALSMESDVT KSIRNVIKACEDDSEFNDYHLVDYLTGDFLEEQYKGQRDLAGKASTLKKLMDRHEALGEFIFDKKLLGIDV	25
SIB 17397 leader sequence gp220-*T. ni* ferritin light chain	MDSKGSSQKGSRLLLLLVVSNLLLPQGVLAEAALLVCQYTIQSLIHLTGEDPGFFNVEIPEFPFYPTCNVCTADVNVTINFDVGGKKH QLDLDFGQLTPHTKAVYQPRGAFGGSENATNLFLLELLGAGELALTMRSKKLPINVTTGEEQQVSLESVDVYFQDVFGTMWCHHAEMQ NPVYLIPETVPYIKWDNCNSTNITAVVRAQGLDVTLPLSLPTSAQDSNFSVKTEMLGNEIDIECIMEDGEISQVLPGDNKFNITCSGY ESHVPSGGILTSTSPVATPIPGTGYAYSLRLTPRPVSRFLGNNSILYVFYSGNGPKASGGDYCIQSNIVFSDEIPASQDMPTNTTDIT YVGDNATYSVPMVTSEDANSPNVTVTAFWAWPNNTETDFKCKWTLTSGTPSGCENISGAFASNRTFDITVSGLGTAPKTLIITRTATN ATTTTHKVIFSKAPEGSADTCYNDVALDCGITSNSLALPRCNAVYGEYGSHGNVATELQAYAKLHLERSYDYLLSAAYFNNYQTNRAG FSKLFKKLSDEAWSKTIDIIKHVTKRGDKMNFDQHSTMKTERKNYTAENHELEALAKALDTQKELAERAFYIHREATRNSQHLHDPEI AQYLEEEFIEDHAEKIRTLAGHTSDLKKFITANNGHDLSLALYVFDEYLQKTV	26
SIB 17398 leader sequence gL_linker_gH-*T. ni* ferritin light chain	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSF FISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGGGGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGAS GGSGGSGAASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSE GSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRV TEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLE MKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKM EELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLL STALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDR	27

	LDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEK EGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGSADTCYNDVALDCGITSNSLALPRCNAVYGEYG SHGNVATELQAYAKLHLERSYDYLLSAAYFNNYQTNRAGFSKLFKKLSDEAWSKTIDIIKHVTKRGDKMNFDQHSTMKTERKNYTAEN HELEALAKALDTQKELAERAFYIHREATRNSQHLHDPEIAQYLEEEFIEDHAEKIRTLAGHTSDLKKFITANNGHDLSLALYVFDEYL QKTV	
16 amino acid linker	GGGGSGGGGSGGGGSG	28
28 amino acid linker	GGSGSGSNSSASSGASSGGASGGSGGSG	29
46 amino acid linker	GGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGASGGSGGSG	30
FR1	GGSGSASAEAAAKEAAAKAGGSGGSG	31
FR2	GGSGSASAEAAAKEAAAKEAAAKASGGSGGSG	32
47 amino acid linker comprising a C for conjugation	SGGGSGSASSGASASGSSCSGSGSGSSSASSGASSGGASGGGSGGSG	33
Gp42	DSKGSSQKGSRLLLLLVVSNLLLPQGVLAYFLPPRVRGGGRVAAAAITWVPKPNVEVWPVDPPPPVNFNKTAEQEYGDKEVKLPHWTP TLHTFQVPQNYTKANCTYCNTREYTFSYKGCCFYFTKKKHTWNGCFQACAELYPCTYFYGPTPDILPVVTRNLNAIESLWVGVYRVGE GNWTSLDGGTFKVYQIFGSHCTYVSKFSTVPVSHHECSFLKPCLCVSQRSNS	34
CpG (phosphorothioate modifications where * is shown)	T*G*A*C*T*G*T*G*A*A*C*G*T*T*C*G*A*G*A*T*G*A	35
Exemplary gL polypeptide	NWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSFFISILKRSSSALTGHLRELLTT LETLYGSFSVEDLFGANLNRYAWHRGG	36
Exemplary gH polypeptide	AASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDA SKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEH VLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCRE PELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKMEELGHLT	37

	TEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSA LEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMI IPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTT YITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERA	
Exemplary gp220 polypeptide	EAALLVCQYTIQSLIHLTGEDPGFFNVEIPEFPFYPTCNVCTADVNVTINFDVGGKKHQLDLDFGQLTPHTKAVYQPRGAFGGSENAT NLFLLELLGAGELALTMRSKKLPINVTTGEEQQVSLESVDVYFQDVFGTMWCHHAEMQNPVYLIPETVPYIKWDNCNSTNITAVVRAQ GLDVTLPLSLPTSAQDSNFSVKTEMLGNEIDIECIMEDGEISQVLPGDNKFNITCSGYESHVPSGGILTSTSPVATPIPGTGYAYSLR LTPRPVSRFLGNNSILYVFYSGNGPKASGGDYCIQSNIVFSDEIPASQDMPTNTTDITYVGDNATYSVPMVTSEDANSPNVTVTAFWA WPNNTETDFKCKWTLTSGTPSGCENISGAFASNRTFDITVSGLGTAPKTLIITRTATNATTTTHKVIFSKAPE	38
Cysteine-Thrombin-His Linker	CLVPRGSLEHHHHHH	39
Lumazine synthase of Aquifex aeolicus (strain VF5)	MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDCIVRHGGREEDITLVRVPGSWEIPVAAGELARKEDIDAVIAIGVLIRGATPH FDYIASEVSKGLANLSLELRKPITFGVITADTLEQAIERAGTKHGNKGWEAALSAIEMANLFKSLR	40
E. coli 6,7-dimethyl-8-ribityllumazine synthase	MNIIEANVATPDARVAITIARFNNFINDSLLEGAIDALKRIGQVKDENITVVWVPGAYELPLAAGALAKTGKYDAVIALGTVIRGGTA HFEYVAGGASNGLAHVAQDSEIPVAFGVLTTESIEQAIERAGTKAGNKGAEAALTALEMINVLKAIKA	41
	Not Used	42-200
bfpFerritin-N19Q/C31S/S26C	ESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMCMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEG LTQIFQKAYEHEQHISESINNIVDHAIKSKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	201
bfpFerritin-N19Q/C31S/S72C	ESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTCISAPEHKFEG LTQIFQKAYEHEQHISESINNIVDHAIKSKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	202
bfpFerritin-N19Q/C31S/A75C	ESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISCPEHKFEG LTQIFQKAYEHEQHISESINNIVDHAIKSKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	203

bfpFerritin-N19Q/C31S/K79C	ESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHCFEGLTQIFQKAYEHEQHISESINNIVDHAIKSKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	204
bfpFerritin-N19Q/C31S/S100C	ESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISECINNIVDHAIKSKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	205
bfpFerritin-N19Q/C31S/S111C	ESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKCKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	206
bfpFerritin-N19Q/C31S/E12C	ESQVRQQFSKDIEKLLNCQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKSKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	207
Exemplary H. pylori Ferritin with bullfrog linker	ESQVRQQFSKDIEKLLNEQVNKEMNSSNLYMSMSSWCYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKSKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	208
Exemplary wild-type H. pylori ferritin (GenBank Accession AAD06160.1) (without bullfrog linker or N-terminal Met)	LSKDIIKLLNEQVNKEMNSSNLYMSMSSWCYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKSKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	209
CpG (ISS-1018)	TGACTGTGAACGTTCGAGATGA	210
Trichoplusia ni heavy chain ferritin	TQCNVNPVQIPKDWITMHRSCRNSMRQQIQMEVGASLQYLAMGAHFSKDVVNRPGFAQLFFDAASEEREHAMKLIEYLLMRGELTNDVSSLLQVRPPTRSSWKGGVEALEHALSMESDVTKSIRNVIKACEDDSEFNDYHLVDYLTGDFLEEQYKGQRDLAGKASTLKKLMDRHEALGEF	211

	IFDKKLLGIDV	
Trichoplusia ni light chain ferritin	ADTCYNDVALDCGITSNSLALPRCNAVYGEYGSHGNVATELQAYAKLHLERSYDYLLSAAYFNNYQTNRAGFSKLFKKLSDEAWSKTI DIIKHVTKRGDKMNFDQHSTMKTERKNYTAENHELEALAKALDTQKELAERAFYIHREATRNSQHLHDPEIAQYLEEEFIEDHAEKIR TLAGHTSDLKKFITANNGHDLSLALYVFDEYLQKTV	212
Pyrococcus furiosus ferritin	MLSERMLKALNDQLNRELYSAYLYFAMAAYFEDLGLEGFANWMKAQAEEEIGHALRFYNY IYDRNGRVELDEIPKPPKEWESPLKAFEAAYEHEKFISKSIYELAALAEEEKDYSTRAFL EWFINEQVEEEASVKKILDKLKFAKDSPQILFMLDKELSARAPKLPGLLMQGGE	213
human heavy chain ferritin	MTTASTSQVRQNYHQDSEAAINRQINLELYASYVYLSMSYYFDRDDVALKNFAKYFLHQSHEEREHAEKLMKLQNQRGGRIFLQDIKK PDCDDWESGLNAMECALHLEKNVQQSLLELHKLATDKNDPHLCDFIETHYLNEQVKAIKELGDHVTNLRKMGAPESGLAEYLFDKHTL GDSDQES	214
human light chain ferritin (signal peptide is underlined)	MDSKGSSQKGSRLLLLLVVSNLLLPQGVLASSQIRQNYSTDVEAAVNSLVNLYLQASYTYLSLGFYFDRDDVALEGVSHFFRELAEEK REGYERLLKMQNQRGGRALFQDIKKPAEDEWGKTPDAMKAAMALEKKLNQALLDLHALGSARTDPHLCDFLETHFLDEEVKLIKKMGD HLTNLHRLGGPEAGLGEYLFERLTLKHD	215
lumazine synthase from Aquifex aeolicus	MQIYEGKLTAEGLRFGIVASRFNHALVDRLVEGAIDCIVRHGGREEDITLVRVPGSWEIPVAAGELARKEDIDAVIAIGVLIRGATPH FDYIASEVSKGLANLSLELRKPITFGVITADTLEQAIERAGTKHGNKGWEAALSAIEMANLFKSLR	216
bullfrog linker	ESQVRQQF	217
Cysteine-Thrombin-His Linker	CLVPRGSLEHHHHHH	218

E. coli 6,7-dimethyl-8-ribityllumazine synthase	MNIIEANVATPDARVAITIARFNNFINDSLLEGAIDALKRIGQVKDENITVVWVPGAYELPLAAGALAKTGKYDAVIALGTVIRGGTA HFEYVAGGASNGLAHVAQDSEIPVAFGVLTTESIEQAIERAGTKAGNKGAEAALTALEMINVLKAIKA	219
16 amino acid linker	GGGGSGGGGSGGGGSG	220
28 amino acid linker	GGSGSGSNSSASSGASSGGASGGSGGSG	221
46 amino acid linker	GGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGASGGSGGSG	222
FR1	GGSGSASAEAAAKEAAAKAGGSGGSG	223
FR2	GGSGSASAEAAAKEAAAKEAAAKASGGSGGSG	224
47 amino acid linker comprising a C for conjugation	SGGGSGSASSGASASGSSCSGSGSGSSSASSGASSGGASGGGSGGSG	225
SIB 15007 gL/gH/gp42-His	MRAVGVFLAICLVTIFVLPTWG*NWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSF FISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGG*GGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGAS GGSGGSG**AASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSE GSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRAHLSYVALSINGDKFQYTGAMTSKFLMGTYKRV TEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLE MKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKM EELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLL STALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDR LDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEK EGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERA**SGGGSGSASSGASASGSSGSGSGSGSSSASSG*LAY*	226

	FLPPRVRGGGRVAAAAITWVPKPNVEVWPVDPPPPVNFNKTAEQEYGDKEVKLPHWTPTLHTFQVPQNYTKANCTYCNTREYTFSYKG CCFYFTKKKHTWNGCFQACAELYPCTYFYGPTPDILPVVTRNLNAIESLWVGVYRVGEGNWTSLDGGTFKVYQIFGSHCTYVSKFSTV PVSHHECSFLKPCLCVSQRSNSGSHHHHHH	
SIB 15008 gH/gL/gp42 NP	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSF FISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGGGGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGAS GGSGGSGAASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSE GSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRAHLSYVALSINGDKFQYTGAMTSKFLMGTYKRV TEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLE MKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKM EELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLL STALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDR LDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEK EGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGGGSGSASSGASASGSSGSGSGSGSSSASSGLAY FLPPRVRGGGRVAAAAITWVPKPNVEVWPVDPPPPVNFNKTAEQEYGDKEVKLPHWTPTLHTFQVPQNYTKANCTYCNTREYTFSYKG CCFYFTKKKHTWNGCFQACAELYPCTYFYGPTPDILPVVTRNLNAIESLWVGVYRVGEGNWTSLDGGTFKVYQIFGSHCTYVSKFSTV PVSHHECSFLKPCLCVSQRSNSGGSGSASSGASASGSSGSGSGSGSSSASSGASSGGASGGSGGSGGGSGSASSGASASGSSGSGSGS GSSSASSGASSGGASGGSGGSGESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIF LNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKCKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNEN HGLYLADQYVKGIAKSRKS	227
SIB 15009 gH/gL/gp42_NP_ C12	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSF FISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGGGGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGAS GGSGGSGAASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSE GSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRAHLSYVALSINGDKFQYTGAMTSKFLMGTYKRV TEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLE MKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKM EELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLL STALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDR LDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEK EGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGGGSGSASSGASASGSSGSGSGSGSSSASSGLAY FLPPRVRGGGRVAAAAITWVPKPNVEVWPVDPPPPVNFNKTAEQEYGDKEVKLPHWTPTLHTFQVPQNYTKANCTYCNTREYTFSYKG CCFYFTKKKHTWNGCFQACAELYPCTYFYGPTPDILPVVTRNLNAIESLWVGVYRVGEGNWTSLDGGTFKVYQIFGSHCTYVSKFSTV PVSHHECSFLKPCLCVSQRSNSGGSGSASSGASASGSSGSGSGSGSSSASSGASSGGASGGSGGSGESQVRQQFSKDIEKLLNEQVNK EMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNI VDHAIKCKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	228
SIB 15010	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSF FISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGGGGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGAS GGSGGSGAASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSE	229

gH/gL/gp42_NP_ C13	GSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRAHLSYVALSINGDKFQYTGAMTSKFLMGTYKRV TEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLE MKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKM EELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLL STALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDR LDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEK EGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGGGSGSASSGASASGSSGSGSGSGSSSASSGLAY FLPPRVRGGGRVAAAAITWVPKPNVEVWPVDPPPPVNFNKTAEQEYGDKEVKLPHWTPTLHTFQVPQNYTKANCTYCNTREYTFSYKG CCFYFTKKKHTWNGCFQACAELYPCTYFYGPTPDILPVVTRNLNAIESLWVGVYRVGEGNWTSLDGGTFKVYQIFGSHCTYVSKFSTV PVSHHECSFLKPCLCVSQRSNSEPEPEPEPEPGGESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAA EEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKCKDHATFNFLQWYVAEQHEEEVLFKD ILDKIELIGNENHGLYLADQYVKGIAKSRKS	
SIB 15011 gH/gL/gp42_NP_ C14	MRAVGVFLAICLVTIFVLPTWGNWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSF FISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGGGGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGAS GGSGGSGAASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSE GSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRAHLSYVALSINGDKFQYTGAMTSKFLMGTYKRV TEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLE MKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKM EELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLL STALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDR LDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEK EGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGGGSGSASSGASASGSSGSGSGSGSSSASSGLAY FLPPRVRGGGRVAAAAITWVPKPNVEVWPVDPPPPVNFNKTAEQEYGDKEVKLPHWTPTLHTFQVPQNYTKANCTYCNTREYTFSYKG CCFYFTKKKHTWNGCFQACAELYPCTYFYGPTPDILPVVTRNLNAIESLWVGVYRVGEGNWTSLDGGTFKVYQIFGSHCTYVSKFSTV PVSHHECSFLKPCLCVSQRSNSGGSGESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKK LIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKCKDHATFNFLQWYVAEQHEEEVLFKDILDKIELI GNENHGLYLADQYVKGIAKSRKS	230
SIB 15012 gH/gL/gp42_NP_ C16	MDSKGSSQKGSRLLLLLVVSNLLLPQGVLANWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDAFSLASLNSPKQGSNQLVISRCA NGLNVVSFFISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGAQLNRYAWHRGGGGSGSASSGASASGSSGGSGSGSGSGSSASS GASSGGASGGSGGSGAASLSEVKLHLDIEGHASHYTIPWTELLAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEP VDIPAVSEGSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKF LMGTYKRVTEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGEYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYV LQKLVLLEMKGGCREPELETETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAA ILMATVKMEELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGP NLALYQLLSTALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSIAPQEATLDQAAVSQAVDGFLGRLSLEREDRDAWHL PAYKCVDRLDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVILNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGF ALLSYDEKEGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERASGGGSGSASSGASASGSSGSGSGSGSS SASSGAITWVPKPNVEVWPVDPPPPVNFNKTAEQEYGDKEVKLPHWTPTLHTFQVPQNYTKANCTYCNTREYTFSYKGCCFYFTKKKH TWQGCFQACAELYPCTYFYGPTPDILPVVTRSLQAIESLWVGVYRVGEGNWTSLDGGTFKVYQIFGSHCTYVSKFSTVPVSHHECSFL	231

	*KPCLCVSQRSNS*GGSGSASSGASASGSSGSGSGSGSSSASSGASSGGASGGSGGSGGGSGSASSGASASGSSGSGSGSGSSSASSGASSGGASGGSGGSGESQVRSQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINQIVDHAIKCKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGQENHGLYLADQYVKGIAKSRKS	
SIB 15013 gH/gL_rigid_NP	MRAVGVFLAICLVTIFVLPTWG*NWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDGFSLASLNSPKNGSNQLVISRCANGLNVVSFFISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGANLNRYAWHRGG*GGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGASGGSGGSG**AASLSEVKLHLDIEGHASHYTIPWTELMAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRAHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGDYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCREPELDTETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKMEELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERA**SGEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPESQVRQQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINNIVDHAIKCKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS	232
46 amino acid linker	GGSGSASSGASASGSSGGSGSGSGSGSSASSGASSGGASGGSGGSG	233
32 amino acid linker	SGGGSGSASSGASASGSSGSGSGSGSSSASSG	234
88 amino acid linker	GGSGSASSGASASGSSGSGSGSGSSSASSGASSGGASGGSGGSGGGSGSASSGASASGSSGSGSGSGSSSASSGASSGGASGGSGGSG	235
44 amino acid linker	GGSGSASSGASASGSSGSGSGSGSSSASSGASSGGASGGSGGSG	236
12 amino acid rigid linker	EPEPEPEPEPGG	237
48 amino acid rigid linker	SGEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEPEP	238

gp42 fusion segment	LAYFLPPRVRGGGRVAAAAITWVPKPNVEVWPVDPPPPVNFNKTAEQEYGDKEVKLPHWTPTLHTFQVPQNYTKANCTYCNTREYTFSYKGCCFYFTKKKHTWNGCFQACAELYPCTYFYGPTPDILPVVTRNLNAIESLWVGVYRVGEGNWTSLDGGTFKVYQIFGSHCTYVSKFSTVPVSHHECSFLKPCLCVSQRSNS	239
gp42 fusion segment 2	AITWVPKPNVEVWPVDPPPPVNFNKTAEQEYGDKEVKLPHWTPTLHTFQVPQNYTKANCTYCNTREYTFSYKGCCFYFTKKKHTWQGCFQACAELYPCTYFYGPTPDILPVVTRSLQAIESLWVGVYRVGEGNWTSLDGGTFKVYQIFGSHCTYVSKFSTVPVSHHECSFLKPCLCVSQRSNS	240
SIB 15014 gH/gL/gp42_ NP_ C17	MDSKGSSQKGSRLLLLLVVSNLLLPQGVLANWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDAFSLASLNSPKQGSNQLVISRCANGLNVVSFFISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGAQLNRYAWHRGGGGSGSASSGASASGSSGGSGSGSGSGSSASSGASSGGASGGSGGSG**AASLSEVKLHLDIEGHASHYTIPWTELLAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGEYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCREPELETETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAILMATVKMEELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSIAPQEATLDQAAVSQAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERA**SGGGSGSASSGASASGSSGSGSGSGSSSASSGAITWVPKPNVEVWPVDPPPPVNFNKTAEQEYGDKEVKLPHWTPTLHTFQVPQNYTKANCTYCNTREYTFSYKGCCFYFTKKKHTWQGCFQACAELYPCTYFYGPTPDILPVVTRNLNAIESLWVGVYRVGEGNWTSLDGGTFKVYQIFGSHCTYVSKFSTVPVSHHECSFLKPCLCVSQRSNSGGSGSASSGASASGSSGSGSGSGSSSASSGASSGGASGGSGGSGGGSGSASSGASASGSSGSGSGSGSSSASSGASSGGASGGSGGSGESQVRSQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINQIVDHAIKCKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGQENHGLYLADQYVKGIAKSRKS	241
SIB 15015 gH/gL/gp42_ NP_ C18	MDSKGSSQKGSRLLLLLVVSNLLLPQGVLANWAYPCCHVTQLRAQHLLALENISDIYLVSNQTCDAFSLASLNSPKQGSNQLVISRCANGLNVVSFFISILKRSSSALTGHLRELLTTLETLYGSFSVEDLFGAQLNRYAWHRGGGGSGSASSGASASGSSGGSGSGSGSGSSASSGASSGGASGGSGGSG**AASLSEVKLHLDIEGHASHYTIPWTELLAKVPGLSPEALWREANVTEDLASMLNRYKLIYKTSGTLGIALAEPVDIPAVSEGSMQVDASKVHPGVISGLNSPACMLSAPLEKQLFYYIGTMLPNTRPHSYVFYQLRCHLSYVALSINGDKFQYTGAMTSKFLMGTYKRVTEKGDEHVLSLVFGKTKDLPDLRGPFSYPSLTSAQSGEYSLVIVTTFVHYANFHNYFVPNLKDMFSRAVTMTAASYARYVLQKLVLLEMKGGCREPELETETLTTMFEVSVAFFKVGHAVGETGNGCVDLRWLAKSFFELTVLKDIIGICYGATVKGMQSYGLERLAAMLMATVKMEELGHLTTEKQEYALRLATVGYPKAGVYSGLIGGATSVLLSAYNRHPLFQPLHTVMRETLFIGSHVVLRELRLNVTTQGPNLALYQLLSTALCSALEIGEVLRGLALGTESGLFSPCYLSLRFDLTRDKLLSMAPQEATLDQAAVSNAVDGFLGRLSLEREDRDAWHLPAYKCVDRLDKVLMIIPLINVTFIISSDREVRGSALYEASTTYLSSSLFLSPVIMNKCSQGAVAGEPRQIPKIQNFTRTQKSCIFCGFALLSYDEKEGLETTTYITSQEVQNSILSSNYFDFDNLHVHYLLLTTNGTVMEIAGLYEERA**SGGGSGSASSGASASGSSGSGSGSGSSSASSGAITWVPKPNVEVWPVDPPPPVNFNKTAEQEYGDKEVKLPHWTPTLHTFQVPQNYTKANCTYCNTREYTFSYKGCCFYFTKKKHTWNGCFQACAELYPCTYFYGPTPDILPVVTRNLNAIESLWVGVYRVGEGNWTSLDGGTFKVYQIFGSHCTYVSKFSTVPVSHHECSFLKPCLCVSQRSNSGGSGSASSGASASGSSGSGSGSGSSSASSGASSGGASGGSGGSGGGSGSASSGASASGSSGSGSGSGSSSASSGASSGGASGGSGGSGESQVRSQFSKDIEKLLNEQVNKEMQSSNLYMSMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVPVQLTSISAPEHKFEGLTQIFQKAYEHEQHISESINQIVDHAIKCKDHATFNFLQWYVAEQHEEEVLFKDILDKIELIGQENHGLYLADQYVKGIAKSRKS	242

EXAMPLES

[00281] The following examples are provided to illustrate certain disclosed embodiments and are not to be construed as limiting the scope of this disclosure in any way.

1. Antigenic EBV polypeptides for eliciting antibodies against EBV

[00282] Antigenic polypeptides that elicit antibodies against EBV were developed. Self-assembling ferritin nanoparticles were developed that display EBV gL and gH polypeptides as a single-chain, and the immunogenicity of these nanoparticles in mice was evaluated.

[00283] Monomeric and trimeric gL/gH constructs were expressed and purified. Figure 1A shows single-chain gL and gH monomer (SEQ ID NO: 6) +/- His-tag cleavage by Coomassie and western blot (anti-His) analysis. Figure 1B shows fractionation of a gL and gH trimer (SEQ ID NO: 11) on a Superose® SEC column as an absorbance trace and by Coomassie, along with a western blot to confirm His-tag cleavage by thrombin protease. The final concentration of samples was 1 mg/mL, the total volume was 15 mL, and the endotoxin level was 1.48 EU/mL for the SEQ ID NO: 6 construct.

[00284] Single-chain gL/gH ferritin nanoparticles (SEQ ID NO: 14) were expressed and purified. Figures 2A-2E show purification and characterization thereof by Superose® 6 SEC fractionation (2A), Coomassie of SEC fractions (2B), western blot of SEC fractions with anti-ferritin primary Ab (2C), dynamic light scattering (DLS, 2D), and electron microscopy (2E).

[00285] Exemplary constructs of single-chain EBV gL and gH fused to ferritin are shown in Figure 3. A conjugation site for an immune-stimulatory moiety, such as a toll-like receptor 7/8 agonist (TLR7/8a), can be present either on the ferritin or in the linker (*see, e.g.*, SEQ ID NOS: 14, 19, 22, 20, 23, and 33 for exemplary sequences).

[00286] gL/gH trimers or nanoparticles with different linkers were injected into mice and immune sera were assessed (Figure 4). Mice were given two 2-µg injections with adjuvant AF03, a squalene emulsion-based adjuvant, with a 3-week interval between doses. Anti-gL/gH antibody endpoint titers were measured by ELISA at week 6. For gH_16_gL, a nanoparticle (SEQ ID NO: 10) outperformed a trimer construct (SEQ ID NO: 16). The gL_28_gH nanoparticle (SEQ ID NO: 13) did not perform significantly differently from the

trimer construct (SEQ ID NO: 11). The gL_46_gH nanoparticle (SEQ ID NO: 14) outperformed the gL_46_gH trimer (SEQ ID NO: 12).

[00287] These data indicate that single-chain gL/gH nanoparticles can elicit a robust immune response against EBV.

2. Bivalent immunization against gL/gH and gp220

[00288] Bivalent immunization was performed using compositions comprising single-chain gL/gH nanoparticles and gp220 nanoparticles. Including the gp220 nanoparticles (SEQ ID NO: 1) had no significant interfering effect on the immune response elicited by single-chain gL/gH nanoparticles (gL-gH_C5 NP [SEQ ID NO: 19]), as measured by an ELISA binding assay using sera from mice vaccinated as described above (Figures 5A-5B, showing measurements at individual dilutions and binding titers, respectively). Similarly, no interference was observed in the response to the immune response to gp220 nanoparticles when administered in combination with the single-chain gL/gH nanoparticles, as measured by ELISA (Figures 6A-6B, showing measurements at individual dilutions and binding titers, respectively).

[00289] Thus, immunization with both a single-chain gL/gH nanoparticle and a gp220 nanoparticle did not decrease the immune response to either polypeptide.

3. Conjugation of adjuvant to ferritin nanoparticles

[00290] Next, conjugation of adjuvants to ferritin nanoparticles was assessed. Figure 7A illustrates a construct in which the ferritin comprises a mutation replacing a surface-exposed amino acid with a cysteine, which is available for conjugation. Figure 7B shows an exemplary immune-stimulatory moiety (SM7/8a, a TLR-7/8 agonist) linked to a PEG4 linker and maleimide. This maleimide can be used to covalently conjugate the linker (itself attached to SM7/8a) to the surface-exposed cysteine of the ferritin. A polypeptide comprising a single-chain gL/gH polypeptide fused to ferritin conjugated to SM7/8a is shown in the electron micrograph of Figure 7C.

[00291] A cysteine resulting from mutation of a surface-exposed amino acid is illustrated in the structure a ferritin molecule in Figure 8A. Conjugation of a CpG adjuvant (SEQ ID NO: 230) to ferritin is illustrated in Figure 8B by juxtaposing the ferritin, linker, and CpG adjuvant, oriented to show the parts of each moiety that become attached to each other in proximity.

[00292] A gL/gH nanoparticle (SEQ ID NO: 19) was reduced using 2mM TCEP and then oxidized via the addition of 1X PBS and using a 100kD microspin column to remove TCEP. SM7/8a was then incubated with the gL/gH nanoparticle for conjugation. Excess SM7/8a was removed from the reaction via a 100kD microspin column. Mass spectrometry (MS) data indicated that about 100% of the polypeptide comprising single-chain gL/gH and ferritin (SEQ ID NO: 19) was conjugated to SM7/8a (Figure 9B) based on shift of the main MS peak relative to the spectrum of the unconjugated polypeptide (Figure 9A). The difference between the mass of the conjugated and unconjugated polypeptide corresponds to the molecular weight of the SM7/8a-linker-maleimide adduct (711 Da).

[00293] A gp220 nanoparticle (SEQ ID NO: 1) was reduced using 2mM TCEP and then oxidized via the addition of 1X PBS and using a 100kD microspin column to remove TCEP. The SM7/8a was then incubated with the gL/gH nanoparticle for conjugation. Excess SM7/8a was removed from the reaction via a 100kD microspin column. MS data indicated that about 100% of a conjugated polypeptide comprising gp220 and ferritin (SEQ ID NO: 1) is conjugated to SM7/8a (Figure 10B) based on shift of the main MS peak relative to the spectrum of the unconjugated polypeptide (Figure 10A).

[00294] Electron microscopy (EM) data also confirmed that conjugation of SM7/8a to polypeptides comprising single-chain gL/gH and ferritin (Figure 11B in comparison to unconjugated sample in Figure 11A) or comprising gp220 and ferritin (Figure 11D in comparison to unconjugated sample in Figure 11C) did not disrupt nanoparticle assembly.

[00295] Antibody responses were assayed by ELISA following immunization with 1 μg of nanoparticles comprising single-chain gL/gH (gL_gH_C5 NP, Figures 12A and 12B) or nanoparticles comprising gp220 (Figures 13A and 13B). Nanoparticles were in combination with 1 μg of naked ferritin and were unconjugated or conjugated to SM7/8a. Unconjugated nanoparticles were administered with or without admixed AF03 adjuvant. Each mouse received 100 μL of the nanoparticle composition as described above. For mice receiving AF03 adjuvant, a 1:1 volume of AF03 was mixed with the nanoparticles. BALB/c mice (n=5/group) were immunized twice with a 3-week interval between doses. A bleed was taken for ELISA analysis at week 5. The most robust ELISA responses were seen for nanoparticles administered in the AF03 adjuvant. Conjugation to SM7/8a produced a more robust ELISA response compared to unconjugated nanoparticles without adjuvant.

[00296] The effect of coadministration of 1 μg each of gL_gH_C5 nanoparticles conjugated to SM7/8a and gp220 nanoparticles conjugated to SM7/8a was also assessed, as

compared to single administration of either nanoparticle accompanied by naked ferritin nanoparticles in Figures 14A-14B and 15A-15B. No interference was observed on the immune response to either single-chain gL/gH (Figures 14A-14B, without and with AF03, respectively) or gp220 (Figures 15A-15B, without and with AF03, respectively).

4. Long-term immunogenicity studies

[00297] Studies were performed to assess immunogenicity at 3 months after dosing with nanoparticles comprising single-chain gL/gH (gL/gH_C5, SEQ ID NO: 19). BALB/c mice (n=5/group) were immunized twice with a 3-week interval between doses. Naked ferritin (i.e., ferritin not conjugated to any polypeptide or adjuvant) was administered at 1 μg with the 1 μg nanoparticles comprising single-chain gL/gH, and the nanoparticles were formulated in the presence or absence of admixed AF03 adjuvant. A bleed was taken for ELISA analysis at week 13. For mice receiving AF03 adjuvant, a 1:1 volume of AF03 was mixed with the nanoparticle composition. Each mouse received 100 μL of the nanoparticle composition described above. Some mice received nanoparticles comprising single-chain gL/gH in which the ferritin was conjugated to SM7/8a ("7/8a" in Figures 16-17).

[00298] As shown in Figure 16, nanoparticles comprising single-chain gL/gH conjugated to SM7/8a produced the greatest immune response when formulated in AF03. A robust immune response was also seen for these nanoparticles without AF03.

[00299] A parallel experiment was performed using gp220 nanoparticles (SEQ ID NO: 1) (with or without conjugation to SM7/8a) in place of the nanoparticles comprising single-chain gL/gH. Similar results were seen for these nanoparticles, wherein the formulation including admixed AF03 produced the most robust response, and a robust immune response was also seen for these nanoparticles without AF03 (Figure 17).

[00300] The immune response elicited by a bivalent composition comprising nanoparticles comprising single-chain gL/gH (gL/gH_C5; SEQ ID NO: 19) and nanoparticles comprising gp220 (SEQ ID NO: 1) was assessed. BALB/c mice (n=5/group) were immunized with a 3-week interval between doses. 100 μL of the nanoparticle composition containing 1 μg of each nanoparticle was administered. For mice receiving AF03 adjuvant, a 1:1 volume of AF03 was mixed with vaccine. A terminal week 13 bleed was taken for ELISA analysis. For immune responses against both single-chain gL/gH (Figure 18) and gp220 (Figure 19), no interference was seen due to administration of the nanoparticles in combination, as compared to administration of either nanoparticle in combination with naked ferritin.

[00301] Further experiments with the gL/gH_C5 nanoparticle (SEQ ID NO: 19) confirmed that long-term immune responses were seen when the nanoparticle was conjugated to SM7/8a (7/8a) or when the nanoparticle was formulated in AF03 (Figure 21). BALB/c mice (n=5/group) were immunized with a 3-week interval between doses. 100 µL of the nanoparticle composition containing 1 µg of nanoparticles was administered. For mice receiving AF03 adjuvant, a 1:1 volume of AF03 was mixed with vaccine. Week 2 (Prime), 5 (Boost), and 13 (Terminal) bleeds were taken for ELISA analysis. A parallel experiment was performed using gp220 nanoparticles (SEQ ID NO: 1) and a similar long-term response was also seen for gp220 nanoparticles (Figure 22).

[00302] A different nanoparticle comprising single-chain gL/gH (gL_gH_C7: SEQ ID NO: 20) was also assessed. The gL_gH_C7 construct comprises a flexible linker between the gH polypeptide and the ferritin with a cysteine as a conjugation site for an immune-stimulatory moiety. The linker may be used with a ferritin lacking a surface-exposed cysteine (as shown in SEQ ID NO: 20). SM7/8a was conjugated to gL_gH_C7 by reducing the protein using 2mM TCEP and then oxidizing by adding 1X PBS and using a 100kD microspin column to remove TCEP. The SM7/8a was then incubated with the gL/gH nanoparticle. Following conjugation, excess SM7/8a was removed from the reaction via a 100kD microspin column.

[00303] Mice received 1 µg of these gL/gH nanoparticles, either conjugated to 7/8a or unconjugated, plus 1 µg of naked ferritin. 100 µL of the nanoparticle composition containing 1 µg of nanoparticles was administered. BALB/c mice (n=5/group) were immunized with a 3-week interval between doses. For mice receiving AF03 adjuvant, a 1:1 volume of AF03 was mixed with the nanoparticle composition. Week 2 (prime), 5 (booster), and 13 (terminal) bleeds were taken for ELISA analysis. These nanoparticles elicited immune responses when formulated in AF03 or when conjugated to SM7/8a as measured by ELISA endpoint titer at prime bleed (Figure 20A). Similar results were seen with booster bleed (Figure 20C) or terminal bleed (Figure 20D) samples. These nanoparticles were also conjugated to a CpG oligodeoxynucleotide, and administered in the same way. Results for the CpG conjugate were similar to unconjugated nanoparticles (Figure 20B) at week 5.

5. Characterization of nanoparticles comprising *Trichoplusia ni* ferritin

[00304] Nanoparticles were also developed comprising *Trichoplusia ni* ferritin and gp220 and/or gL/gH polypeptides. *Trichoplusia ni* ferritin nanoparticles contain heavy and light chains self-assembled at a 1:1 ratio. It was found that combining one non-ferritin

polypeptide with the light chain and another non-ferritin polypeptide on the heavy chain allowed presentation of two distinct polypeptides on the surface of individual nanoparticles. Thus, for example, a self-assembled *Trichoplusia ni* ferritin nanoparticle could present both gp220 and gL/gH.

[00305] A *Trichoplusia ni* ferritin nanoparticle was produced and purified with the heavy chain fused to either gp220 (SEQ ID NO: 24) or single-chain gL/gH (SEQ ID NO: 25) and the light chain fused to either gp220 (SEQ ID NO: 26) or single-chain gL/gH (SEQ ID NO: 27) (constructs illustrated in Figure 23B and visualized by Coomassie gel staining in Figure 23A, showing the expected increase in molecular weight relative to light and heavy chains alone). The combination of a light chain and a heavy chain fused to gL/gH and gp220, respectively or vice versa, generated an individual multivalent nanoparticle that can present two different EBV polypeptides.

[00306] Two *T. ni* ferritin nanoparticles with either only gp220 in both the heavy and light chains (as shown in Figure 24E) or gp220 in the heavy chain and gH_gL in the light chain (as shown in Figure 25E) were also produced. The purification followed two steps: The first purification step was an ion exchange chromatographic step (Q column, see Figure 24A with Coomassie results in Figure 24C and Figure 25A with Coomassie results shown in Figure 25C). This step was followed by size exclusion chromatography (see Figure 24B with Coomassie results in Figure 24D and 25B with Coomassie results in Figure 25D).

[00307] Nanoparticles comprising *Trichoplusia ni* light and heavy chain fused to gp220 (SEQ ID NOs: 24 and 26; illustrated in Figure 26B) showed a profile consistent with formation of a nanoparticle comprising the heterologous gp220 polypeptide, based on Coomassie staining (Figure 26A), an increase in DLS radius (Figure 26D) relative to naked *T. ni* ferritin (Figure 27C), and EM analysis (Figure 26C) in which additional peripheral density around the nanoparticle core appeared relative to the naked nanoparticles (Figure 27B). Similar results indicative of the presence of heterologous gL/gH and gp220 polypeptides in the nanoparticles were seen for SEQ ID NOs: 24 and 27 (*Trichoplusia ni* light chains with a gL/gH polypeptide and heavy chains with a gp220 polypeptide; see Figures 26E-26H for visualization by Coomassie staining, an illustration of the construct, an electron micrograph, and characterization by DLS, respectively). For comparison, Figures 27A-27C show Coomassie staining (Figure 27A), DLS radius (Figure 27B), and EM analysis (Figure 27C) for naked *T. ni* ferritin (i.e., not conjugated to any polypeptide).

[00308] Thus, use of *T. ni* ferritin allows presentation of 2 polypeptides on individual nanoparticles.

6. **gH/gL/gp42 Constructs**

[00309] A cartoon of a single-chain construct of gH/gL/gp42 fused to ferritin (as in each of SEQ ID NOs: 227-231 and 241-242) is shown in FIG 35A. The fusion between each protein is via a flexible amino acid linker or a rigid amino acid linker. The single-chain gH/gL/gp42 molecule provides a 1:1:1 ratio of heterotrimer formation on the nanoparticle.

[00310] The crystal structure of a gH/gL/gp42 His-tagged fusion (SEQ ID NO: 226) has been solved to show that the single-chain gH/gL/gp42 can adopt a heterotrimer conformation similar to wild-type gH, gL, and gp42 proteins found in nature (FIGs 34 and 35B). In FIG 34 and 35B, Gp42 (in dark gray and indicated with arrows in FIG 34) interacts with the gH/gL heterodimer. FIG 35C is a model of how this single-chain gH/gL/gp42 heterotrimer fused to ferritin is displayed on a nanoparticle. There are twenty-four copies of the single-chain gH/gL/gp42 that will be displayed on a single nanoparticle.

[00311] A gH/gL/gp42 NP construct (SEQ ID NO: 227) was expressed in 293 expi cells and purified (FIG 28A). gH/gL/gp42 NP purified from CHO pools had a dynamic light scattering radius of around 26.2nm (FIG 28B).

[00312] The immune responses elicited by a monovalent (gH/gL/gp42 NP + naked ferritin nanoparticle) or bivalent (gH/gL/gp42 NP + gp220 NP) composition were assessed. The gH/gL/gp42 NP had the sequence of SEQ ID NO: 227 and the gp220 NP had the sequence of SEQ ID NO: 1. BALB/c mice (n=5/group) were immunized with a 3-week interval between doses. 100 μL of the nanoparticle composition containing 1 μg of each nanoparticle was administered with an AF03 adjuvant (1:1 volume of AF03 mixed with vaccine). The boost indicates the sera collected at week 5 after the second immunization. EBV viral neutralizing assay analysis in B cells (FIG 29A) and in epithelial cells (FIG 29B) was done using sera collected at week 5 from the mice. No interference was seen due to administration of the nanoparticles in bivalent formulation, as compared to administration of the monovalent form (gH/gL/gp42 with naked ferritin).

[00313] Bivalent immunization of ferrets was performed using compositions comprising single-chain gL/gH nanoparticles (gL_gH_C137A_bfpFerr Nanoparticle N19Q/C31S/S111C [SEQ ID NO: 22]) and gp220 nanoparticles (SEQ ID NO: 1) in the presence of adjuvant AF03 (FIGs 30A-30B) or gL/gH/gp42 NP (SEQ ID NO: 227) and gp220 nanoparticles (SEQ ID NO: 1) in the presence of adjuvant AF03 (FIGs 30C-30E). Inj.

1 = injection one (sera collected from 6 ferrets at week 2 post Inj. 1). Inj. 2 = injection 2 (sera collected from 6 ferrets at week 2 post Inj. 2). An ELISA binding assay measured endpoint binding titers against the antigens indicated in FIGs 30A-30E. FIGs 30F-G shows an EBV viral neutralizing assay (in B cells and epithelial cells, respectively) of sera from ferrets receiving bivalent vaccination of gL/gH/gp42 NP (SEQ ID NO: 227) and gp220 nanoparticles (SEQ ID NO: 1) in the presence of adjuvant AF03. Prime = Inj. 1 and Boost = Inj. 2.

[00314] gH/gL/gp42_NP_ C12 (SEQ ID NO: 228) was expressed and purified using Superose 6 size exclusion chromatography (FIG 31A). A dynamic light scattering analysis of the sample in FIG 31A showed a particle size radius of 20.6 nm (FIG 31B).

[00315] gH/gL/gp42_NP_ C13 (SEQ ID NO: 229) was expressed and purified using Superose 6 size exclusion chromatography (FIG 32A). A dynamic light scattering analysis of the sample in FIG 32A showed a particle size radius of 17.1 nm (FIG 32B).

[00316] gH/gL/gp42_NP_ C14 (SEQ ID NO: 230) was expressed and purified using Superose 6 size exclusion chromatography (FIG 33A). A dynamic light scattering analysis of the sample in FIG 33A showed a particle size radius of 16.9 nm (FIG 33B).

[00317] FIG 35D shows the purification of SEQ ID NO: 227 after expression in 293Expi cells. A denaturing SDS coomassie gel shows the gH/gL/gp42 fused to ferritin to be above 150kD with glycosylation. Negative stain electron microscopy analysis of the purified product shows the single-chain gH/gL/gp42 fused to ferritin can successfully form nanoparticles displaying the gH/gL/gp42 antigens on the surface (FIG 35E). Through temperature, oxidation, and/ or deamidation stress test on days 0, 3, 7, or 14, potential labile sequences have been identified via sequence analysis or mass spectrometry for the single-chain gH/gL/gp42 nanoparticle of SEQ ID NO: 227. To improve vaccine stability, expression, and/or immunogenicity of this vaccine construct, conservative amino acid substitution mutations will be made to SEQ ID NO: 227 in different combinations, particularly at the sites listed in Table 1. Conservative amino acid mutations at the respective location in the particular gene will also be tested in SEQ ID NOs: 228-230, which differ from SEQ ID NOs. 227 only by the linker sequence that fuses the C-terminus of gp42 with the N-terminus of the ferritin sequence.

We claim:

1. An antigenic EBV polypeptide comprising an Epstein Barr Virus (EBV) gL polypeptide and an EBV gH polypeptide, wherein a linker having a length of at least 15 amino acids separates the EBV gL polypeptide and the EBV gH polypeptide.
2. An antigenic EBV polypeptide comprising an Epstein Barr Virus (EBV) gL polypeptide, an EBV gH polypeptide, and an EBV gp42 polypeptide, wherein a linker having a length of at least 15 amino acids separates the EBV gL polypeptide and the EBV gH polypeptide.
3. The antigenic EBV polypeptide of claim 1 or claim 2, further comprising a ferritin.
4. An antigenic EBV polypeptide comprising an EBV polypeptide and a ferritin protein, wherein the ferritin protein comprises a mutation replacing a surface-exposed amino acid with a cysteine.
5. The antigenic EBV polypeptide of claim 4, wherein the EBV polypeptide comprises an EBV gL polypeptide, an EBV gH polypeptide, or an EBV gp220 polypeptide.
6. The antigenic EBV polypeptide of claim 5, wherein the EBV polypeptide comprises a gL polypeptide and the polypeptide further comprises an EBV gH polypeptide.
7. The antigenic EBV polypeptide of any one of claims 1 or 3-6, wherein the polypeptide further comprises an EBV gp42 polypeptide.
8. A composition comprising a first antigenic EBV polypeptide and a second antigenic EBV polypeptide, wherein the first antigenic EBV polypeptide comprises a ferritin heavy chain and a first EBV polypeptide, the second antigenic EBV polypeptide comprises a ferritin light chain and a second EBV polypeptide, and the first and second EBV polypeptides are different.
9. The composition of claim 8, wherein the first EBV polypeptide or the second EBV polypeptide comprises a gp220 polypeptide.
10. The composition of any one of claims 8 to 9, wherein (i) the first antigenic EBV polypeptide comprises one or both of a gL polypeptide and a gH polypeptide and the second antigenic EBV polypeptide comprises a gp220 polypeptide, or (ii) the first antigenic EBV polypeptide comprises a gp220 polypeptide and the second antigenic EBV polypeptide comprises one or both of a gL polypeptide and a gH polypeptide.
11. The composition of any one of claims 8 to 10, wherein the first antigenic EBV polypeptide comprises a gL polypeptide and a gH polypeptide; or the second antigenic EBV polypeptide comprises a gL polypeptide and an EBV gH polypeptide.

12. The composition of claim 10 or 11, wherein the antigenic EBV polypeptide comprising a gL polypeptide and/or a gH polypeptide further comprises a gp42 polypeptide.
13. The antigenic EBV polypeptide or composition of any one of claims 1-12, comprising a gH and gL polypeptide, wherein the gH polypeptide is C-terminal to the gL polypeptide, optionally comprising a gp42 polypeptide, wherein the gp42 polypeptide is C-terminal to the gH polypeptide.
14. The antigenic EBV polypeptide or composition of any one of claims 1-13, comprising a gp42 polypeptide, wherein the gp42 polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 239 or 240.
15. The antigenic EBV polypeptide or composition of any one of claims 1-14, comprising an EBV gH polypeptide and an EBV gp42 polypeptide, wherein a linker having a length of at least 15 amino acids separates the EBV gH polypeptide and the EBV gp42 polypeptide, optionally wherein the linker has a length of 15 to 60 amino acids, 20 to 60 amino acids, 30 to 60 amino acids, 40 to 60 amino acids, 30 to 50 amino acids, or 40 to 50 amino acids, further optionally wherein the linker comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 234.
16. The antigenic EBV polypeptide or composition of any one of the preceding claims, comprising a linker, wherein the linker has a length of at least 15 amino acids, optionally wherein the linker separates a first EBV polypeptide and a second EBV polypeptide.
17. The antigenic EBV polypeptide or composition of claim 16, wherein the linker has a length of 15 to 60 amino acids, 20 to 60 amino acids, 30 to 60 amino acids, 40 to 60 amino acids, 30 to 50 amino acids, or 40 to 50 amino acids.
18. The antigenic EBV polypeptide or composition of any one of the preceding claims, wherein the EBV polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 36.
19. The antigenic EBV polypeptide or composition of any one of the preceding claims, wherein the EBV polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 37.
20. The antigenic EBV polypeptide or composition of any one of the preceding claims, wherein the polypeptide comprises a linker comprising an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 30,

optionally wherein the linker separates a first EBV polypeptide and a second EBV polypeptide.

21. The antigenic EBV polypeptide or composition of any one of the preceding claims, wherein the EBV polypeptide comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 38.
22. The antigenic EBV polypeptide or composition of any one of claims 3-21, further comprising a further linker that separates the EBV polypeptide and the ferritin.
23. The antigenic EBV polypeptide or composition of any one of claims 3-22, comprising an EBV gp42 polypeptide located N-terminal to the ferritin and C-terminal to the gH polypeptide, wherein a linker separates the EBV gp42 polypeptide and the ferritin, optionally wherein the linker has a length of at least 15 amino acids or has a length of 15 to 60 amino acids, 20 to 60 amino acids, 30 to 60 amino acids, 40 to 60 amino acids, 30 to 50 amino acids, or 40 to 50 amino acids, further optionally wherein the linker comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to any one of SEQ ID NOs: 233, 234, 235, 236, 237, or 238.
24. The antigenic EBV polypeptide or composition of claim 22 or 23, wherein the linker comprises a cysteine.
25. The antigenic EBV polypeptide or composition of any one of claims 22-24, wherein the linker comprises an amino acid sequence with at least 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 33.
26. The antigenic EBV polypeptide or composition of claim 24-25, wherein the cysteine is conjugated to an immune-stimulatory moiety, optionally wherein the immune-stimulatory moiety is an agonist of TLR2, TLR7/8, TLR9, or STING.
27. The antigenic EBV polypeptide or composition of any one of claims 3-26, wherein the ferritin comprises one or more of E12C, S26C, S72C, A75C, K79C, S100C, and S111C mutations of *H. pylori* ferritin or one or more corresponding mutations in a non-*H. pylori* ferritin as determined by pairwise or structural alignment.
28. The antigenic EBV polypeptide or composition of any one of claims 3-27, wherein the ferritin comprises a mutation replacing a surface-exposed asparagine with a non-asparagine amino acid, optionally wherein the asparagine is at position 19 of *H. pylori* ferritin, or an analogous position in a non- *H. pylori* ferritin as determined by pairwise or structural alignment.

29. The antigenic EBV polypeptide or composition of any one of claims 3-28, wherein the ferritin comprises a mutation replacing an internal cysteine with a non-cysteine amino acid, optionally wherein the internal cysteine is at position 31 of *H. pylori* ferritin, or a position that corresponds to position 31 of *H. pylori* ferritin as determined by pair-wise or structural alignment.
30. The antigenic EBV polypeptide or composition of any one of claims 3-29, wherein the ferritin comprises an amino acid sequence with 80%, 85%, 90%, 95%, 98%, or 99% identity to any one of SEQ ID NOs: 201-207 or 211-215.
31. The antigenic EBV polypeptide or composition of any one of claims 1-31, wherein the antigenic EBV polypeptide comprises a sequence with at least 80%, 85%, 90%, 95%, 98%, or 99% identity to amino acids 23-1078 of SEQ ID NO: 226.
32. The antigenic EBV polypeptide or composition of any one of claims 1-31, wherein the antigenic EBV polypeptide comprises a sequence with at least 80%, 85%, 90%, 95%, 98%, or 99% identity to any one of SEQ ID NOs: 226-231 or 241-242, optionally lacking the leader sequence.
33. A ferritin particle comprising the antigenic EBV polypeptide or the first and second polypeptides of any one of claims 3-32.
34. A composition comprising the antigenic EBV polypeptide(s) or ferritin particle of any one of the preceding claims and a pharmaceutically acceptable carrier.
35. The composition of claim 34, wherein the ferritin particle comprises an EBV gL polypeptide and an EBV gH polypeptide, and the composition further comprises a second ferritin particle comprising a gp220 polypeptide.
36. The antigenic EBV polypeptide, ferritin particle, or composition of any one of the preceding claims for use in a method of eliciting an immune response to influenza or in protecting a subject against infection with EBV.
37. A method of eliciting an immune response to EBV or protecting a subject against infection with EBV comprising administering any one or more antigenic EBV polypeptide, ferritin particle, or composition of any one of the preceding claims to a subject.
38. The antigenic EBV polypeptide, ferritin particle, composition, or method of any one of claims 36-37, wherein the subject is human.
39. A nucleic acid encoding the antigenic EBV polypeptide of any one of claims 1-32, optionally wherein the nucleic acid is an mRNA.

Construct: SEQ ID NO: 6 Monomer

Coomassie Western Anti-His

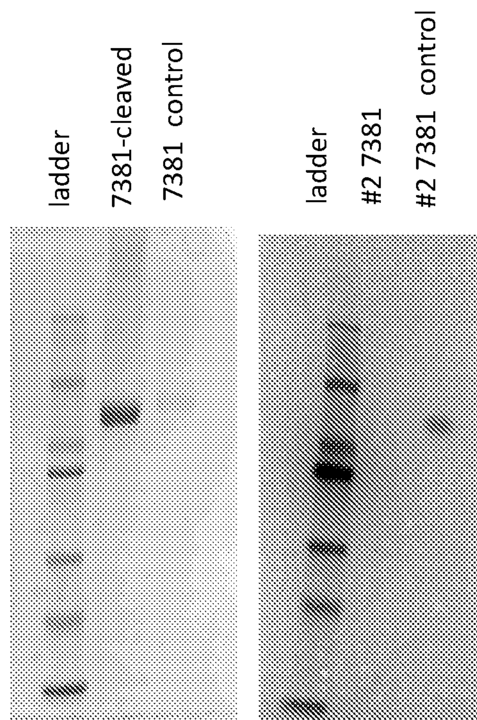


Fig. 1A

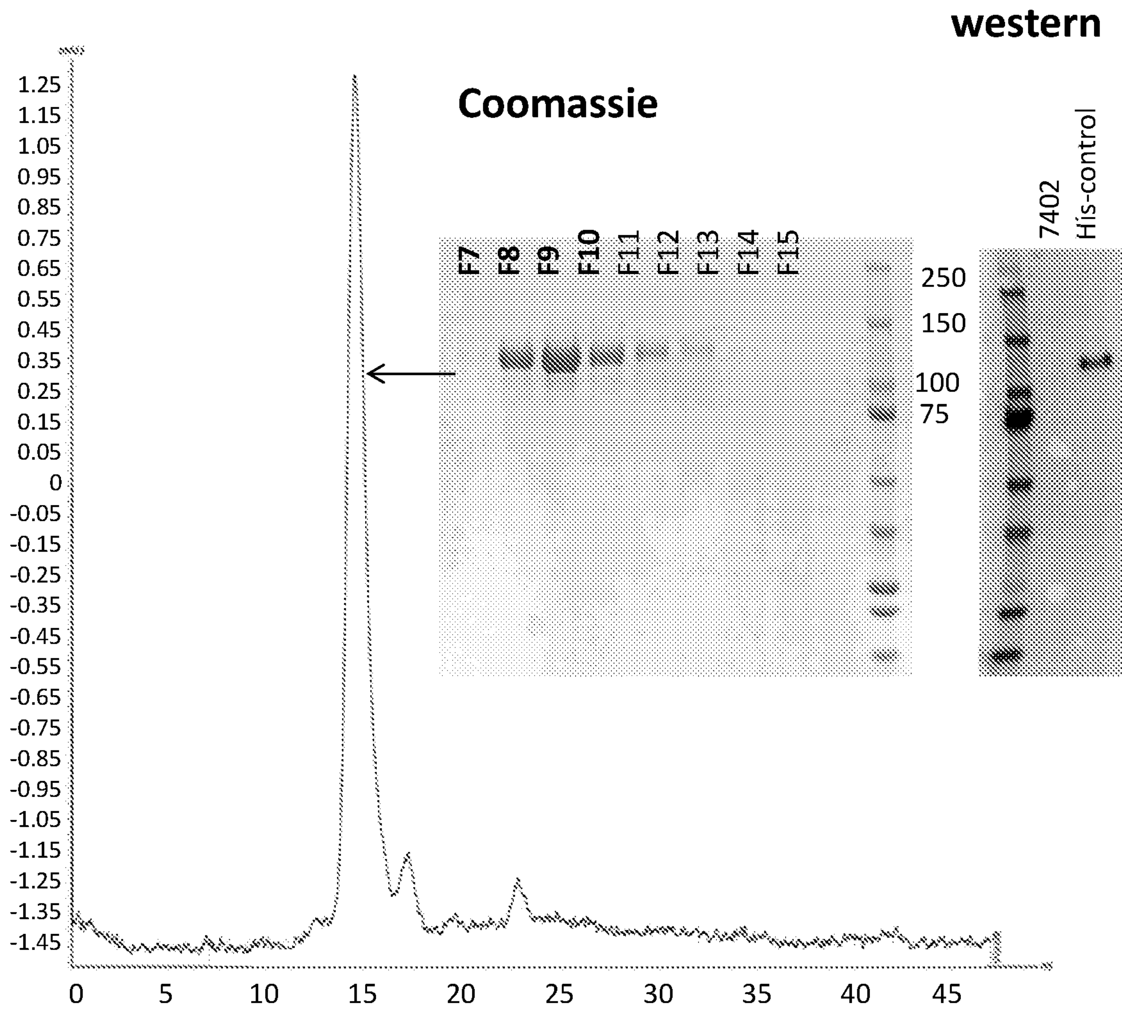


Fig. 1B

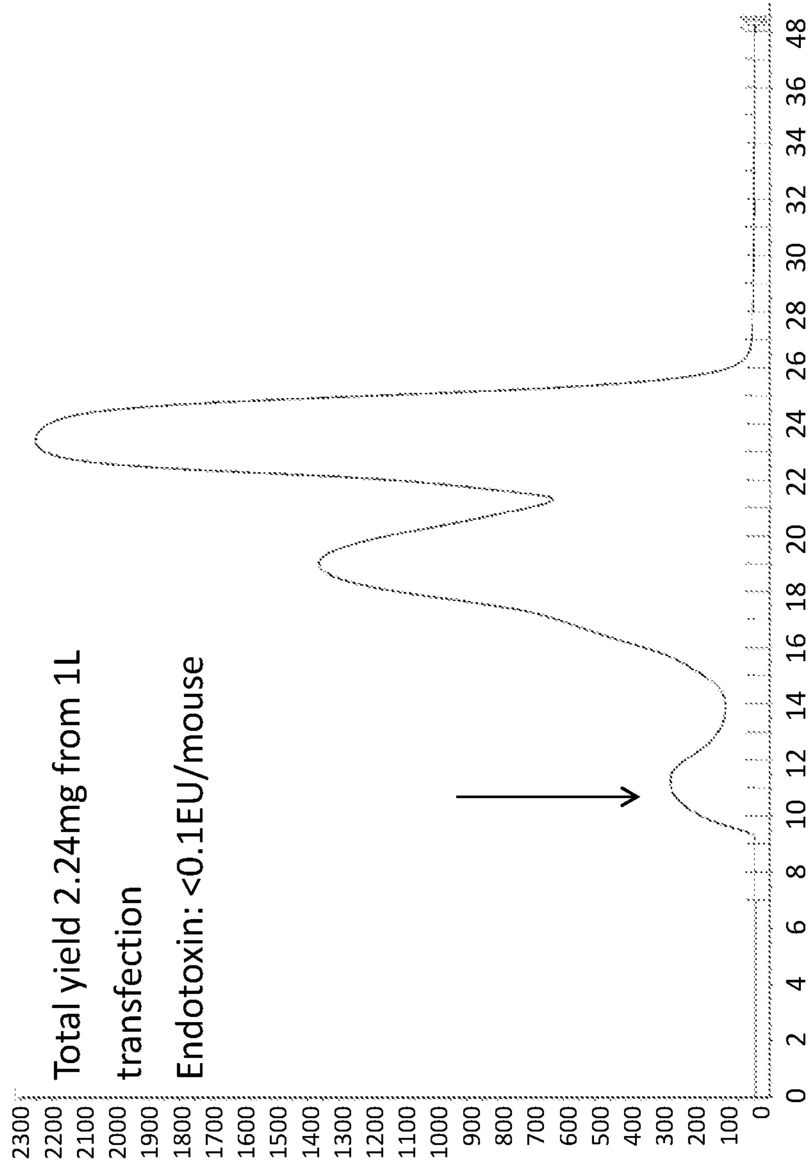


Fig. 2A

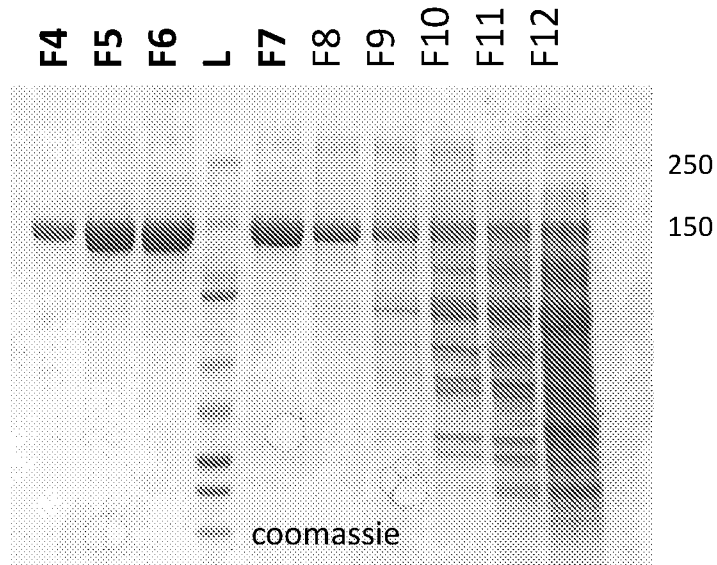


Fig. 2B

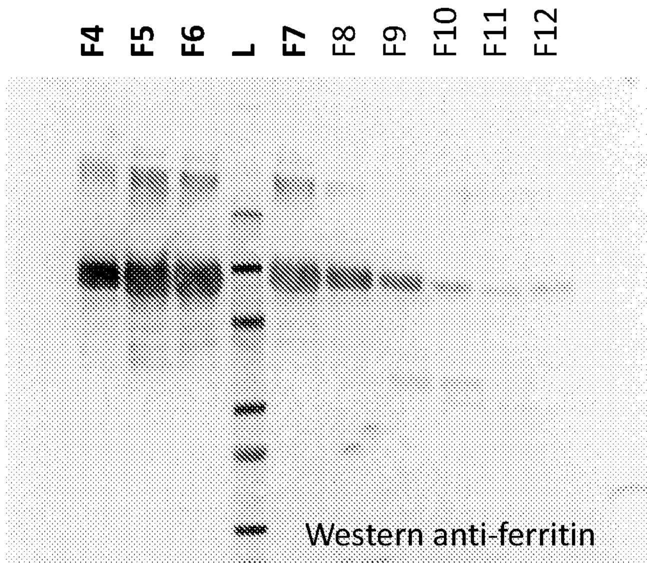
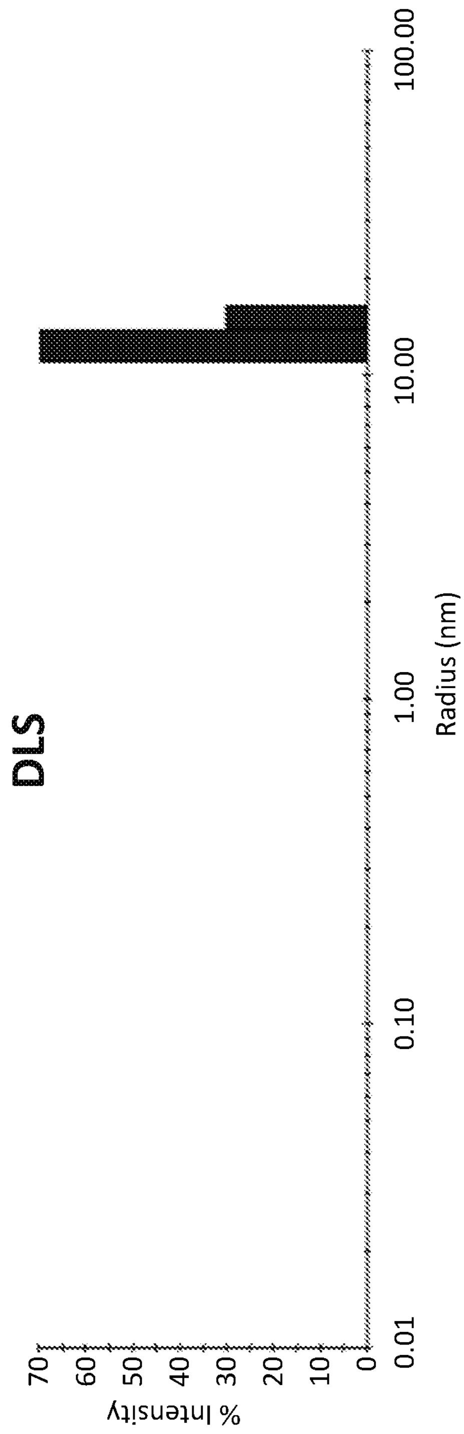


Fig. 2C



Intensity Distribution	Radius (nm)	%Pd	Mw-R (kDa)	%Intensity	%Mass
☒ Peak 1	13.3	11.4	1433	100.0	100.0

Fig. 2D

Negative stain EM image of gL and gH nanoparticle

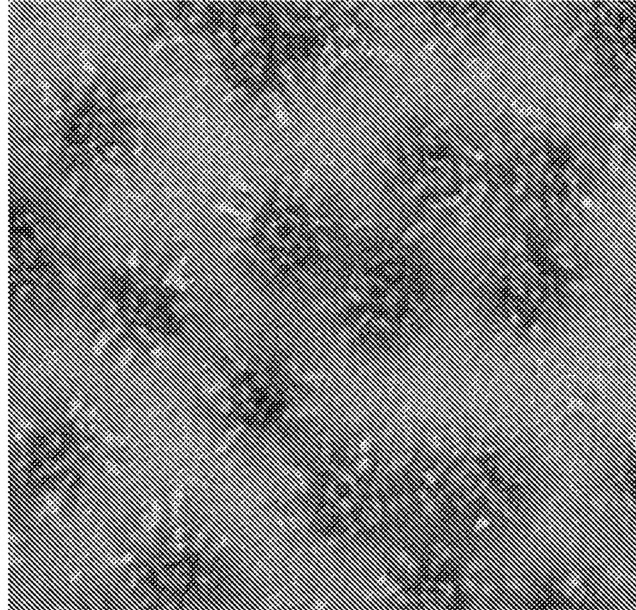


Fig. 2E

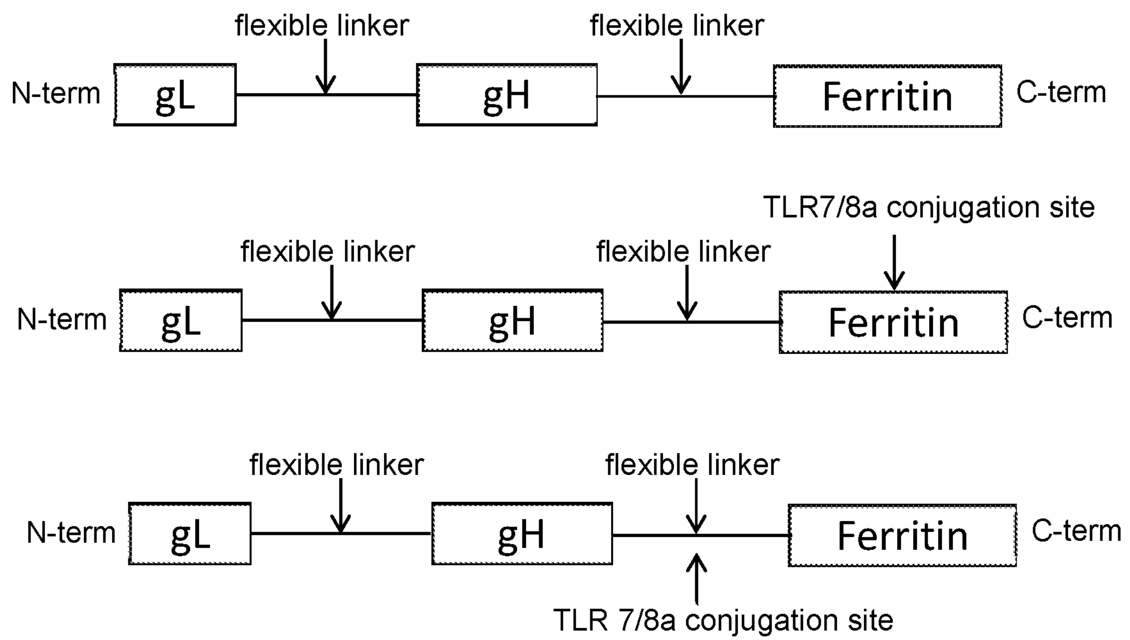


Fig. 3

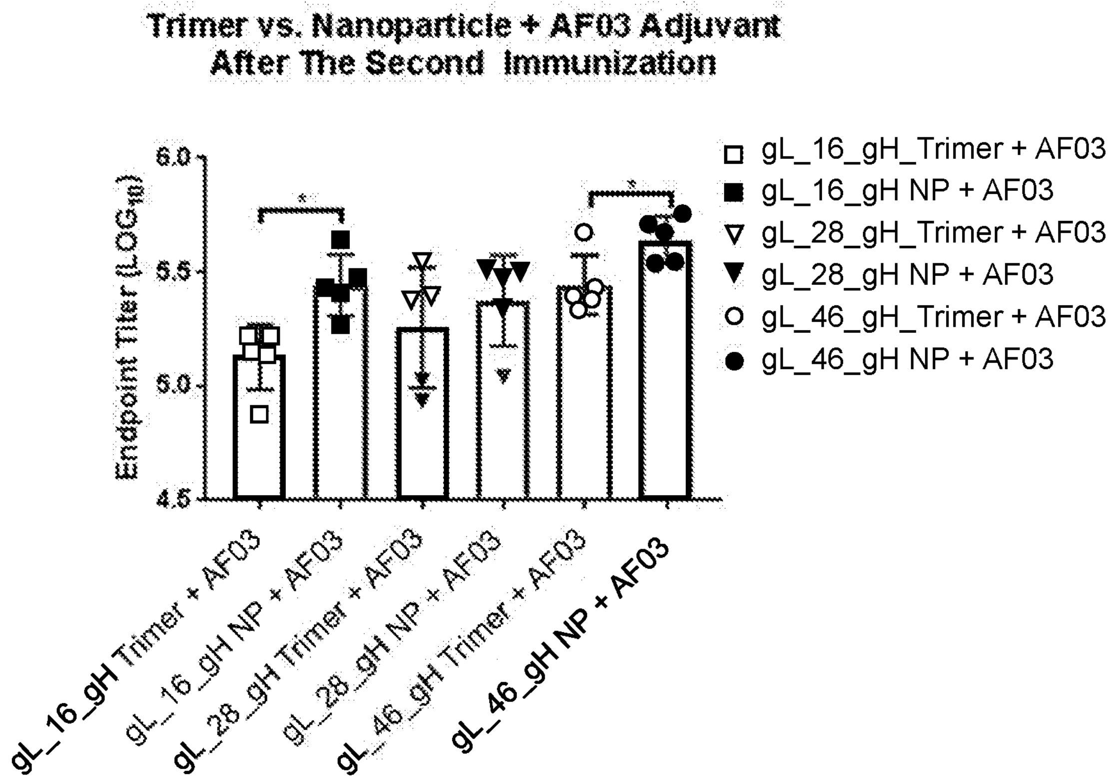


Fig. 4

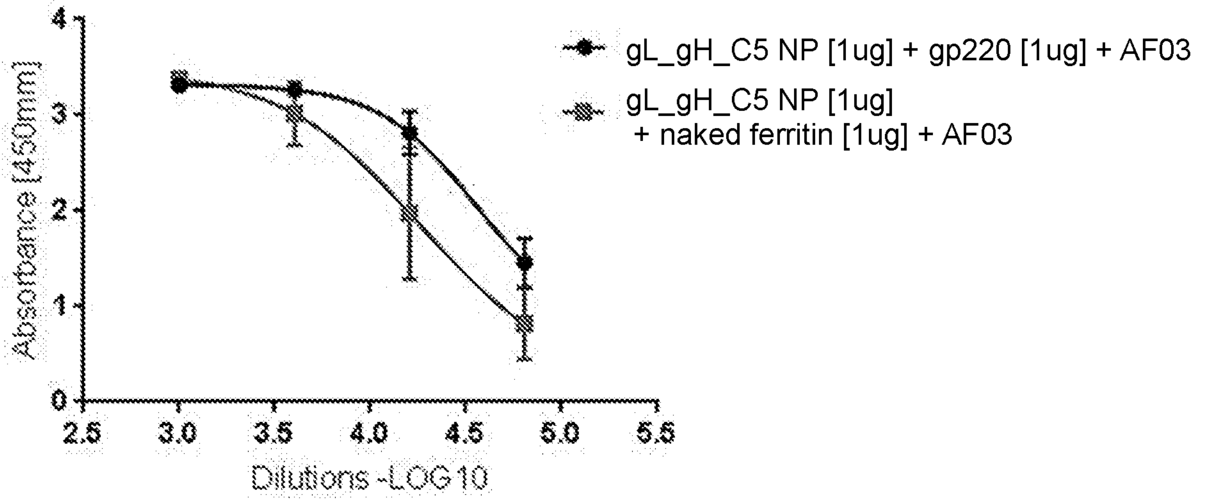


Fig. 5A

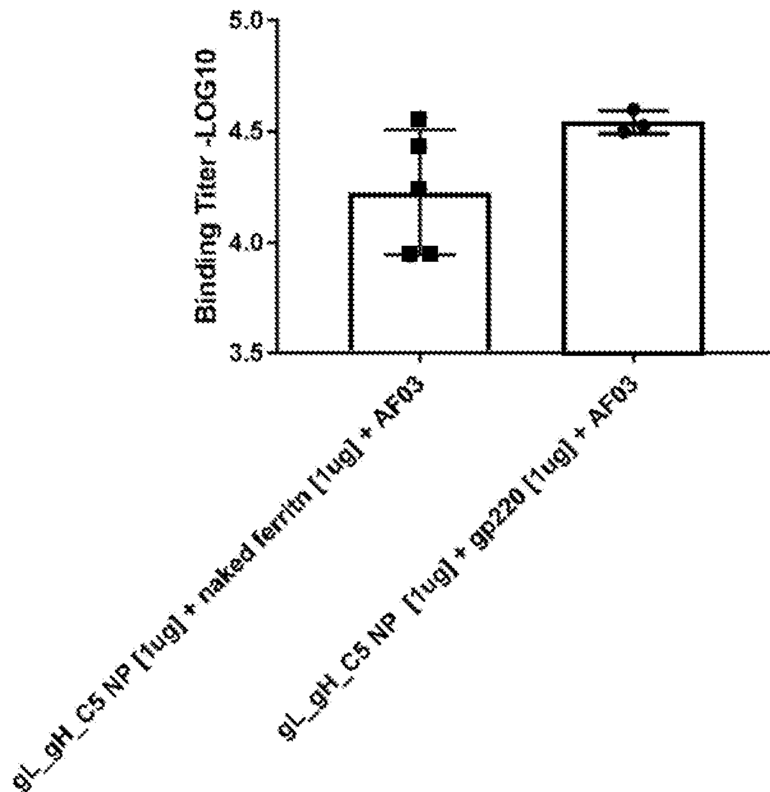


Fig. 5B

ELISA binding assay against gp220

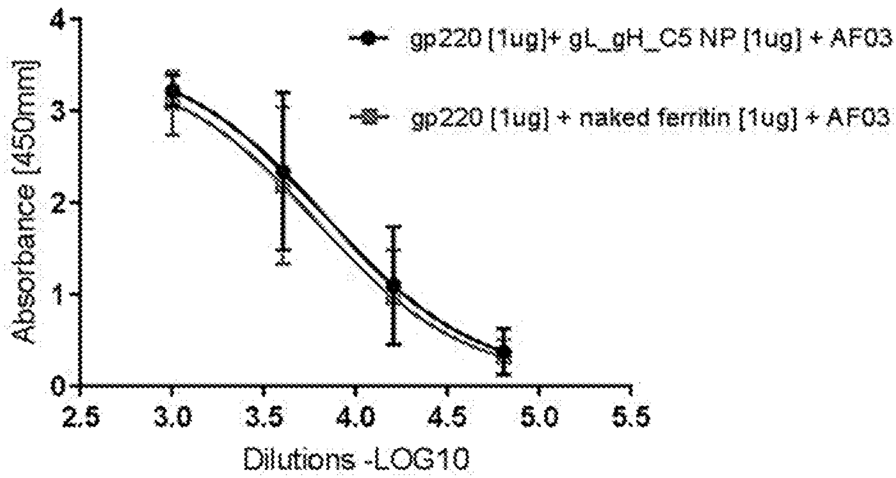


Fig. 6A

ELISA binding assay against gp220

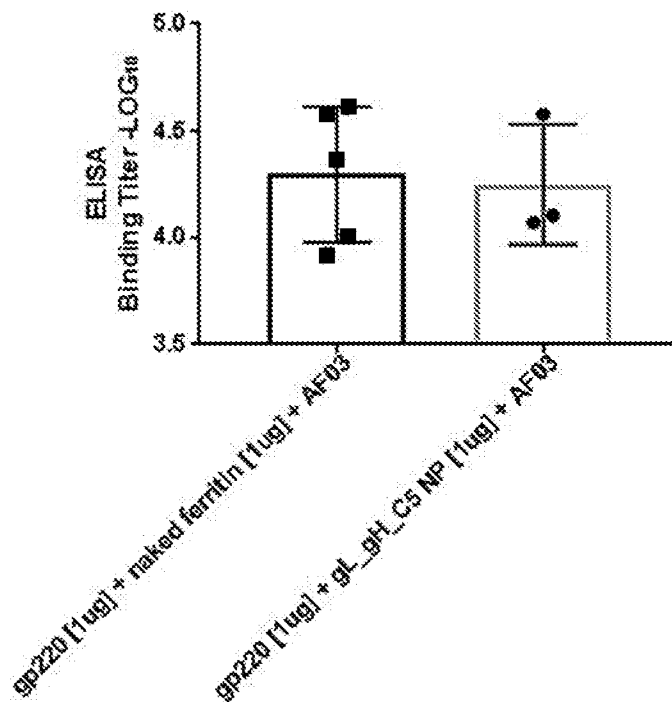


Fig. 6B

Flexible amino acid linker

gL gH Ferritin

Cysteine for TLR agonist conjugation

Fig. 7A

TLR agonist

SM7/8 a-PEG4-Maleimide

Fig. 7B

gL and gH nanoparticle 7/8a

Fig. 7C

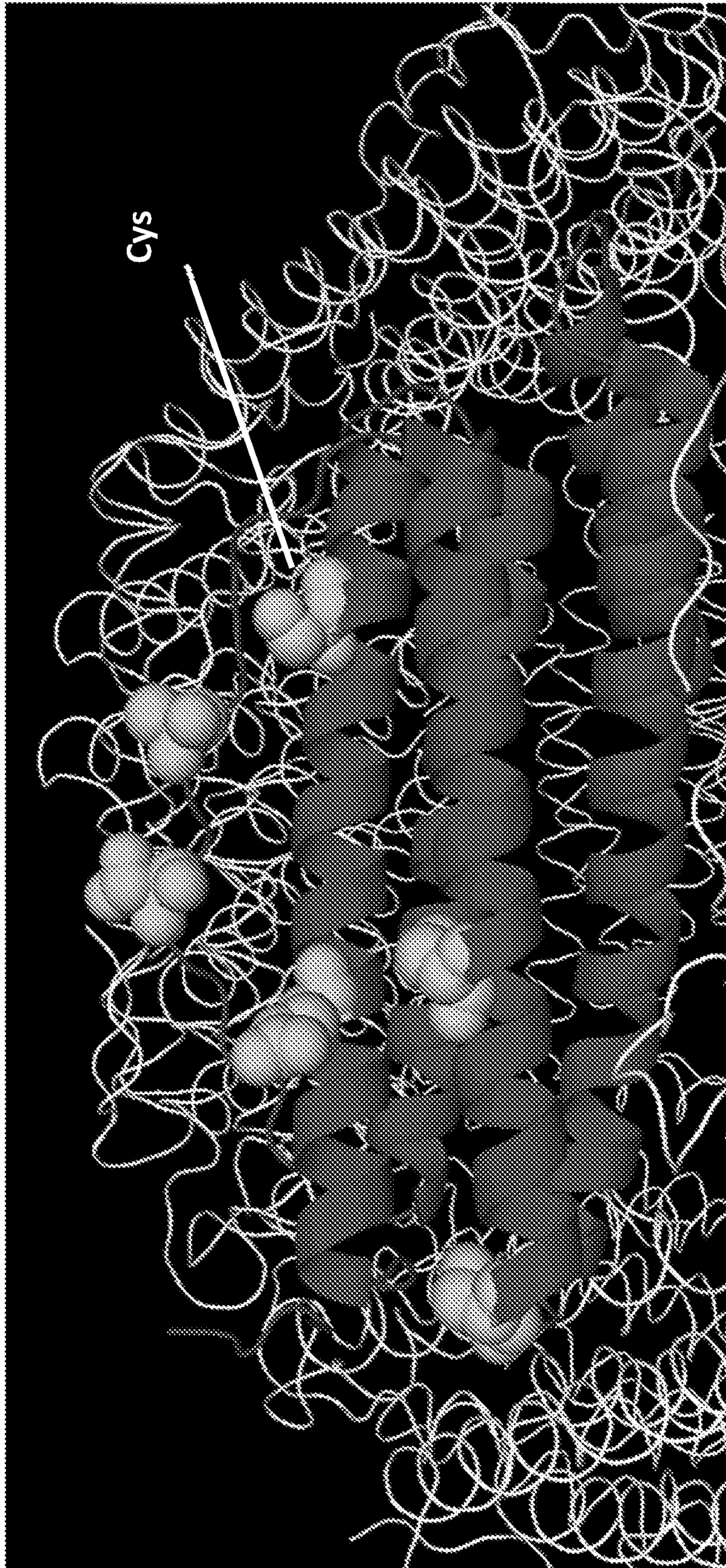


Fig. 8A

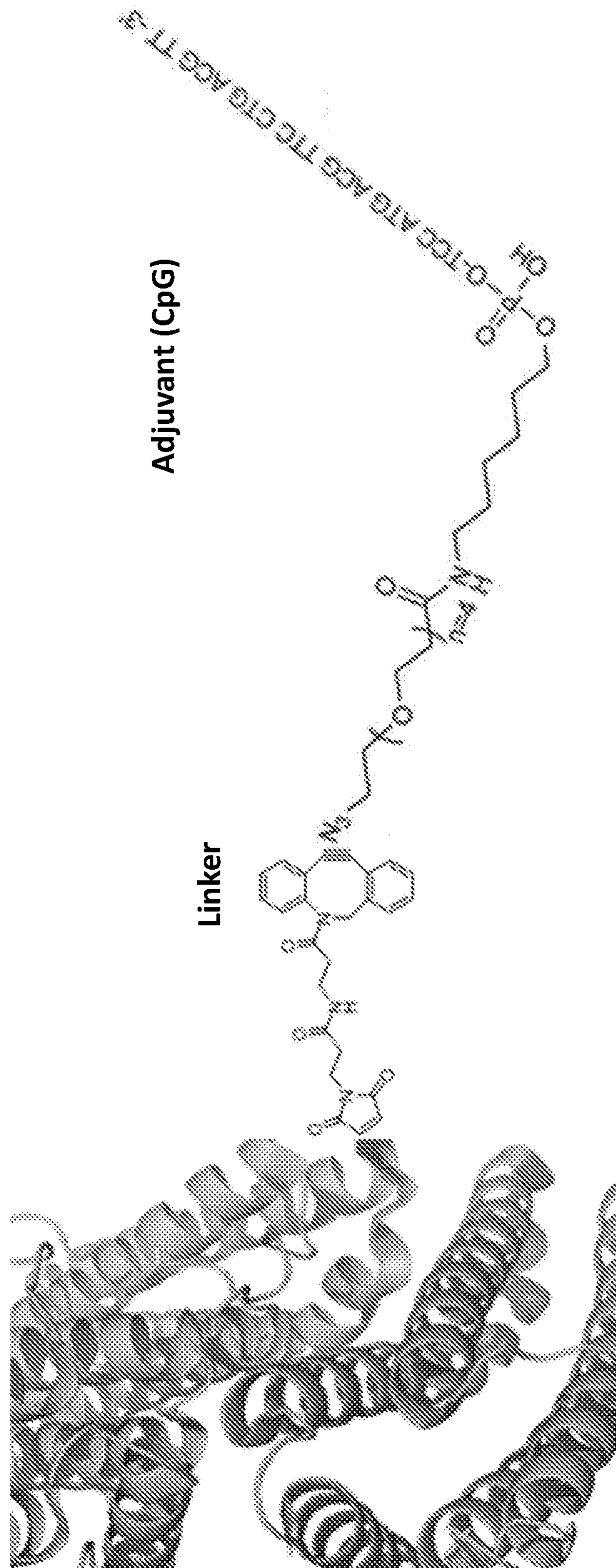


Fig. 8B

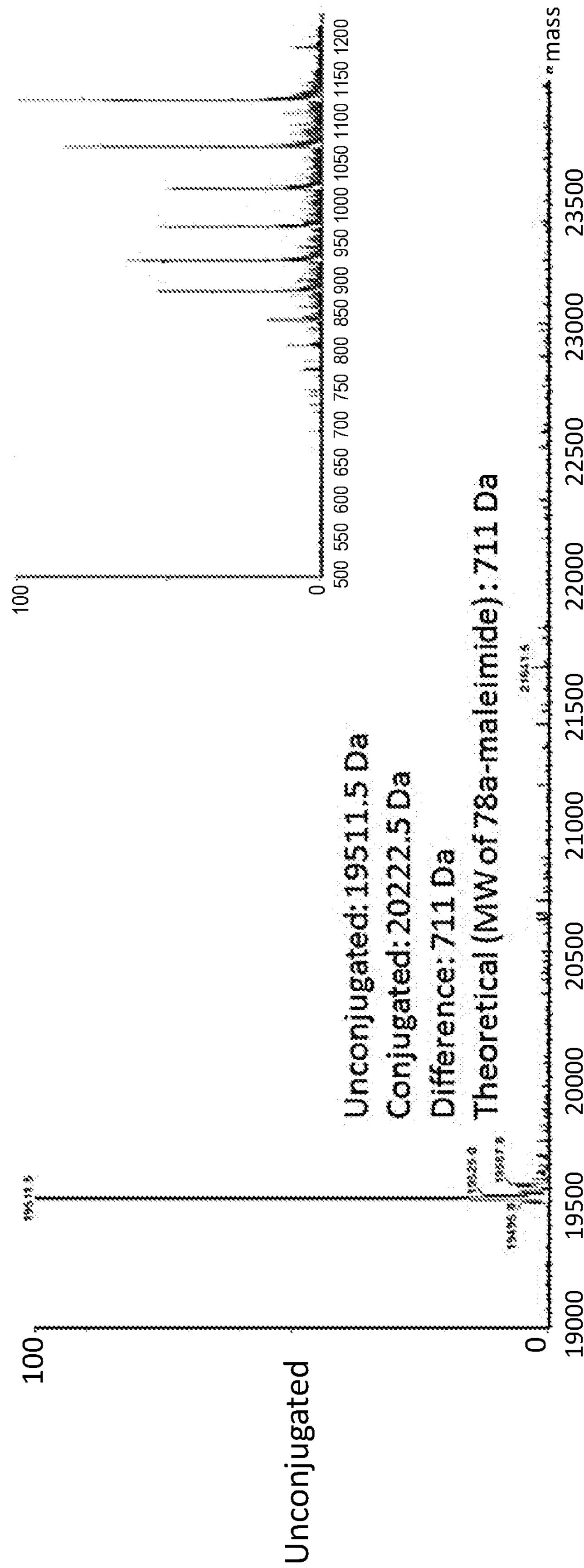


Fig. 9A

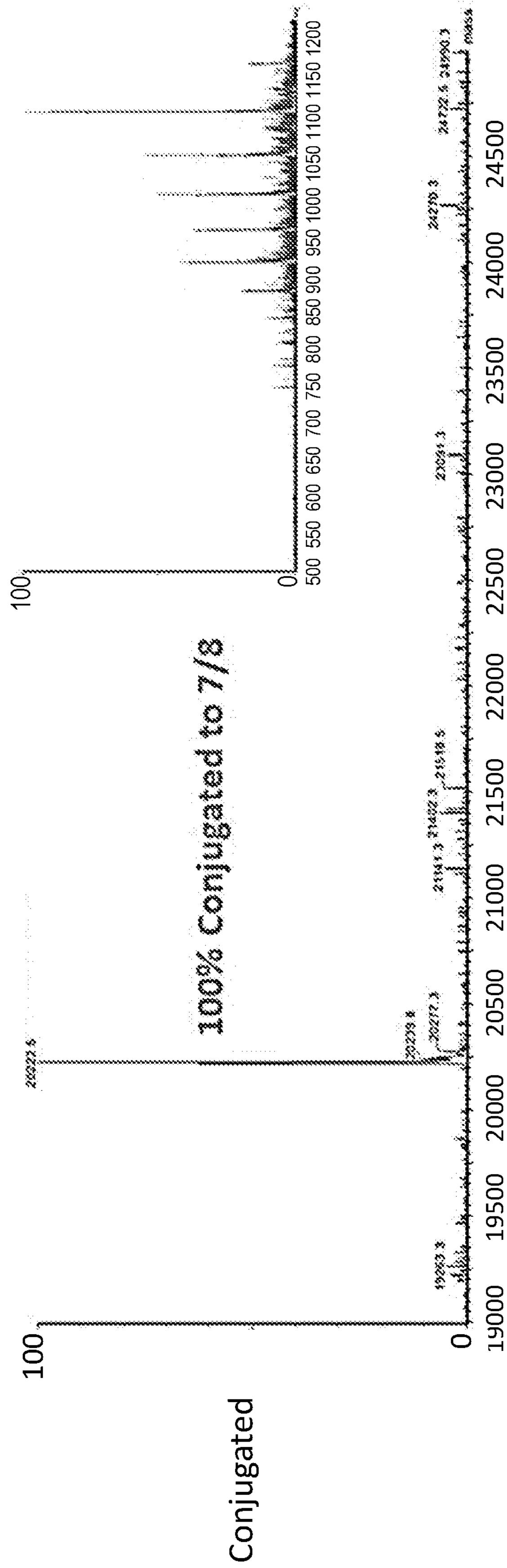


Fig. 9B

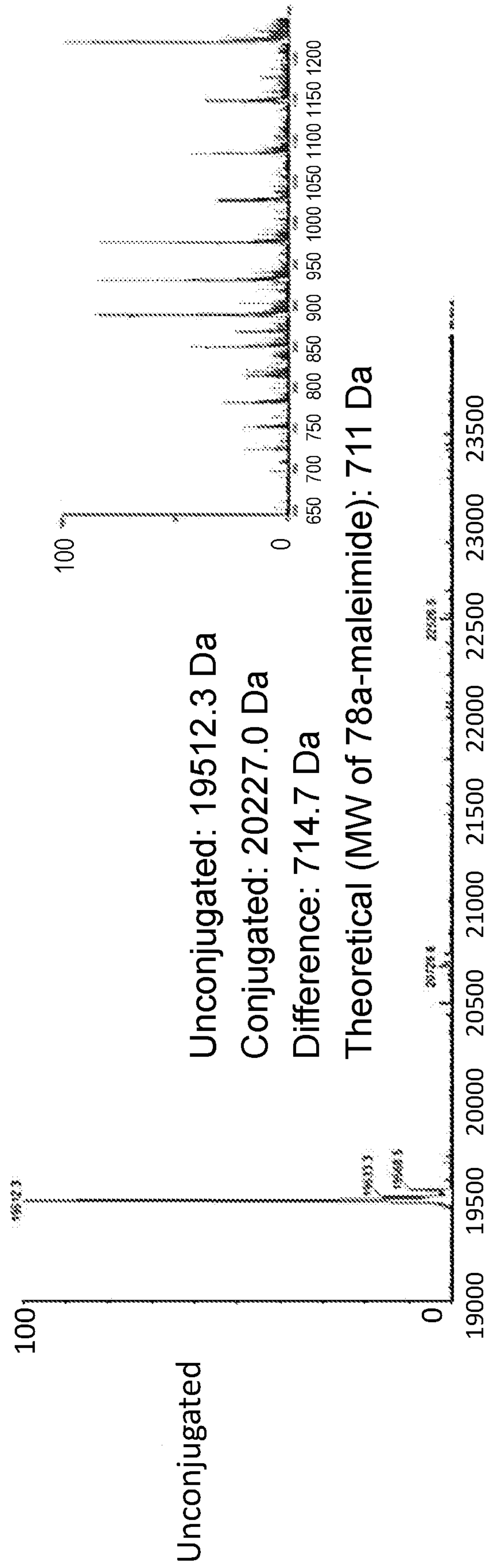


Fig. 10A

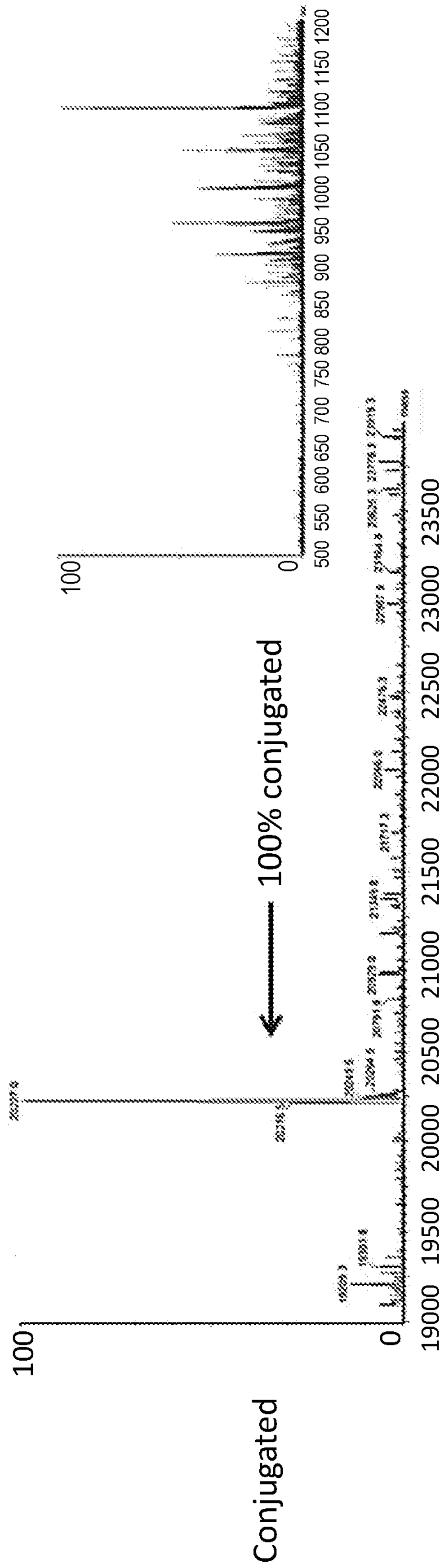


Fig. 10B

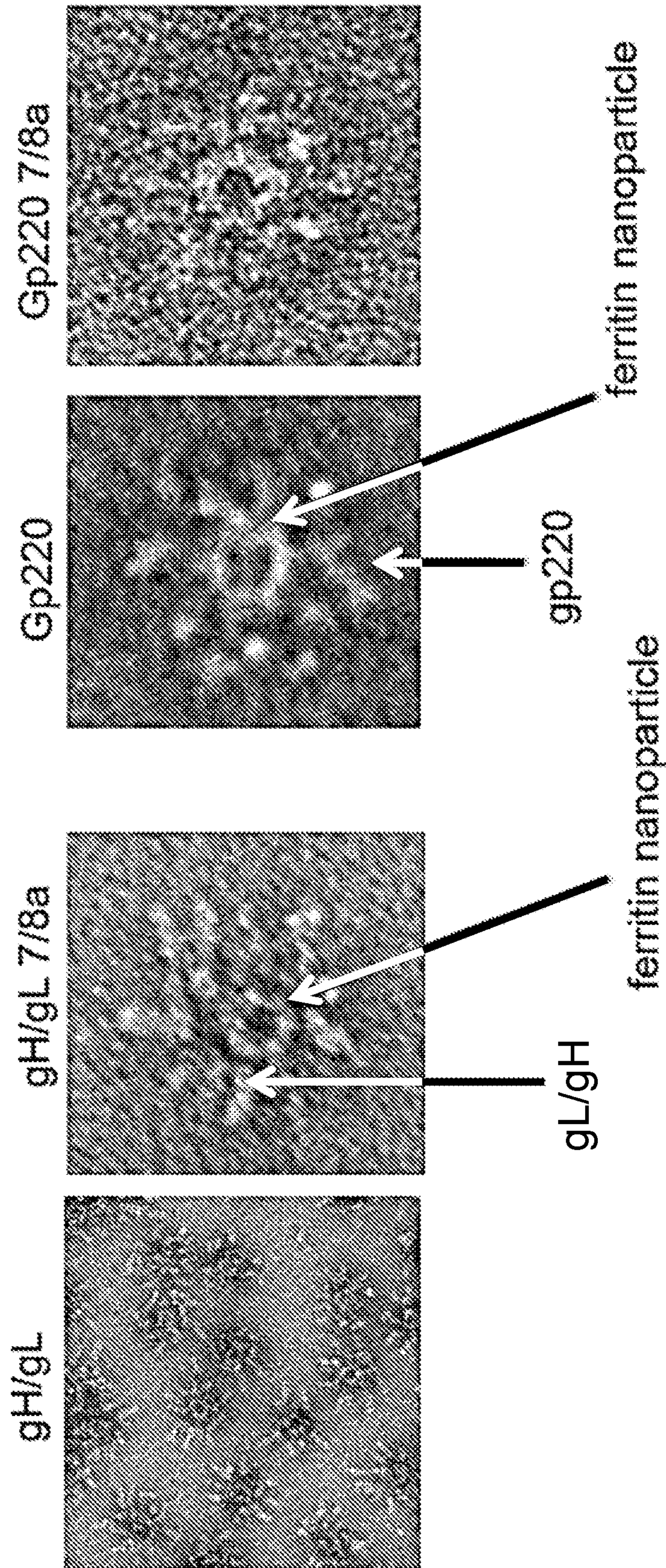


Fig. 11A

Fig. 11B

Fig. 11C

Fig. 11D

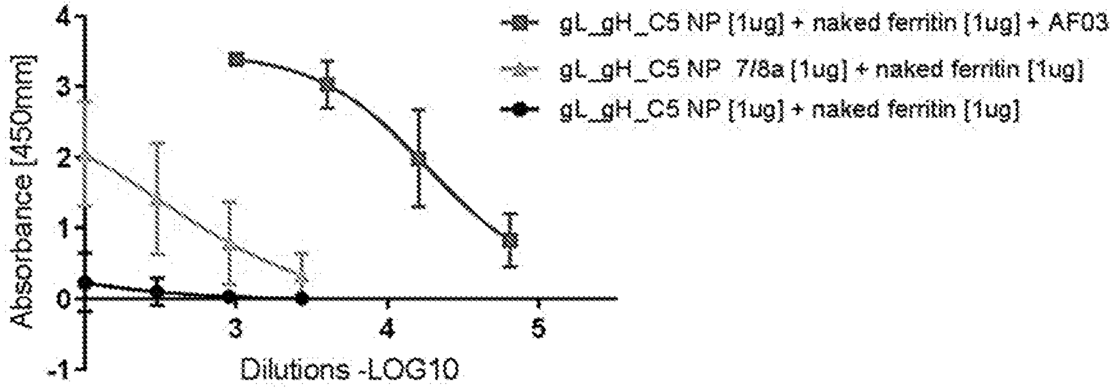


Fig. 12A

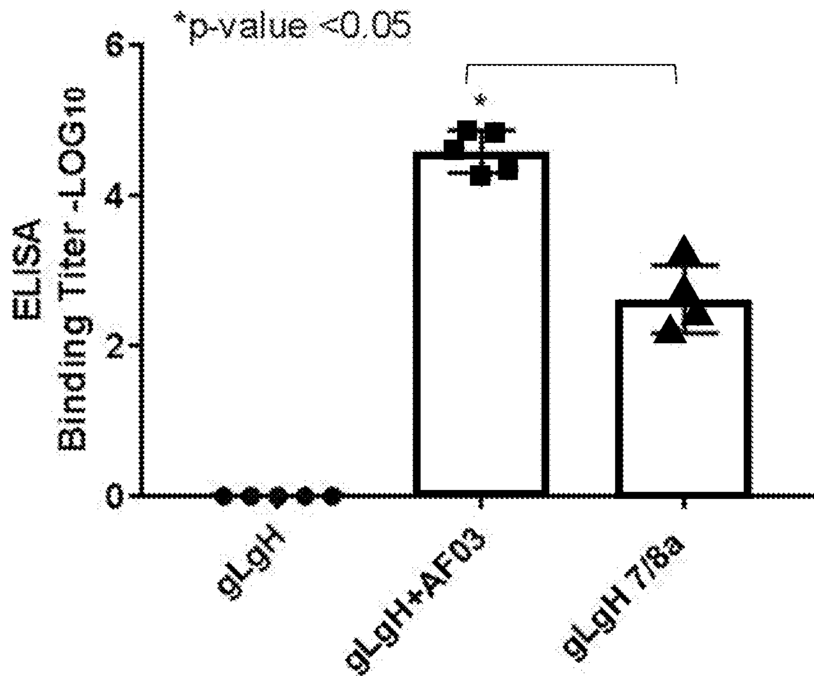


Fig. 12B

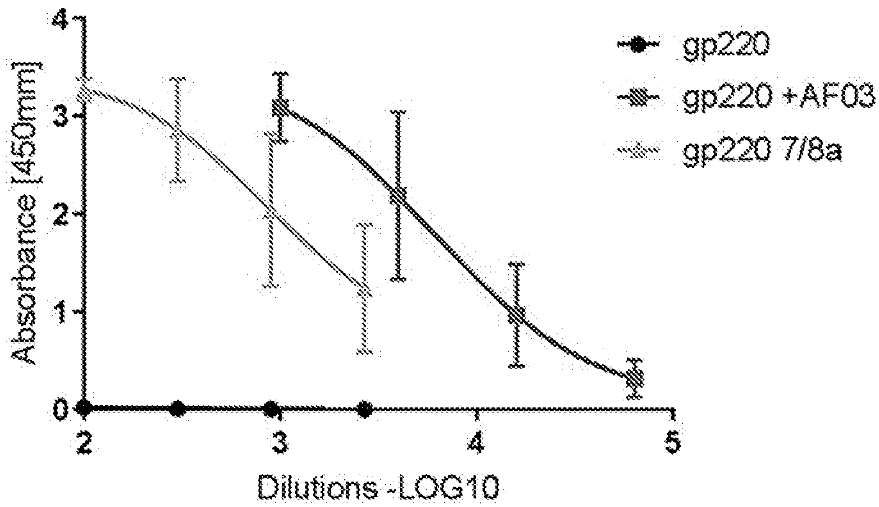


Fig. 13A

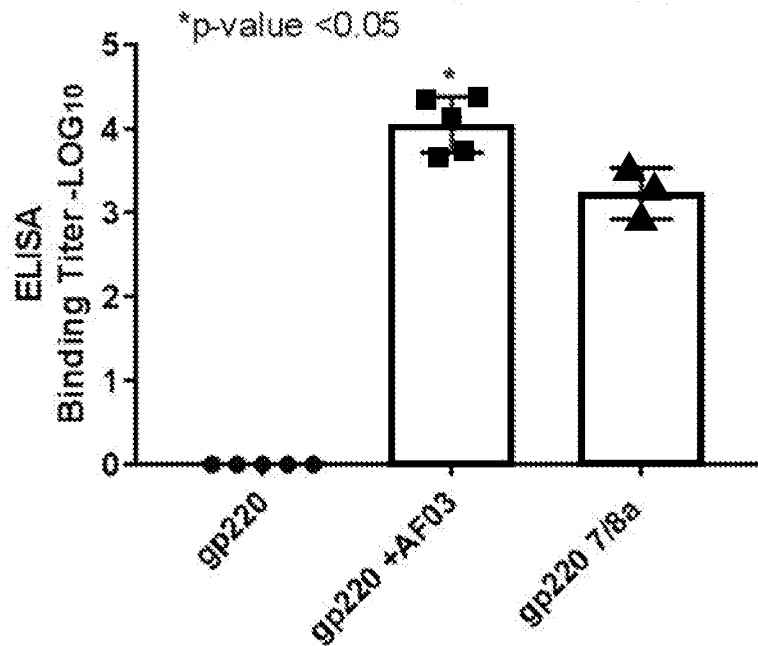


Fig. 13B

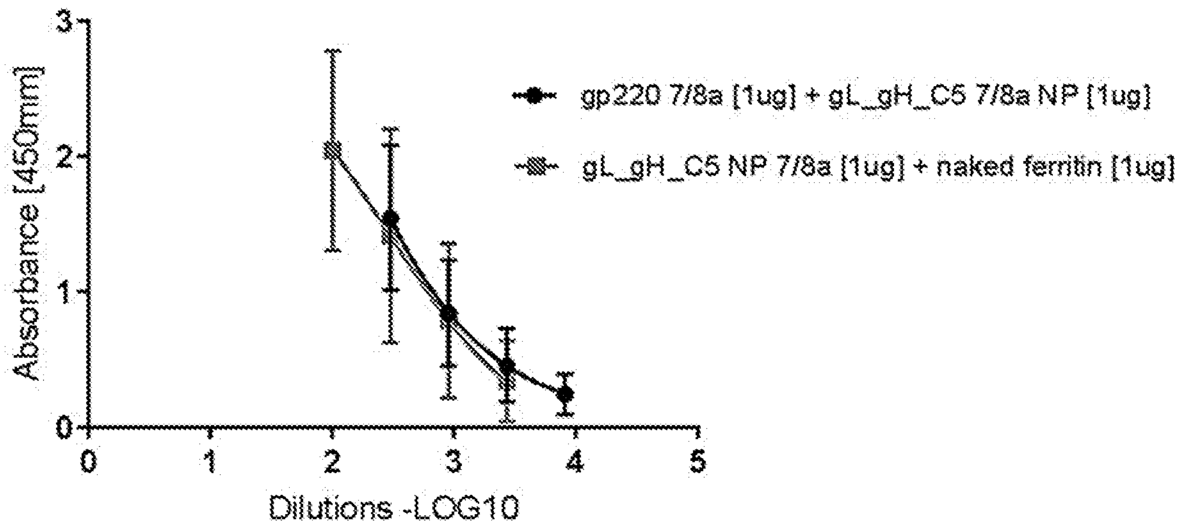


Fig. 14A

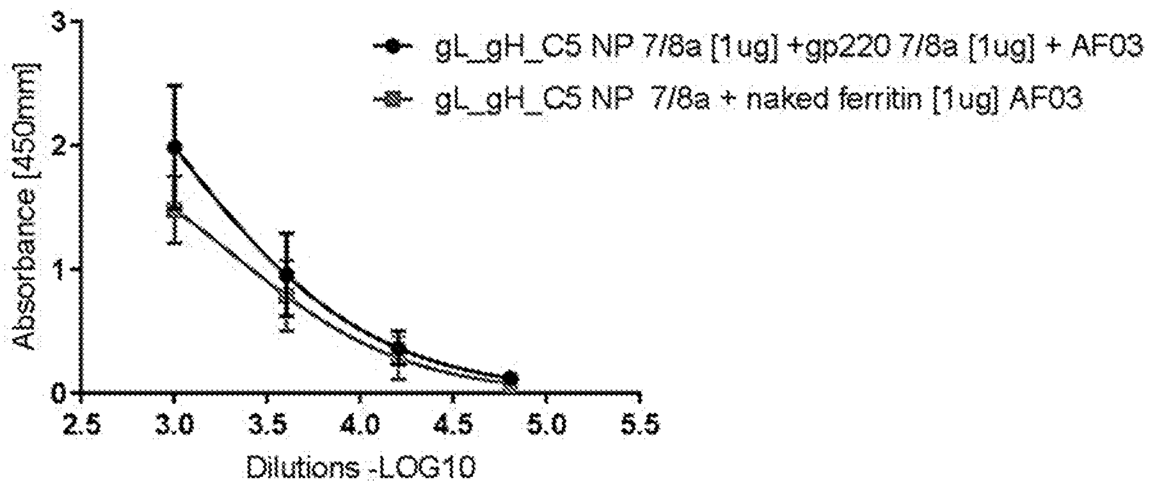


Fig. 14B

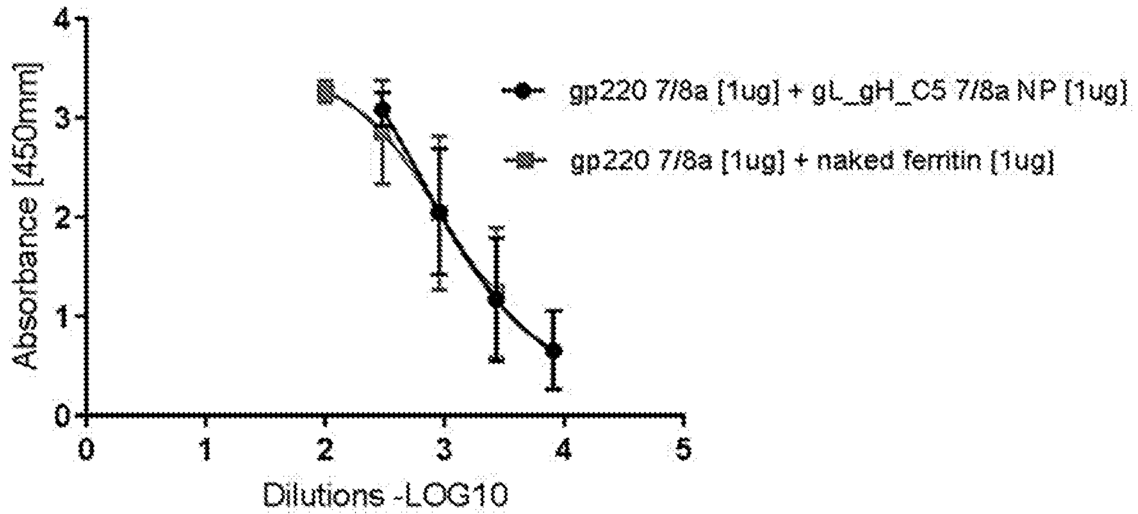


Fig. 15A

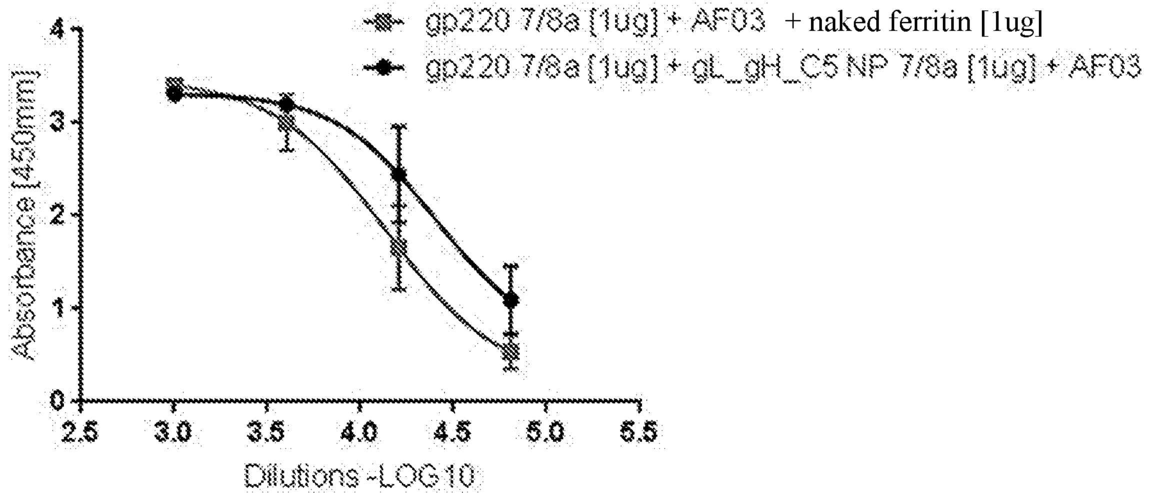


Fig. 15B

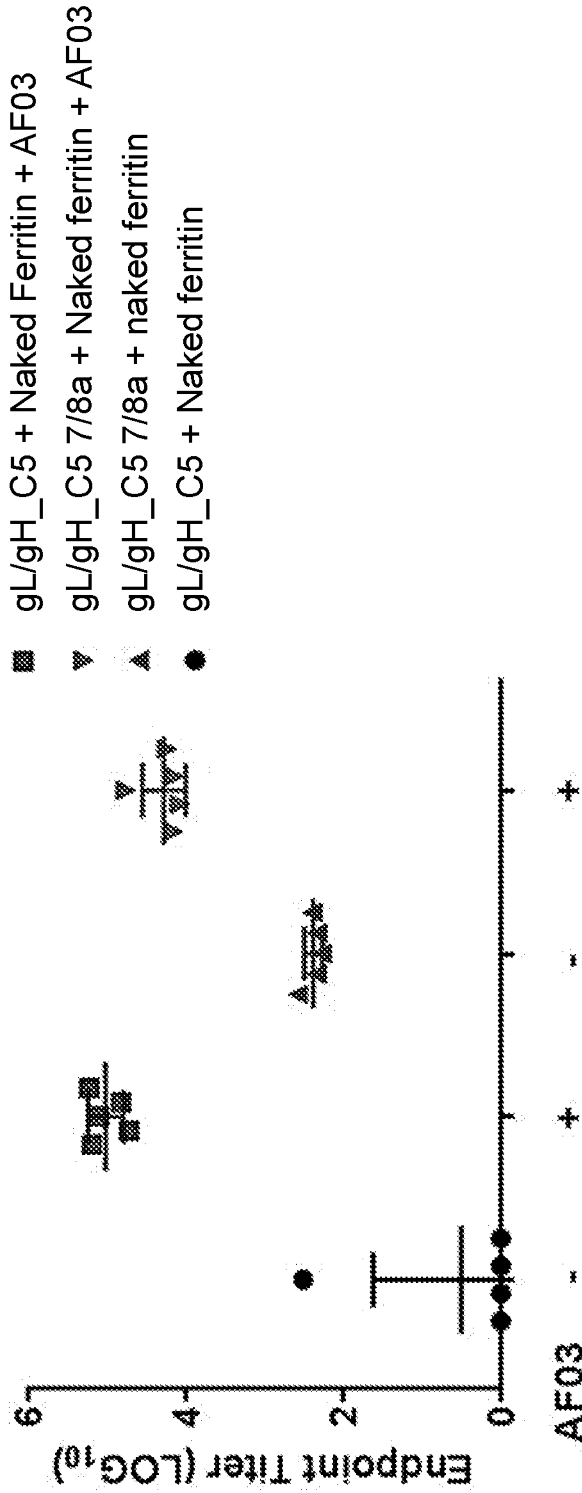


Fig. 16

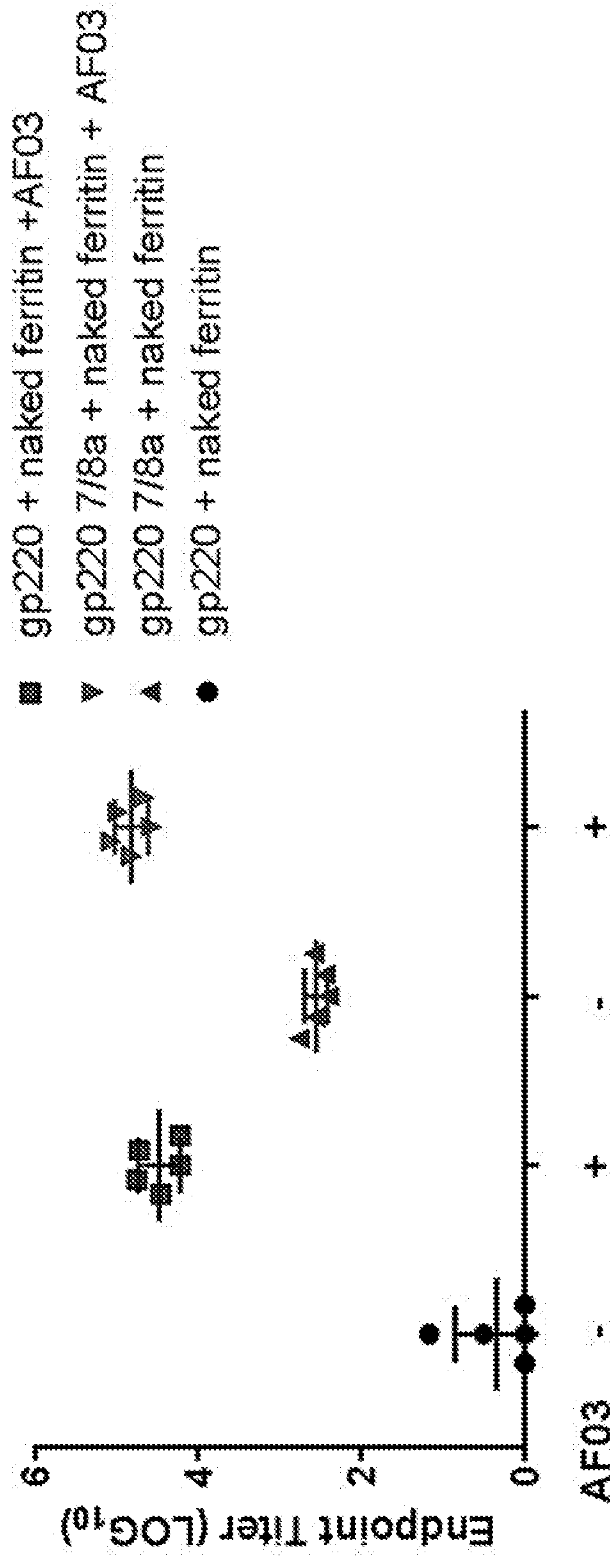


Fig. 17

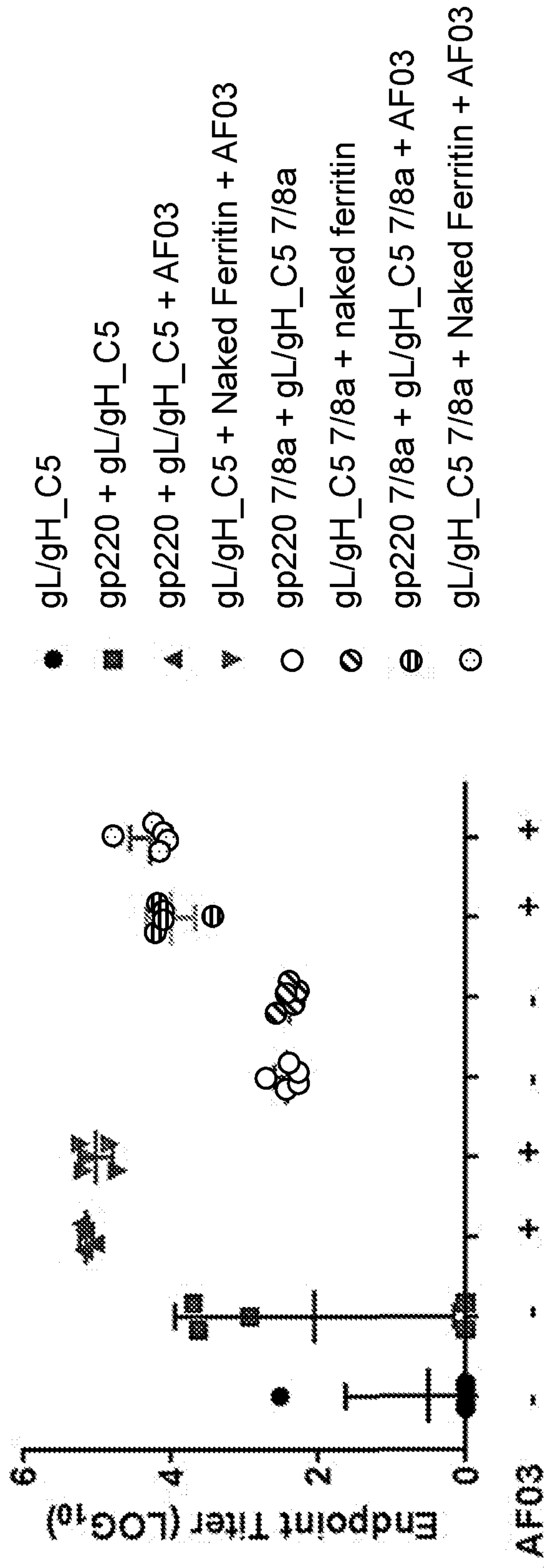


Fig. 18

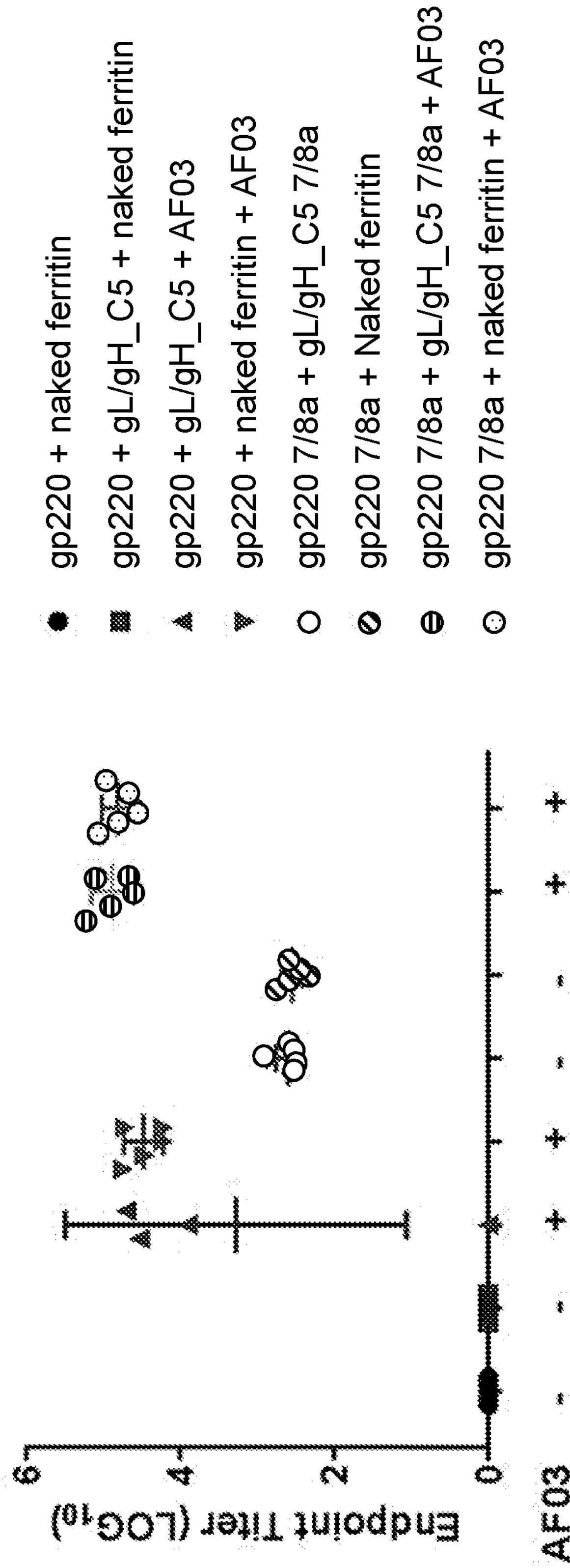


Fig. 19

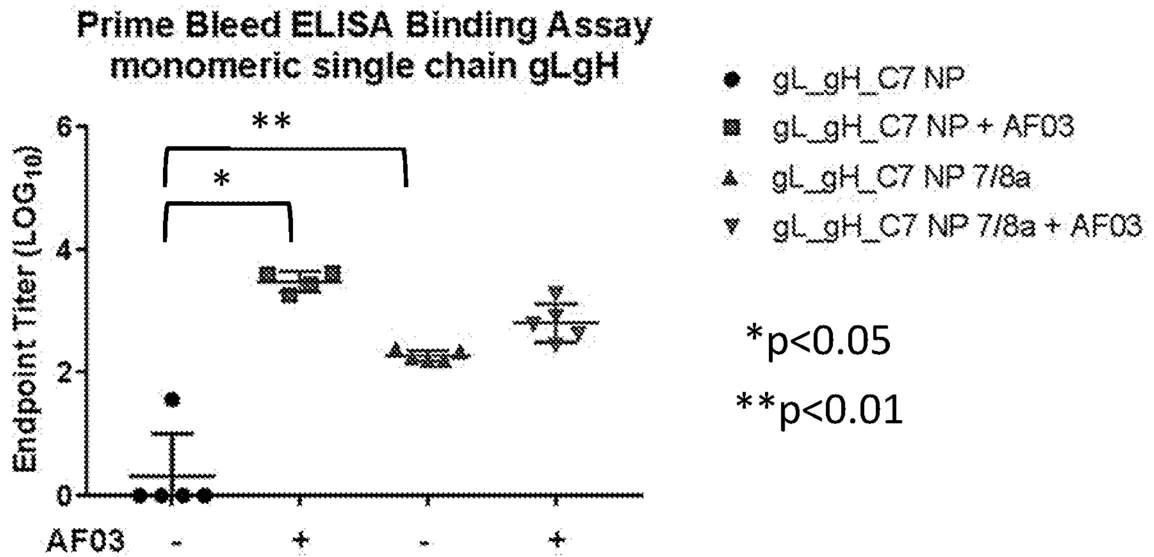


Fig. 20A

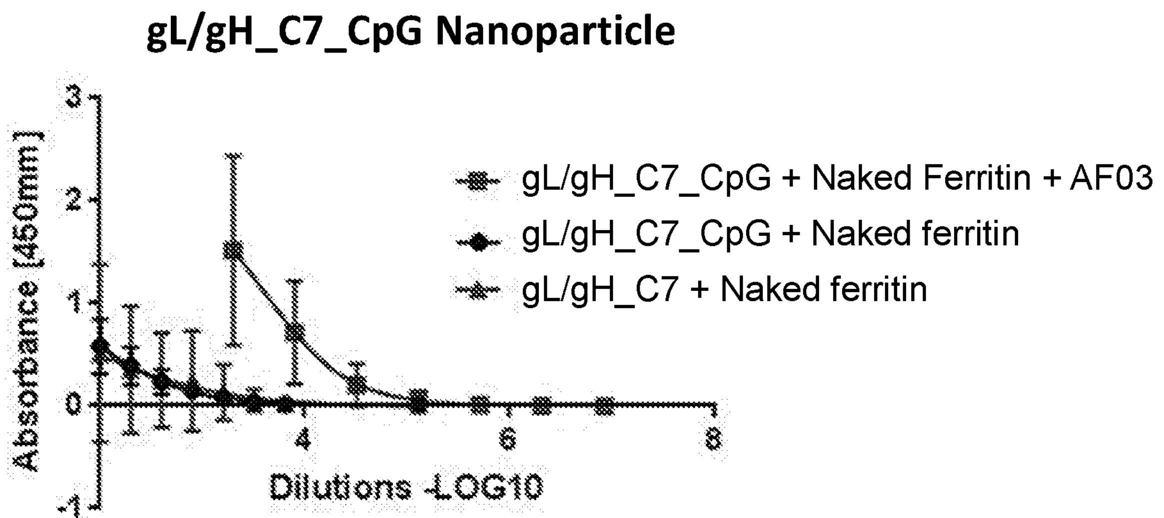


Fig. 20B

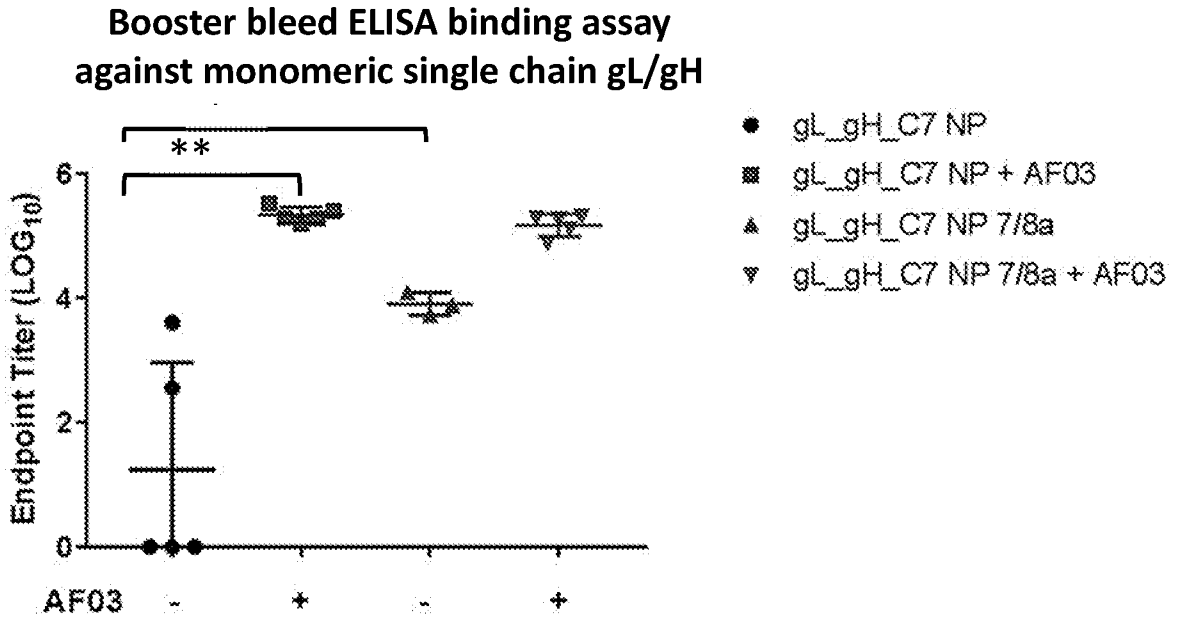


Fig. 20C

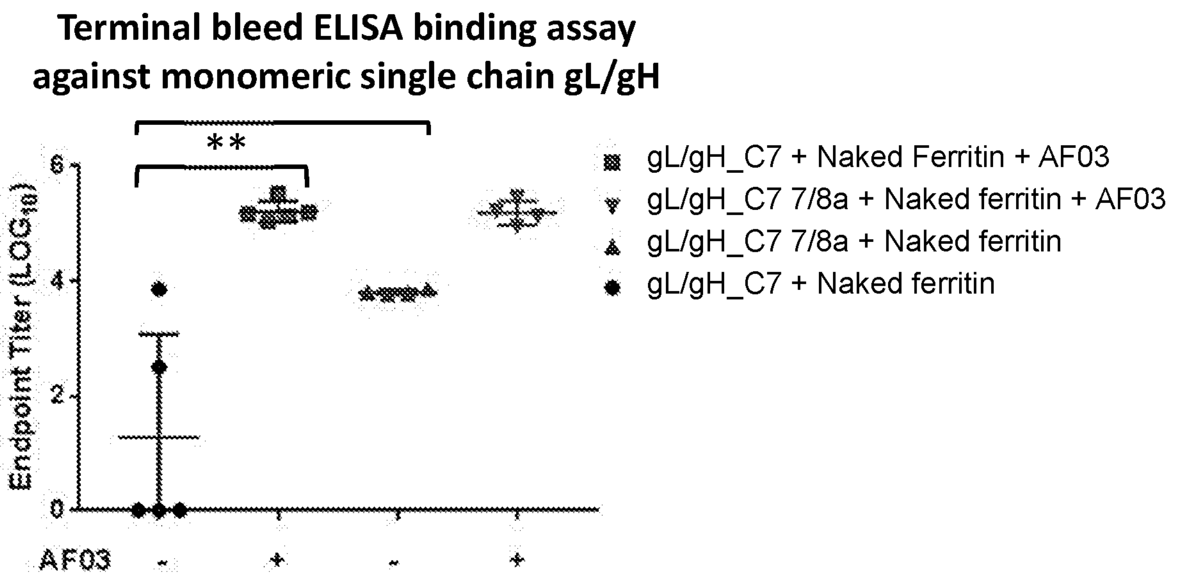


Fig. 20D

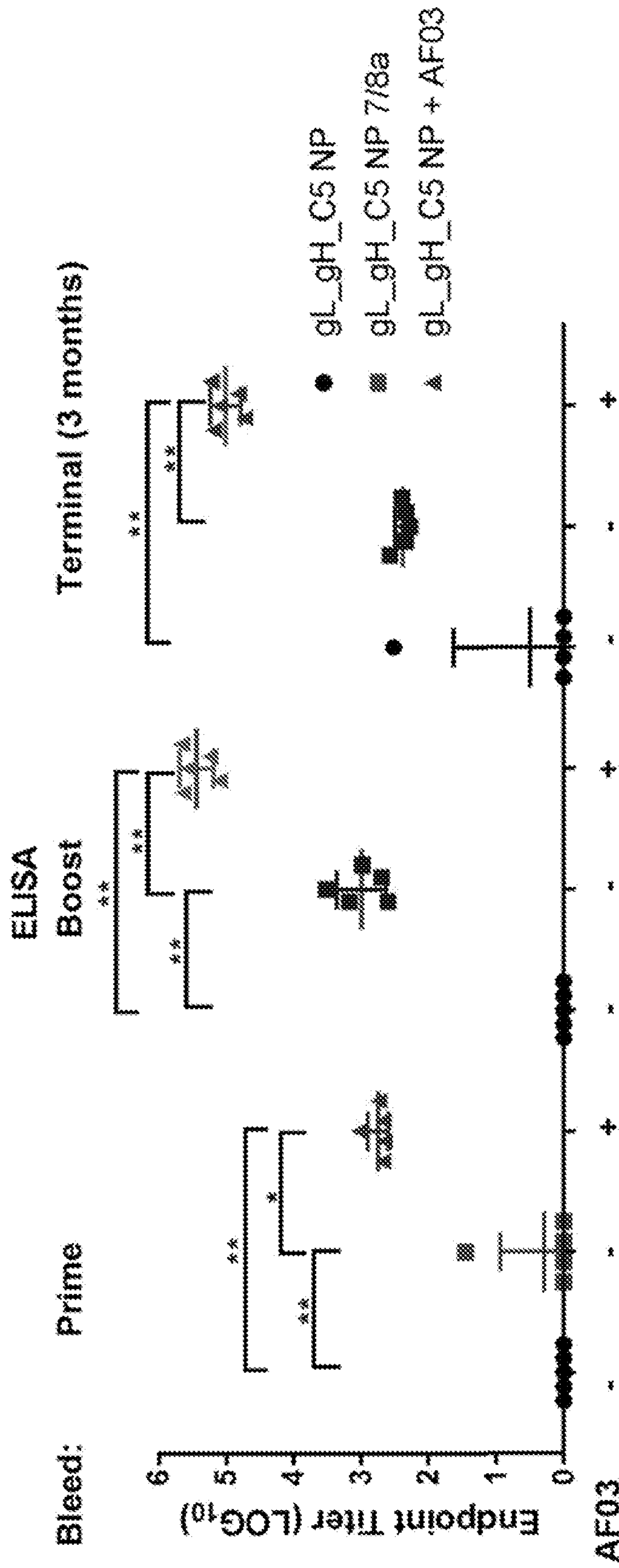


Fig. 21

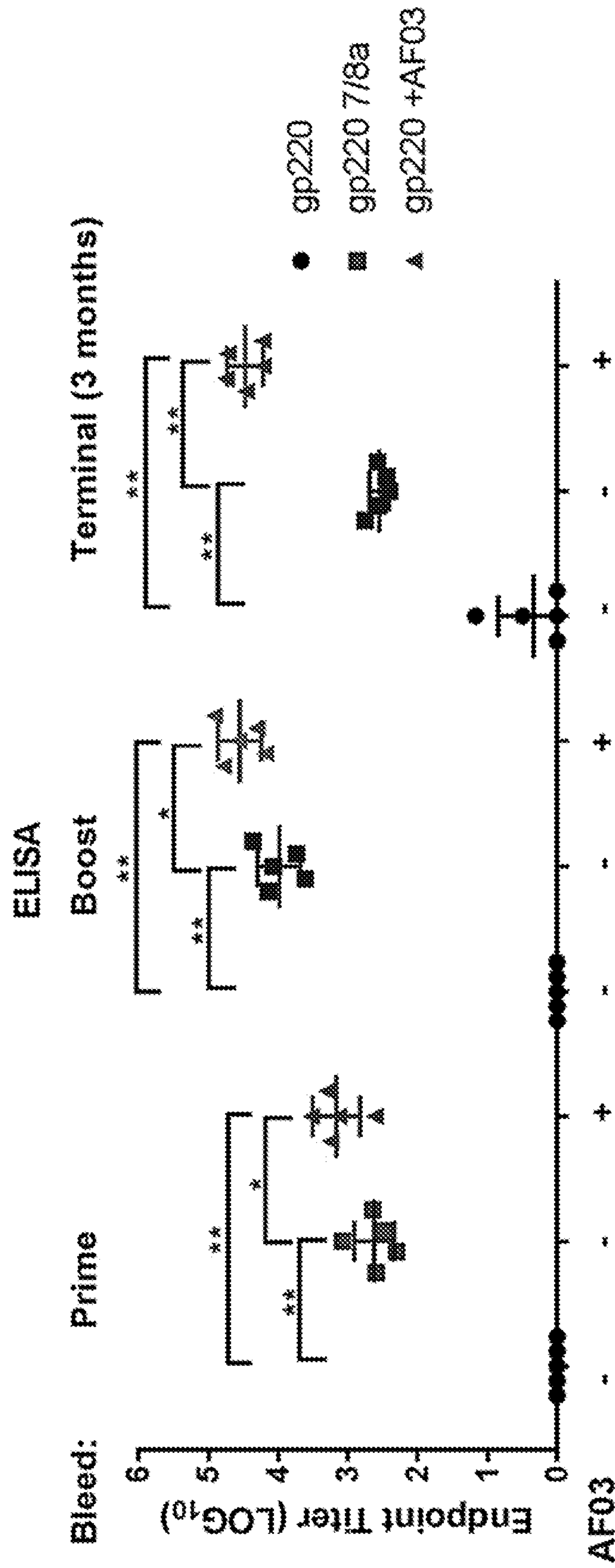
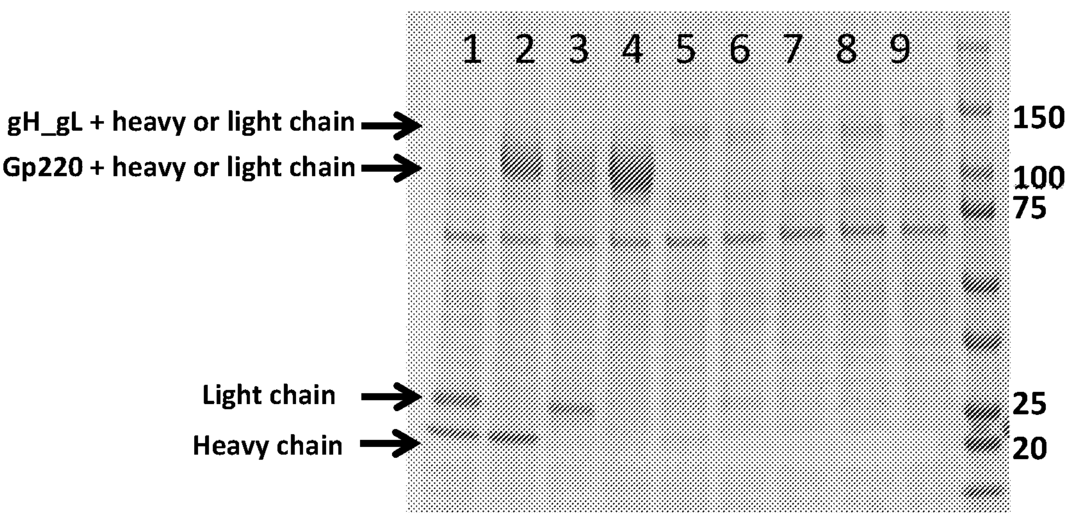


Fig. 22



1 – Light + heavy chain

2 – heavy chain + (gp220-light)

3 – (gp220-heavy) + light chain

4- (gp220-heavy) + (gp220-light)

5 – heavy chain + (gL_gH-light)

6 – (gL_gH-heavy) + light chain)

7 – (gL_gH-heavy) + (gL_gH-light)

8 – (gp220-heavy) + (LH_gH-light)

9 – (gL_gH-heavy) + (gp220-light)

Fig. 23A

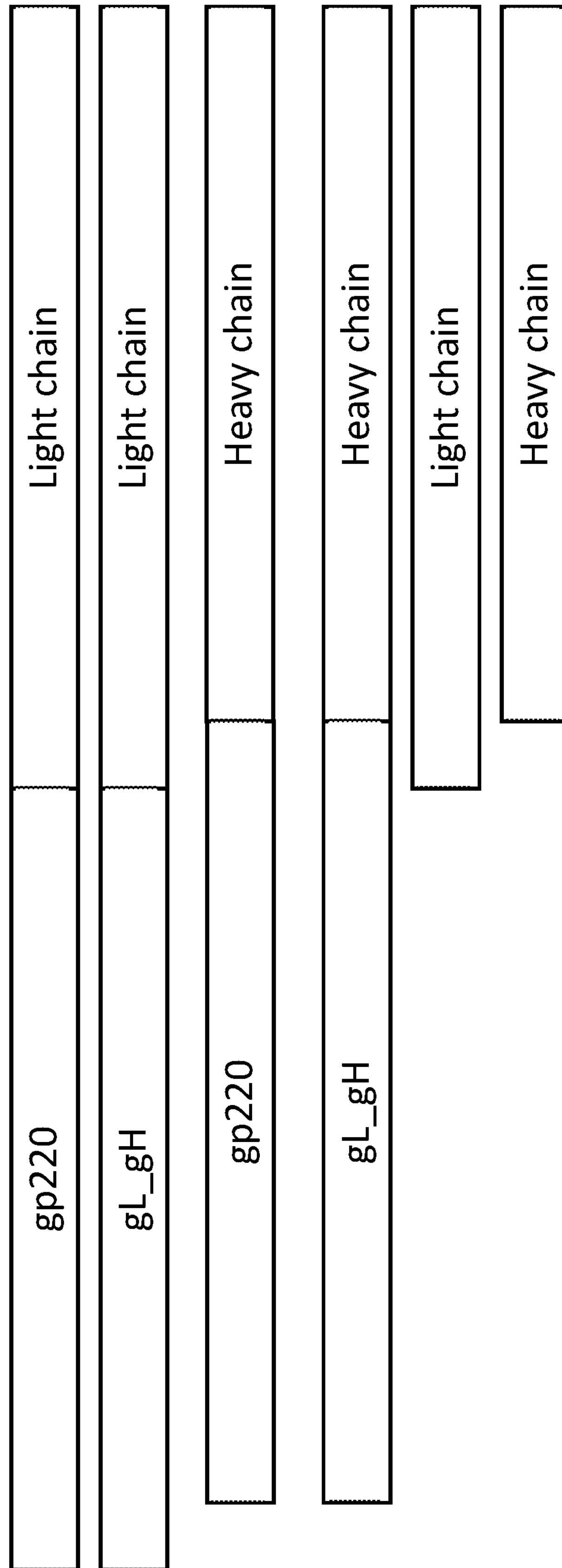


Fig. 23B

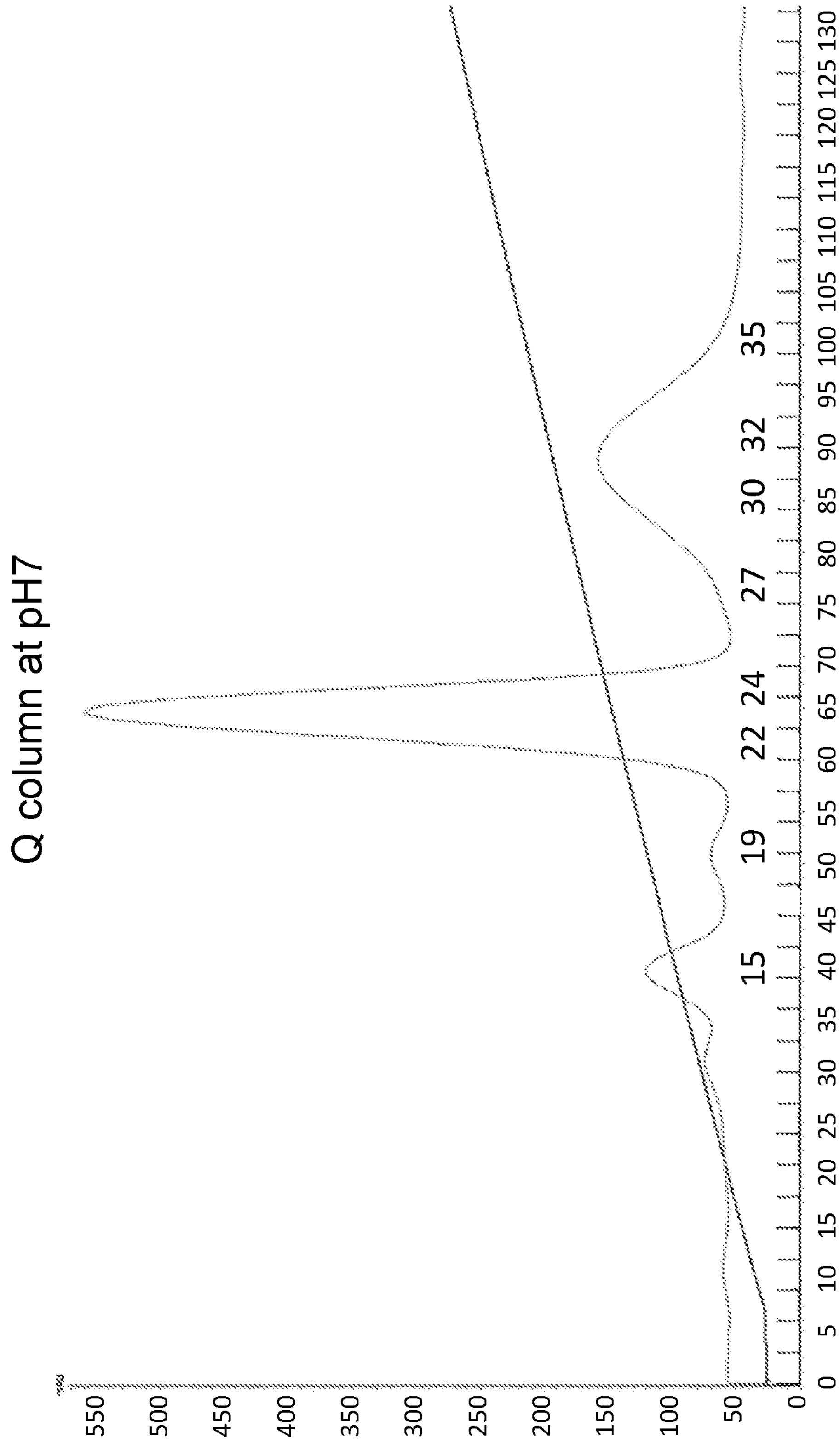


Fig. 24A

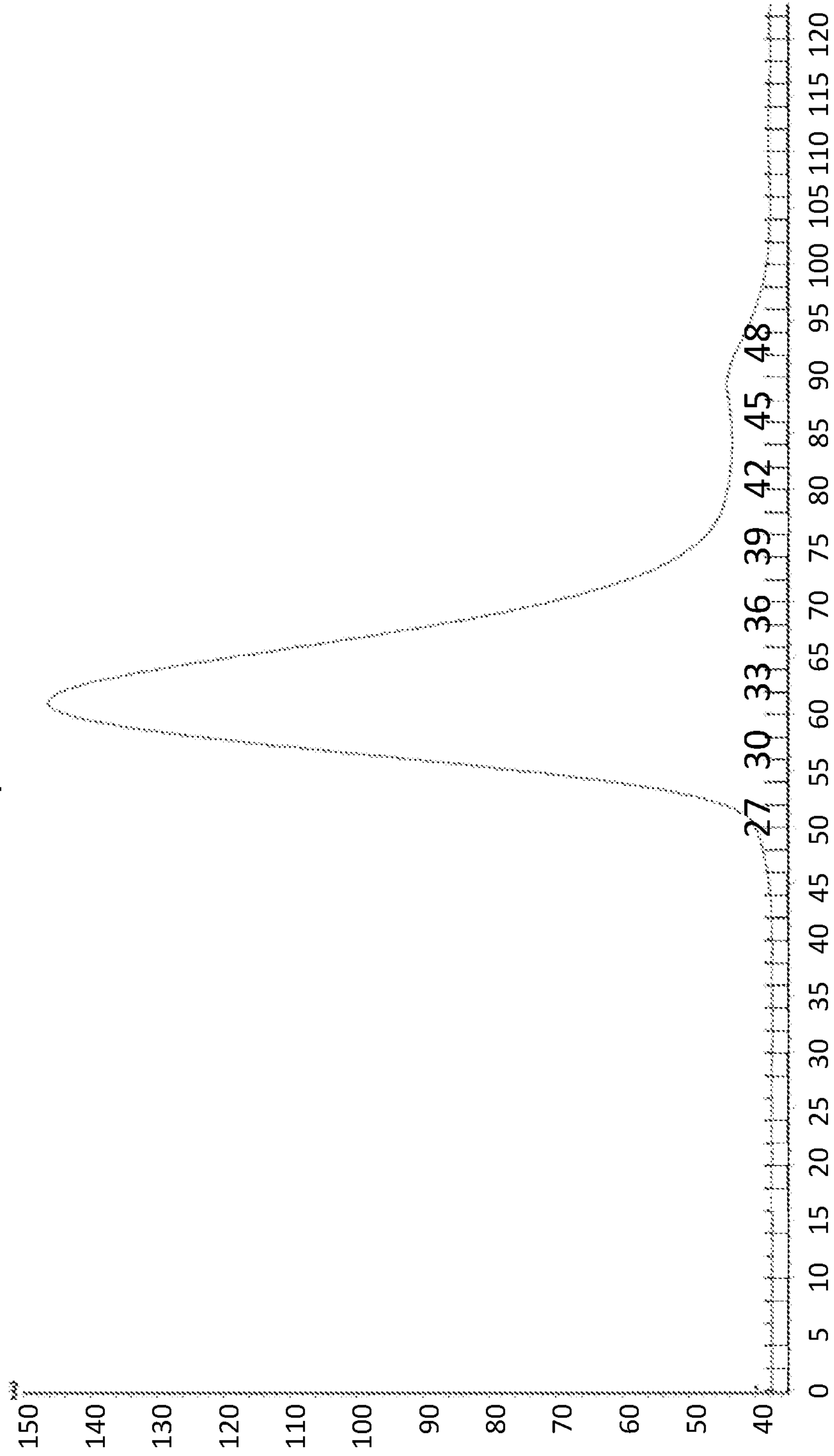


Fig. 24B

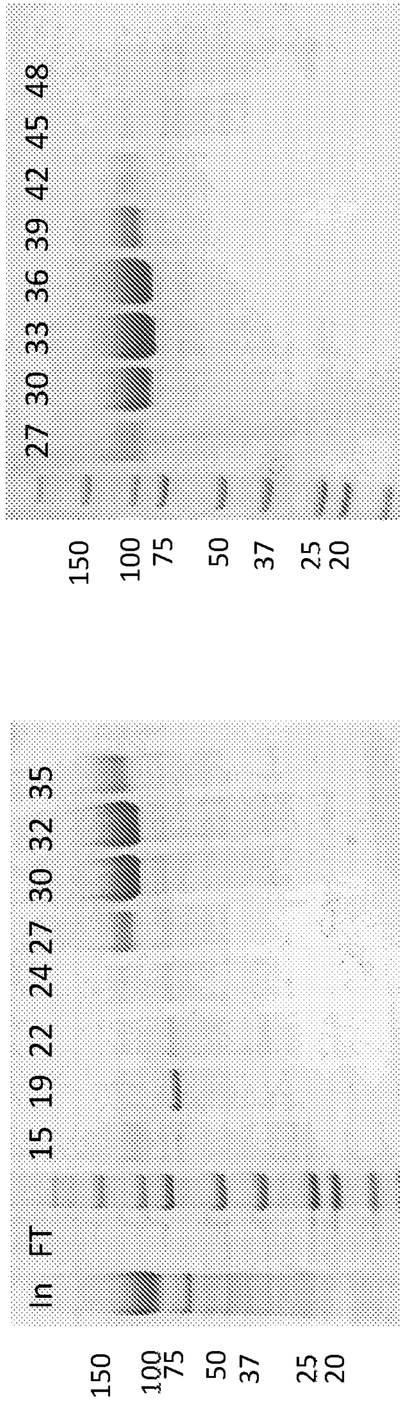


Fig. 24C

Fig. 24D

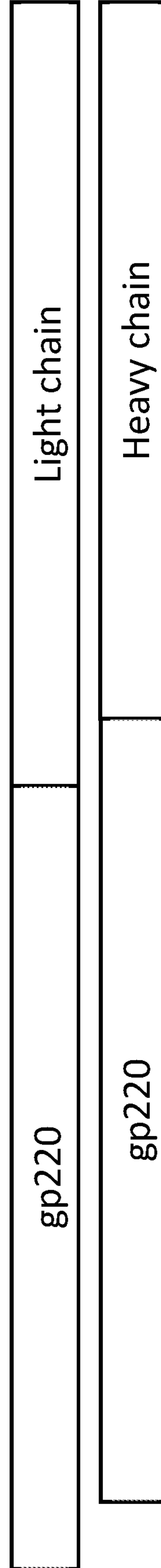


Fig. 24E

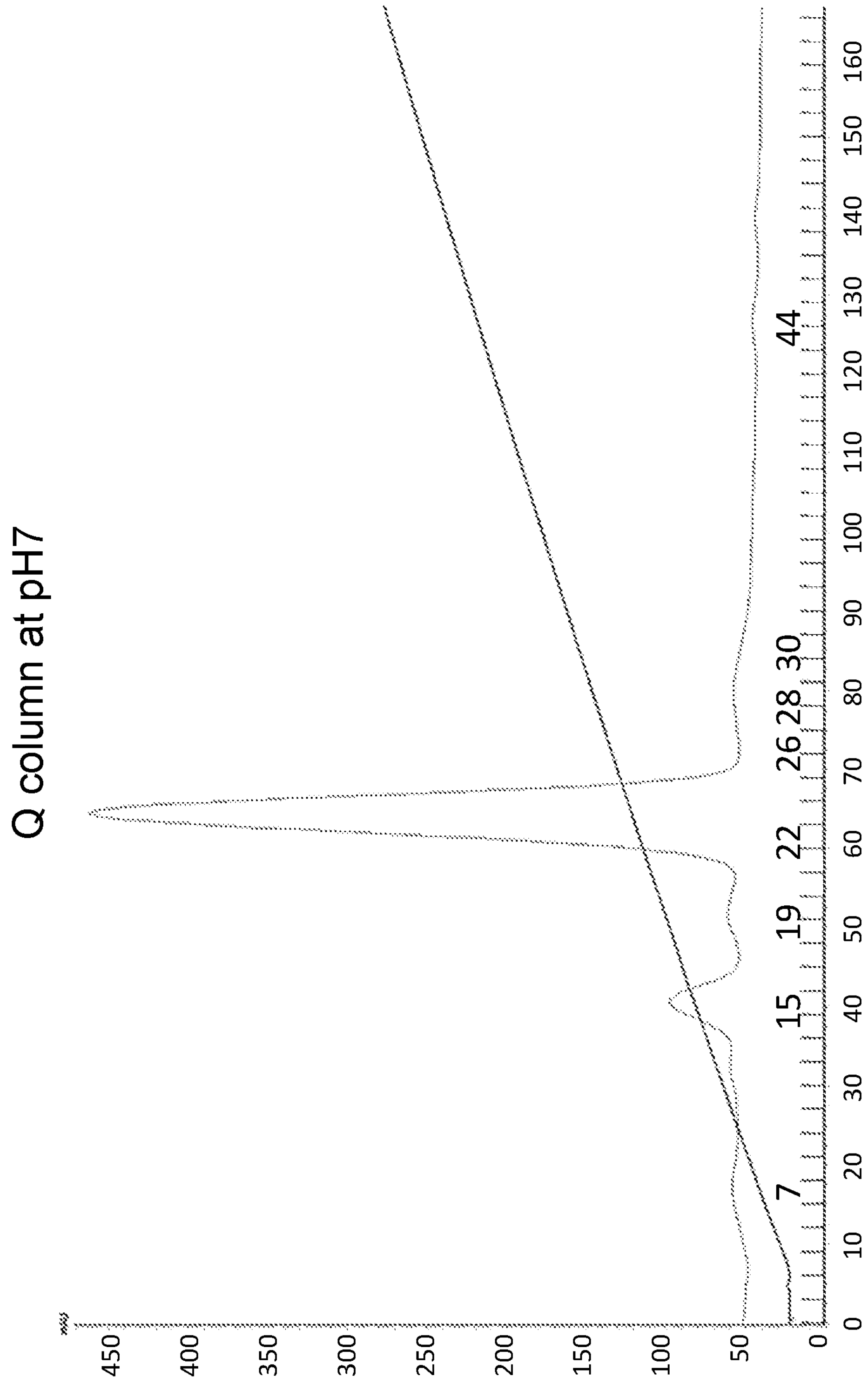


Fig. 25A

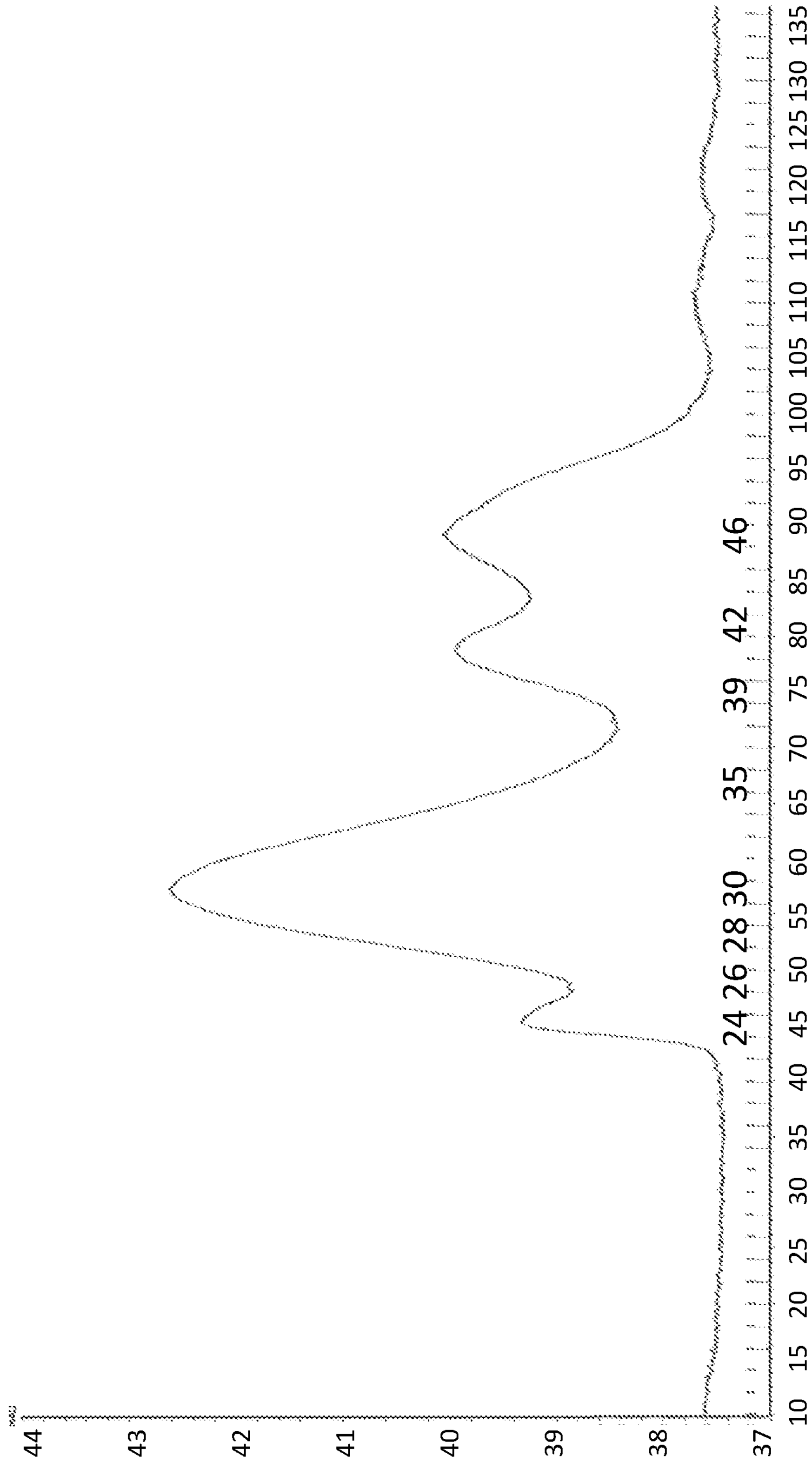


Fig. 25B

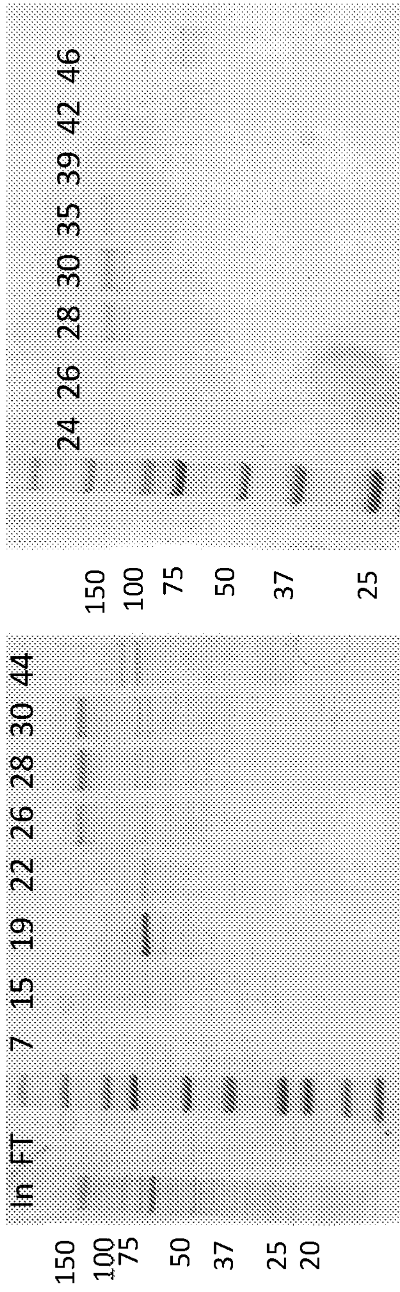


Fig. 25C

Fig. 25D

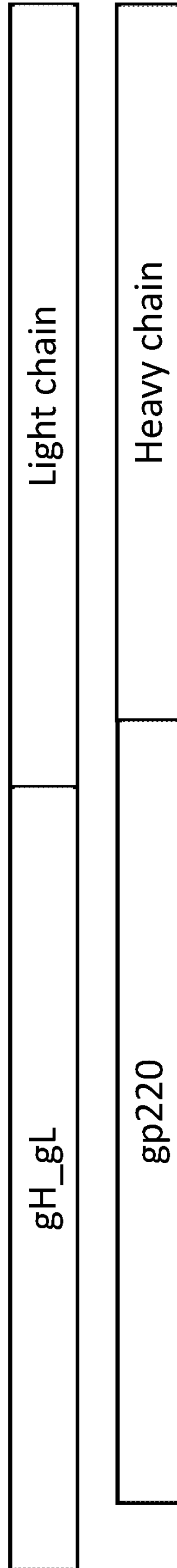


Fig. 25E

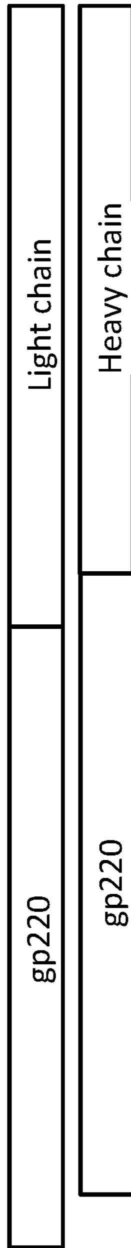
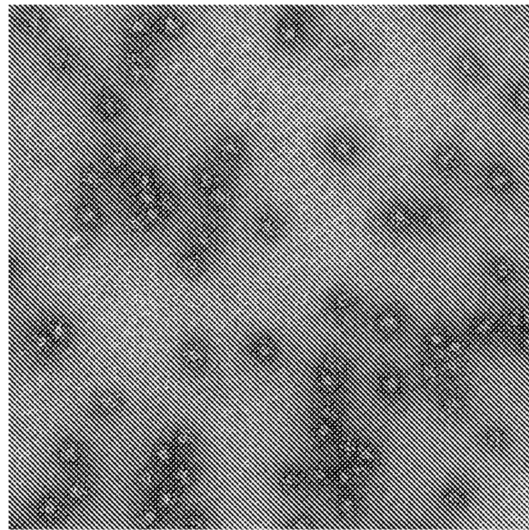


Fig. 26B



50nm
HV=80.0kV
Direct Mag: 68000x

Fig. 26C

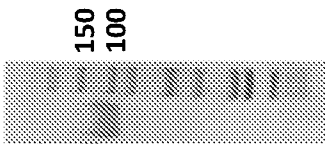


Fig. 26A

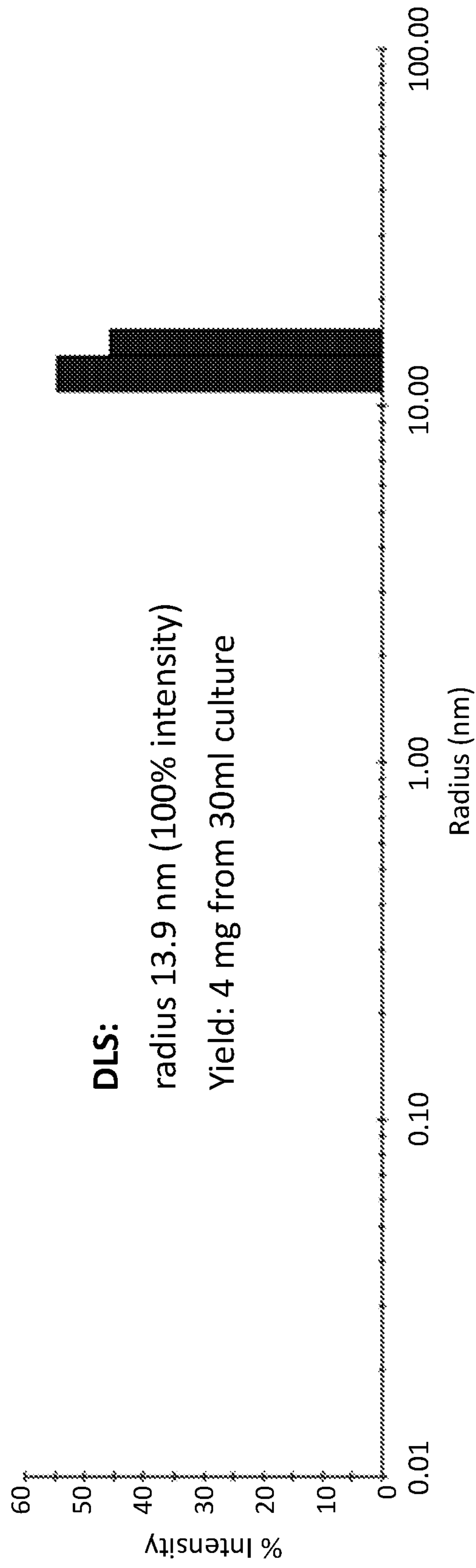


Fig. 26D

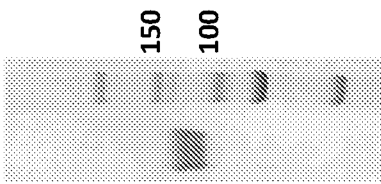


Fig. 26E

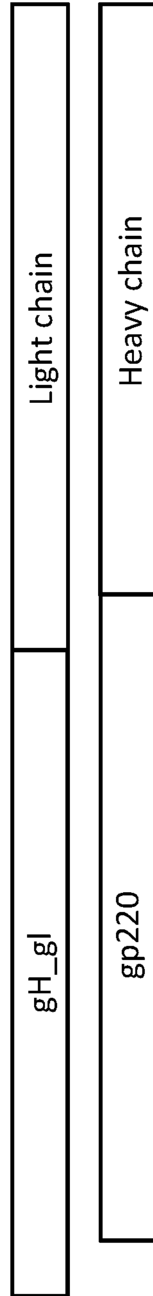


Fig. 26F

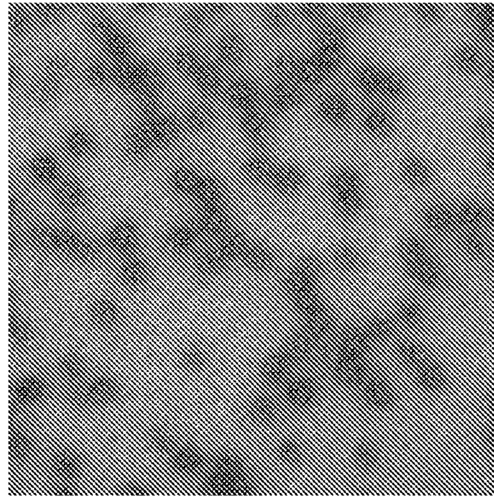


Fig. 26G

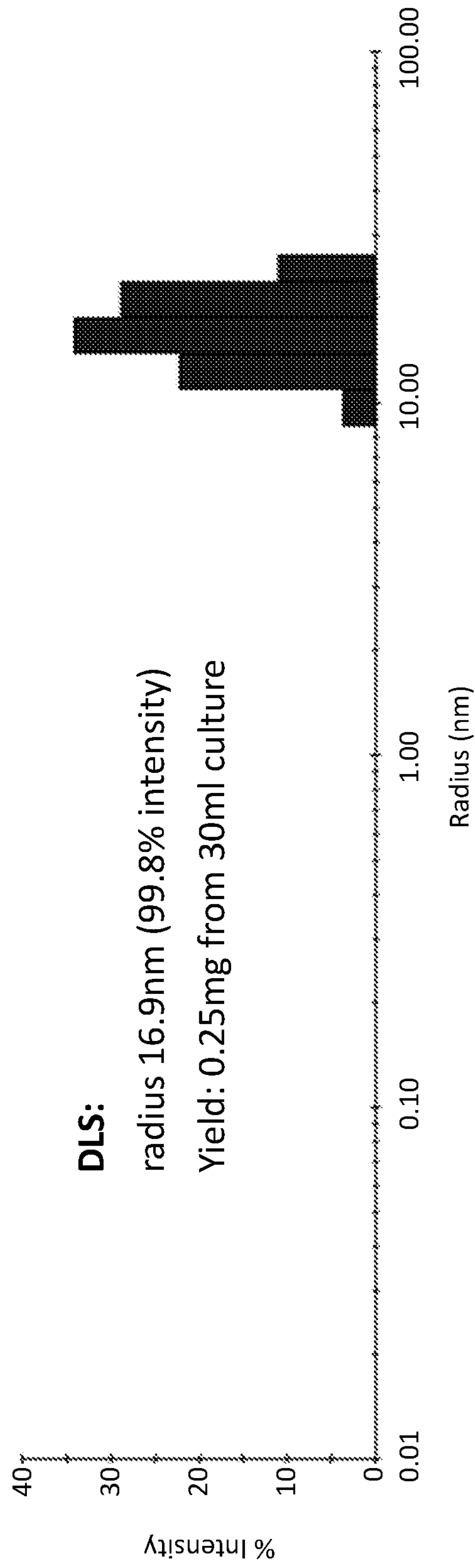


Fig. 26H

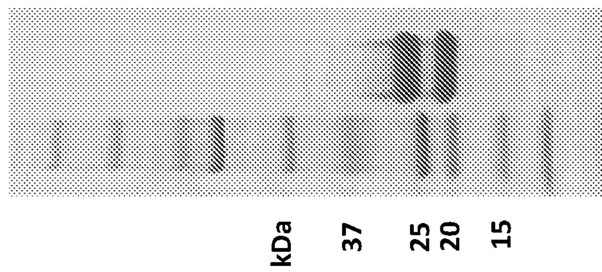
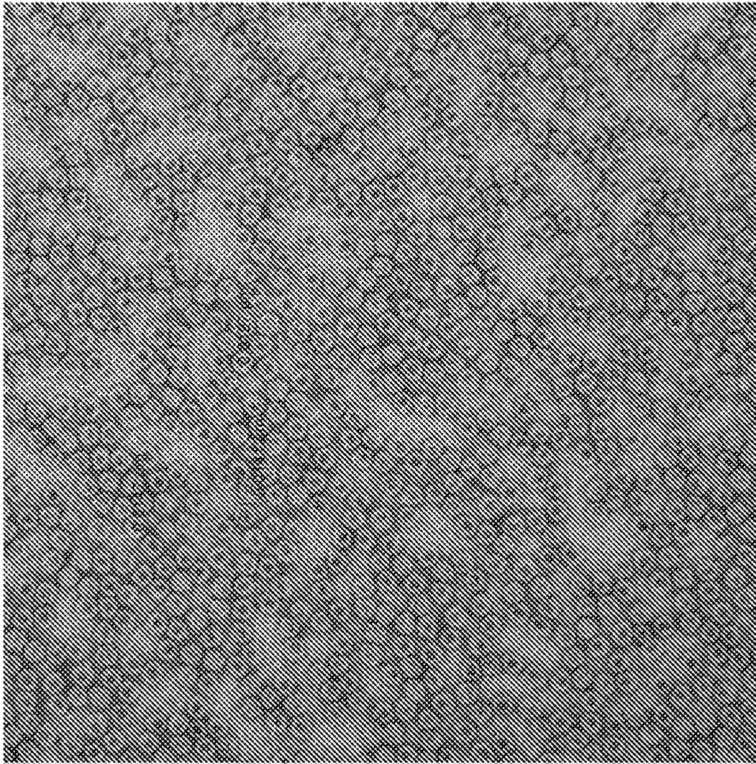
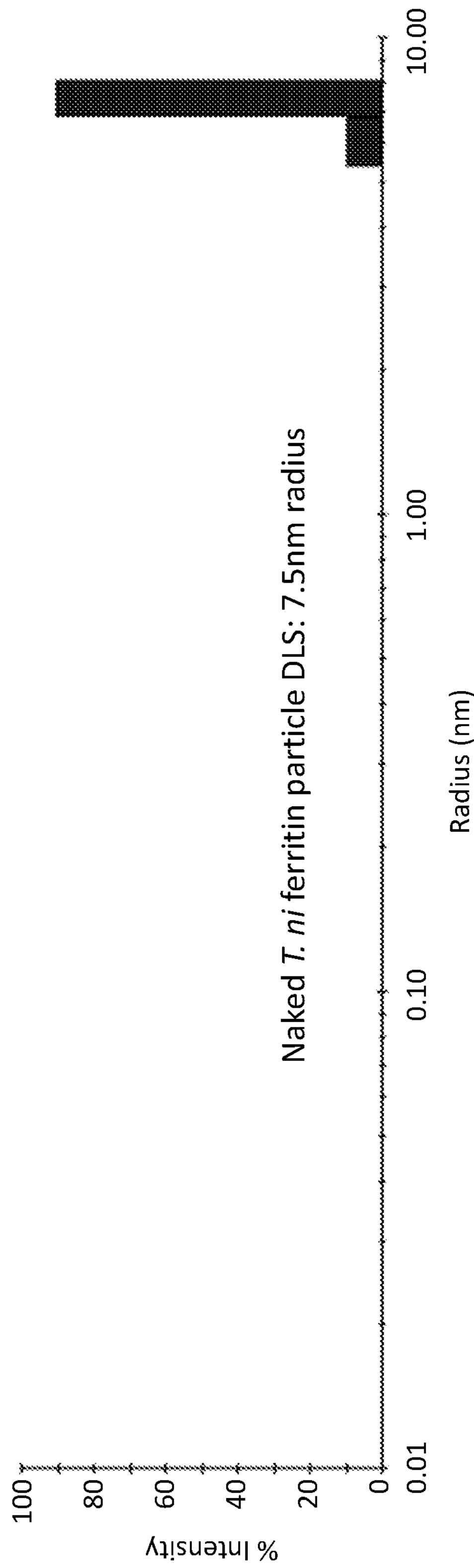


Fig. 27A



Naked Trichoplusia ni ferritin particle

Fig. 27B



Intensity Distribution	Radius (nm)	%Pd	Mw-R (kDa)	%Intensity	%Mass
☒ Peak 1	7.5	6.4	379	100.0	100.0

Fig. 27C

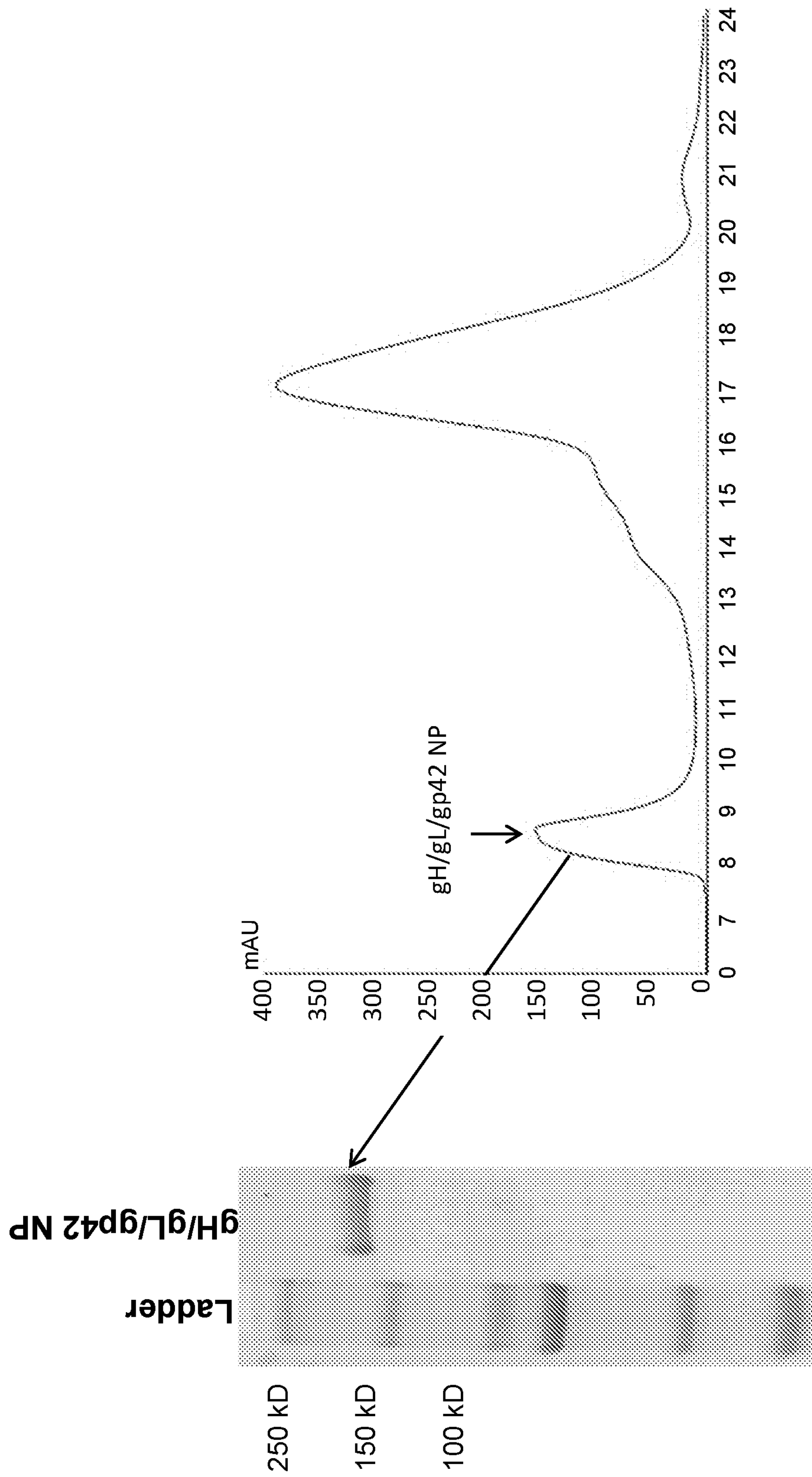
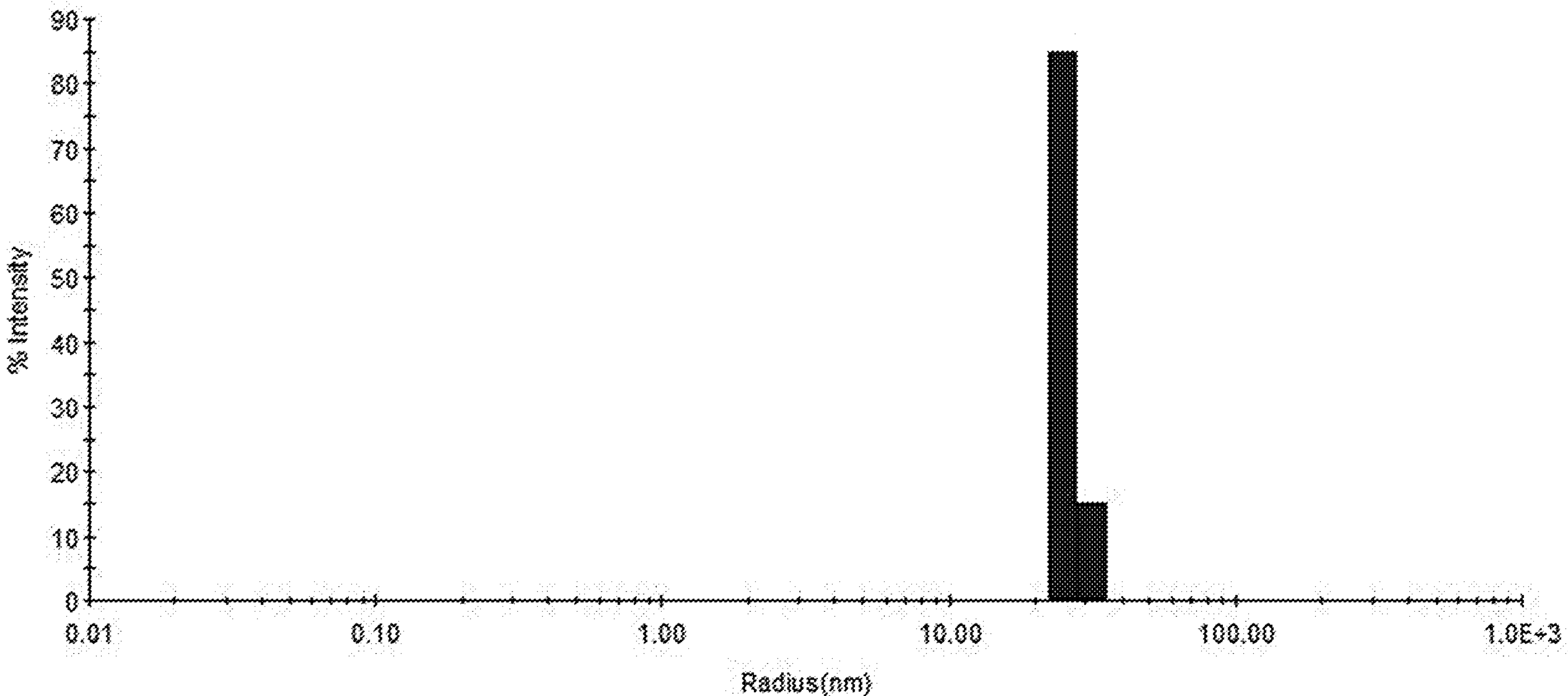


Fig. 28A



Intensity Distribution	Radius (nm)	%Pd	Mw-R (kDa)	%Intensity	%Mass
☒ Peak 1	26.2	9.2	7023	100.0	100.0
☐ Peak 2	459.9	10.2	5717160	----	----

Fig. 28B

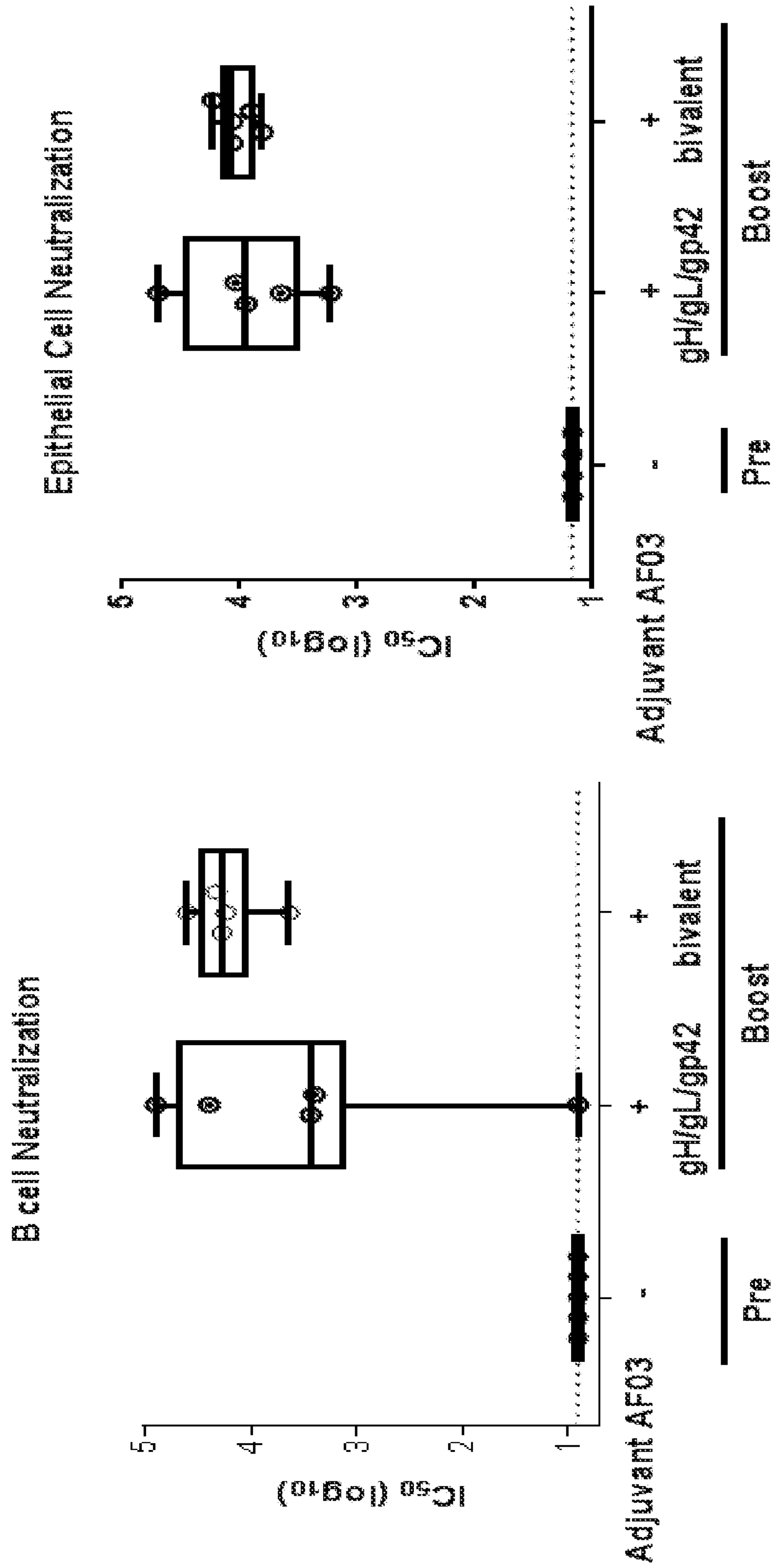


Fig. 29A

Fig. 29B

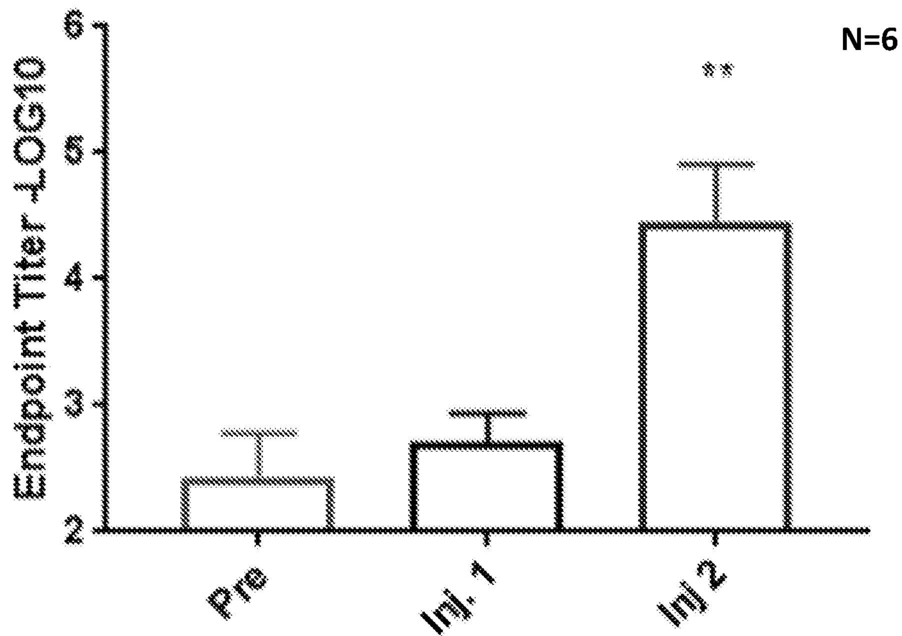


Fig. 30A

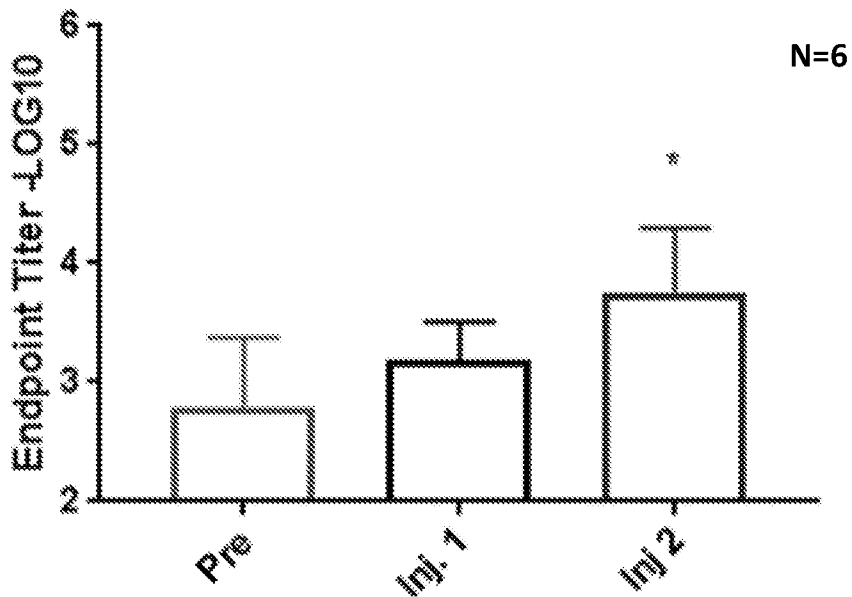


Fig. 30B

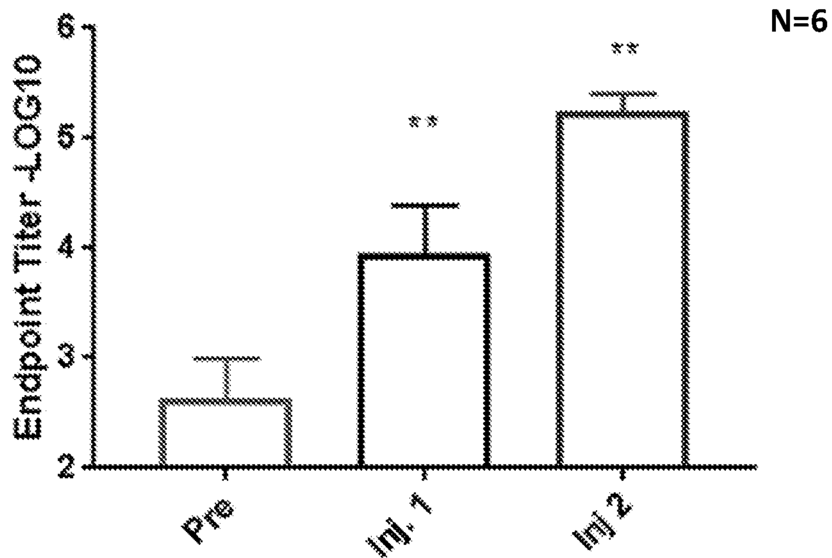


Fig. 30C

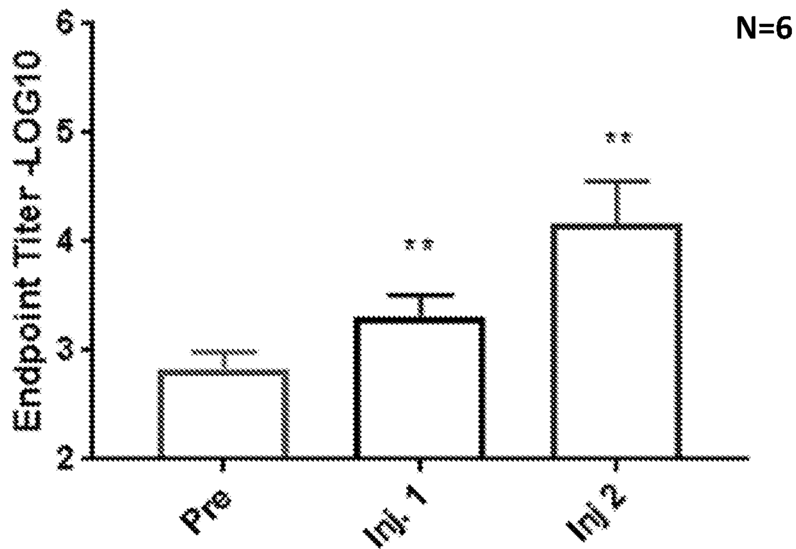


Fig. 30D

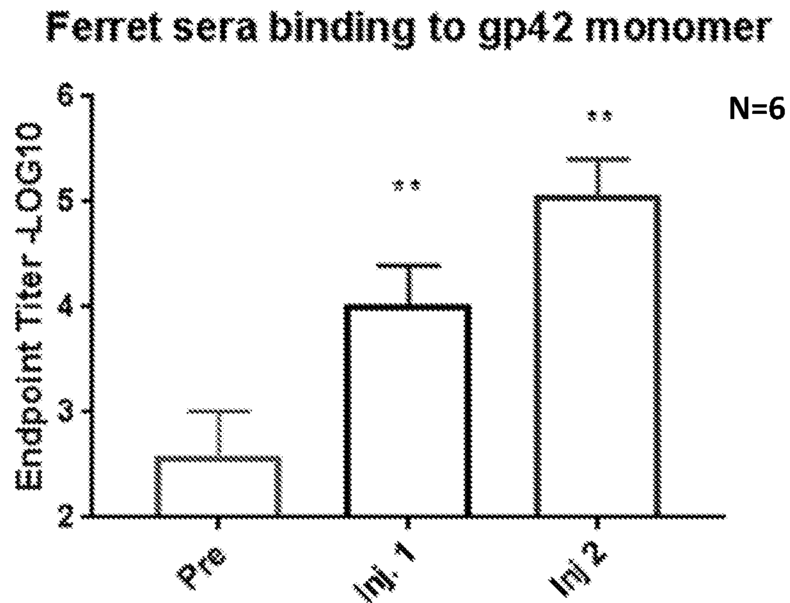


Fig. 30E

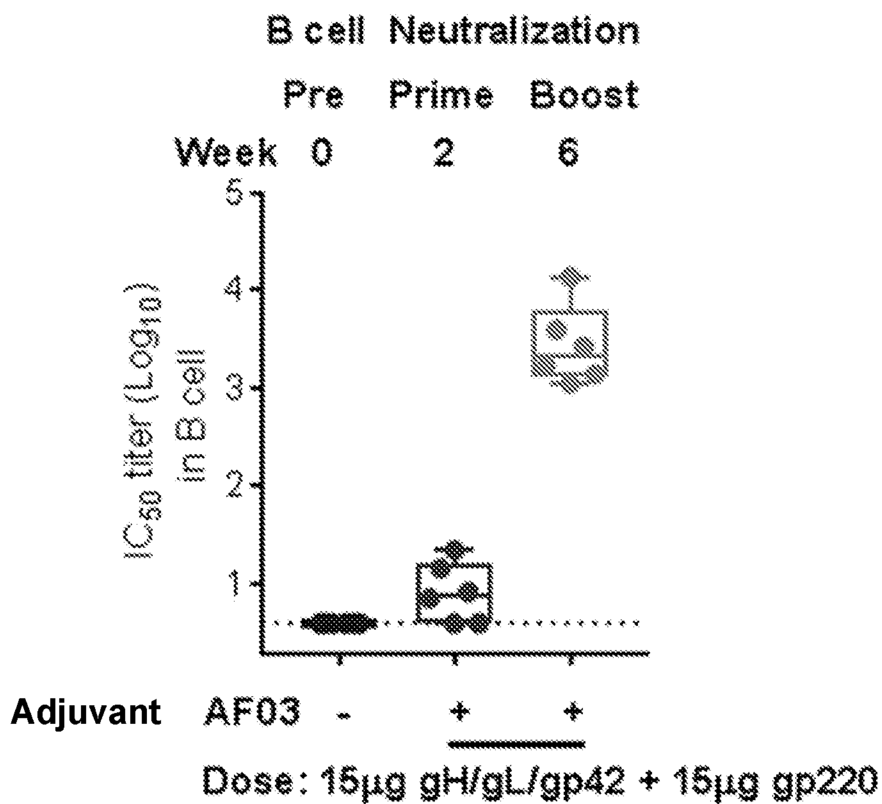


Fig. 30F

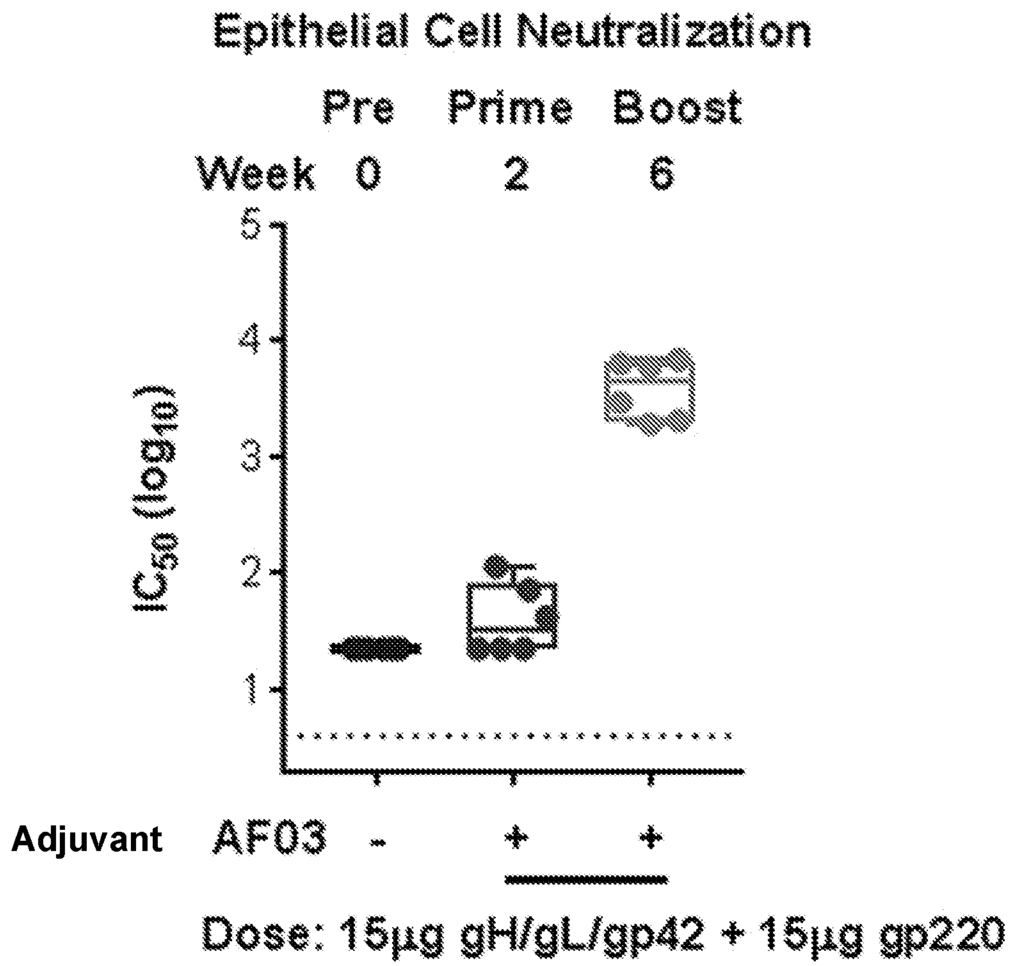


Fig. 30G

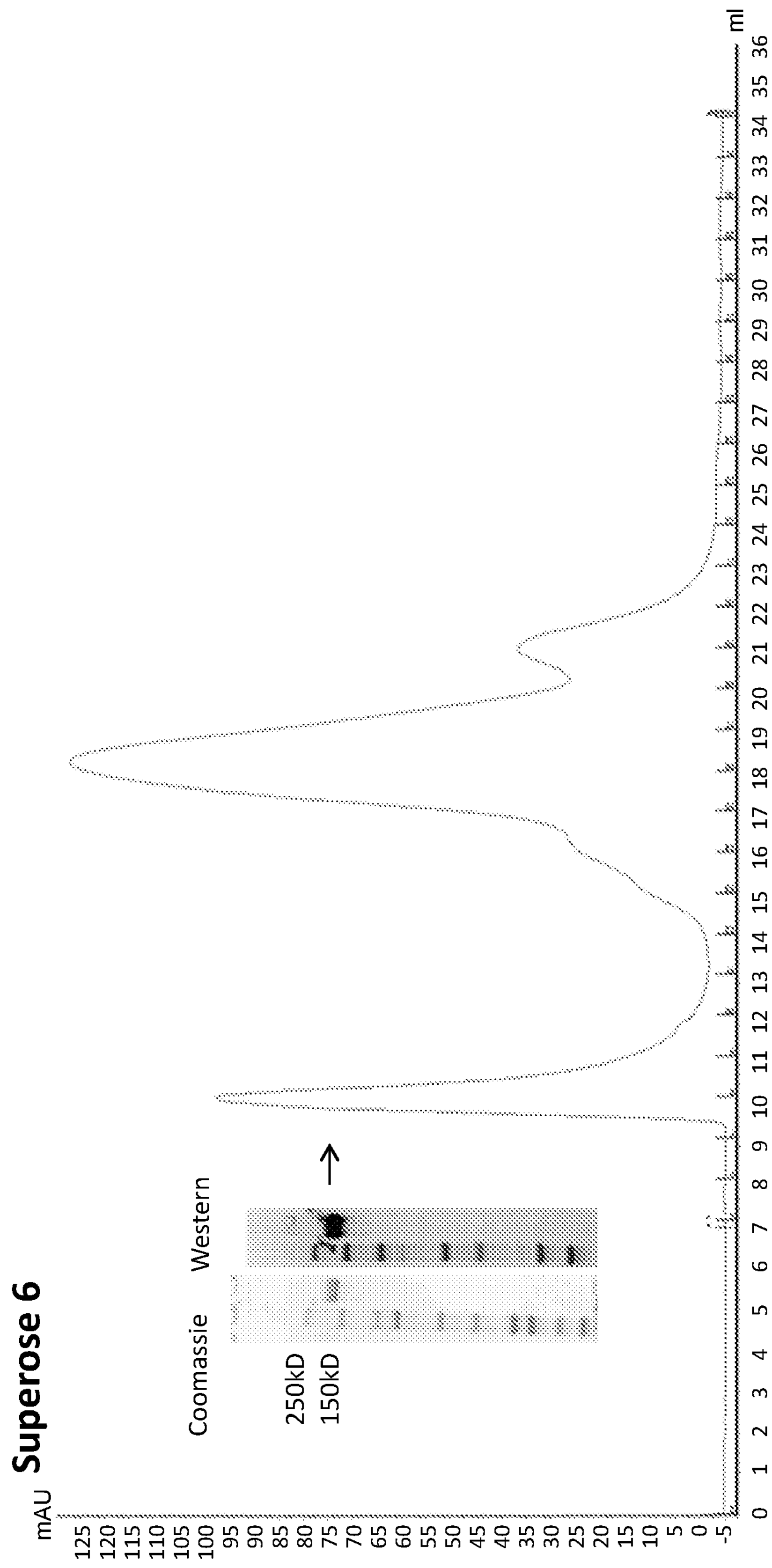
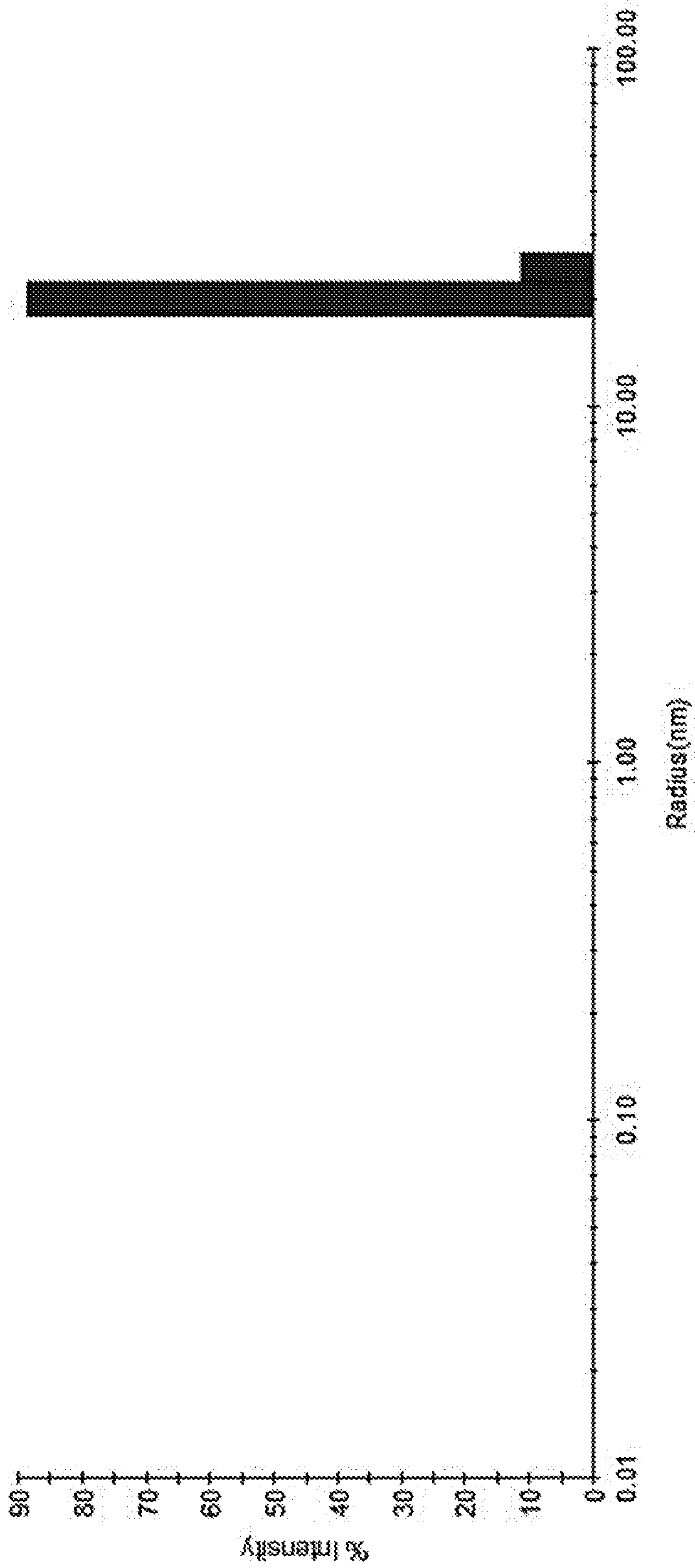


Fig. 31A



Intensity Distribution	Radius (nm)	%Pd	Mw-R (kDa)	%Intensity	%Mass
☒ Peak 1	0.8	9.3	2	0.2	96.7
☒ Peak 2	20.6	8.3	4015	99.8	3.3

Fig. 31B

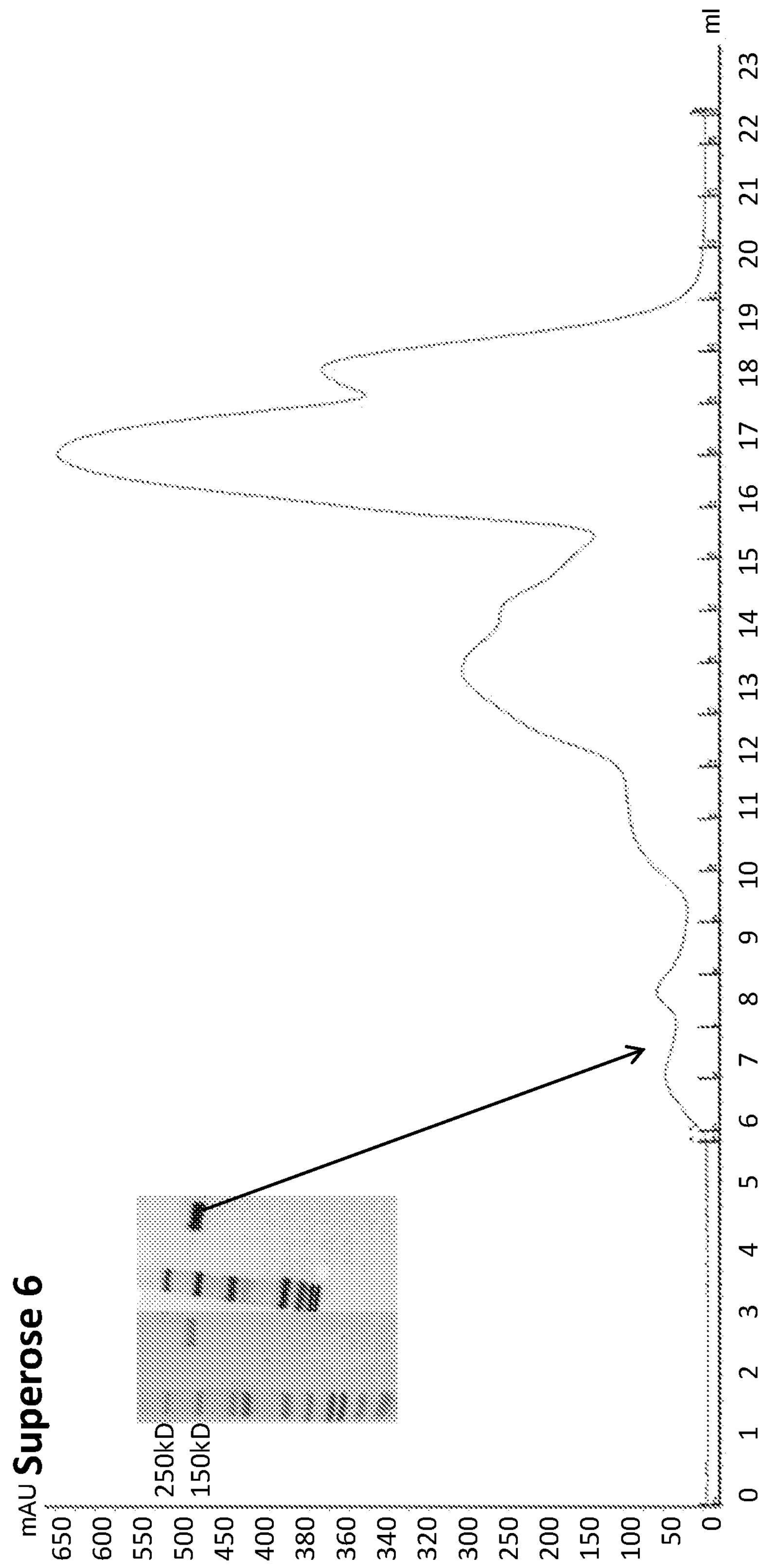
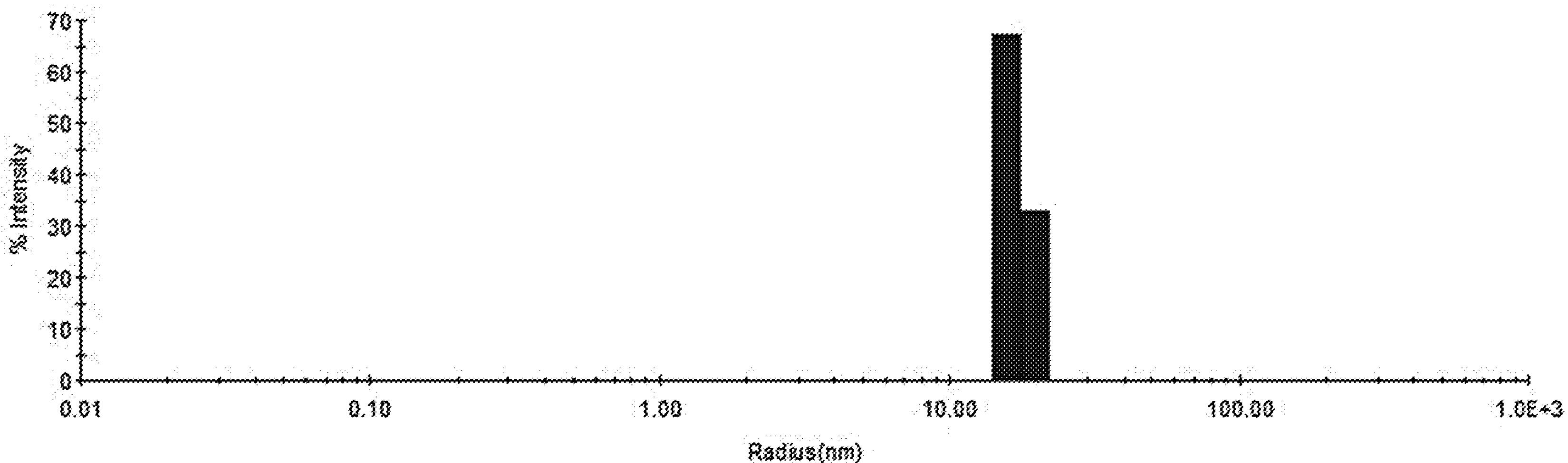


Fig. 32A



Intensity Distribution	Radius (nm)	%Pd	Mw-R (kDa)	%Intensity	%Mass
☒ Peak 1	17.1	11.6	2582	100.0	100.0

Fig. 32B

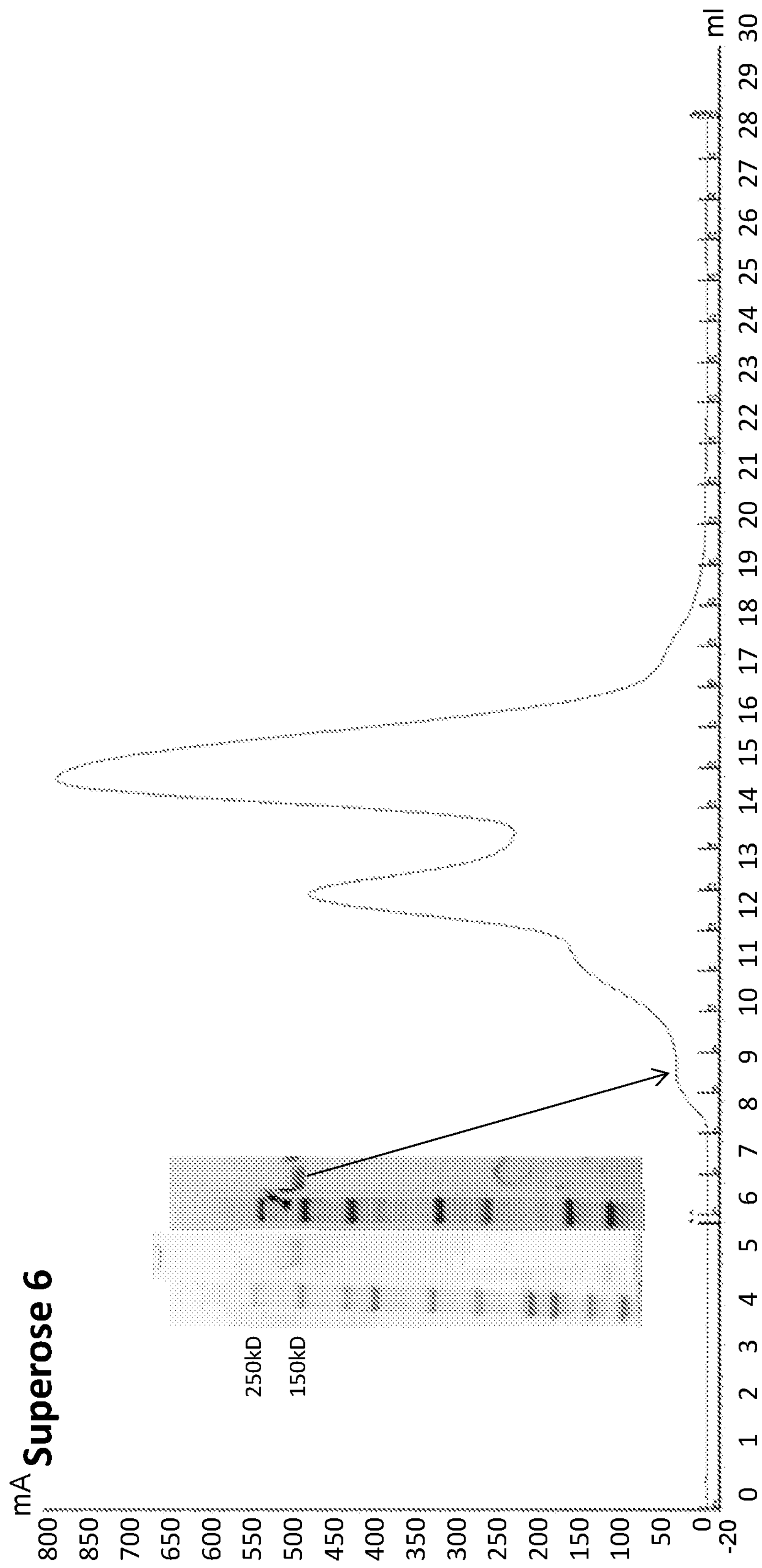
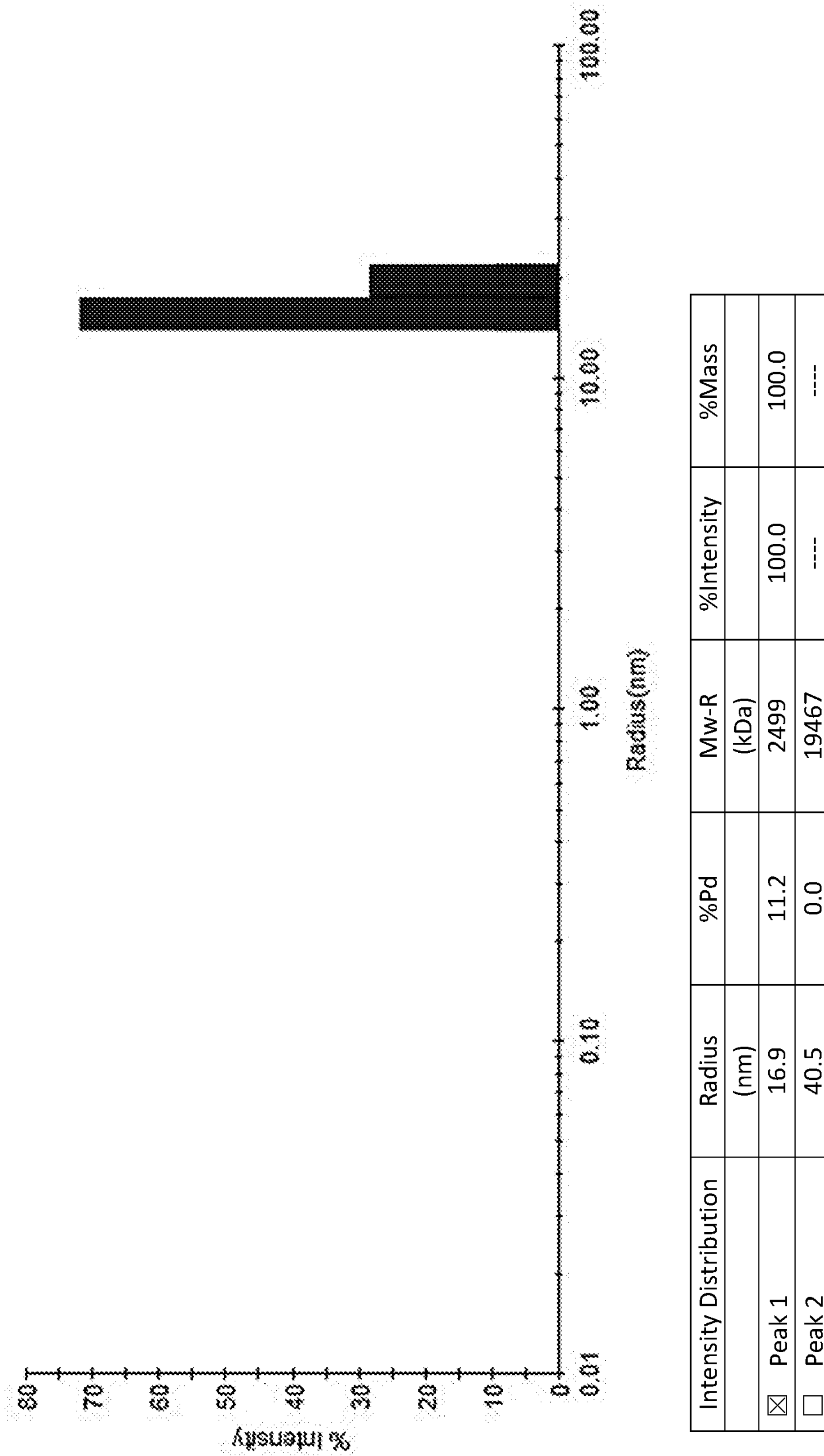


Fig. 33A



Intensity Distribution	Radius (nm)	%Pd	Mw-R (kDa)	%Intensity	%Mass
☒ Peak 1	16.9	11.2	2499	100.0	100.0
☐ Peak 2	40.5	0.0	19467	----	----

Fig. 33B

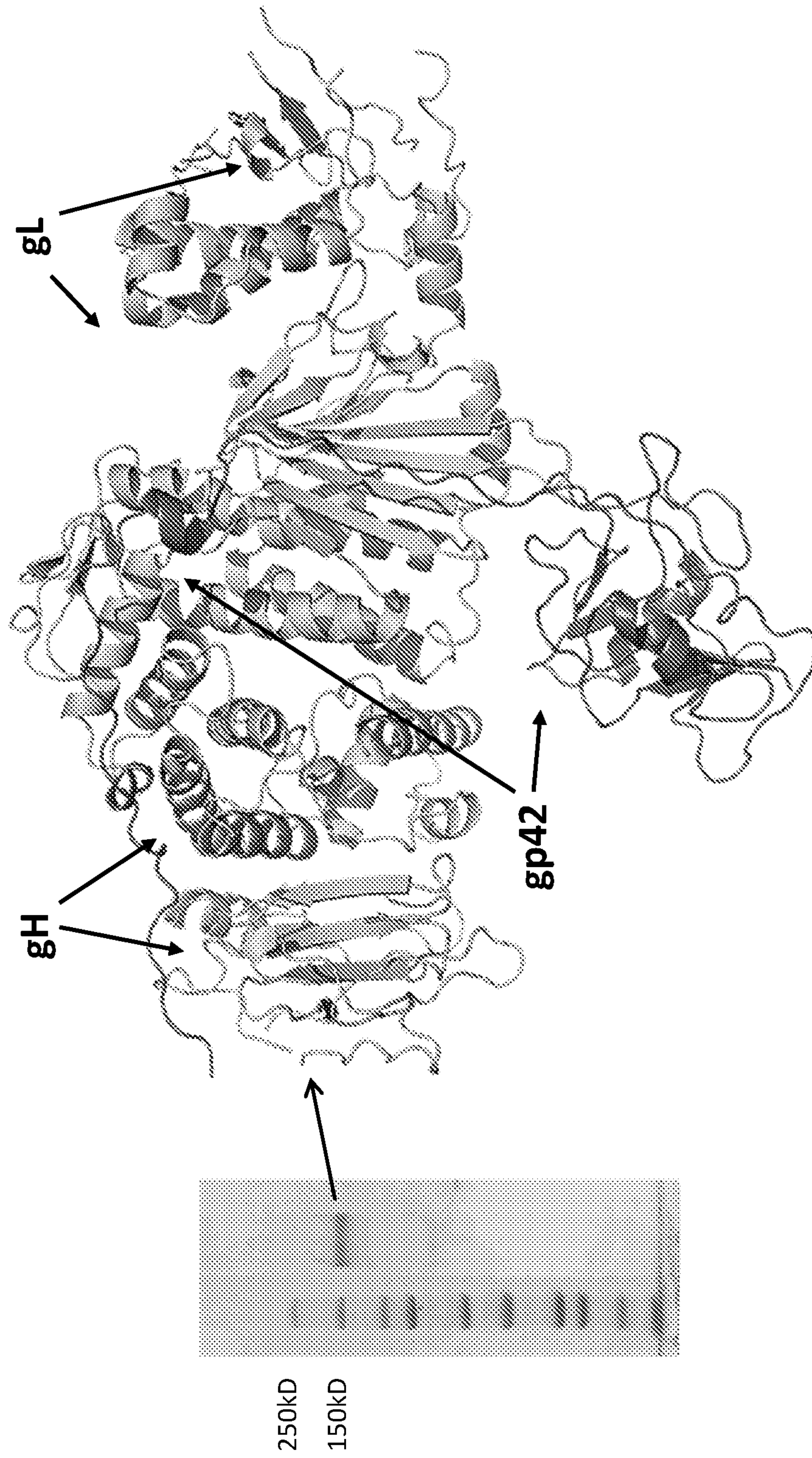


Fig. 34

Fig. 35A

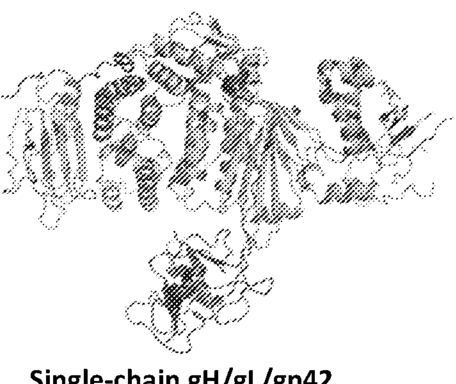


Fig. 35B

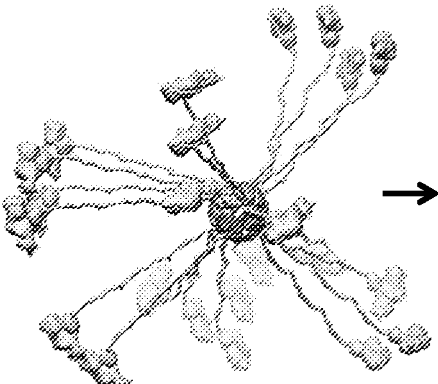


Fig. 35C

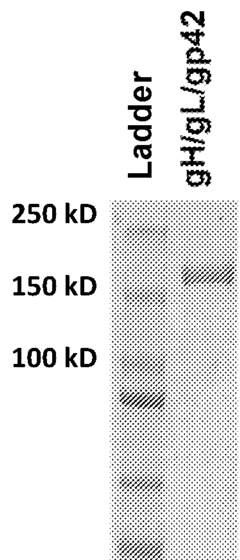


Fig. 35D

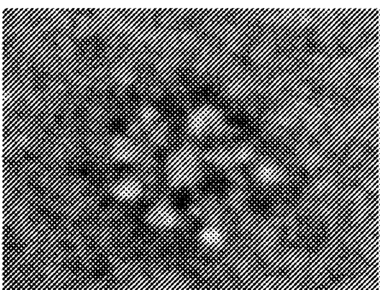


Fig. 35E