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(54) Title: ENGINEERED ANTIBODY FUSION PROTEINS WITH MODULABLE BINDING CONSTANTS AND THEIR APPLICATIONS

(57) Abstract: Compositions and methods of making engineered antibody fusion proteins with redox-sensitive domains with modifiable binding affinity and devices for the continuous in vivo detection of target molecules are described.

ENGINEERED ANTIBODY FUSION PROTEINS WITH MODULABLE BINDING CONSTANTS AND THEIR APPLICATIONS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority from U.S. Provisional Application No. 63/506,971, filed June 8, 2023, which is hereby incorporated by reference in its entirety.

REFERENCE TO A SEQUENCE LISTING

[0002] The Sequence Listing written in file name 614159SEQLIST.xml is 55.8 kilobytes, was created on June 7, 2024, and is hereby incorporated by reference.

BACKGROUND

[0003] Sensors capable of continuously monitoring biologically relevant molecules such as metabolites, hormones, and therapeutic compounds *in vivo* would provide highly useful medical information which could improve treatments and outcomes for many patients. However, clinically useful continuous sensors are only available for glucose monitoring, employing either an enzyme, such as glucose oxidase, or an abiotic synthetic receptor harboring a fluorescent probe. For continuous monitoring, the sensing molecule must be regenerated after the recognition of its target, which has limited the development of continuous sensors, particularly for hormones, such as insulin.

[0004] Antibodies can be generated to selectively bind to a wide range of biologically relevant molecules and can be used for detection and quantification of their antigens through a variety of immunoassays. The high binding affinity that antibodies typically have for their antigens (e.g., K_d ranges 10^{-8} to 10^{-12}) enables high selectivity and sensitivity, but also keeps antibodies tightly and irreversibly bound to their antigen under physiological conditions. Thus, the ligand binding site of an antibody can only be regenerated under harsh physical or chemical conditions (e.g., extreme changes in pH, temperature, or denaturing conditions). Due to these characteristics, the use of antibodies for the detection of targets such as insulin has only been performed in *in vitro* immunoassays, and antibodies have been used for single use monitoring, not for continuous

biosensing applications *in vivo*. The antibody fusion proteins disclosed herein address the need for antibody-based biosensors capable of continuously monitoring targets *in vivo* and *in situ*.

BRIEF SUMMARY

[0005] Compositions and methods of making engineered antibody fusion proteins with redox-sensitive domains with modulable binding affinity are provided and their application in devices for the continuous *in vivo* detection of target molecules.

[0006] One embodiment is an engineered antibody fusion protein, the protein comprising a variable region of an immunoglobulin heavy chain (V_H), a variable region of an immunoglobulin light chain (V_L), and a redox-sensitive domain, wherein the redox-sensitive domain is capable of undergoing a conformational change upon exposure to an external signal, and wherein the conformational change modulates an affinity of the antibody fusion protein for a target molecule upon exposure to the external signal.

[0007] In some embodiments, the amino acid sequence of the redox-sensitive domain is located at the C-terminal end of the antibody fusion protein.

[0008] In other embodiments, the amino acid sequence of the redox-sensitive domain is located at the N-terminal end of the antibody fusion protein.

[0009] In some embodiments, the V_H region and the V_L region are adjacent and comprise a single chain variable region (scFv), optionally wherein a linker sequence is between the amino acid sequence of the V_H region and the amino acid sequence of the V_L region, wherein the linker sequence comprises one or more glycine residues.

[0010] In other embodiments, the amino acid sequence of the redox-sensitive domain is located between the amino acid sequence of the V_H region and the amino acid sequence of the V_L region. In some embodiments, the amino acid sequence of the V_H region is located at the C-terminal end of the antibody fusion protein and the amino acid sequence of the V_L region is located at the N-terminal end of the antibody fusion protein. In some embodiments, the amino acid sequence of the V_H region is located at the N-terminal end of the antibody fusion protein and the amino acid sequence of the V_L region is located at the C-terminal end of the antibody fusion protein.

[0011] In embodiments, the target molecule is a metabolite, a hormone, and/or a therapeutic compound. In some embodiments, the target molecule is selected from the group consisting of insulin, glucagon, and glucagon-like peptide 1. In one embodiment, the target molecule is insulin and/or insulin analogues.

[0012] In some embodiments, the V_H region and the V_L region are anti-insulin.

[0013] In some embodiments, the scFv comprises an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to SEQ ID NO: 1. In one embodiment, the scFv comprises an amino acid sequence set forth in SEQ ID NO: 1.

[0014] In embodiments, the redox-sensitive domain comprises a heme cofactor or a flavin cofactor. In some embodiments, the redox-sensitive domain is selected from the group consisting of a bacterium-derived cytochrome c-like molecule (CYTc) domain, a light-oxygen-voltage-sensing (LOV) domain, and a cGMP-specific phosphodiesterases, adenylyl cyclases, FhlA (GAF) domain. In some embodiments, wherein the LOV domain is a second LOV domain of a phototropin (LOV2). In one embodiment, the LOV2 domain is derived from *Avena sativa* (asLOV2).

[0015] In some embodiments, the redox-sensitive domain comprises an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 36. In one embodiment, the redox-sensitive domain comprises an amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 36.

[0016] In some embodiments, the amino acid sequence of the V_H region comprises an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to SEQ ID NO: 6. In one embodiment, the amino acid sequence of the V_H region comprises an amino acid sequence set forth in SEQ ID NO: 6.

[0017] In some embodiments, the amino acid sequence of the V_L region comprises an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9%

sequence identity to SEQ ID NO: 7. In one embodiment, the amino acid sequence of the V_L region comprises an amino acid sequence set forth in SEQ ID NO: 7.

[0018] In some embodiments, the antibody fusion protein comprises an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to any one of SEQ ID NOs: 3-5.

[0019] In some embodiments, the antibody fusion protein comprises an amino acid sequence set forth in any one of SEQ ID NO: 3-5 and 16-20.

[0020] In embodiments, a sensitivity towards the target molecule is modulated by exposure to the external signal. In some embodiments, the sensitivity of the antibody fusion protein towards the target molecule is at least 2-fold, at least 5-fold, at least 10-fold, at least 100-fold, or at least 1000-fold different upon exposure to the external signal. In embodiments, a limit of detection (LOD) of the target molecule is modulated by exposure to the external signal. In embodiments, a specific binding ability of the antibody fusion protein towards the target molecule is modulated by exposure to the external signal. In some embodiments, the specific binding ability of the antibody fusion protein towards the target molecule is at least 2-fold, at least 5-fold, at least 10-fold, at least 100-fold, or at least 1000-fold different upon exposure to the external signal.

[0021] In embodiments, the dissociation constant (K_d) of the antibody fusion protein towards the target molecule is modulated by exposure to the external signal. In some embodiments, the K_d of the antibody fusion protein is at least 10, at least 10^2 , at least 10^3 , at least 10^4 , at least 10^5 , at least 10^6 , at least 10^7 , at least 10^8 , at least 10^9 , or at least 10^{10} times different upon exposure to the external signal.

[0022] In embodiments, the external signal is redox potential or light. In some embodiments, the external signal is blue light. In some embodiments, the light has a wavelength of 400-700 nm.

[0023] Another embodiment is an electrode comprising said antibody fusion protein immobilized on an electroconductive material.

[0024] A further embodiment is a device for detection of the target molecule, comprising said engineered antibody fusion protein. In some embodiments, the detection is continuous. In

some embodiments, the detection is performed *in vivo*. In some embodiments, the target molecule detected by said device is insulin.

[0025] An embodiment is a polynucleotide encoding said antibody fusion protein. In some embodiments, said polynucleotide comprises a nucleotide sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to any one of SEQ ID NOs: 8-10 and 31-35. In some embodiments, said polynucleotide comprises a nucleotide sequence set forth in any one of SEQ ID NOs: 8-10 and 31-35.

[0026] Another embodiment is a recombinant plasmid comprising a promoter operably linked to said polynucleotide sequence and prepared by inserting the nucleotide sequence into an expression vector. In some embodiments, the expression vector is a pET30c vector.

[0027] A further embodiment is a method of producing a recombinant antibody fusion protein, the method comprising: i) introducing said recombinant plasmid into a host cell and ii) expressing the antibody fusion protein. In some embodiments, the host cell is *E. coli*.

BRIEF DESCRIPTION OF FIGURES

[0028] **Figure 1A** shows the structure of a redox-sensitive light-oxygen-voltage-sensing domain derived from *Avena sativa* (asLOV2) and **Figure 1B** shows its flavin mononucleotide (FMN) cofactor, which binds to the Cys966 upon excitation with blue light, which leads to the undocking of the J α -helix.¹

[0029] **Figure 2** shows the structural model of an engineered antibody fusion protein with an anti-insulin single-chain variable fragment (scFV) domain and an asLOV2 domain at the C-terminus (cLOV) as predicted by AlphaFold 2.

[0030] **Figure 3** shows a recombinant plasmid for the expression of an antibody fusion protein with an anti-insulin scFV domain and an asLOV2 domain at the C-terminus (cLOV) in a pET30c expression vector.

[0031] **Figure 4A** shows the chromatogram tracking at 280 nm (UV280) and 450 nm (UV450) absorptions, and **Figure 4B** shows SDS-PAGE of the eluted soluble antibody fusion

protein with an anti-insulin scFV domain and an asLOV2 domain at the C-terminus (cLOV), which was expressed in *Escherichia coli* Shuffle T7 Express™ cells.

[0032] **Figure 5** shows results of an insulin ELISA with the crude soluble extract of the antibody fusion protein with an anti-insulin scFV domain and an asLOV2 domain at the C-terminus (cLOV).

[0033] **Figures 6A-6B** show spectroscopic analyses of an asLOV2 protein (**Figure 6A**) and the antibody fusion protein (cLOV) with an anti-insulin scFV domain and an asLOV2 domain at the C-terminus (cLOV) (**Figure 6B**) in the dark and in the presence of light. The light exposure was performed using an LED array at 470 nm.

[0034] **Figure 7A** shows a schematic overview of the insulin ELISA used to measure the binding affinity of the antibody fusion protein with an anti-insulin scFV domain and an asLOV2 domain. **Figure 7B** shows the results of the ELISA of the antibody fusion protein with an anti-insulin scFV domain and an asLOV2 domain at the C-terminus (cLOV) in the dark and in the presence of light at various insulin concentrations. For the ELISA assays, the entire procedure including all washing and incubation steps were performed either in a dark room, or under an LED array with constant 470 nm light exposure.

[0035] **Figures 8A-8D** show further spectroscopic analyses of an asLOV protein (**Figures 8A-8B**) and an antibody fusion protein with an anti-insulin scFV domain and an asLOV2 domain at the C-terminus (cLOV; **Figures 8C-8D**) by measuring the intrinsic fluorescence of the flavin mononucleotide (FMN) cofactor in light and dark conditions. **Figures 8A** and **8C** show fluorescence of asLOV2 and the cLOV antibody fusion protein in light and dark conditions. **Figure 8B** and **8D** show recovery of the fluorescence signal in the dark at 30 second intervals for two minutes after light exposure.

[0036] **Figure 9** shows the structural model of an engineered antibody fusion protein with an anti-insulin single-chain variable fragment (scFV) domain and an asLOV2 domain at the N-terminus (nLOV) as predicted by AlphaFold 2.

[0037] **Figure 10** shows a recombinant plasmid for the expression of an antibody fusion protein with an anti-insulin scFV domain and an asLOV2 domain at the N-terminus (nLOV) in a pET30c expression vector.

[0038] **Figure 11A** shows the chromatogram tracking at 280 nm (UV280) and 450 nm (UV450) absorptions, and **Figure 11B** shows SDS-PAGE of the eluted soluble antibody fusion protein with an anti-insulin scFV domain and an asLOV2 domain at the N-terminus (nLOV), which was expressed in *E. coli* Shuffle T7 Express™ cells.

[0039] **Figure 12** shows the intrinsic fluorescence of an antibody fusion protein with an anti-insulin scFV domain and an asLOV2 domain at the N-terminus (nLOV), measured in both dark and light conditions. Recovery is shown by measuring the fluorescence signal in the dark at 30 second intervals for six minutes after light exposure.

[0040] **Figure 13** shows the structural model of an antibody fusion protein with an anti-insulin single-chain variable fragment (scFV) domain and a truncated asLOV2 domain at the N-terminus (ntLOV) as predicted by AlphaFold 2.

[0041] **Figure 14** shows a recombinant plasmid for the expression of an antibody fusion protein with an anti-insulin scFV domain and a truncated asLOV2 domain at the N-terminus (ntLOV) in a pET30c expression vector.

[0042] **Figure 15A** shows the chromatogram tracking at 280 nm (UV280) and 450 nm (UV450) absorptions, and **Figure 15B** shows SDS-PAGE of the eluted soluble antibody fusion protein with an anti-insulin scFV domain and a truncated asLOV2 domain at the N-terminus (ntLOV), which was expressed in *E. coli* Shuffle T7 Express™ cells.

[0043] **Figure 16** shows the intrinsic fluorescence of an antibody fusion protein with an anti-insulin scFV domain and a truncated asLOV2 domain at the N-terminus (ntLOV), measured in both dark and light conditions. Recovery is shown by measuring the fluorescence signal in the dark at 30 second intervals for six minutes after light exposure.

[0044] **Figure 17** shows the structural model of an engineered antibody fusion protein with an asLOV2 domain between an anti-insulin V_H region at the N-terminus and an anti-insulin V_L region at the C-terminus with glycine-rich linkers (gsLOV) as predicted by AlphaFold 2.

[0045] **Figure 18** shows a recombinant plasmid for the expression of an antibody fusion protein with an asLOV2 domain between an anti-insulin V_H region at the N-terminus and an anti-insulin V_L region at the C-terminus (gsLOV) in a pET30c expression vector.

[0046] **Figure 19A** shows SDS-PAGE of eluted soluble antibody fusion proteins with an asLOV2 domain between an anti-insulin V_H region at the N-terminus and an anti-insulin V_L region at the N-terminus (gsLOV) which were expressed in *E. coli* Shuffle T7 Express™ cells. Variants of the antibody fusion proteins were prepared with mutations to fix the proteins in a “dark state” (C190A) or a “light state” (I272E, A276E). **Figures 19B-19D** show the chromatogram tracking at 280 nm (UV280) and 450 nm (UV450) absorptions for the gsLOV (**Figure 19B**), the gsLOV C190A “dark” variant (**Figure 19C**), and the gsLOV I272E, A276E “light” variant (**Figure 19D**) antibody fusion proteins.

[0047] **Figure 20** shows the intrinsic fluorescence of an antibody fusion protein with an asLOV2 domain between an anti-insulin V_H region at the N-terminus and an anti-insulin V_L region at the N-terminus (gsLOV), measured in both dark and light conditions. Recovery is shown by measuring the fluorescence signal in the dark at 30 second intervals for six minutes after light exposure.

[0048] **Figure 21A** shows a schematic of an antibody fusion protein binding to insulin as used in a Biolayer Interferometry (BLI) assay. **Figures 21B-21E** show BLI sensor grams for the wild type anti-insulin (**Figure 21B**), the gsLOV fusion protein (**Figure 21C**), the gsLOV I272E, A276E fusion protein fixed in a “light state” (**Figure 21D**), and the gsLOV C190A fusion protein fixed in a “dark state” (**Figure 21E**).

[0049] **Figure 22** shows the structural model of an engineered antibody fusion protein with an asLOV2 domain between an anti-insulin V_H region at the N-terminus and an anti-insulin V_L region at the N-terminus without glycine-rich linkers between the domains (nolinkLOV) as predicted by AlphaFold 2.

[0050] **Figure 23** shows a recombinant plasmid for the expression of an antibody fusion protein with an asLOV2 domain between an anti-insulin V_H region at the N-terminus and an anti-insulin V_L region at the N-terminus without glycine-rich linkers between the domains (nolinkLOV) in a pET30c expression vector.

[0051] **Figure 24A** shows SDS-PAGE of eluted soluble antibody fusion proteins with an asLOV2 domain between an anti-insulin V_H region at the N-terminus and an anti-insulin V_L region at the N-terminus without glycine-rich linkers between the domains (nolinkLOV) which were

expressed in *E. coli* Shuffle T7 Express™ cells. Variants of the antibody fusion proteins were prepared with mutations to fix the proteins in a “dark state” (C185A) or a “light state” (I267E, A271E). **Figures 24B-24D** show the chromatogram tracking at 280 nm (UV280) and 450 nm (UV450) absorptions for the nolinkLOV (**Figure 24B**), the nolinkLOV C185A “dark” variant (**Figure 24C**), and the nolinkLOV I267E, A271E “light” variant (**Figure 24D**) antibody fusion proteins.

[0052] **Figures 25A-25D** show BLI sensor grams for wild type anti-insulin (**Figure 25A**), the nolinkLOV fusion protein (**Figure 25B**), the nolinkLOV C185A fusion protein fixed in a “dark state” (**Figure 25C**), and the nolinkLOV I267E, A271E fusion protein fixed in a “light state” (**Figure 25D**).

DETAILED DESCRIPTION

[0053] Antibodies capable of selectively binding their antigens can be easily generated for a vast range of relevant targets and are highly useful in many biomedical applications, including immunoassays. However, the high affinity of an antibody for its antigen (e.g., K_d ranges 10^{-8} to 10^{-12}) makes the binding essentially irreversible, thus the antibody binding site cannot be regenerated under physiological conditions. These physical properties have made it impossible to use antibodies for continuous *in vivo* detection of target molecules. The development of an antibody with modutable binding affinity would solve this problem by being able to release the antigen from the antibody upon exposure to an external signal. Disclosed herein are engineered antibody fusion proteins with redox-sensitive domains which undergo a conformational change to modulate binding affinity upon exposure to an external signal.

[0054] The presently disclosed subject matter will now be described more fully hereinafter. However, many modifications and other embodiments of the presently disclosed subject matter set forth herein will come to mind to one skilled in the art to which the presently disclosed subject matter pertains having the benefit of the teachings presented in the foregoing descriptions. Therefore, it is to be understood that the presently disclosed subject matter is not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims. In other words, the subject matter described

herein covers all alternatives, modifications, and equivalents. In the event that one or more of the incorporated literature, patents, and similar materials differs from or contradicts this application, including but not limited to defined terms, term usage, described techniques, or the like, this application controls. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in this field. All publications, patent applications, patents and other references mentioned herein are incorporated by reference into their entirety.

I. Definitions

[0055] Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. It will be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the present application and relevant art and should not be interpreted in an idealized or overly formal sense unless expressly so defined herein. The terminology used in the description of the invention herein is for the purpose of describing particular aspects only and is not intended to be limiting of the invention. In case of a conflict in terminology, the present specification is controlling.

[0056] As used herein, the term “physiological conditions” refers to the range of conditions of temperature, pH, and tonicity (or osmolality) normally encountered within tissues in the body of a living human.

[0057] The term “*in vitro*” refers to artificial environments and to processes or reactions that occur within an artificial environment (e.g., a test tube).

[0058] The term “*in vivo*” refers to natural environments (e.g., a cell or organism or body) and to processes or reactions that occur within a natural environment.

[0059] As used herein, the term “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (“or”).

[0060] The term “or” refers to any one member of a particular list.

[0061] Unless otherwise apparent from the context, the term “about” encompasses values within a standard margin of error of measurement (e.g., SEM) of a stated value or variations $\pm$ 0.5%, 1%, 5%, or 10% from a specified value.

[0062] The singular forms of the articles “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a protein” or “at least one protein” can include a plurality of proteins, including mixtures thereof.

[0063] Compositions or methods “comprising” or “including” one or more recited elements may include other elements not specifically recited. For example, a composition that “comprises” or “includes” a protein may contain the protein alone or in combination with other ingredients.

[0064] As used herein, “about” means within a statistically meaningful range of a value or values such as a stated concentration, length, molecular weight, pH, sequence identity, time frame, temperature or volume. Such a value or range can be within an order of magnitude, typically within 20%, more typically within 10%, and even more typically within 5% of a given value or range. The allowable variation encompassed by “about” will depend upon the particular system under study, and can be readily appreciated by one of skill in the art.

II. Compositions

Engineered Antibody Fusion Proteins

[0065] In one embodiment, an isolated engineered antibody fusion protein with a redox-sensitive domain and modifiable binding affinity for a target molecule is provided. In embodiments, the antibody fusion protein undergoes a conformational change upon exposure to an external signal and exhibits a different binding affinity to a target molecule.

[0066] As used herein, “isolated,” with respect to a polypeptide (and also a polynucleotide), means a molecule (e.g., polypeptide, protein or polynucleotide) isolated from its natural environment or prepared using synthetic methods such as those known to one of skill in the art. Complete purification is not required in either case. The molecules described herein can be isolated and purified from normally associated material in conventional ways, such that in the purified preparation the molecule is the predominant species in the preparation. At the very least, the degree

of purification is such that extraneous material in the preparation does not interfere with use of the molecule in the manner disclosed herein. The molecule is at least about 85% pure; alternatively, at least about 90% pure, alternatively, at least about 95% pure; and alternatively, at least about 99% pure.

[0067] Disclosed herein are engineered antibody fusion proteins comprising a variable region of an immunoglobulin heavy chain (V_H), a variable region of an immunoglobulin light chain (V_L), and a redox-sensitive domain.

[0068] The V_H region and the V_L region are the variable regions of an immunoglobulin which are capable of binding to a target molecule.

[0069] The redox-sensitive domain (RSD) undergoes a conformational change upon exposure to an external signal. The redox-sensitive domain may be part of the Per-Arnt-Sim (PAS) superfamily of domains. The redox-sensitive domain may comprise a heme cofactor or a flavin cofactor. The flavin cofactor may be flavin mononucleotide (FMN). The redox-sensitive domain may be a bacterium-derived cytochrome c-like molecule (CYTc) domain, a light-oxygen-voltage-sensing (LOV) domain, or a cGMP-specific phosphodiesterases, adenylyl cyclases, formate hydrogen lysate transcriptional activator (FhlA) (GAF) domain. In some embodiments, the redox-sensitive domain is a second LOV domain of a phototropin (LOV2). In some embodiments, the redox-sensitive domain is an LOV2 domain derived from *Avena sativa* (asLOV2).

[0070] The external signal may be redox potential or light. In some embodiments, the external signal is blue light. In some embodiments, the blue light has a wavelength of about 400-500 nm. In some embodiments, the blue light has a wavelength of about 450-490 nm, about 460-480 nm, about 465-475 nm, or about 470 nm. In other embodiments, the external signal is light with a wavelength between about 500-700 nm.

[0071] The V_H region and V_L region may be adjacent in the amino acid sequence of the antibody fusion protein. There may be a short peptide linker between the V_H region and the V_L region and the peptide linker may comprise glycine residues and/or serine residues. The linker may be between about 5 amino acids to about 30 amino acids in length. An amino acid sequence comprising a V_H region and a V_L region, optionally wherein a linker sequence is between the V_H region and the V_L region, may be referred to as single chain variable region (scFv) domain.

[0072] In some embodiments, the antibody fusion protein comprises a scFv domain (e.g., a V_H region and a V_L region optionally connected by a linker) and a redox-sensitive domain. In some embodiments, the redox-sensitive domain is located at the C-terminal end of the antibody fusion protein. In other embodiments, the redox-sensitive domain is located at the N-terminal end of the antibody fusion protein.

[0073] Peptides and proteins are said to have a C-terminal end (e.g., a C-terminus) and a N-terminal end (e.g., N-terminus). The C-terminus has a terminal amino acid residue with a free carboxylic acid (e.g., CO₂H) which is not bound to another amino acid residue as part of a peptide bond. The N-terminus has a free amino group (e.g., NH₂) which is not bound to another amino acid residue as part of a peptide bond.

[0074] In some embodiments, the redox-sensitive domain is located between the V_H region and the V_L region. In one embodiment, the V_H region is located at the C-terminal end and the V_L region is located at the N-terminal end of the antibody fusion protein. In another embodiment, the V_H region is located at the N-terminal end and the V_L region is located at the C-terminal end of the antibody fusion protein.

[0075] The target molecule to which the V_H region and the V_L regions are capable of binding may be any biologically relevant molecule of interest. In embodiments, the target molecule is a metabolite, a hormone, and/or a therapeutic compound. Target molecules may be sugars, small molecules, peptides, or proteins. Target molecules may originate from any species of interest, including but not limited to proteins from human, monkey, mouse, rat, rabbit, dog, cat, horse, donkey, goat, pig, cow, bird, reptile, amphibian, fish, plant, fungi, bacteria, or Archean species. In some embodiments, the target molecule is insulin, glucagon, and glucagon-like peptide 1.

[0076] In an embodiment, the target molecule is insulin and the V_H region and the V_L region of the antibody fusion protein are anti-insulin. In some embodiments, the antibody fusion protein comprises an anti-human insulin scFv domain and/or an asLOV2 domain. In some embodiments, the antibody fusion protein has an scFv domain comprising an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity

to SEQ ID NO: 1. In one embodiment, the antibody fusion protein comprises the amino acid sequence set forth in SEQ ID NO: 1.

[0077] *Anti-human insulin scFv (Clone HB125)*

MAQIQLVQSGPELKKPGETVKISCKASGYTFTDYSMHWVKQAPGKGLKWMDWI
NTETGVPTYADDFKGRFAFSLETSANTAYLQINDLKNEDTATYFCTRGYGKGYF
DVWGAGTTVTVSSAKSTPPSVYPLAPGSGGGGSGGGGSGGGGSQIVLTQSPTIMS
ASLGERVTMTCTASSSVSSSYLHWYQQKPGSSPKLWIYSTSNLASGVPARFSGSG
SGTSYSLTISSMEAEDAATYYCHQYHRSPPTFGAGTKLELKRADAA (SEQ ID NO:
1).

[0078] In some embodiments, the antibody fusion protein comprises an LOV2 domain from *Avena sativa* (asLOV2) as the redox sensitive domain. In some embodiments, the antibody fusion protein has a redox-sensitive domain comprising an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to SEQ ID NO: 2. In one embodiment, the antibody fusion protein comprises the amino acid sequence set forth in SEQ ID NO: 2.

[0079] *LOV2 derived from Avena sativa (asLOV2)*

GEFLATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPET
DRATVRKIRDAIDNQTEVTQVLINYTEKSGKKFWNLFHLQPMRDQKGDVQYFIGV
QLDGTEHVRDAAEREAVMLIKKTAAEIDEAAKEL (SEQ ID NO: 2)

[0080] In some embodiments, the antibody fusion protein comprises a truncated LOV2 domain from *Avena sativa* as the redox sensitive domain (tLOV2). In some embodiments, the antibody fusion protein has a redox-sensitive domain comprising an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to SEQ ID NO: 36. In one embodiment, the antibody fusion protein comprises the amino acid sequence set forth in SEQ ID NO: 36.

[0081] *truncated LOV2 derived from Avena sativa (tLOV2)*

GEFLATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPET
 DRATVRKIRDAIDNQTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIGV
 QLDGTEHVRDAAEREAVMLIKKTA (SEQ ID NO: 36)

[0082] In some embodiments the antibody fusion protein comprises an anti-human insulin scFv domain and an asLOV2 domain. In one embodiment, the asLOV2 domain is at the C-terminal end. In one embodiment, the antibody fusion protein comprises an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to SEQ ID NO: 3 or SEQ ID NO: 16. In one embodiment, the antibody fusion protein comprises an amino acid sequence set forth in SEQ ID NO: 3 or SEQ ID NO: 16.

[0083] *Anti-human insulin scFV fusion with asLOV2 at C-terminus (cLOV)*

MAQIQLVQSGPELKKPGETVKISCKASGYTFTDYSMHWVKQAPGKGLKWMDWI
 NTETGVPTYADDFKGRFAFSLETSANTAYLQINDLKNEEDTATYFCTRGYGKGYF
 DVWGAGTTVTVSSAKSTPPSVYPLAPGSGGGGSGGGGSGGGGSGQIVLTQSPTIMS
 ASLGERVTMTCTASSSVSSSYLHWYQQKPGSSPKLWIYSTSNLASGVPARFSGSG
 SGTSYSLTISSMEAEDAATYYCHQYHRSPPTFGAGTKLELKRADAAGGGGSGEF
 LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRA
 TVRKIRDAIDNQTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLD
 GTEHVRDAAEREAVMLIKKTAAEIDEAAKEL (SEQ ID NO: 3); or
 MAQIQLVQSGPELKKPGETVKISCKASGYTFTDYSMHWVKQAPGKGLKWMDWI
 NTETGVPTYADDFKGRFAFSLETSANTAYLQINDLKNEEDTATYFCTRGYGKGYFD
 VWGAGTTVTVSSAKSTPPSVYPLAPGSGGGGSGGGGSGGGGSGQIVLTQSPTIMSA
 SLGERVTTCTASSSVSSSYLHWYQQKPGSSPKLWIYSTSNLASGVPARFSGSGSG
 TSYSYSLTISSMEAEDAATYYCHQYHRSPPTFGAGTKLELKRADAAGGGGSGEF
 LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRA
 TVRKIRDAIDNQTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLD
 GTEHVRDAAEREAVMLIKKTAAEIDEAAKEL (SEQ ID NO: 16)

[0084] In another embodiment, the antibody fusion protein comprises an anti-human insulin scFv domain and an asLOV2 domain, wherein the asLOV2 domain is at the N-terminal

end. In one embodiment, the antibody fusion protein comprises an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to SEQ ID NO: 4 or SEQ ID NO: 17. In one embodiment, the antibody fusion protein comprises an amino acid sequence set forth in SEQ ID NO: 4 or SEQ ID NO: 17.

[0085] *Anti-human insulin scFV fusion with asLOV2 at N-terminus (nLOV)*

MGEFLATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPE
 TDRATVRKIRDAIDNQTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIG
 VQLDGTEHVRDAAEREAVMLIKKTAEEIDEAAGGGGSAQIQLVQSGPELKKPGPE
 TVKISCKASGYTFTDYSMHWVKQAPGKGLKWMDWINTETGVPTYADDFKGRF
 AFSLETSANTAYLQINDLKNEDTATYFCTRGYGKGYFDVWGAGTTVTVSSAKST
 PPSVYPLAPGSGGGGSGGGGSGGGGSGQIVLTQSPTIMSASLGERVTMTCTASSSV
 SSSYLHWYQQKPGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDA
 ATYYCHQYHRSPPTFGAGTKLELKRADAA (SEQ ID NO: 4); or
 MGEFLATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPE
 TDRATVRKIRDAIDNQTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIG
 VQLDGTEHVRDAAEREAVMLIKKTAEEIDEAAKELAQIQLVQSGPELKKPGETV
 KISCKASGYTFTDYSMHWVKQAPGKGLKWMDWINTETGVPTYADDFKGRFAFS
 LETSANTAYLQINDLKNEDTATYFCTRGYGKGYFDVWGAGTTVTVSSAKSTPPS
 VYPLAPGSGGGGSGGGGSGGGGSGQIVLTQSPTIMSASLGERVTMTCTASSSVSS
 YLHWYQQKPGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAATY
 YCHQYHRSPPTFGAGTKLELKRADAAKL (SEQ ID NO: 17)

[0086] In some embodiments, the antibody fusion protein comprises a truncated asLOV2 domain at the N-terminus. In one embodiment, the antibody fusion protein has an amino acid sequence comprising at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to SEQ ID NO: 18. In one embodiment, the antibody fusion protein comprises an amino acid sequence set forth in SEQ ID NO: 18.

[0087] *Anti-human insulin scFV fusion with truncated asLOV2 at N-terminus (ntLOV)*

MGEFLATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPE
 TDRATVRKIRDAIDNQTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIG
 VQLDGTEHV RDAAEREAVMLIKKTAAQIQLVQSGPELKKPGETVKISCKASGYT
 FTDYSMHWVKQAPGKGLKWMDWINTETGVPTYADDFKGRFAFSLETSANTAY
 LQINDLKNEDTATYFCTRGYGKGYFDVWGAGTTVTVSSAKSTPPSVYPLAPGSG
 GGGSGGGGSGGGGSGQIVLTQSPTIMSASLGERVTMTCTASSSVSSSYLHWYQQK
 PGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAATYYCHQYHRSP
 PTFGAGTKLELKRADA AKL (SEQ ID NO: 18)

[0088] In some embodiments, the antibody fusion protein comprises a V_H region and a V_L region, which are not directly adjacent to each other. In some embodiments, the redox-sensitive domain is between the V_H region and a V_L region. In one embodiment, the V_H region and the V_L region are anti-human insulin. In some embodiments, the V_H region comprises an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to SEQ ID NO: 6. In one embodiment, the V_H region comprises an amino acid sequence set forth in SEQ ID NO: 6.

[0089] *V_H region of anti-human insulin*

AQIQLVQSGPELKKPGETVKISCKASGYTFTDYSMHWVKQAPGKGLKWMDWIN
 TETGVPTYADDFKGRFAFSLETSANTAYLQINDLKNEDTATYFCTRGYGKGYFD
 VWGAGTTVTVSSAKSTPPSVYPLAPGS (SEQ ID NO: 6)

[0090] In some embodiments, the V_L region comprises an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to SEQ ID NO: 7. In one embodiment, the V_H region comprises an amino acid sequence set forth in SEQ ID NO: 7.

[0091] *V_L region of anti-human insulin*

QIVLTQSPTIMSASLGERVTMTCTASSSVSSSYLHWYQQKPGSSPKLWIYSTSNLA
 SGVPARFSGSGSGTSYSLTISSMEAEDAATYYCHQYHRSPPTFGAGTKLELKRAD
 AA (SEQ ID NO: 7)

[0092] In some embodiments, the antibody fusion protein has an amino acid sequence comprising: i) a V_H region with at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to SEQ ID NO: 6; ii) a V_L region with at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to SEQ ID NO: 7; and iii) an asLOV2 redox-sensitive domain with at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 36. In one embodiment, the antibody fusion protein has an amino acid sequence comprising: i) a V_H region comprising the amino acid sequence set forth in SEQ ID NO: 6; ii) a V_H region comprising the amino acid sequence set forth in SEQ ID NO: 7; and iii) an asLOV2 domain comprising the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 36.

[0093] In one embodiment, the antibody fusion protein has an amino acid sequence comprising at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to SEQ ID NO: 5 or SEQ ID NO: 19. In one embodiment, the antibody fusion protein comprises an amino acid sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 19.

[0094] *Anti-human insulin V_H-asLOV2-V_L with glycine-rich linkers (gsLOV)*

MAQIQLVQSGPELKKPGETVKISCKASGYTFTDYSMHWVKQAPGKGLKWMDWI
 NTETGVPTYADDFKGRFAFSLETSANTAYLQINDLKNEDTATYFCTRGRYGGKGYF
 DVWGAGTTVTVSSAKSTPPSVYPLAPGSGGGSGEFLATTLERIEKNFVITDPRLP
 DNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATVRKIRDAIDNQTEVTVQL
 INYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTSHVRDAAEREAVMLIK
 KTAEEIDEAAGGGGSQIVLTQSPTIMSASLGERVTMTCTASSSVSSSYLHWYQQK
 PGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAATYYCHQYHRSP
 PTFGAGTKLELKRADAA (SEQ ID NO: 5);
 MAQIQLVQSGPELKKPGETVKISCKASGYTFTDYSMHWVKQAPGKGLKWMDWI
 NTETGVPTYADDFKGRFAFSLETSANTAYLQINDLKNEDTATYFCTRGRYGGKGYF

DVWGAGTTVTVSSAKSTPPSVYPLAPGSGGGSGEFLATTLERIEKNFVITDPRLP
 DNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATVRKIRDAIDNQTEVTVQL
 INYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTEHVRDAAEREAVMLIK
 KTAEEIDEAAKELGGGGSQIVLTQSPTIMSASLGERVTMTCTASSSVSSSYLHWY
 QQKPGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAATYYCHQY
 HRSPTFGAGTKLELKRADA AKL (SEQ ID NO: 19).

[0095] In one embodiment, the antibody fusion protein has an amino acid sequence comprising at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to SEQ ID NO: 20. In one embodiment, the antibody fusion protein comprises an amino acid sequence set forth in SEQ ID NO: 20.

[0096] *Anti-human insulin V_{II} -asLOV2- V_L without glycine-rich linkers (nolinkLOV)*

MAQIQLVQSGPELKKPGETVKISCKASGYTFTDYSMHWVKQAPGKGLKWMDWI
 NTETGVPTYADDFKGRFAFSLETSANTAYLQINDLKNEDTATYFCTRGYGKGYF
 DVWGAGTTVTVSSAKSTPPSVYPLAPGSGEFLATTLERIEKNFVITDPRLPDNPIIF
 ASDSFLQLTEYSREEILGRNCRFLQGPETDRATVRKIRDAIDNQTEVTVQLINYTK
 SGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTEHVRDAAEREAVMLIKKTAEE
 IDEAAKELQIVLTQSPTIMSASLGERVTMTCTASSSVSSSYLHWYQQKPGSSPKL
 WIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAATYYCHQYHRSPTFGAG
 TKLELKRADA AKL (SEQ ID NO: 20)

[0097] Variants of antibody fusion proteins can be prepared to fix the proteins in a “dark state” or a “light state” by mutating residues of the redox-sensitive domain (e.g., LOV2). These variants can be used in order to test and compare the binding affinities of the antibody fusion protein in the different states. Provided are amino acid sequences for various antibody fusion proteins fixed in light and dark states.

[0098] *Anti-human insulin scFV fusion with asLOV2 at C-terminus (cLOV) fixed in “dark state” (mutant of SEQ ID NO: 3)*

MAQIQLVQSGPELKKPGETVKISCKASGYTFTDYSMHWVKQAPGKGLKWMDWI
 NTETGVPTYADDFKGRFAFSLETSANTAYLQINDLKNEDTATYFCTRGYGKGYF

DVWGAGTTVTVSSAKSTPPSVYPLAPGSGGGGSGGGGSGGGGSGQIVLTQSPTIMS
 ASLGERVTMTCTASSSVSSSYLHWYQKPGSSPKLWIYSTSNLASGVPARFSGSGS
 GTSYSLTISSMEAEDAATYYCHQYHRSPPTFGAGTKLELKRADAAGGGGSGEFL
 ATLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNARFLQGPETDRATV
 RKIRDAIDNQTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGT
 EHVRDAAEREAVMLIKKTAEIDEAAKELKL (SEQ ID NO: 21)

[0099] *Anti-human insulin scFV fusion with asLOV2 at C-terminus (cLOV) fixed in “light state” (mutant of SEQ ID NO: 3)*

MAQIQLVQSGPELKKPGETVKISCKASGYTFTDYSMHWVKQAPGKGLKWMDWI
 NTETGVPTYADDFKGRFAFSLETSANTAYLQINDLKNEEDTATYFCTRGYGKGYF
 DVWGAGTTVTVSSAKSTPPSVYPLAPGSGGGGSGGGGSGGGGSGQIVLTQSPTIMS
 ASLGERVTMTCTASSSVSSSYLHWYQQKPGSSPKLWIYSTSNLASGVPARFSGSG
 SGTSYSLTISSMEAEDAATYYCHQYHRSPPTFGAGTKLELKRADAAGGGGSGEF
 LATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRA
 TVRKIRDAIDNQTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLD
 GTEHVRDAAEREAVMLEKKTEEEIDEAAKELKL (SEQ ID NO: 22)

[0100] *Anti-human insulin scFV fusion with asLOV2 at N-terminus (nLOV) fixed in a “dark state” (mutant of SEQ ID NO: 17)*

MGEFLATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNARFLQGP
 ETDTRATVRKIRDAIDNQTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIG
 VQLDGTGTEHVRDAAEREAVMLIKKTAEIDEAAKELAQIQLVQSGPELKKPGETV
 KISCKASGYTFTDYSMHWVKQAPGKGLKWMDWINTETGVPTYADDFKGRFAFS
 LETSANTAYLQINDLKNEEDTATYFCTRGYGKGYFDVWGAGTTVTVSSAKSTPPS
 VYPLAPGSGGGGSGGGGSGGGGSGQIVLTQSPTIMSASLGERVTMTCTASSSVSS
 SYLHWYQQKPGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAATY
 YCHQYHRSPPTFGAGTKLELKRADA AKL (SEQ ID NO: 23)

[0101] *Anti-human insulin scFV fusion with asLOV2 at N-terminus (nLOV) fixed in a “light state” (mutant of SEQ ID NO: 17)*

MGEFLATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPE
 TDRATVRKIRDAIDNQTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIG
 VQLDGTEHVRDAAEREAVMLEKKTEEEIDEAAKELAQIQLVQSGPELKKPGETV
 KISCKASGYTFTDYSMHWVKQAPGKGLKWMDWINTETGVPTYADDFKGRFAFS
 LETSANTAYLQINDLKNEEDTATYFCTRGYGKGYFDVWGAGTTVTVSSAKSTPPS
 VYPLAPGSGGGGSGGGGSGGGGSGQIVLTQSPTIMSASLGERVTMTCTASSSVSSS
 YLHWYQQKPGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAATY
 YCHQYHRSPPTFGAGTKLELKRADA AKL (SEQ ID NO: 24)

[0102] *Anti-human insulin scFV fusion with truncated asLOV2 at N-terminus (ntLOV) fixed in a “dark state” (mutant of SEQ ID NO: 18)*

MGEFLATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNARFLQGPE
 TDRATVRKIRDAIDNQTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIGV
 QLDGTEHVRDAAEREAVMLIKKTAAQIQLVQSGPELKKPGETVKISCKASGYTFT
 DYSMHWVKQAPGKGLKWMDWINTETGVPTYADDFKGRFAFSLETSANTAYLQI
 NDLKNEEDTATYFCTRGYGKGYFDVWGAGTTVTVSSAKSTPPSVYPLAPGSGGG
 GSGGGGSGGGGSGQIVLTQSPTIMSASLGERVTMTCTASSSVSSSYLHWYQQKPGSS
 PKLWIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAATYYCHQYHRSPPTFG
 AGTKLELKRADA AKL (SEQ ID NO: 25)

[0103] *Anti-human insulin scFV fusion with truncated asLOV2 at N-terminus (ntLOV) fixed in a “light state” (mutant of SEQ ID NO: 18)*

MGEFLATTLERIEKNFVITDPRLPDNPIIFASDSFLQLTEYSREEILGRNCRFLQGPE
 TDRATVRKIRDAIDNQTEVTVQLINYTKSGKKFWNLFHLQPMRDQKGDVQYFIG
 VQLDGTEHVRDAAEREAVMLEKKTEAQIQLVQSGPELKKPGETVKISCKASGYT
 FTDYSMHWVKQAPGKGLKWMDWINTETGVPTYADDFKGRFAFSLETSANTAY
 LQINDLKNEEDTATYFCTRGYGKGYFDVWGAGTTVTVSSAKSTPPSVYPLAPGSG
 GGGSGGGGSGGGGSGQIVLTQSPTIMSASLGERVTMTCTASSSVSSSYLHWYQQK
 PGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAATYYCHQYHRSP
 PTFGAGTKLELKRADA AKL (SEQ ID NO: 26)

[0104] *Anti-human insulin V_H -asLOV2- V_L with glycine-rich linkers (gsLOV) fixed in a “dark state” (mutant of SEQ ID NO: 19)*

MAQIQLVQSGPELKKPGETVKISCKASGYTFTDYSMHWVKQAPGKGLKWMDWI
NTETGVPTYADDFKGRFAFSLETSANTAYLQINDLKNEDTATYFCTRGYGKGYF
DVWGAGTTVTVSSAKSTPPSVYPLAPGSGGGGSGEFLATTLERIEKNFVITDPRLP
DNPIIFASDSFLQLTEYSREEILGRNARFLQGPETDRATVRKIRDAIDNQTEVTVQL
INYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTEHVRDAAEREAVMLIK
KTAEIDEAAKELGGGGSQIVLTQSPTIMSASLGERVTMTCTASSSVSSSYLHWY
QQKPGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAATYYCHQY
HRSPPTFGAGTKLELKRADA AKL (SEQ ID NO: 27)

[0105] *Anti-human insulin V_H -asLOV2- V_L with glycine-rich linkers (gsLOV) fixed in a “light state” (mutant of SEQ ID NO: 19)*

MAQIQLVQSGPELKKPGETVKISCKASGYTFTDYSMHWVKQAPGKGLKWMDWI
NTETGVPTYADDFKGRFAFSLETSANTAYLQINDLKNEDTATYFCTRGYGKGYF
DVWGAGTTVTVSSAKSTPPSVYPLAPGSGGGGSGEFLATTLERIEKNFVITDPRLP
DNPIIFASDSFLQLTEYSREEILGRNCRFLQGPETDRATVRKIRDAIDNQTEVTVQL
INYTKSGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTEHVRDAAEREAVMLEK
KTEEEIDEAAKELGGGGSQIVLTQSPTIMSASLGERVTMTCTASSSVSSSYLHWY
QQKPGSSPKLWIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAATYYCHQY
HRSPPTFGAGTKLELKRADA AKL (SEQ ID NO: 28)

[0106] *Anti-human insulin V_H -asLOV2- V_L without glycine-rich linkers (nolinkLOV) fixed in a “dark state” (mutant of SEQ ID NO: 20)*

MAQIQLVQSGPELKKPGETVKISCKASGYTFTDYSMHWVKQAPGKGLKWMDWI
NTETGVPTYADDFKGRFAFSLETSANTAYLQINDLKNEDTATYFCTRGYGKGYF
DVWGAGTTVTVSSAKSTPPSVYPLAPGSGEFLATTLERIEKNFVITDPRLPDNPIIF
ASDSFLQLTEYSREEILGRNARFLQGPETDRATVRKIRDAIDNQTEVTVQLINYTK
SGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGTEHVRDAAEREAVMLIKKTAE
IDEAAKELQIVLTQSPTIMSASLGERVTMTCTASSSVSSSYLHWYQQKPGSSPKL

WIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAATYYCHQYHRSPPTFGAG
TKLELKRADA AKL (SEQ ID NO: 29)

[0107] *Anti-human insulin V_H-asLOV2-V_L without glycine-rich linkers (nolinkLOV) fixed in a “light state” (mutant of SEQ ID NO: 20)*

MAQIQLVQSGPELKKPGETVKISCKASGYTFTDYSMHWVKQAPGKGLKWMDWI
NTETGVPTYADDFKGRFAFSLETSANTAYLQINDLKNEDTATYFCTRGYGKGYF
DVWGAGTTVTVSSAKSTPPSVYPLAPGSGEFLATTLERIEKNFVITDPRLPDNPIIF
ASDSFLQLTEYSREEILGRNCRFLQGPETDRATVRKIRDAIDNQTEVTVQLINYTK
SGKKFWNLFHLQPMRDQKGDVQYFIGVQLDGT EHV RDAAEREA VMLEKKTEEE
IDEAAKELQIVLTQSPTIMSASLGERVTMTCTASSSVSSSYLHWYQQKPGSSPKL
WIYSTSNLASGVPARFSGSGSGTSYSLTISSMEAEDAATYYCHQYHRSPPTFGAG
TKLELKRADA AKL (SEQ ID NO: 30)

[0108] “Sequence identity” or “identity” in the context of two polynucleotides or polypeptide sequences refers to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have “sequence similarity” or “similarity.” Means for making this adjustment are well known to those of skill in the art. Typically, this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

[0109] “Percentage of sequence identity” refers to the value determined by comparing two optimally aligned sequences (greatest number of perfectly matched residues) over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity. Unless otherwise specified (e.g., the shorter sequence includes a linked heterologous sequence), the comparison window is the full length of the shorter of the two sequences being compared.

[0110] Unless otherwise stated, sequence identity/similarity values refer to the value obtained using GAP Version 10 using the following parameters: % identity and % similarity for a nucleotide sequence using GAP Weight of 50 and Length Weight of 3, and the nwsgapdna.cmp scoring matrix; % identity and % similarity for an amino acid sequence using GAP Weight of 8 and Length Weight of 2, and the BLOSUM62 scoring matrix; or any equivalent program thereof. “Equivalent program” includes any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide or amino acid residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by GAP Version 10.

[0111] The term “conservative amino acid substitution” refers to the substitution of an amino acid that is normally present in the sequence with a different amino acid of similar size, charge, or polarity. Examples of conservative substitutions include the substitution of a non-polar (hydrophobic) residue such as isoleucine, valine, or leucine for another non-polar residue. Likewise, examples of conservative substitutions include the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, or between glycine and serine. Additionally, the substitution of a basic residue such as lysine, arginine, or histidine for another, or the substitution of one acidic residue such as aspartic acid or glutamic acid for another acidic residue are additional examples of conservative

substitutions. Examples of nonconservative substitutions include the substitution of a non-polar (hydrophobic) amino acid residue such as isoleucine, valine, leucine, alanine, or methionine for a polar (hydrophilic) residue such as cysteine, glutamine, glutamic acid or lysine and/or a polar residue for a non-polar residue. Typical amino acid categorizations are summarized below.

Alanine	Ala	A	Nonpolar	Neutral	1.8
Arginine	Arg	R	Polar	Positive	-4.5
Asparagine	Asn	N	Polar	Neutral	-3.5
Aspartic acid	Asp	D	Polar	Negative	-3.5
Cysteine	Cys	C	Nonpolar	Neutral	2.5
Glutamic acid	Glu	E	Polar	Negative	-3.5
Glutamine	Gln	Q	Polar	Neutral	-3.5
Glycine	Gly	G	Nonpolar	Neutral	-0.4
Histidine	His	H	Polar	Positive	-3.2
Isoleucine	Ile	I	Nonpolar	Neutral	4.5
Leucine	Leu	L	Nonpolar	Neutral	3.8
Lysine	Lys	K	Polar	Positive	-3.9
Methionine	Met	M	Nonpolar	Neutral	1.9
Phenylalanine	Phe	F	Nonpolar	Neutral	2.8
Proline	Pro	P	Nonpolar	Neutral	-1.6
Serine	Ser	S	Polar	Neutral	-0.8
Threonine	Thr	T	Polar	Neutral	-0.7
Tryptophan	Trp	W	Nonpolar	Neutral	-0.9
Tyrosine	Tyr	Y	Polar	Neutral	-1.3
Valine	Val	V	Nonpolar	Neutral	4.2

[0112] It may be desired with respect to biosensors to engineer an antibody capable of modulating its binding affinity to its target molecule (e.g., antigen) based on physicochemical conditions (e.g., irradiation with light) to be able to regenerate the antigen-binding sites of the antibody *in situ* for continuous monitoring applications. The antibody fusion proteins disclosed herein have a binding affinity towards a target molecule which is modulated upon exposure to an external signal (e.g., redox potential or light).

[0113] In some embodiments, a sensitivity of the antibody fusion protein towards its target molecule is modulated upon exposure to the external signal. In some embodiments, the sensitivity of the antibody fusion protein towards the target molecule is at least 2-fold, at least 5-fold, at least

10-fold, at least 100-fold, or at least 1000-fold different upon exposure to the external signal. In some embodiments, a limit of detection (LOD) of the antibody fusion protein is modulated upon exposure to the external signal. In some embodiments, a specific binding ability of the antibody fusion protein towards its target molecule is modulated by exposure to the external signal. In some embodiments, the target molecule is at least 2-fold, at least 5-fold, at least 10-fold, at least 100-fold, or at least 1000-fold different upon exposure to the external signal. In some embodiments, the dissociation constant (K_d) of the antibody fusion protein towards the target molecule is modulated by exposure to the external signal. In some embodiments, the K_d of the antibody fusion protein is at least 10, at least 10^2 , at least 10^3 , at least 10^4 , at least 10^5 , at least 10^6 , at least 10^7 , at least 10^8 , at least 10^9 , or at least 10^{10} times different upon exposure to the external signal.

Polynucleotides Encoding Antibody Fusion Proteins

[0114] In one embodiment, an isolated polynucleotide that encodes for an engineered antibody fusion protein is described herein. In some embodiments, the polynucleotide comprises a nucleotide sequence encoding an amino acid sequence comprising any one of SEQ ID NO: 3-5, and 16-20.

[0115] The terms “nucleic acid” and “polynucleotide,” used interchangeably herein, refer to polymeric forms of nucleotides of any length, including ribonucleotides, deoxyribonucleotides, or analogs or modified versions thereof. They include single-, double-, and multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, and polymers comprising purine bases, pyrimidine bases, or other natural, chemically modified, biochemically modified, non-natural, or derivatized nucleotide bases.

[0116] Nucleic acids are said to have “5’ ends” and “3’ ends” because mononucleotides are reacted to make oligonucleotides in a manner such that the 5’ phosphate of one mononucleotide pentose ring is attached to the 3’ oxygen of its neighbor in one direction via a phosphodiester linkage. An end of an oligonucleotide is referred to as the “5’ end” if its 5’ phosphate is not linked to the 3’ oxygen of a mononucleotide pentose ring. An end of an oligonucleotide is referred to as the “3’ end” if its 3’ oxygen is not linked to a 5’ phosphate of another mononucleotide pentose ring. A nucleic acid sequence, even if internal to a larger

oligonucleotide, also may be said to have 5' and 3' ends. In either a linear or circular DNA molecule, discrete elements are referred to as being "upstream" or 5' of the "downstream" or 3' elements.

[0117] In some embodiments, the polynucleotide encoding the antibody fusion protein comprises a nucleotide sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to any one of SEQ ID NOs: 8-10, and 21-25. In some embodiments, the polynucleotide encoding the antibody fusion protein comprises a nucleotide sequence set forth in any one of SEQ ID NOs: 8-10, and 21-25.

[0118] *Anti-human insulin fusion with scFV-asLOV2 at C-terminus (cLOV)*

ATGGCACAGATCCAGTTGGTGCAGTCTGGACCTGAACTGAAGAAGCCTGGAG
 AGACAGTCAAGATCTCCTGCAAGGCTTCTGGTTATACCTTCACAGACTATTCA
 ATGCACTGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGGATGGACTGG
 ATAAACACTGAGACTGGTGTGCCAACCTATGCAGATGACTTCAAGGGACGGT
 TTGCCTTCTCTTTGGAAACCTCTGCTAACACTGCCTATTTGCAGATCAACGAC
 CTCAAAAATGAGGACACGGCTACATATTTCTGTACTAGAGGGTATGGAAAGG
 GGTACTTCGATGTCTGGGGCGCAGGGACCACGGTCACCGTCTCCTCAGCCAA
 AAGCACACCCCCATCCGTTTATCCATTGGCCCCCTGGAAGCGGCGGAGGCGGA
 TCAGGAGGAGGAGGATCAGGCGGAGGAGGATCACAAATTGTTCTCACCCAGT
 CTCCAACAATCATGTCTGCATCTCTAGGGGAACGGGTACCATGACCTGCAC
 TGCCAGCTCAAGTGTAAGTTCCAGTTACTTGCAGTGGTACCAGCAGAAGCCA
 GGATCCTCCCCCAAACCTCTGGATTTATAGTACATCCAACCTGGCTTCTGGAGT
 CCCAGCTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCACAATCA
 GCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCACCAGTATCATCG
 TTCCCCACCCACGTTTCGGTGCTGGGACCAAGCTGGAGCTGAAACGGGGCTGAT
 GCTGCAGGCGGAGGCGGATCAGGCGAATTTCTGGCGACCACCCTGGAACGCA
 TTGAAAAAACTTTGTGATTACCGATCCGCGCCTGCCGGATAACCCGATTATT
 TTTGCGAGCGATAGCTTTCTGCAGCTGACCGAATATAGCCGCGAAGAAATTC

TGGGCCGCAACTGCCGCTTTCTGCAGGGCCCCGGAAACCGATCGCGCGACCGT
GCGCAAAATTTCGCGATGCGATTGATAACCAG (SEQ ID NO: 8); or
CATATGGCACAGATCCAGTTGGTGCAGTCTGGACCTGAACTGAAGAAGCCTG
GAGAGACAGTCAAGATCTCCTGCAAGGCTTCTGGTTATACCTTCACAGACTAT
TCAATGCACTGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGGATGGACT
GGATAAACACTGAGACTGGTGTGCCAACCTATGCAGATGACTTCAAGGGACG
GTTTGCCTTCTCTTTGGAAACCTCTGCTAACACTGCCTATTTGCAGATCAACG
ACCTCAAAAATGAGGACACGGCTACATATTTCTGTACTAGAGGGTATGGAAA
GGGGTACTTCGATGTCTGGGGCGCAGGGACCACGGTCACCGTCTCCTCAGCC
AAAAGCACACCCCCATCCGTTTATCCATTGGCCCCTGGAAGCGGCGGAGGCG
GATCAGGAGGAGGAGGATCAGGCGGAGGAGGATCACAAATTGTTCTCACCC
AGTCTCCAACAATCATGTCTGCATCTCTAGGGGAACGGGTACCATGACCTG
CACTGCCAGCTCAAGTGTAAGTTCCAGTTACTTGCACTGGTACCAGCAGAAG
CCAGGATCCTCCCCAACTCTGGATTTATAGTACATCCAACCTGGCTTCTGG
AGTCCCAGCTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCACAA
TCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCACCAGTATCA
TCGTTCCCCACCCACGTTTCGGTGCTGGGACCAAGCTGGAGCTGAAACGGGCT
GATGCTGCAGGCGGAGGCGGATCAGGCGAATTTCTGGCGACCACCCTGGAAC
GCATTGAAAAAACTTTGTGATTACCGATCCGCGCCTGCCGGATAACCCGAT
TATTTTTGCGAGCGATAGCTTTCTGCAGCTGACCGAATATAGCCGCGAAGAA
ATTCTGGGCCGCAACTGCCGCTTTCTGCAGGGCCCCGGAAACCGATCGCGCGA
CCGTGCGCAAAATTTCGCGATGCGATTGATAACCAGACCGAAGTGACCGTGCA
GCTGATTA ACTATAACCAAAGCGGCAAAAAATTTTGGAACCTGTTTCATCTGC
AGCCGATGCGCGATCAGAAAGGCGATGTGCAGTATTTTATTGGCGTGCACT
GGATGGCACCGAACATGTGCGCGATGCGGCGGAACGCGAAGCGGTGATGCT
GATTAAAAAAACCGCGGAAGAAATTGATGAAGCGGCGAAAGAACTGAAGCT
T (SEQ ID NO: 31; encoding SEQ ID NO: 16).

[0119] *Anti-human insulin scFV fusion with asLOV2 at N-terminus (nLOV)*

ATGGGCGAATTTCTGGCGACCACCCTGGAACGCATTGAAAAAACTTTGTGA
TTACCGATCCGCGCCTGCCGGATAACCCGATTATTTTTGCGAGCGATAGCTTT
CTGCAGCTGACCGAATATAGCCGCGAAGAAATTCTGGGCCGCAACTGCCGCT
TTCTGCAGGGCCCGGAAACCGATCGCGCGACCGTGCGCAAAATTCGCGATGC
GATTGATAACCAGACCGAAGTGACCGTGCGAGCTGATTAATAACCAAAAGC
GGCAAAAATTTTGGAACCTGTTTCATCTGCAGCCGATGCGCGATCAGAAAG
GCGATGTGCAGTATTTTATTGGCGTGCGAGCTGGATGGCACCGAACATGTGCG
CGATGCGGCGGAACGCGAAGCGGTGATGCTGATTAAAAAAACCGCGGAAGA
AATTGATGAAGCGGCGAAAGAACTGGGTAGTGGTGCACAGATCCAGTTGGTG
CAGTCTGGACCTGAACTGAAGAAGCCTGGAGAGACAGTCAAGATCTCCTGCA
AGGCTTCTGGTTATACCTTCACAGACTATTCAATGCACTGGGTGAAGCAGGCT
CCAGGAAAGGGTTTAAAGTGGATGGACTGGATAAACACTGAGACTGGTGTGC
CAACCTATGCAGATGACTTCAAGGGACGGTTTGCCTTCTCTTTGGAAACCTCT
GCTAACACTGCCTATTTGCAGATCAACGACCTCAAAAATGAGGACACGGCTA
CATATTTCTGTACTAGAGGGTATGGAAAGGGGTACTTCGATGTCTGGGGCGC
AGGGACCACGGTCACCGTCTCCTCAGCCAAAAGCACACCCCCATCCGTTTAT
CCATTGGCCCCTGGAAGCGGCGGAGGCGGATCAGGAGGAGGAGGATCAGGC
GGAGGAGGATCACAAATTGTTCTCACCCAGTCTCCAACAATCATGTCTGCATC
TCTAGGGGAACGGGTACCATGACCTGCACTGCCAGCTCAAGTGTAAGTTCC
AGTTACTTGCACTGGTACCAGCAGAAGCCAGGATCCTCCCCAACTCTGGA
TTTATAGTACATCCAACCTGGCTTCTGGAGTCCCAGCTCGCTTCAGTGGCAGT
GGGTCTGGGACCTCTTACTCTCTCACAATCAGCAGCATGGAGGCTGAAGATG
CTGCCACTTATTACTGCCACCAGTATCATCGTTCCCCACCCACGTTCCGGTGCT
GGGACCAAGCTGGAGCTGAAACGGGCTGATGCTGC (SEQ ID NO: 9); or
CATATGGGCGAATTTCTGGCGACCACCCTGGAACGCATTGAAAAAACTTTG
TGATTACCGATCCGCGCCTGCCGGATAACCCGATTATTTTTGCGAGCGATAGC
TTTCTGCAGCTGACCGAATATAGCCGCGAAGAAATTCTGGGCCGCAACTGCC
GCTTTCTGCAGGGCCCGGAAACCGATCGCGCGACCGTGCGCAAAATTCGCGA
TGCGATTGATAACCAGACCGAAGTGACCGTGCGAGCTGATTAATAACCAAA

AGCGGCAAAAAATTTTGGAACCTGTTTCATCTGCAGCCGATGCGCGATCAGA
 AAGGCGATGTGCAGTATTTTATTGGCGTGCAGCTGGATGGCACCGAACATGT
 GCGCGATGCGGCGGAACGCGAAGCGGTGATGCTGATTAAAAAACCGCGGA
 AGAAATTGATGAAGCGGCGAAAGAACTGGCACAGATCCAGTTGGTGCAGTCT
 GGACCTGAACTGAAGAAGCCTGGAGAGACAGTCAAGATCTCCTGCAAGGCTT
 CTGGTTATACCTTCACAGACTATTCAATGCACTGGGTGAAGCAGGCTCCAGG
 AAAGGGTTTAAAGTGGATGGACTGGATAAACACTGAGACTGGTGTGCCAACC
 TATGCAGATGACTTCAAGGGACGGTTTGCCTTCTCTTTGGAAACCTCTGCTAA
 CACTGCCTATTTGCAGATCAACGACCTCAAAAATGAGGACACGGCTACATAT
 TTCTGTACTAGAGGGTATGGAAAGGGGTACTTCGATGTCTGGGGCGCAGGGA
 CCACGGTCACCGTCTCCTCAGCCAAAAGCACACCCCCATCCGTTTATCCATTG
 GCCCTGGAAGCGGCGGAGGCGGATCAGGAGGAGGAGGATCAGGCGGAGGA
 GGATCACAAATTGTTCTCACCCAGTCTCCAACAATCATGTCTGCATCTCTAGG
 GGAACGGGTCACCATGACCTGCACTGCCAGCTCAAGTGTAAGTTCCAGTTAC
 TTGCACTGGTACCAGCAGAAGCCAGGATCCTCCCCAAACTCTGGATTTATA
 GTACATCCAACCTGGCTTCTGGAGTCCCAGCTCGCTTCAGTGGCAGTGGGTCT
 GGGACCTCTTACTCTCTCACAATCAGCAGCATGGAGGCTGAAGATGCTGCCA
 CTTATTACTGCCACCAGTATCATCGTTCCCCACCCACGTTCCGTGCTGGGACC
 AAGCTGGAGCTGAAACGGGCTGATGCTGCAAAGCTT (SEQ ID NO: 32; encoding
 SEQ ID NO: 17)

[0120] *Anti-human insulin scFV fusion with truncated asLOV2 at N-terminus (ntLOV)*

CATATGGGCGAATTTCTGGCGACCACCCTGGAACGCATTGAAAAAACTTTG
 TGATTACCGATCCGCGCCTGCCGGATAACCCGATTATTTTTCGAGCGATAGC
 TTTCTGCAGCTGACCGAATATAGCCGCGAAGAAATTCTGGGCCGCAACTGCC
 GCTTTCTGCAGGGCCCGGAAACCGATCGCGCGACCGTGCGCAAAATTTCGCGA
 TGCGATTGATAACCAGACCGAAGTGACCGTGCAGCTGATTAATAACCAAA
 AGCGGCAAAAAATTTTGGAACCTGTTTCATCTGCAGCCGATGCGCGATCAGA
 AAGGCGATGTGCAGTATTTTATTGGCGTGCAGCTGGATGGCACCGAACATGT
 GCGCGATGCGGCGGAACGCGAAGCGGTGATGCTGATTAAAAAACCGCGGC

ACAGATCCAGTTGGTGCAGTCTGGACCTGAACTGAAGAAGCCTGGAGAGACA
GTCAAGATCTCCTGCAAGGCTTCTGGTTATACCTTCACAGACTATTCAATGCA
CTGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGGATGGACTGGATAAA
CACTGAGACTGGTGTGCCAACCTATGCAGATGACTTCAAGGGACGGTTTGCC
TTCTCTTTGGAAACCTCTGCTAACACTGCCTATTTGCAGATCAACGACCTCAA
AAATGAGGACACGGCTACATATTTCTGTACTAGAGGGTATGGAAAGGGGTAC
TTCGATGTCTGGGGCGCAGGGACCACGGTCACCGTCTCCTCAGCCAAAAGCA
CACCCCCATCCGTTTATCCATTGGCCCCCTGGAAGCGGCGGAGGCGGATCAGG
AGGAGGAGGATCAGGCGGAGGAGGATCACAAATTGTTCTCACCCAGTCTCCA
ACAATCATGTCTGCATCTCTAGGGGAACGGGTACCATGACCTGCACTGCCA
GCTCAAGTGTAAGTTCCAGTTACTTGCCTGGTACCAGCAGAAGCCAGGATC
CTCCCCCAAACCTCTGGATTTATAGTACATCCAACCTGGCTTCTGGAGTCCCAG
CTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCACAATCAGCAGC
ATGGAGGCTGAAGATGCTGCCACTTATTACTGCCACCAGTATCATCGTTCCCC
ACCCACGTTTCGGTGCTGGGACCAAGCTGGAGCTGAAACGGGCTGATGCTGCA
AAGCTT (SEQ ID NO: 33; encoding SEQ ID NO: 18)

[0121] *Anti-human insulin V_H-asLOV2-V_L with glycine-rich linkers (gsLOV)*

ATGGCACAGATCCAGTTGGTGCAGTCTGGACCTGAACTGAAGAAGCCTGGAG
AGACAGTCAAGATCTCCTGCAAGGCTTCTGGTTATACCTTCACAGACTATTCA
ATGCACTGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGGATGGACTGG
ATAAACACTGAGACTGGTGTGCCAACCTATGCAGATGACTTCAAGGGACGGT
TTGCCTTCTCTTTGGAAACCTCTGCTAACACTGCCTATTTGCAGATCAACGAC
CTCAAAAATGAGGACACGGCTACATATTTCTGTACTAGAGGGTATGGAAAGG
GGTACTTCGATGTCTGGGGCGCAGGGACCACGGTCACCGTCTCCTCAGCCAA
AAGCACACCCCCATCCGTTTATCCATTGGCCCCCTGGAAGCGGCGGAGGCGGA
TCAGGCGAATTTCTGGCGACCACCCTGGAACGCATTGAAAAAACTTTGTGA
TTACCGATCCGCGCCTGCCGGATAACCCGATTATTTTTGCGAGCGATAGCTTT
CTGCAGCTGACCGAATATAGCCGCGAAGAAATTCTGGGCCGCAACTGCCGCT
TTCTGCAGGGCCCCGGAACCGATCGCGCGACCGTGCGCAAAATTCGCGATGC

GATTGATAACCAGACCGAAGTGACCGTGCAGCTGATTA ACTATAACCAAAAGC
GGCAAAAAATTTTGGAACCTGTTTCATCTGCAGCCGATGCGCGATCAGAAAG
GCGATGTGCAGTATTTTATTGGCGTGCAGCTGGATGGCACCGAACATGTGCG
CGATGCGGCGGAACGCGAAGCGGTGATGCTGATTA AAAAAAACCGCGGAAGA
AATTGATGAAGCGGCGGGCGGAGGAGGATCACAAATTGTTCTCACCCAGTCT
CCAACAATCATGTCTGCATCTCTAGGGGAACGGGTCACCATGACCTGCACTG
CCAGCTCAAGTGTAAGTTCCAGTTACTTGCACTGGTACCAGCAGAAGCCAGG
ATCCTCCCCCAA ACTCTGGATTTATAGTACATCCAACCTGGCTTCTGGAGTCC
CAGCTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTACAATCAGC
AGCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCACCAGTATCATCGTTC
CCCACCCACGTTTCGGTGCTGGGACCAAGCTGGAGCTGAAACGGGCTGATGCT
GCA (SEQ ID NO: 10); or

CATATGGCACAGATCCAGTTGGTGCAGTCTGGACCTGAACTGAAGAAGCCTG
GAGAGACAGTCAAGATCTCCTGCAAGGCTTCTGGTTATACCTTCACAGACTAT
TCAATGCACTGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGGATGGACT
GGATAAACACTGAGACTGGTGTGCCAACCTATGCAGATGACTTCAAGGGACG
GTTTGCCTTCTCTTTGGAAACCTCTGCTAACACTGCCTATTTGCAGATCAACG
ACCTCAAAAATGAGGACACGGCTACATATTTCTGTACTAGAGGGTATGGAAA
GGGGTACTTCGATGTCTGGGGCGCAGGGACCACGGTCACCGTCTCCTCAGCC
AAAAGCACACCCCCATCCGTTTATCCATTGGCCCCTGGAAGCGGCGGAGGCG
GATCAGGCGAATTTCTGGCGACCACCCTGGAACGCATTGAAAAAACTTTGT
GATTACCGATCCGCGCCTGCCGGATAACCCGATTATTTTTGCGAGCGATAGCT
TTCTGCAGCTGACCGAATATAGCCGCGAAGAAATTCTGGGCCGCAACTGCCG
CTTTCTGCAGGGCCCGGAAACCGATCGCGCGACCGTGCGCAAAATTCGCGAT
GCGATTGATAACCAGACCGAAGTGACCGTGCAGCTGATTA ACTATAACCAAAA
GCGGCAAAAAATTTTGGAACCTGTTTCATCTGCAGCCGATGCGCGATCAGAA
AGGCGATGTGCAGTATTTTATTGGCGTGCAGCTGGATGGCACCGAACATGTG
CGCGATGCGGCGGAACGCGAAGCGGTGATGCTGATTA AAAAAAACCGCGGAA
GAAATTGATGAAGCGGCGAAAGAACTGGGCGGAGGCGGATCACAAATTGTT

CTCACCCAGTCTCCAACAATCATGTCTGCATCTCTAGGGGAACGGGTCACCAT
GACCTGCACTGCCAGCTCAAGTGTAAGTTCCAGTTACTTGCACTGGTACCAGC
AGAAGCCAGGATCCTCCCCCAAACCTCTGGATTTATAGTACATCCAACCTGGC
TTCTGGAGTCCCAGCTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTC
TCACAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCACCA
GTATCATCGTTCCCCACCCACGTTCCGGTGCTGGGACCAAGCTGGAGCTGAAA
CGGGCTGATGCTGCAAAGCTT (SEQ ID NO: 34; encoding SEQ ID NO: 19)

[0122] *Anti-human insulin V_H-asLOV2-V_L without glycine-rich linkers (nolinkLOV)*

CATATGGCACAGATCCAGTTGGTGCAGTCTGGACCTGAACTGAAGAAGCCTG
GAGAGACAGTCAAGATCTCCTGCAAGGCTTCTGGTTATACCTTCACAGACTAT
TCAATGCACTGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGGATGGACT
GGATAAACACTGAGACTGGTGTGCCAACCTATGCAGATGACTTCAAGGGACG
GTTTGCCTTCTCTTTGGAAACCTCTGCTAACACTGCCTATTTGCAGATCAACG
ACCTCAAAAATGAGGACACGGCTACATATTTCTGTACTAGAGGGTATGGAAA
GGGGTACTTCGATGTCTGGGGCGCAGGGACCACGGTCACCGTCTCCTCAGCC
AAAAGCACACCCCCATCCGTTTATCCATTGGCCCCTGGAAGCGGCGAATTTCT
GGCGACCACCCTGGAACGCATTGAAAAAACTTTGTGATTACCGATCCGCGC
CTGCCGGATAACCCGATTATTTTTGCGAGCGATAGCTTTCTGCAGCTGACCGA
ATATAGCCGCGAAGAAATTCTGGGCCGCAACTGCCGCTTTCTGCAGGGCCCG
GAAACCGATCGCGCGACCGTGCGCAAAATTCGCGATGCGATTGATAACCAGA
CCGAAGTGACCGTGCAGCTGATTAACCTATACCAAAAAGCGGCAAAAAATTTTG
GAACCTGTTTCATCTGCAGCCGATGCGCGATCAGAAAGGCGATGTGCAGTAT
TTTATTGGCGTGCAGCTGGATGGCACCGAACATGTGCGCGATGCGGCGGAAC
GCGAAGCGGTGATGCTGATTAAAAAAACCGCGGAAGAAATTGATGAAGCGG
CGAAAGAAGTGCAAATTGTTCTCACCCAGTCTCCAACAATCATGTCTGCATCT
CTAGGGGAACGGGTCACCATGACCTGCACTGCCAGCTCAAGTGTAAGTTCCA
GTTACTTGCACTGGTACCAGCAGAAGCCAGGATCCTCCCCCAAACCTCTGGAT
TTATAGTACATCCAACCTGGCTTCTGGAGTCCCAGCTCGCTTCAGTGGCAGTG
GGTCTGGGACCTCTTACTCTCTCACAATCAGCAGCATGGAGGCTGAAGATGC

TGCCACTTATTACTGCCACCAGTATCATCGTTCCCCACCCACGTTCCGGTGCTG
GGACCAAGCTGGAGCTGAAACGGGCTGATGCTGCAAAGCTT (SEQ ID NO: 35;
encoding SEQ ID NO: 20)

[0123] The nucleotide sequence encoding the anti-insulin scFv disclosed herein is provided in SEQ ID NO: 12. The nucleotide sequence encoding the V_H region of the anti-insulin scFv disclosed herein is provided in SEQ ID NO: 13. The nucleotide sequence encoding the V_L region of the anti-insulin scFv disclosed herein is provided in SEQ ID NO: 14.

[0124] *Anti-Insulin scFv (Clone HB125)*

ATGGCACAGATCCAGTTGGTGCAGTCTGGACCTGAACTGAAGAAGCCTGGAG
AGACAGTCAAGATCTCCTGCAAGGCTTCTGGTTATACCTTCACAGACTATTCA
ATGCACTGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGGATGGACTGG
ATAAACACTGAGACTGGTGTGCCAACCTATGCAGATGACTTCAAGGGACGGT
TTGCCTTCTCTTTGGAAACCTCTGCTAACACTGCCTATTTGCAGATCAACGAC
CTCAAAAATGAGGACACGGCTACATATTTCTGTACTAGAGGGTATGGAAAGG
GGTACTTCGATGTCTGGGGCGCAGGGACCACGGTCACCGTCTCCTCAGCCAA
AAGCACACCCCCATCCGTTTATCCATTGGCCCCTGGAAGCGGCGGAGGCGGA
TCAGGAGGAGGAGGATCAGGCGGAGGAGGATCACAAATTGTTCTCACCCAGT
CTCCAACAATCATGTCTGCATCTCTAGGGGAACGGGTCACCATGACCTGCAC
TGCCAGCTCAAGTGTAAGTTCCAGTTACTTGCACTGGTACCAGCAGAAGCCA
GGATCCTCCCCCAAACCTCTGGATTTATAGTACATCCAACCTGGCTTCTGGAGT
CCCAGCTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCACAATCA
GCAGCATGGAGGCTGAAGATGCTGCCACTTATTACTGCCACCAGTATCATCG
TTCCCCACCCACGTTCCGGTGCTGGGACCAAGCTGGAGCTGAAACGGGCTGAT
GCTGCA (SEQ ID NO: 12)

[0125] *V_H region of anti-human insulin (Clone HB125)*

ATGGCACAGATCCAGTTGGTGCAGTCTGGACCTGAACTGAAGAAGCCTGGAG
AGACAGTCAAGATCTCCTGCAAGGCTTCTGGTTATACCTTCACAGACTATTCA
ATGCACTGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGGATGGACTGG
ATAAACACTGAGACTGGTGTGCCAACCTATGCAGATGACTTCAAGGGACGGT

TTGCCTTCTCTTTGGAAACCTCTGCTAACACTGCCTATTTGCAGATCAACGAC
CTCAAAAATGAGGACACGGCTACATATTTCTGTACTAGAGGGTATGGAAAGG
GGTACTTCGATGTCTGGGGCGCAGGGACCACGGTCACCGTCTCCTCAGCCAA
AAGCACACCCCCATCCGTTTATCCATTGGCCCCTGGAAGC (SEQ ID NO: 13)

[0126] *V_L region of anti-human insulin (Clone HB125)*

CAAATTGTTCTCACCCAGTCTCCAACAATCATGTCTGCATCTCTAGGGGAACG
GGTCACCATGACCTGCACTGCCAGCTCAAGTGTAAGTTCCAGTTACTTGCACT
GGTACCAGCAGAAGCCAGGATCCTCCCCAAACTCTGGATTTATAGTACATC
CAACCTGGCTTCTGGAGTCCCAGCTCGCTTCAGTGGCAGTGGGTCTGGGACCT
CTTACTCTCTCACAATCAGCAGCATGGAGGCTGAAGATGCTGCCACTTATTAC
TGCCACCAGTATCATCGTTCCCCACCCACGTTCGGTGCTGGGACCAAGCTGG
AGCTGAAACGGGCTGATGCTGCA (SEQ ID NO: 14)

[0127] The nucleotide sequence encoding asLOV2 is provided in SEQ ID NO: 15.

[0128] *LOV2 derived from Avena sativa (asLOV2)*

GGCGAATTTCTGGCGACCACCCTGGAACGCATTGAAAAAACTTTGTGATTA
CCGATCCGCGCCTGCCGGATAACCCGATTATTTTTCGAGCGATAGCTTTCTG
CAGCTGACCGAATATAGCCGCGAAGAAATTCTGGGCCGCAACTGCCGCTTTC
TGCAGGGCCCCGGAAACCGATCGCGCGACCGTGCGCAAAATTCGCGATGCGAT
TGATAACCAGACCGAAGTGACCGTGCAGCTGATTAATAACCAAAAGCGGC
AAAAAATTTTGGAACTGTTCATCTGCAGCCGATGCGCGATCAGAAAGGCG
ATGTGCAGTATTTTATTGGCGTGCAGCTGGATGGCACCGAACATGTGCGCGA
TGCGGCGGAACGCGAAGCGGTGATGCTGATTAATAAAAAACCGCGGAAGAAAT
TGATGAAGCGGCGAAGAACTG (SEQ ID NO: 15)

Vectors and Host Cells

[0129] In another embodiment, provided herein is a vector comprising the engineered antibody fusion protein-encoding polynucleotide or a host cell expressing the vector comprising the engineered antibody fusion protein-encoding polynucleotide. Engineered antibody fusion proteins may be prepared by inserting an engineered or mutant polynucleotide into an appropriate

expression vector and introducing the vector into an appropriate host cell, such as, for example, *Escherichia coli*. The transformant is cultured and the engineered antibody fusion protein expressed in the transformant may be collected from the cells or culture medium by any known technique.

[0130] In some embodiments the expression vector is a pET-30c vector (SEQ ID NO: 11).

[0131] *pET-30c Expression Vector*

ATCCGGATATAGTTCCTCCTTTTCAGCAAAAAACCCCTCAAGACCCGTTTAGAG
GCCCCAAGGGGTTATGCTAGTTATTGCTCAGCGGTGGCAGCAGCCAACTCAG
CTTCCTTTCGGGCTTTGTTAGCAGCCGGATCTCAGTGGTGGTGGTGGTGGTGC
TCGAGTGCGGCCGCAAGCTTGTGACGGAGCTCGAATTCGGATCCACAGATA
TCCCATGGCCTTGTGTCGTCGTCGGTACCCAGATCTGGGCTGTCCATGTGCT
GGCGTTTCGAATTTAGCAGCAGCGGTTTCTTTCATACCAGAACCGCGTGGCACC
AGACCAGAAGAATGATGATGATGATGGTGCATATGTATATCTCCTTCTTAAA
GTTAAACAAAATTATTTCTAGAGGGGAATTGTTATCCGCTCACAATTCCCCTA
TAGTGAGTCGTATTAATTTTCGCGGGATCGAGATCGATCTCGATCCTCTACGCC
GGACGCATCGTGGCCGGCATCACCGGCGCCACAGGTGCGGTTGCTGGCGCCT
ATATCGCCGACATCACCGATGGGGAAGATCGGGCTCGCCACTTCGGGCTCAT
GAGCGCTTGTTTCGGCGTGGGTATGGTGGCAGGCCCCGTGGCCGGGGGACTG
TTGGGCGCCATCTCCTTGCATGCACCATTCCTTGCGGCGGCGGTGCTCAACGG
CCTCAACCTACTACTGGGCTGCTTCCTAATGCAGGAGTCGCATAAGGGAGAG
CGTCGAGATCCCGGACACCATCGAATGGCGCAAAACCTTTCGCGGTATGGCA
TGATAGCGCCCGGAAGAGAGTCAATTCAGGGTGGTGAATGTGAAACCAGTAA
CGTTATACGATGTGCGCAGAGTATGCCGGTGTCTCTTATCAGACCGTTTCCCGC
GTGGTGAACCAGGCCAGCCACGTTTCTGCGAAAACGCGGGAAAAAGTGGA
GCGGCGATGGCGGAGCTGAATTACATTCCCAACCGCGTGGCACAACAACCTGG
CGGGCAAACAGTCGTTGCTGATTGGCGTTGCCACCTCCAGTCTGGCCCTGCAC
GCGCCGTCGCAAATTGTCGCGGCGATTAAATCTCGCGCCGATCAACTGGGTG
CCAGCGTGGTGGTGTGATGGTAGAACGAAGCGGCGTCGAAGCCTGTAAAGC
GGCGGTGCACAATCTTCTCGCGCAACGCGTCAGTGGGCTGATCATTAACCTATC

CGCTGGATGACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTTCC
GGCGTTATTTCTTGATGTCTCTGACCAGACACCCATCAACAGTATTATTTTCTC
CCATGAAGACGGTACGCGACTGGGCGTGGAGCATCTGGTCGCATTGGGTCAC
CAGCAAATCGCGCTGTTAGCGGGCCCATTAAGTTCTGTCTCGGCGCGTCTGCG
TCTGGCTGGCTGGCATAAATATCTCACTCGCAATCAAATTCAGCCGATAGCG
GAACGGGAAGGCGACTGGAGTGCCATGTCCGGTTTTCAACAAACCATGCAAA
TGCTGAATGAGGGCATCGTTCCCACTGCGATGCTGGTTGCCAACGATCAGAT
GGCGCTGGGCGCAATGCGCGCCATTACCGAGTCCGGGCTGCGCGTTGGTGCG
GACATCTCGGTAGTGGGATACGACGATACCGAAGACAGCTCATGTTATATCC
CGCCGTTAACCACCATCAAACAGGATTTTCGCCTGCTGGGGCAAACCAGCGT
GGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTG
TTGCCCCTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAA
CCGCCTCTCCCCGCGCGTGGCCGATTCAATTAATGCAGCTGGCACGACAGGTT
TCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTAAGTTAGCTC
ACTCATTAGGCACCGGGATCTCGACCGATGCCCTTGAGAGCCTTCAACCCAG
TCAGCTCCTTCCGGTGGGCGCGGGGCATGACTATCGTCGCCGCACTTATGACT
GTCTTCTTTATCATGCAACTCGTAGGACAGGTGCCGGCAGCGCTCTGGGTCAT
TTTCGGCGAGGACCGCTTTCGCTGGAGCGCGACGATGATCGGCCTGTGCTTG
CGGTATTCGGAATCTTGACGCCCCTCGCTCAAGCCTTCGTCACTGGTCCCGCC
ACCAAACGTTTCGGCGAGAAGCAGGCCATTATCGCCGGCATGGCGGGCCCCAC
GGGTGCGCATGATCGTGCTCCTGTCGTTGAGGACCCGGCTAGGCTGGCGGGG
TTGCCTTACTGGTTAGCAGAATGAATCACCGATACGCGAGCGAACGTGAAGC
GACTGCTGCTGCAAAACGTCTGCGACCTGAGCAACAACATGAATGGTCTTCG
GTTTCCGTGTTTCGTAAAGTCTGGAAACGCGGAAGTCAGCGCCCTGCACCATT
ATGTTCCGGATCTGCATCGCAGGATGCTGCTGGCTACCCTGTGGAACACCTAC
ATCTGTATTAACGAAGCGCTGGCATTGACCCTGAGTGATTTTTCTCTGGTCCC
GCCGCATCCATACCGCCAGTTGTTTACCCTCACAACGTTCCAGTAACCGGGCA
TGTTTCATCATCAGTAACCCGTATCGTGAGCATCCTCTCTCGTTTCATCGGTATC
ATTACCCCCATGAACAGAAATCCCCCTTACACGGAGGCATCAGTGACCAAAC

AGGAAAAAACCGCCCTTAACATGGCCCGCTTTATCAGAAGCCAGACATTAAC
GCTTCTGGAGAACTCAACGAGCTGGACGCGGATGAACAGGCAGACATCTGT
GAATCGCTTCACGACCACGCTGATGAGCTTTACCGCAGCTGCCTCGCGCGTTT
CGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACA
GCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAG
CGGGTGTTGGCGGGTGTGCGGGGCGCAGCCATGACCCAGTCACGTAGCGATAG
CGGAGTGTATACTGGCTTAACATATGCGGCATCAGAGCAGATTGTACTGAGAG
TGCACCATATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATAC
CGCATCAGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCTG
TCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCC
ACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAA
AAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCC
GCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAA
ACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGT
GCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCC
TTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGG
TGTAGGTTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCC
GACCGCTGCGCCTTATCCGGTAACATATCGTCTTGAGTCCAACCCGGTAAGAC
ACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAG
GTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTAC
ACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGG
AAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGT
GGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAG
AAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACCTCA
CGTTAAGGGATTTTGGTCATGAACAATAAACTGTCTGCTTACATAAACAGT
AATACAAGGGGTGTTATGAGCCATATTCAACGGGAAACGTCTTGCTCTAGGC
CGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGC
GATAATGTCGGGCAATCAGGTGCGACAATCTATCGATTGTATGGGAAGCCCG
ATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGT

TACAGATGAGATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCG
ACCATCAAGCATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCCTGC
GATCCCCGGGAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGT
GAAAATATTGTTGATGCGCTGGCAGTGTTCTGCGCCGGTTGCATTTCGATTCC
TGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTTCGTCTCGCTCAGGCGC
AATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCG
TAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCA
TTCTCACC GGATTTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATT
TTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCG
CAGACCGATACCAGGATCTTGCCATCCTATGGAAGTGCCTCGGTGAGTTTTCT
CCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATAT
GAATAAATTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAAGAATTAATTC
ATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTC
CGCGCACATTTCCCCGAAAAGTGCCACCTGAAATTGTAAACGTTAATATTTTG
TTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACCAATAGGC
CGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTG
AGTGTTGTTCCAGTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCA
ACGTCAAAGGGCGAAAAACCGTCTATCAGGGCGATGGCCCACTACGTGAACC
ATCACCTAATCAAGTTTTTTGGGGTCGAGGTGCCGTAAAGCACTAAATCGG
AACCCTAAAGGGAGCCCCCGATTTAGAGCTTGACGGGGAAAGCCGGCGAAC
GTGGCGAGAAAGGAAGGAAGAAAGCGAAAGGAGCGGGCGCTAGGGCGCT
GGCAAGTGTAGCGGTCACGCTGCGCGTAACCACCACACCCGCCGCGCTTAAT
GCGCCGCTACAGGGCGCGTCCCATTGCGCA (SEQ ID NO: 11)

[0132] In embodiments, the engineered antibody fusion protein thus obtained may be purified by any of the known purification techniques including, but not limited to, ion exchange column chromatography, affinity chromatography, liquid chromatography, filtration, ultrafiltration, salt precipitation, solvent precipitation, immunoprecipitation, gel electrophoresis, isoelectric electrophoresis and dialysis.

[0133] In embodiments, provided herein are isolated or purified polypeptides, proteins and polynucleotides for an engineered antibody fusion protein, a vector comprising the polynucleotide encoding the engineered antibody fusion protein, a host cell transformed with such a vector, and a method for preparing the engineered antibody fusion protein by culturing the transformant, collecting and purifying the engineered antibody fusion protein from the culture.

III. Devices

[0134] In another embodiment, a device for assaying a target molecule in a sample is provided, where the device includes an engineered antibody fusion protein described herein.

[0135] Disclosed herein are biosensors comprising an engineered antibody fusion protein to detect a target molecule, wherein the binding affinity of the antibody fusion protein towards the target is modulated upon exposure to an external signal. The modulable binding affinity of the antibody fusion proteins disclosed herein allow for reversible binding to the target molecule, enabling continuous, *in vivo* monitoring applications.

[0136] As used herein, “biosensor” means a device containing bio-recognition elements (e.g., enzymes, aptamers, antibodies, etc.) that react with a target molecule and then transduce the biological signal into an electrical signal that can be analyzed.

[0137] In embodiments, an electrode comprises an antibody fusion protein disclosed herein immobilized on an electroconductive material. Examples of means for immobilizing molecules such as the engineered antibody fusion protein include, but are not limited to, cross-linking, encapsulating into a macromolecular matrix, coating with a dialysis membrane, optical cross-linking polymer, electroconductive polymer, oxidation-reduction polymer, and any combination thereof. The electroconductive material may be any material known in the art for biosensing applications.

[0138] The term “immobilized” refers to a compound which is attached to a surface (e.g., electrode). Immobilization can be performed through adsorption, cross-linking, covalent bonding and/or affinity-tag binding.

[0139] The term “cross-linking” refers to a polymerization reaction of a cross-linking reagent. A “cross-linking reagent” refers to a molecule that contains two or more reactive ends capable of forming covalent bonds.

[0140] The term “electroconductive material” refers to a substance capable of transmitting electricity.

[0141] In some embodiments, the biosensor comprising the antibody fusion protein disclosed herein includes a source of light to irradiate the antibody fusion protein to modulate the binding affinity of the antibody fusion protein for the target molecule. In some embodiments, the light source irradiates blue light, wherein the wavelength of the light may be about 400-700 nm.

[0142] In one embodiment, the biosensor detects a target molecule in a continuous manner. In one embodiment, the target molecule is insulin.

REFERENCES

1. Kopka, B., et al. (2017). Electron transfer pathways in a light, oxygen, voltage (LOV) protein devoid of the photoactive cysteine. *Scientific Reports*, 7(1), 1–16. <https://doi.org/10.1038/s41598-017-13420-1>

EXAMPLES

Example 1: Characterization of an antibody fusion protein with a redox-sensitive domain

[0143] For improved biosensing properties, antibody fusion proteins were designed with a single chain variable fragment (scFv) of an immunoglobulin and a redox-sensitive domain capable of undergoing a conformational change upon exposure to light. The light oxygen voltage domain (LOV) derived from *Avena sativa* (asLOV2) was used as the initial redox-sensitive domain, as it has been extensively characterized and has been used for protein switches. The crystal structure of asLOV2 is shown in **Figure 1A**, which includes a flavin mononucleotide (FMN) cofactor. As shown in **Figure 1B**, upon irradiation with blue light the Cys966 residue of asLOV2 can form a covalent bond with the FMN, which results in the undocking of the J α -helix of the C-terminus. Anti-insulin scFv (clone HB125) was chosen as a model target for this

system, as insulin would be a desirable hormone to monitor with a biosensor for patients with diabetes.

[0144] An antibody fusion protein was designed with an anti-human insulin scFv and asLOV2 domain at the C-terminus (cLOV). For the initial model, the antibody fusion protein was produced with the anti-human insulin scFv consisting of an N-terminal V_H region, followed by a glycine-rich linker, followed by the V_L region and the asLOV2 domain at the C-terminus (SEQ ID NO: 3). The structure of this antibody fusion protein as predicted by AlphaFold 2 is shown in **Figure 2**, where the asLOV2 domain with its alpha helices is seen at the bottom and the V_H and V_L regions are seen on the top of the structure. A pET30c vector with NdeI and HindIII restriction sites and a His tag was used to produce the recombinant antibody fusion protein in *E. coli* Shuffle T7 Express™ cells (**Figure 3**). The antibody fusion protein was collected as a soluble fraction and its expression was confirmed by SDS-PAGE (**Figure 4B**). The crude protein was purified by affinity chromatography using the His tag and by size exclusion chromatography (**Figure 4A**).

[0145] An insulin sandwich ELISA was performed using the antibody fusion protein (SEQ ID NO: 3) or the anti-insulin scFv (SEQ ID NO: 1) and an anti-His-tag IgG antibody conjugated to HRP for detection. The ELISA confirmed that the antibody fusion protein retained its ability to insulin (**Figure 5**). Absorbance of the antibody fusion protein was measured in both dark and light conditions (**Figure 6A**) and compared to the absorbance of an asLOV2 protein (**Figure 6B**) without the scFV domain. For the dark condition, the absorbance was measured in a dark room. For the light condition, light exposure was performed using an LED array at 470 nm. This spectroscopic analysis revealed that the antibody fusion protein exhibited similar characteristic absorbance spectra to the asLOV2 protein, thus showing the antibody fusion protein retained its FMN activity.

[0146] Then, another insulin sandwich ELISA (**Figure 7A**) was performed with the antibody fusion protein in both light and dark conditions. For the ELISA assay, the entire procedure including all washing and incubation steps were performed either in a dark room, or under a LED array with constant 470 nm light exposure. Significantly different binding ability was observed in light vs. dark conditions at various concentrations of antibody, which indicates

that the antibody fusion protein has a different binding affinity towards its substrate in the presence of light, which activates the LOV domain. **Figure 7B** shows the sensitivity of the antibody fusion protein changed by approximately 2-fold in the presence of light, as observed by the slope of insulin detected by the concentration of the antibody. These results validate the hypothesis that an antibody fusion protein can have modifiable binding towards its substrate based on the conformational change which occurs by activating a redox-sensitive domain with an external signal (e.g., blue light).

[0147] **Figure 8** shows further spectroscopic analysis of an asLOV protein and the antibody fusion with an anti-insulin scFv domain and an asLOV2 domain at the C-terminus (cLOV). Here, the intrinsic FMN fluorescence is measured. The antibody fusion protein (cLOV) exhibited a similar characteristic FMN spectra to the asLOV2 protein thus showing the antibody fusion protein retained its FMN activity.

[0148] A second antibody fusion protein was designed with an anti-human insulin scFv and an asLOV2 domain at the N-terminus (nLOV). **Figure 9** shows the structure of the second engineered antibody fusion protein (nLOV) as predicted by AlphaFold 2. The alpha helix involved in the conformational change of the asLOV2 domain is shown in dark gray. **Figure 10** shows a pET30c vector with NdeI and HindIII restriction sites and a His tag was used to produce the second recombinant antibody fusion protein (nLOV) protein in *E. coli* Shuffle T7 Express™ cells (SEQ ID NO: 17). **Figure 11A** shows the chromatogram tracking at 280 nm (UV280) and 450 nm (UV450) absorptions of the second antibody fusion (nLOV), and **Figure 11B** shows SDS-PAGE of the eluted soluble antibody fusion protein with an anti-insulin scFV domain and an asLOV2 domain, which was expressed in *E. coli* Shuffle T7 Express™ cells.

[0149] **Figure 12** shows the intrinsic FMN fluorescence of the antibody fusion protein (nLOV), measured in both dark and light conditions. For the dark condition, the absorbance was measured in a dark room. For the light condition, light exposure was performed using an LED array at 470 nm. The intrinsic FMN fluorescence signal was reversible over the course of three minutes, indicating the LOV protein retains its activity.

[0150] A third antibody fusion protein was designed with an anti-insulin single-chain variable fragment (scFV) domain and a truncated asLOV2 domain at the N-terminus. **Figure 13**

shows the structure of the third engineered antibody fusion protein (ntLOV) as predicted by AlphaFold 2. The alpha helix involved in the conformational change of the asLOV2 domain is shown in dark gray. **Figure 14** shows a pET30c vector with NdeI and HindIII restriction sites and a His tag used to produce the third recombinant antibody fusion (ntLOV) protein in *E. coli* Shuffle T7 Express™ cells (SEQ ID NO: 18). **Figure 15A** shows the chromatogram tracking at 280 nm (UV280) and 450 nm (UV450) absorptions of the third antibody fusion (ntLOV), and **Figure 15B** shows SDS-PAGE of the eluted soluble third antibody fusion (ntLOV) expressed in *E. coli* Shuffle T7 Express™ cells. **Figure 16** shows the intrinsic FMN Fluorescence of the third antibody fusion protein (ntLOV), measured in both dark and light conditions. For the dark condition, the fluorescence was measured in a dark room. For the light condition, light exposure was performed using an LED array at 470 nm. The intrinsic FMN fluorescence signal was reversible over the course of three minutes, indicating the LOV protein retains its activity in this fusion.

[0151] A fourth antibody fusion protein was designed with an asLOV2 domain between an anti-insulin V_H region at the N-terminus and an anti-insulin V_L region at the C-terminus with glycine-rich linkers between the domains (gsLOV). **Figure 17** shows the structure of the fourth engineered antibody fusion protein (gsLOV) as predicted by AlphaFold 2. The alpha helix involved in the conformational change of the asLOV2 domain is shown in dark gray. **Figure 18** shows a pET30c vector with NdeI and HindIII restriction sites and a His tag was used to produce the fourth recombinant antibody fusion protein (gsLOV) in *E. coli* Shuffle T7 Express™ cells (SEQ ID NO: 19). Variants of the gsLOV fusion proteins were prepared with mutations to fix the proteins in a “dark state” (C190A; SEQ ID NO: 27) or a “light state” (I272E, A276E; SEQ ID NO: 26). **Figure 19A** shows SDS-PAGE of the eluted soluble gsLOV antibody fusion proteins expressed in *E. coli* Shuffle T7 Express™ cells. **Figures 19B-19D** show the chromatogram tracking at 280 nm (UV280) and 450 nm (UV450) absorptions for the gsLOV (**Figure 19B**), the gsLOV C190A “dark” variant (**Figure 19C**), and the gsLOV I272E, A276E “light” variant (**Figure 19D**) antibody fusion proteins. **Figure 20** shows the intrinsic FMN Fluorescence of the gsLOV antibody fusion protein, measured in both dark and light conditions. For the dark condition, the absorbance was measured in a dark room. For the light condition, light exposure was performed

using an LED array at 470 nm. The intrinsic FMN fluorescence signal was reversible over the course of three minutes, indicating the LOV protein retains its activity in this fusion.

[0152] A Biolayer Interferometry (BLI) assay was performed to determine the binding affinity of the gsLOV antibody fusion proteins to insulin, as shown in **Figure 21A**. **Figures 21B-21E** show representative sensor grams from the BLI assay for the wild type anti-insulin (**Figure 21B**), the gsLOV fusion protein (**Figure 21C**), the gsLOV I272E, A276E fusion protein fixed in a “light state” (**Figure 21D**), and the gsLOV C190A fusion protein fixed in a “dark state” (**Figure 21E**). The corresponding calculated affinities, shown in Table 1, reveal that the binding affinity of the dark mimetic fusion is twice that of the light mimetic fusion, which is comparable to the wild-type anti-insulin.

[0153] Table 1. Binding affinities of antibody fusion proteins with an asLOV2 domain between an anti-insulin V_H region at the N-terminus and an anti-insulin V_L region at the C-terminus with glycine-rich linkers (gsLOV)

Sample	K _D (nM)	K _{on} (M ⁻¹ * min ⁻¹)	K _{off} (min ⁻¹)
WT anti-insulin (HB125)	55.2	26790	0.001479
gsLOV	100.4	19645	0.001972
gsLOV C190A “dark”	103.7	23397	0.002426
gsLOV I272E, A276E “light”	63.84	29514	0.001885

[0154] A fifth antibody fusion protein was designed with an asLOV2 domain between an anti-insulin V_H region at the N-terminus and an anti-insulin V_L region at the C-terminus without glycine-rich linkers between the domains (nolinkLOV). **Figure 22** shows the structure of the fifth engineered antibody fusion protein (nolinkLOV) as predicted by AlphaFold 2. The alpha helix involved in the conformational change of the asLOV2 domain is shown in dark gray. **Figure 23** shows a pET30c vector with NdeI and HindIII restriction sites and a His tag was used to produce the fifth recombinant antibody fusion protein (nolinkLOV) in *E. coli* Shuffle T7 Express™ cells (SEQ ID NO: 20). Variants of the nolinkLOV fusion proteins were prepared with mutations to fix the proteins in a “dark state” (C185A; SEQ ID NO: 29) or a “light state” (I267E, A271E; SEQ ID NO: 30). **Figure 24A** shows SDS-PAGE of the eluted soluble nolinkLOV antibody fusion proteins

expressed in E. coli Shuffle T7 Express™ cells. **Figures 24B-24D** show the chromatogram tracking at 280 nm (UV280) and 450 nm (UV450) absorptions for the nolinkLOV (**Figure 24B**), the nolinkLOV C185A “dark” variant (**Figure 24C**), and nolinkLOV I267E, A271E “light” variant (**Figure 24D**) antibody fusion proteins.

[0155] A Biolayer Interferometry (BLI) assay was performed to determine the binding affinity of the nolinkLOV antibody fusion proteins to insulin, as shown in **Figure 21A**. **Figures 25A-25D** shows the results of the fifth antibody fusion binding to insulin via BLI (**Figure 21A**). **Figures 25A-25D** show BLI sensor grams for wild type anti-insulin (**Figure 25A**), the nolinkLOV fusion protein (**Figure 25B**), the nolinkLOV C185A fusion protein fixed in a “dark state” (**Figure 25C**) the nolinkLOV I267E, A271E fusion protein fixed in a “light state” (**Figure 25D**). The corresponding calculated affinities shown in **Table 2**, indicating that the binding affinity of the dark mimetic fusion is almost four times that of the light mimetic fusion, which is comparable to the WT anti-insulin.

[0156] Table 2. Binding affinities of antibody fusion proteins with an asLOV2 domain between an anti-insulin V_H region at the N-terminus and an anti-insulin V_L region at the C-terminus without glycine-rich linkers (nolinkLOV)

Sample	K _D (nM)	K _{on} (M ⁻¹ * min ⁻¹)	K _{off} (min ⁻¹)
WT anti-insulin (HB125)	55.2	26790	0.001479
nolinkLOV	78.48	11236	0.0008819
nolinkLOV C185A “dark”	198	7333	0.001452
nolinkLOV I267E, A271E “light”	68.69	25802	0.001772

[0157] Many modifications and other embodiments of the inventions set forth herein will come to mind to one skilled in the art to which the inventions pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings. Therefore, it is to be understood that the inventions are not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the

appended claims. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

CLAIMS

What is claimed is:

1. An engineered antibody fusion protein, the protein comprising a variable region of an immunoglobulin heavy chain (V_H), a variable region of an immunoglobulin light chain (V_L), and a redox-sensitive domain,

wherein the redox-sensitive domain is capable of undergoing a conformational change upon exposure to an external signal, and

wherein the conformational change modulates an affinity of the antibody fusion protein for a target molecule upon exposure to the external signal.

2. The antibody fusion protein of claim 1, wherein the amino acid sequence of the redox-sensitive domain is located at the C-terminal end of the antibody fusion protein.

3. The antibody fusion protein of claim 1, wherein the amino acid sequence of the redox-sensitive domain is located at the N-terminal end of the antibody fusion protein.

4. The antibody fusion protein of any one of claims 1-3, wherein the V_H region and the V_L region are adjacent and comprise a single chain variable region (scFv),

optionally wherein a linker sequence is between the amino acid sequence of the V_H region and the amino acid sequence of the V_L region, wherein the linker sequence comprises one or more glycine residues.

5. The antibody fusion protein of claim 1, wherein the amino acid sequence of the redox-sensitive domain is located between the amino acid sequence of the V_H region and the amino acid sequence of the V_L region.

6. The antibody fusion protein of claim 5, wherein the amino acid sequence of the V_H region is located at the C-terminal end of the antibody fusion protein and the amino acid sequence of the V_L region is located at the N-terminal end of the antibody fusion protein.

7. The antibody fusion protein of claim 5, wherein the amino acid sequence of the V_H region is located at the N-terminal end of the antibody fusion protein and the amino acid sequence of the V_L region is located at the C-terminal end of the antibody fusion protein.

8. The antibody fusion protein of any one of claims 1-7, wherein the target molecule is a metabolite, a hormone, and/or a therapeutic compound.

9. The antibody fusion protein of claim 8, wherein the target molecule is selected from the group consisting of insulin, glucagon, and glucagon-like peptide 1.

10. The antibody fusion protein of claim 9, wherein the target molecule is insulin.

11. The antibody fusion protein of claim 10, wherein the V_H region and the V_L region are anti-insulin.

12. The antibody fusion protein of claim 11, wherein the scFv comprises an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to SEQ ID NO: 1.

13. The antibody fusion protein of claim 12, wherein the scFv comprises an amino acid sequence set forth in SEQ ID NO: 1.

14. The antibody fusion protein of any one of claims 1-13, wherein the redox-sensitive domain comprises a heme cofactor or a flavin cofactor.

15. The antibody fusion protein of any one of claims 1-14, wherein the redox-sensitive domain is selected from the group consisting of a bacterium-derived cytochrome c-like molecule (CYTc) domain, a light-oxygen-voltage-sensing (LOV) domain, and a cGMP-specific phosphodiesterases, adenylyl cyclases, FhlA (GAF) domain.

16. The antibody fusion protein of claim 15, wherein the LOV domain is a second LOV domain of a phototropin (LOV2).

17. The antibody fusion protein of claim 16, wherein the LOV2 domain is derived from *Avena Sativa* (asLOV2).

18. The antibody fusion protein of any one of claims 1-17, wherein the redox-sensitive domain comprises an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to SEQ ID NO: 2 or SEQ ID NO: 36.

19. The antibody fusion protein of claim 18, wherein the redox-sensitive domain comprises an amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 36.

20. The antibody fusion protein of any one of claims 1-19, wherein the amino acid sequence of the V_H region comprises an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to SEQ ID NO: 6.

21. The antibody fusion protein of claim 20, wherein the amino acid sequence of the V_H region comprises an amino acid sequence set forth in SEQ ID NO: 6.

22. The antibody fusion protein of any one of claims 1-21, wherein the amino acid sequence of the V_L region comprises an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to SEQ ID NO: 7.

23. The antibody fusion protein of claim 22, wherein the amino acid sequence of the V_L region comprises an amino acid sequence set forth in SEQ ID NO: 7.

24. The antibody fusion protein of any of claims 1-23, wherein the antibody fusion protein comprises an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to any one of SEQ ID NOs: 3-5, and 16-20.

25. The antibody fusion protein of claim 24, wherein the antibody fusion protein comprises an amino acid sequence set forth in any one of SEQ ID NO: 3-5, and 16-20.

26. The antibody fusion protein of any one of claims 1-25, wherein a sensitivity towards the target molecule is modulated by exposure to the external signal.

27. The antibody fusion protein of claims 26, wherein the sensitivity of the antibody fusion protein towards the target molecule is at least 2-fold, at least 5-fold, at least 10-fold, at least 100-fold, or at least 1000-fold different upon exposure to the external signal.

28. The antibody fusion protein of any one of claims 1-27, wherein a limit of detection (LOD) of the target molecule is modulated by exposure to the external signal.

29. The antibody fusion protein of any one of claims 1-28, wherein a specific binding ability of the antibody fusion protein towards the target molecule is modulated by exposure to the external signal.

30. The antibody fusion protein of claim 29, wherein the specific binding ability of the antibody fusion protein towards the target molecule is at least 2-fold, at least 5-fold, at least 10-fold, at least 100-fold, or at least 1000-fold different upon exposure to the external signal.

31. The antibody fusion protein of any one of claims 1-30, wherein the dissociation constant (K_d) of the antibody fusion protein towards the target molecule is modulated by exposure to the external signal.

32. The antibody fusion protein of claim 31, wherein the K_d of the antibody fusion protein is at least 10, at least 10^2 , at least 10^3 , at least 10^4 , at least 10^5 , at least 10^6 , at least 10^7 , at least 10^8 , at least 10^9 , or at least 10^{10} times different upon exposure to the external signal.

33. The antibody fusion protein of any one of claims 1-32, wherein the external signal is redox potential or light.

34. The antibody fusion protein of claim 33, wherein the external signal is blue light.

35. The antibody fusion protein of claim 34, wherein the blue light has a wavelength of 400-500nm.

36. An electrode comprising the antibody fusion protein of any of claims 1-35 immobilized on an electroconductive material.

37. A device for detection of the target molecule, comprising the engineered antibody fusion protein of any one of claims 1-35.

38. The device of claim 37, wherein the detection is continuous.

39. The device of claim 37 or 38, wherein the detection is performed *in vivo*.

40. The device of any one of claims 37-39, wherein the target molecule is insulin.

41. A polynucleotide encoding the antibody fusion protein of any one of claims 1-35.

42. The polynucleotide of claim 40, further comprising a nucleotide sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% sequence identity to any one of SEQ ID NOs: 8-10, and 31-35.

43. The polynucleotide of claim 42, comprising a nucleotide sequence set forth in any one of SEQ ID NOs: 8-10, and 31-35.

44. A recombinant plasmid comprising a promoter operably linked to the polynucleotide sequence of any one of claims 41-43, and prepared by inserting the nucleotide sequence into an expression vector.

45. The recombinant plasmid of claim 44, wherein the expression vector is a pET30c vector.

46. A method of producing a recombinant antibody fusion protein, the method comprising:

- i) introducing the recombinant plasmid of claim 44 or 45 into a host cell
 - ii) expressing the antibody fusion protein.
47. The method of claim 46, wherein the host cell is *E. coli*.

asLOV2 Domain (PDB 2V0U, 2V0W)

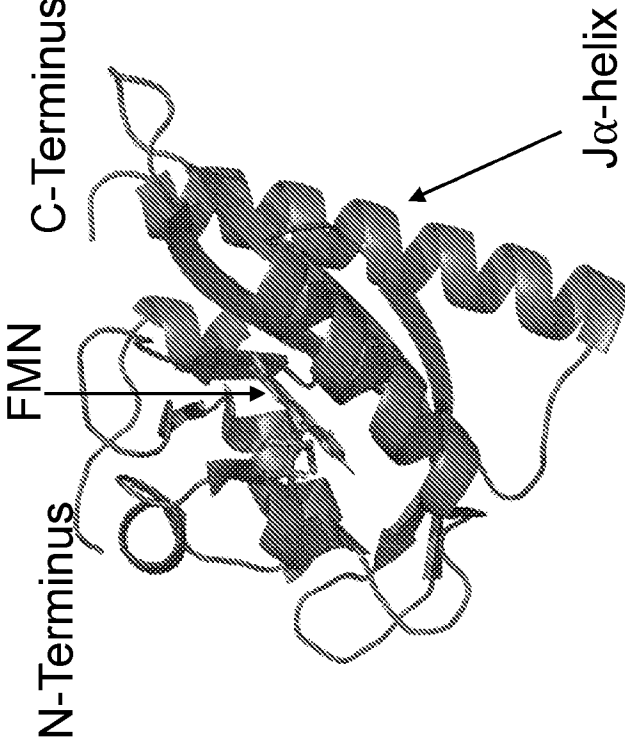


Fig. 1A

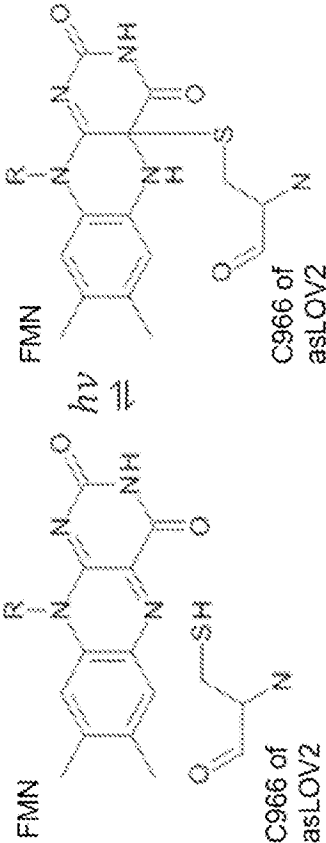


Fig. 1B

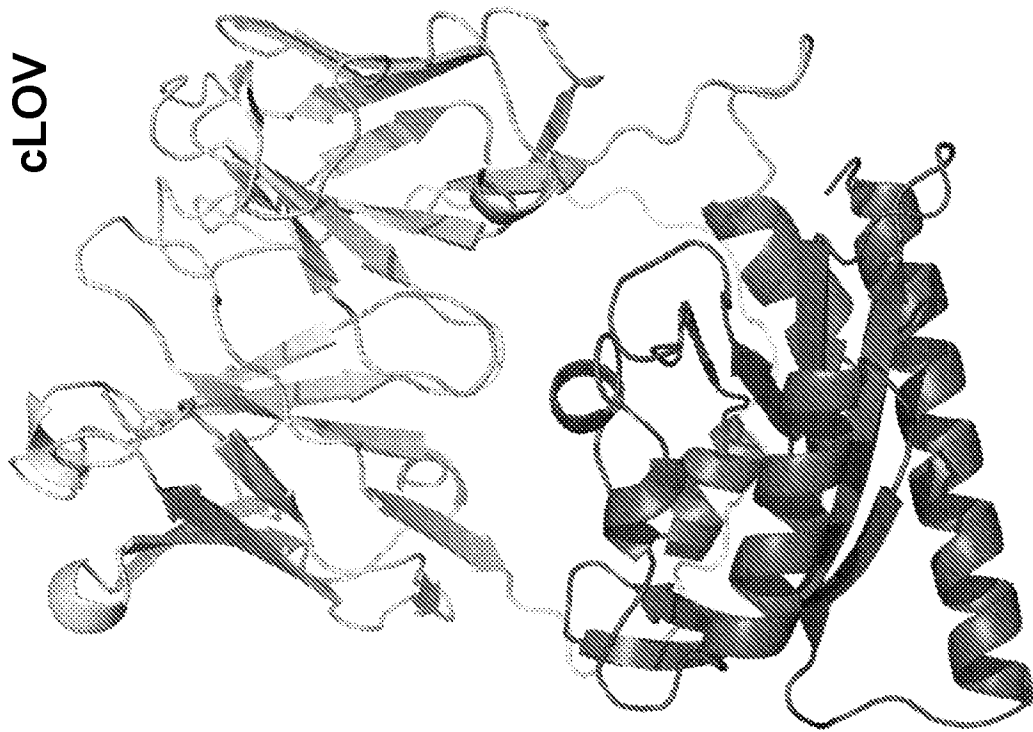


Fig. 2

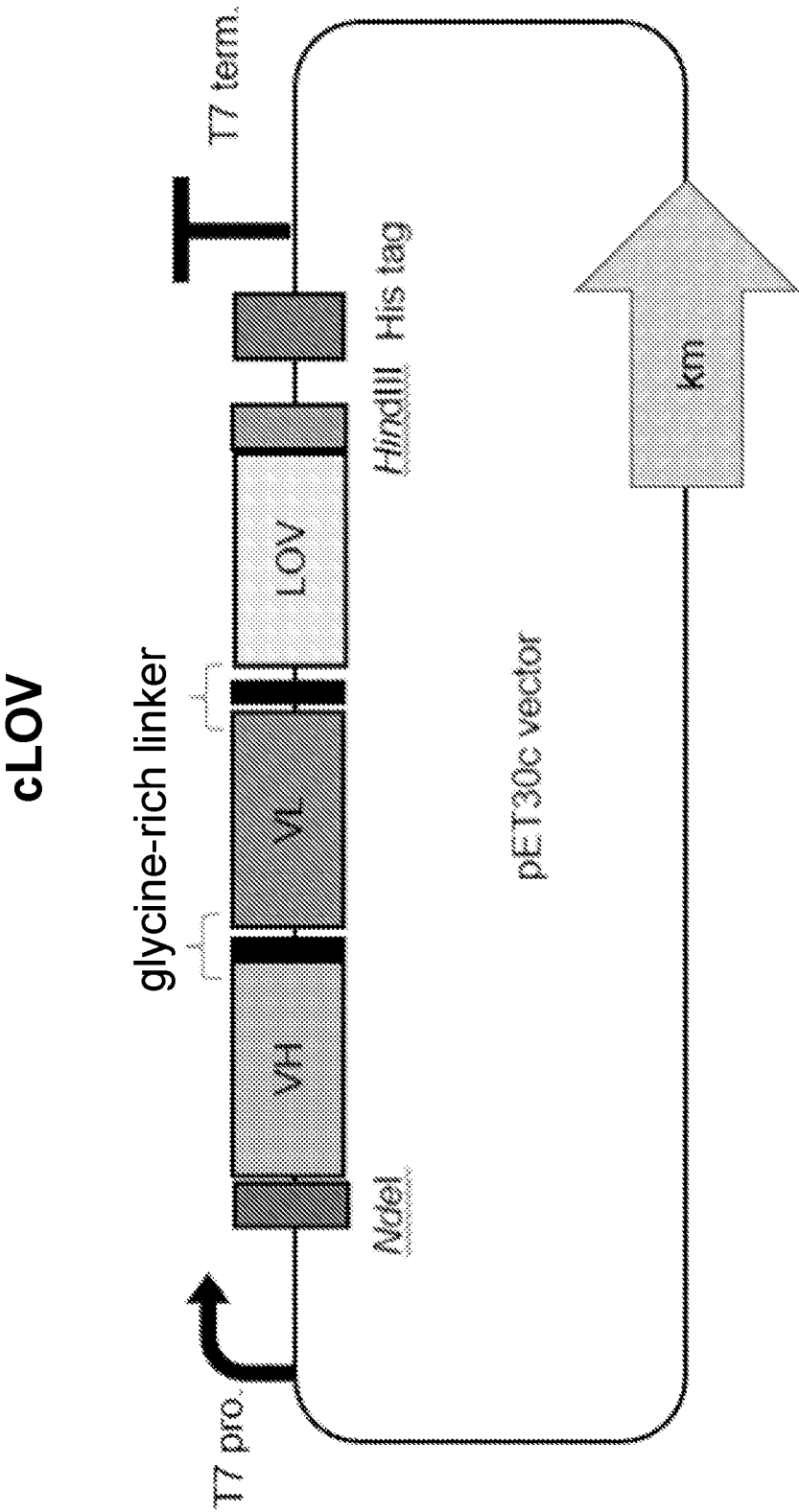


Fig. 3

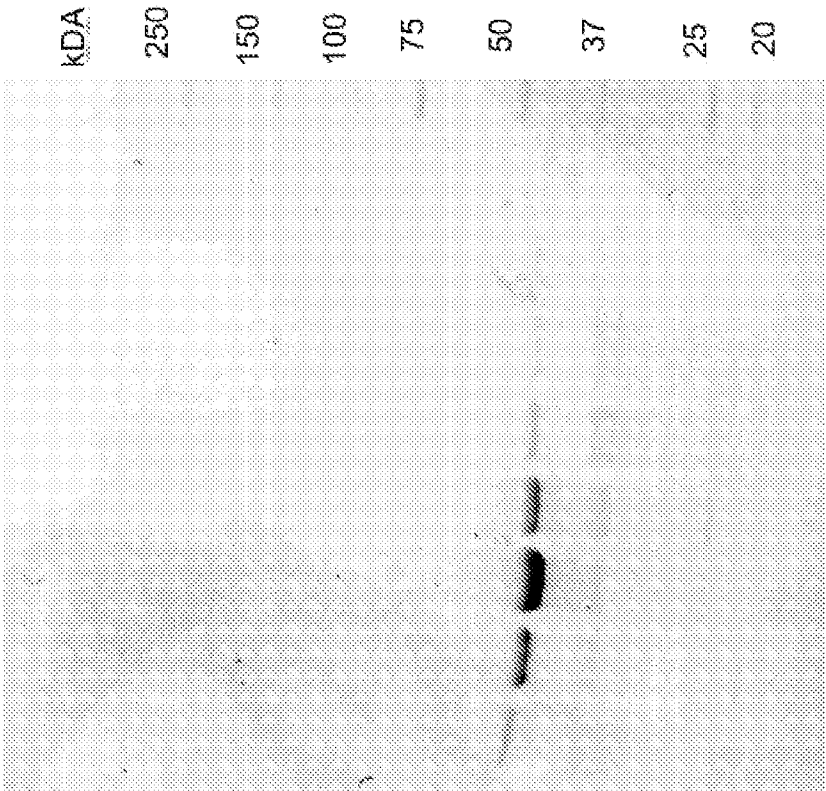


Fig. 4B

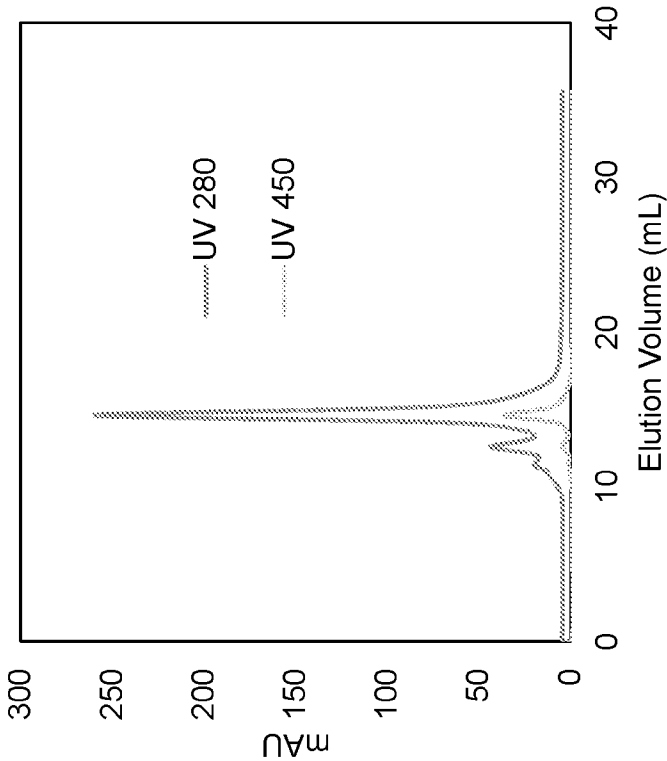


Fig. 4A

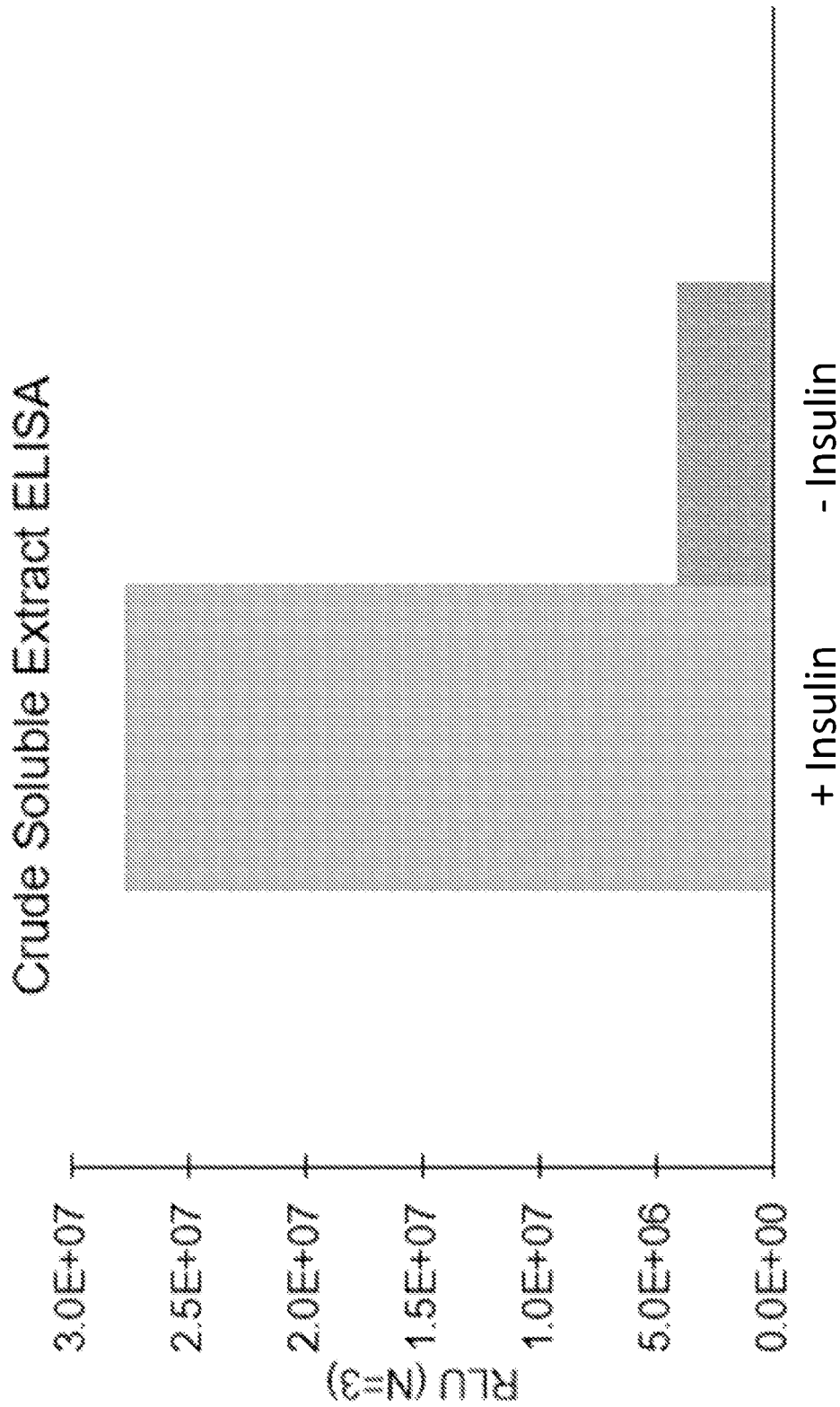


Fig. 5

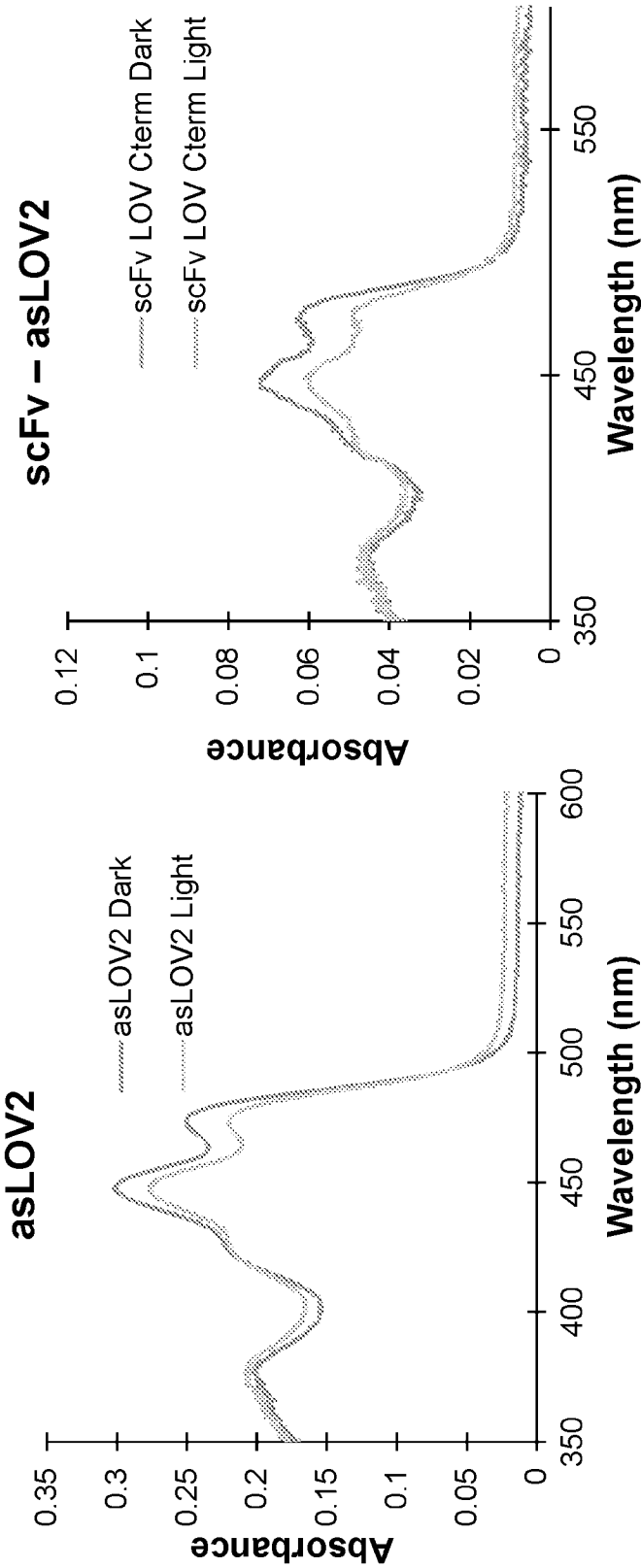


Fig. 6A

Fig. 6B

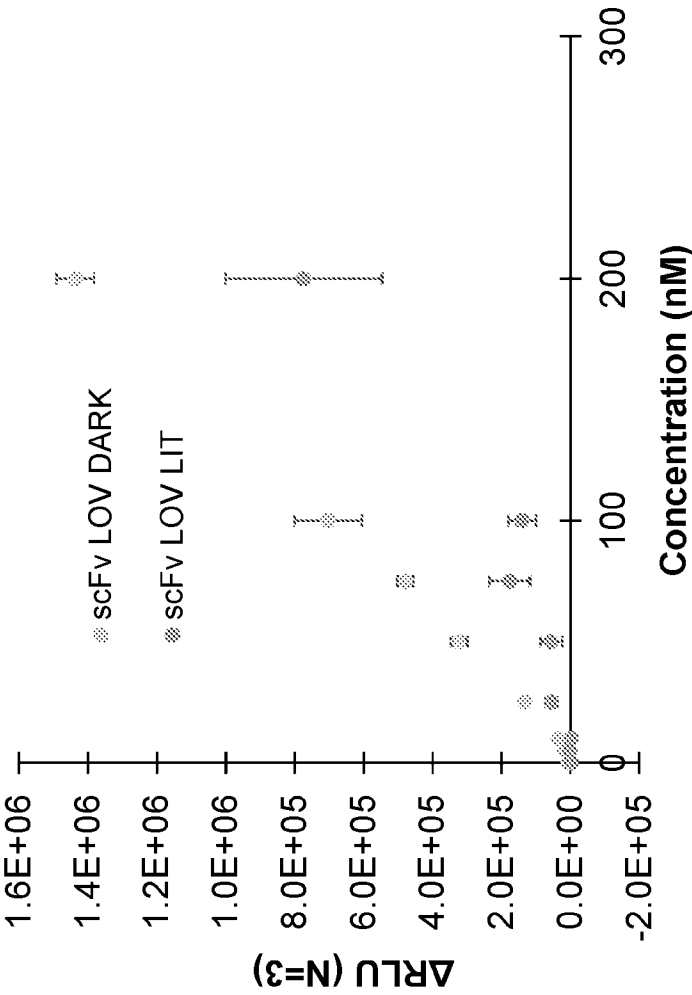


Fig. 7B

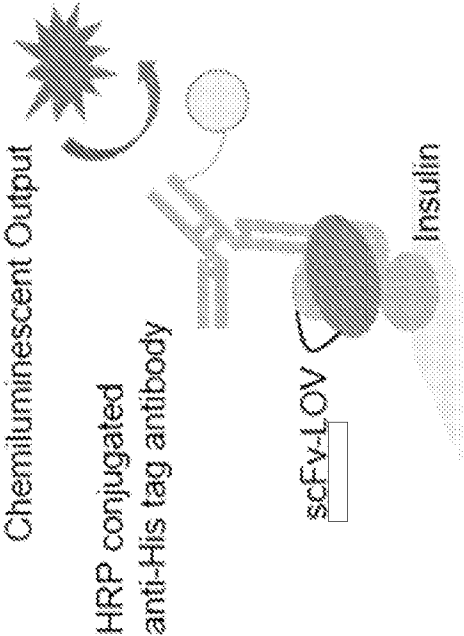


Fig. 7A

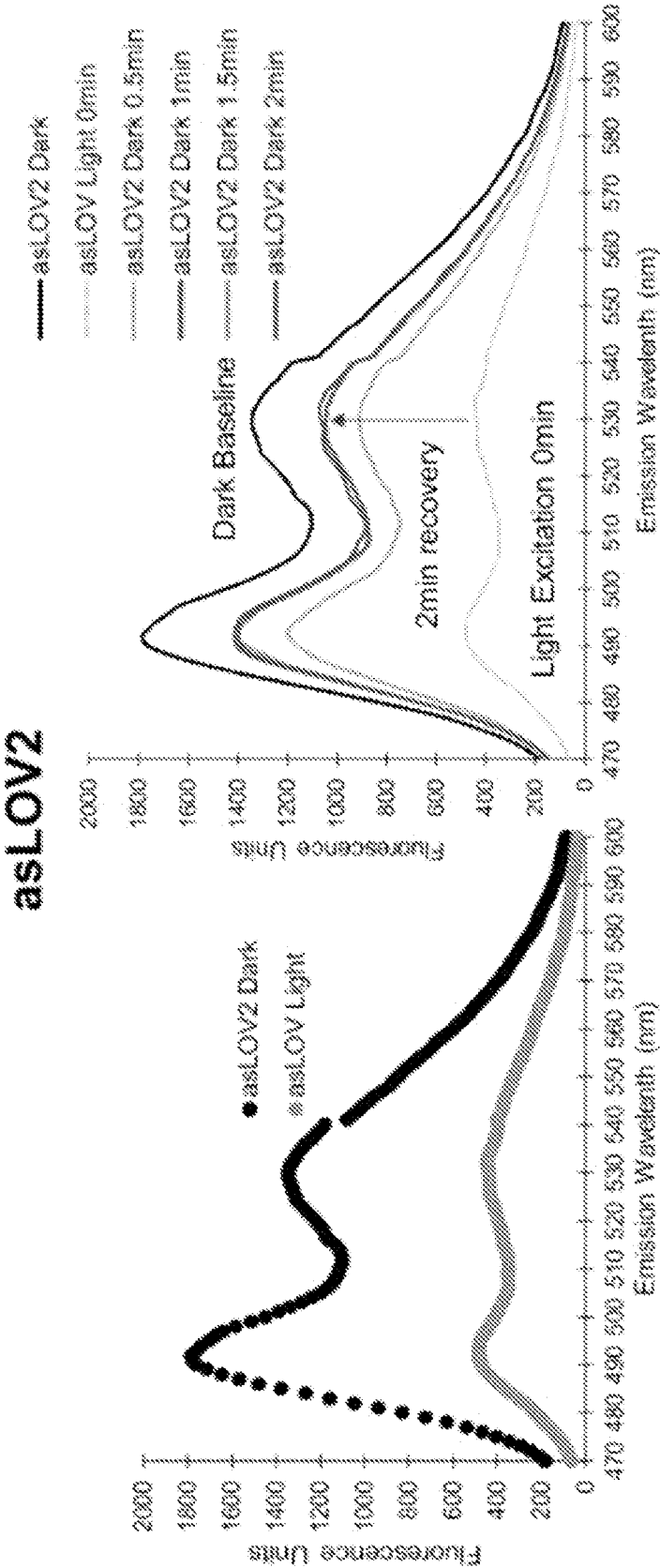


Fig. 8B

Fig. 8A

scFv-asLOV2

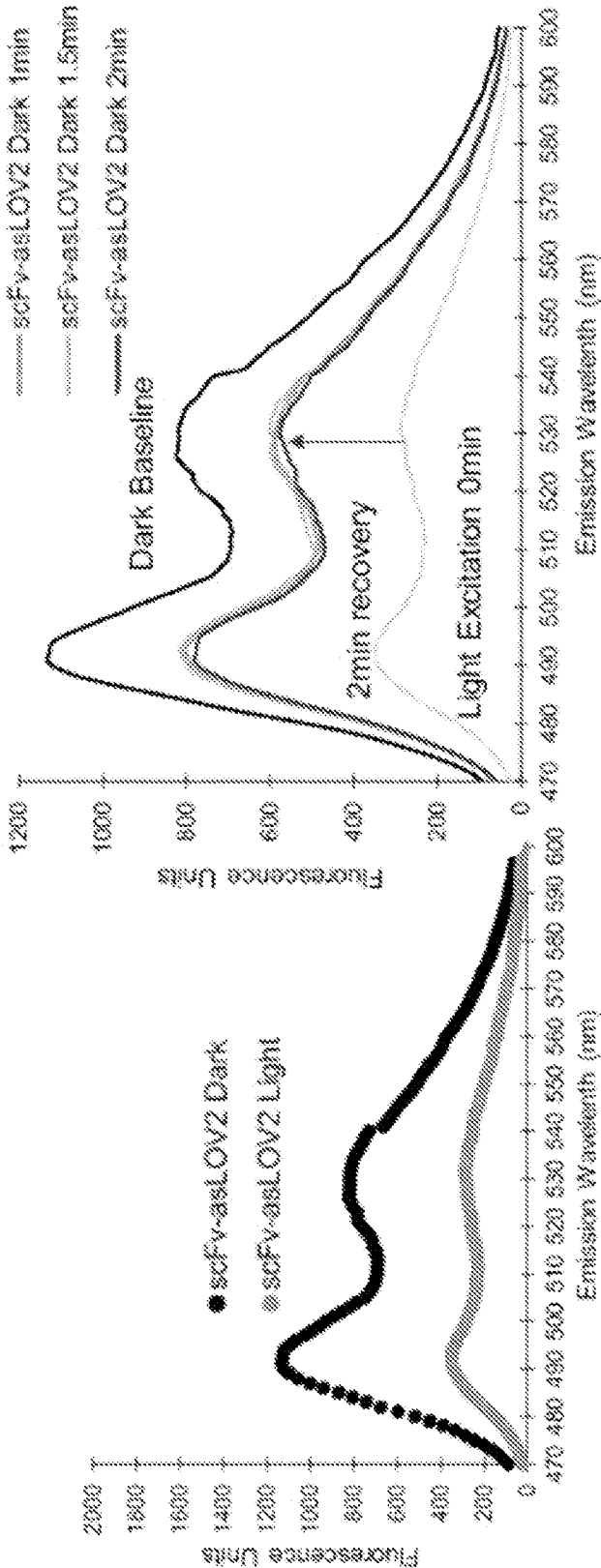


Fig. 8C

Fig. 8D

nLOV



Fig. 9

nLOV

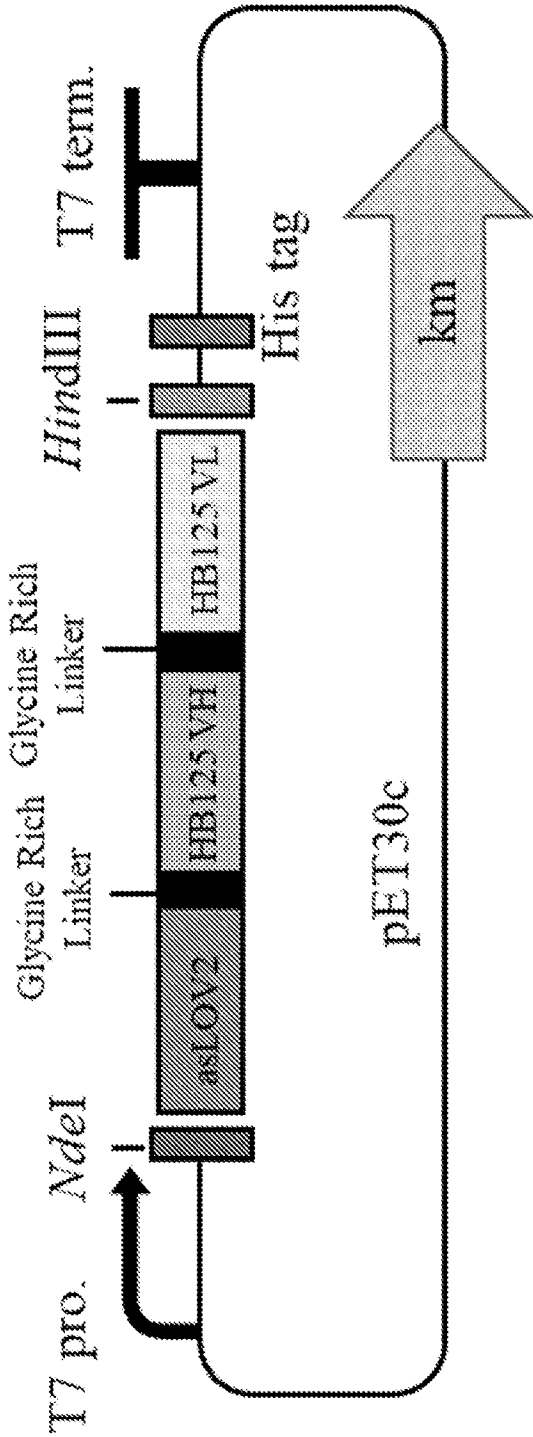


Fig. 10

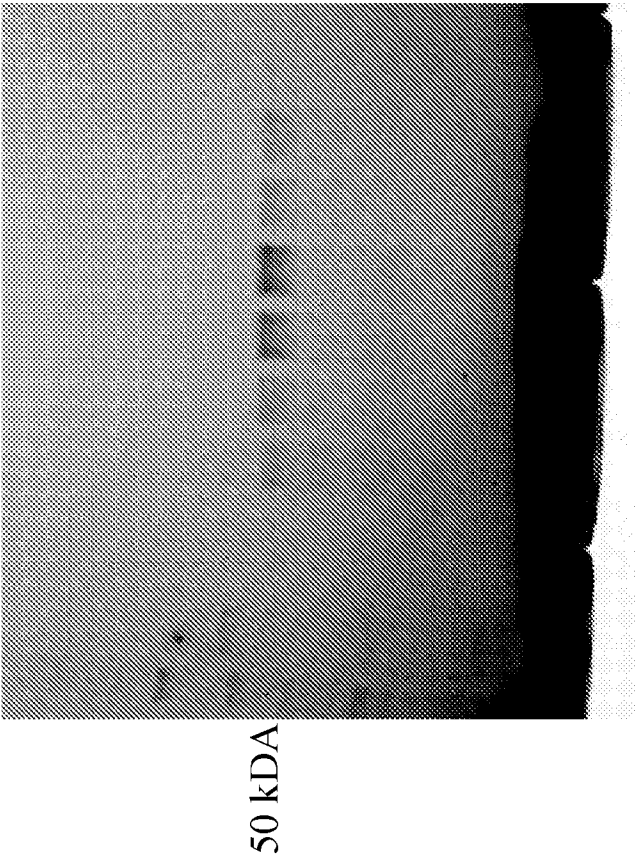


Fig. 11B

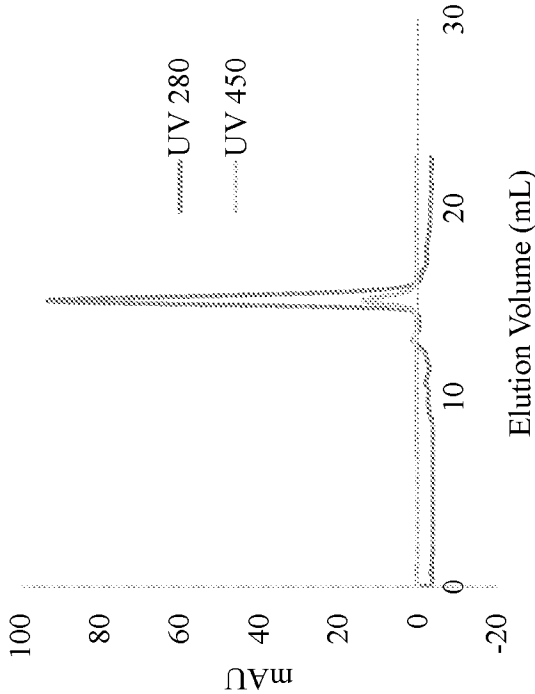


Fig. 11A

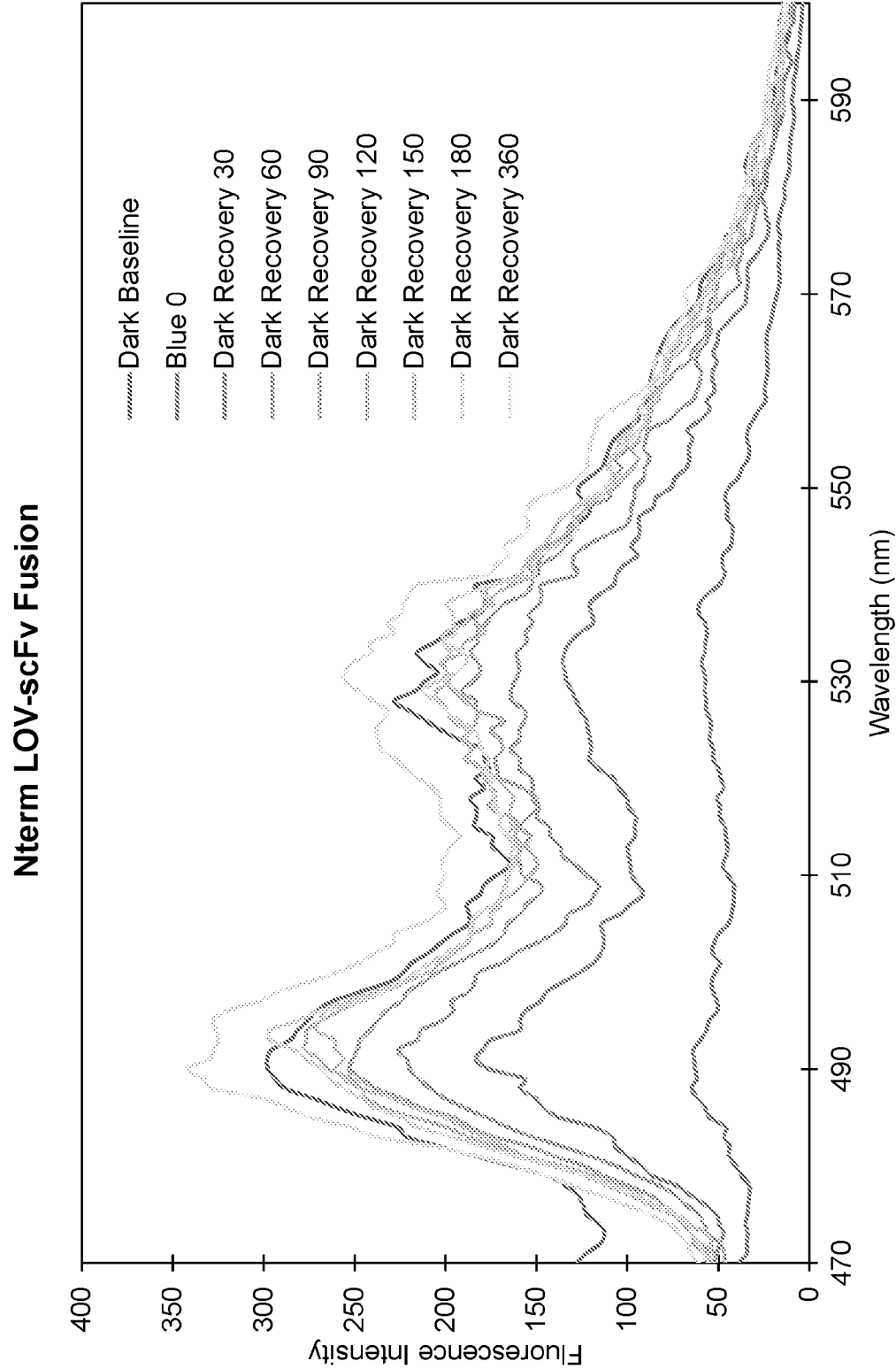


Fig. 12

ntLOV



Fig. 13

ntLOV

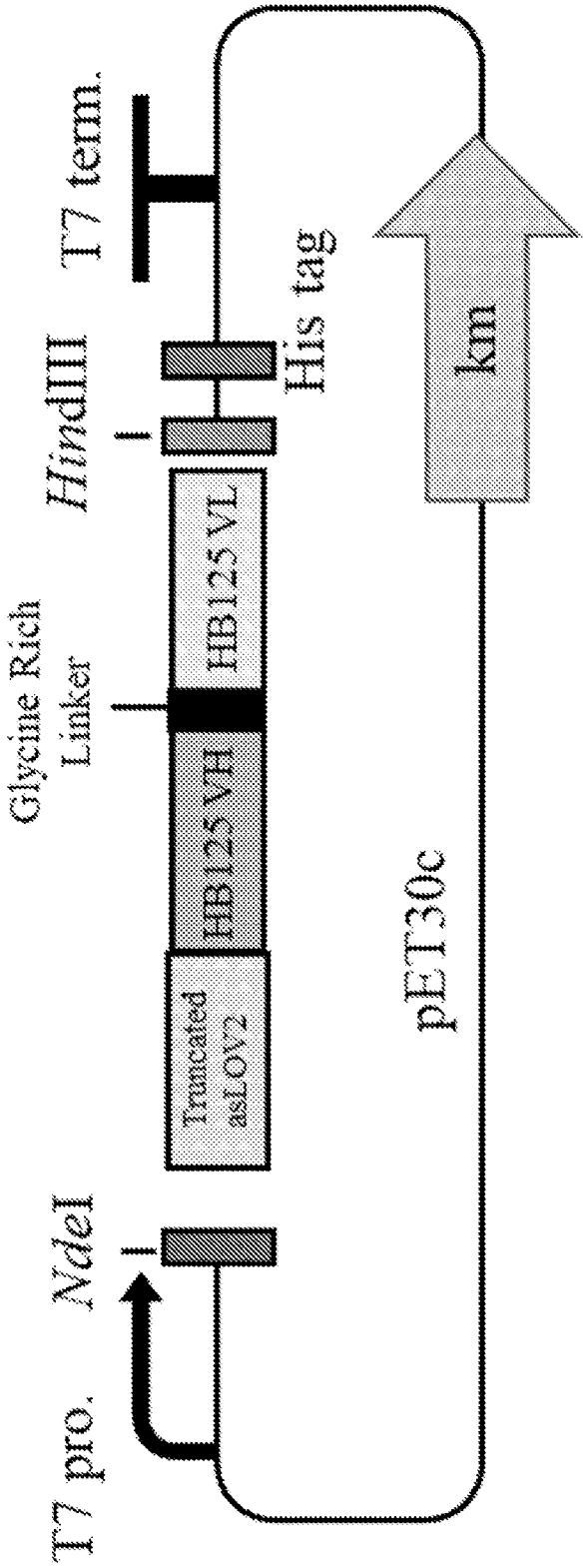


Fig. 14

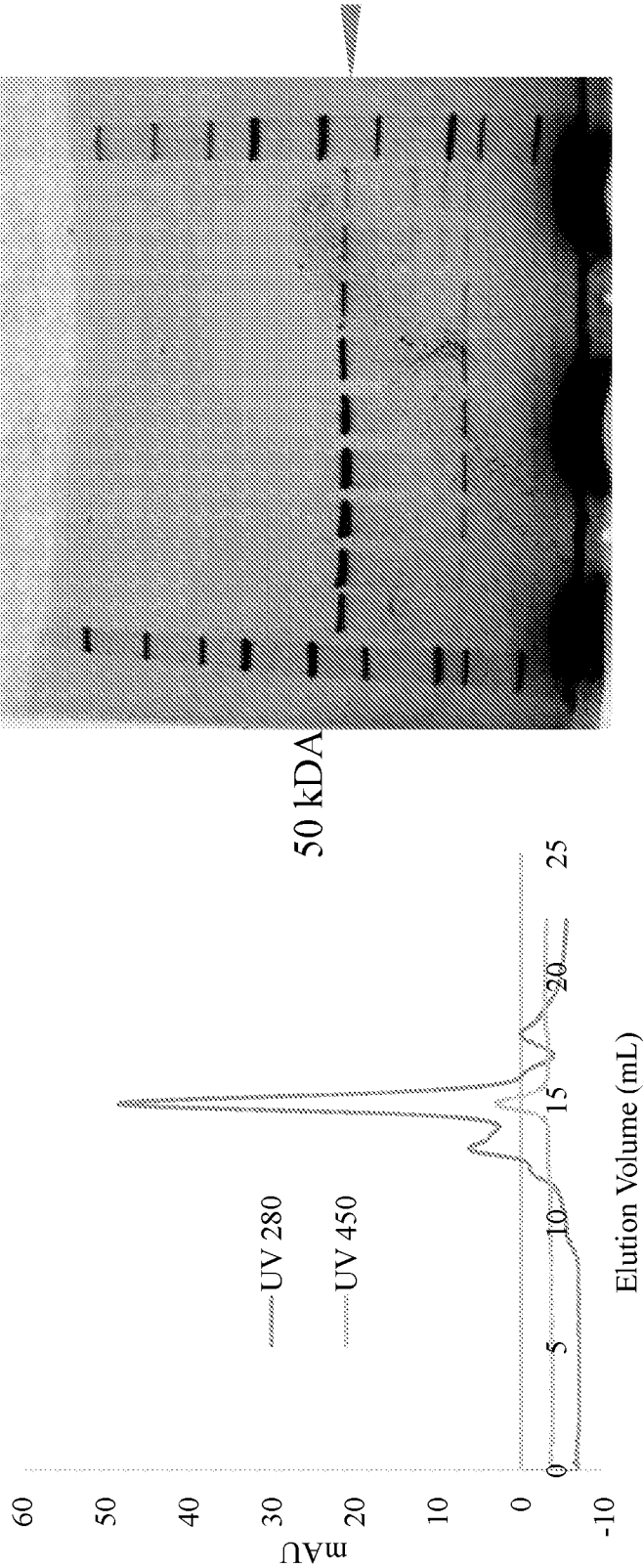


Fig. 15B

Fig. 15A

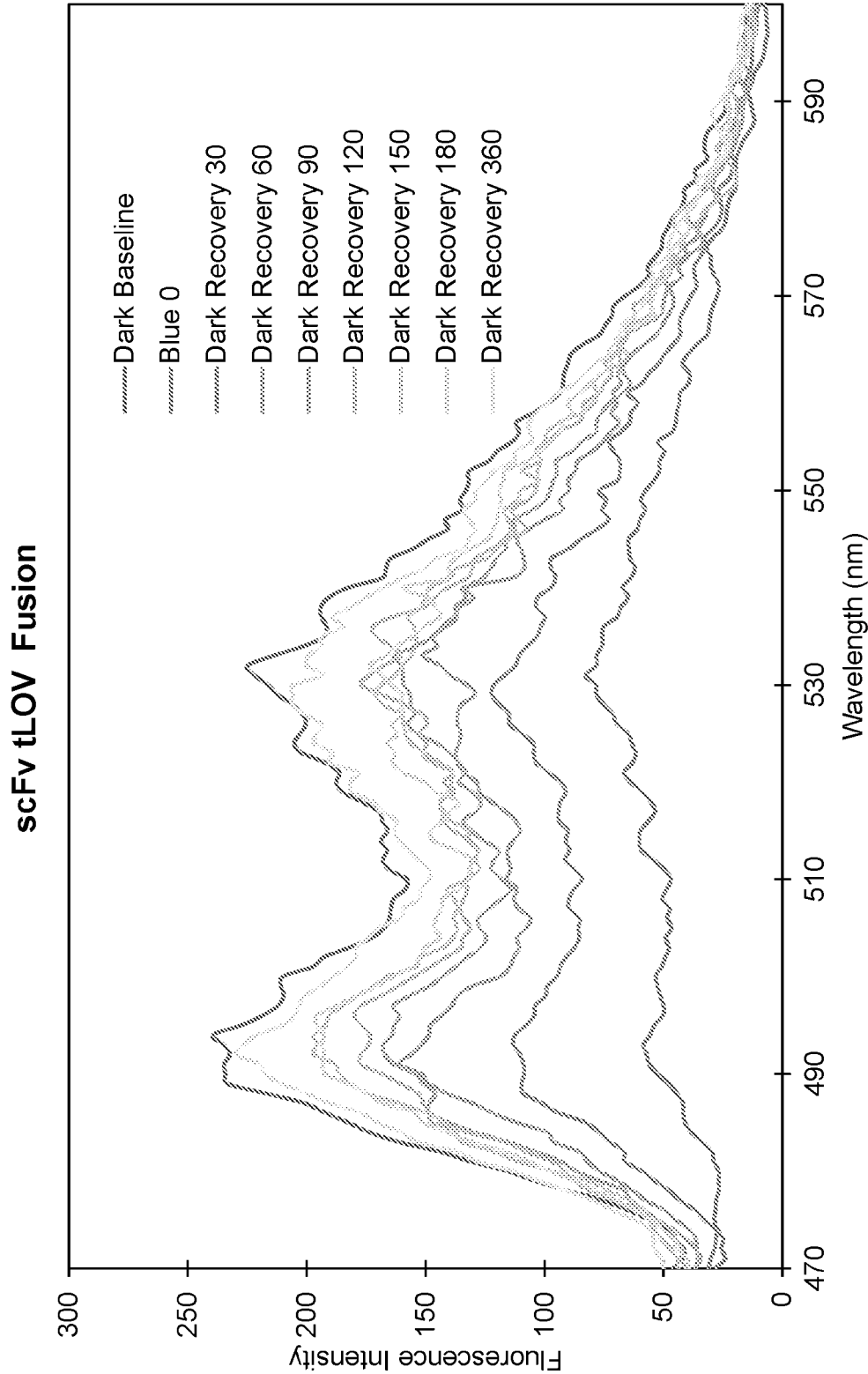


Fig. 16

gsLOV



Fig. 17

gsLOV

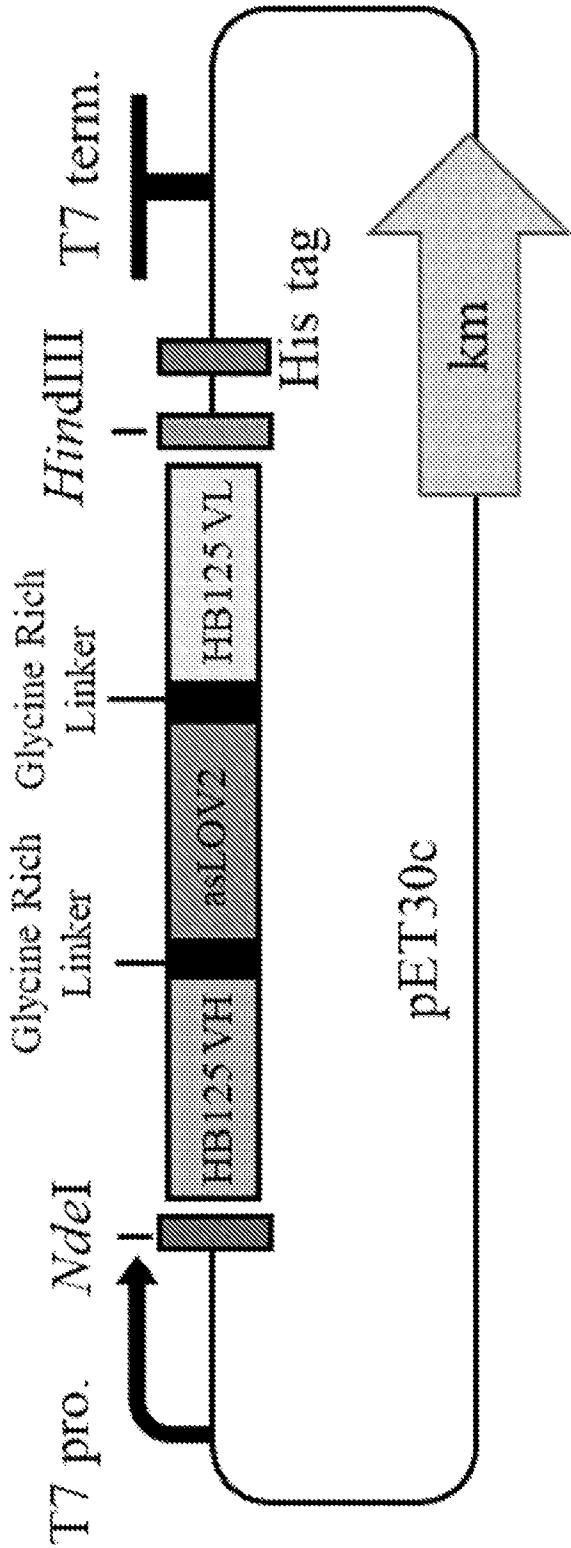


Fig. 18

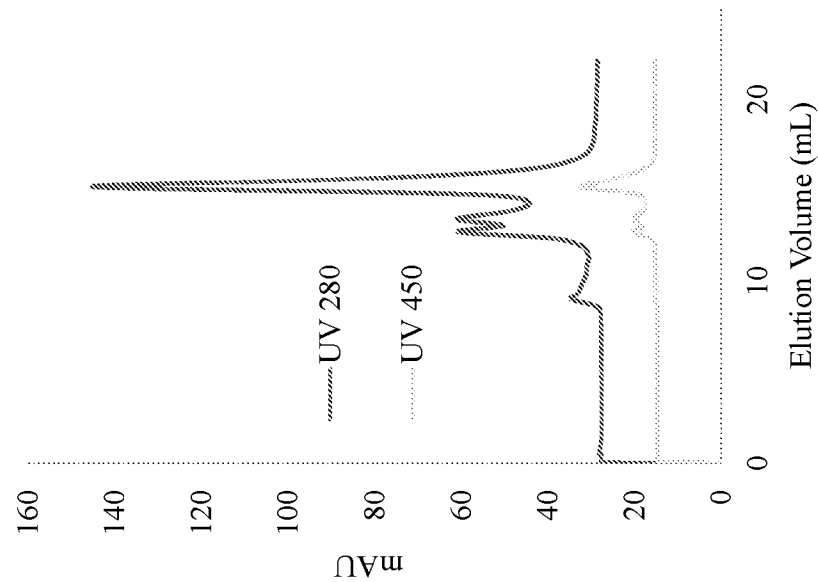


Fig. 19B

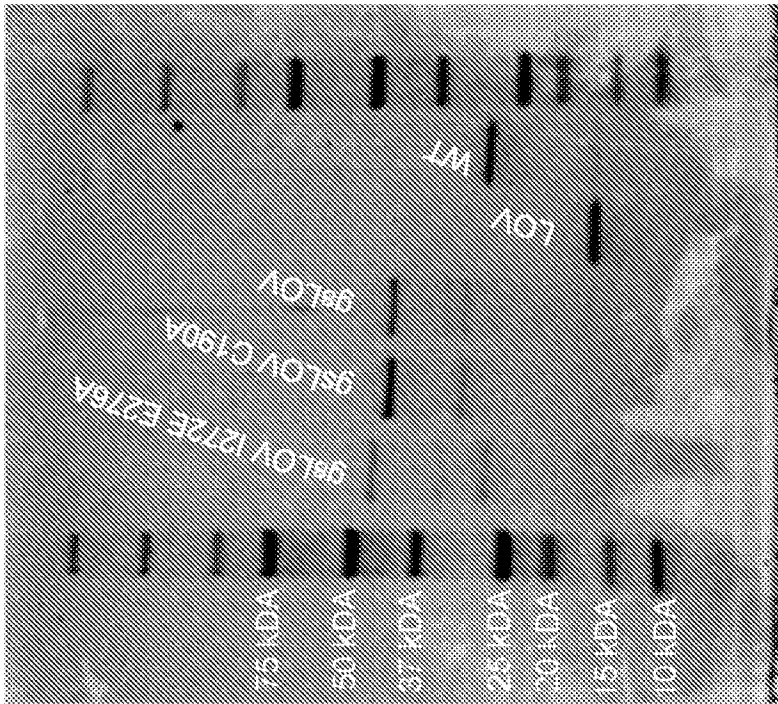


Fig. 19A

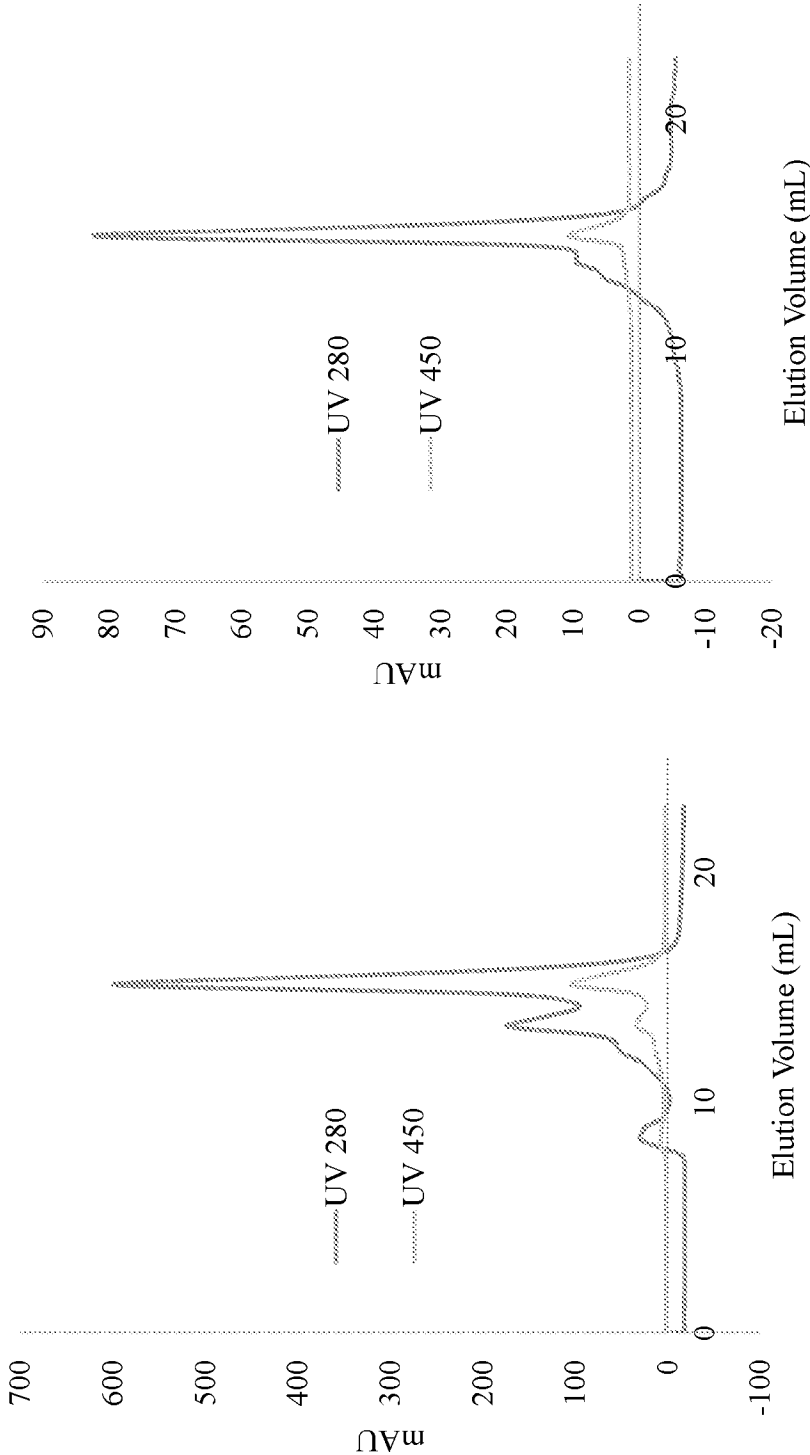


Fig. 19D

Fig. 19C

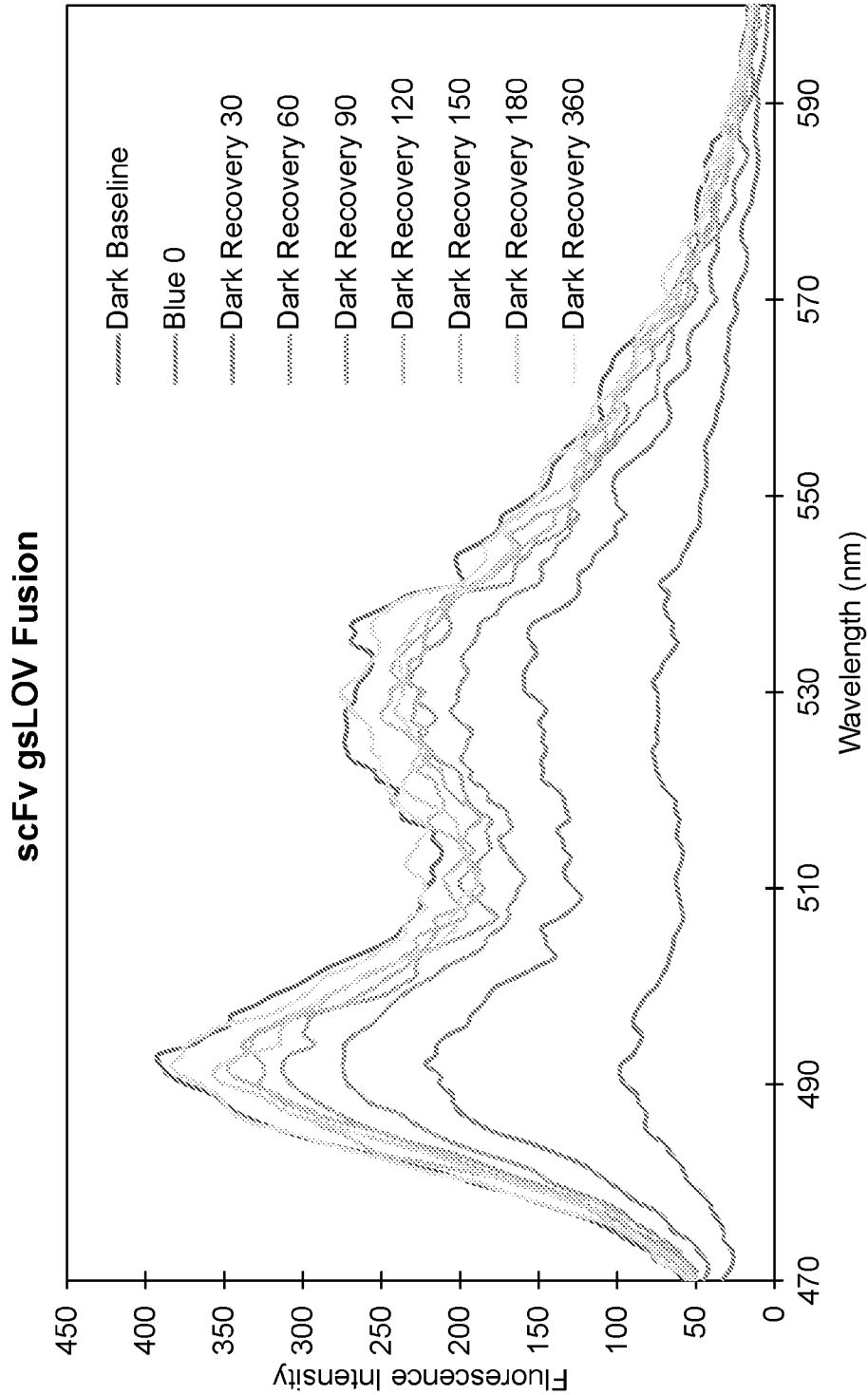


Fig. 20

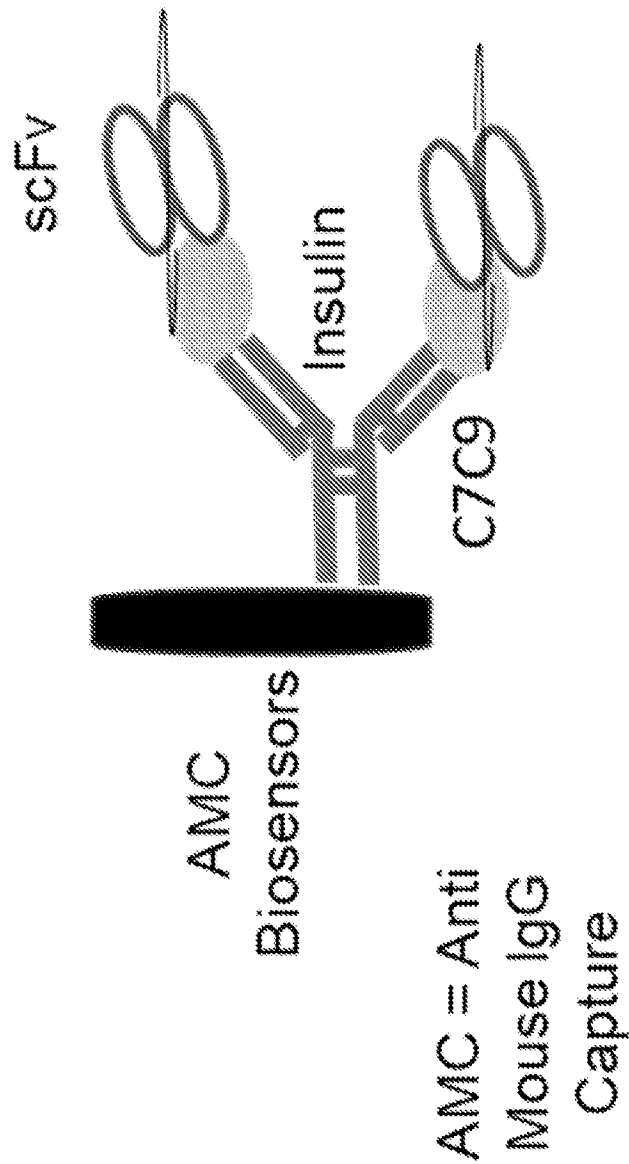


Fig. 21A

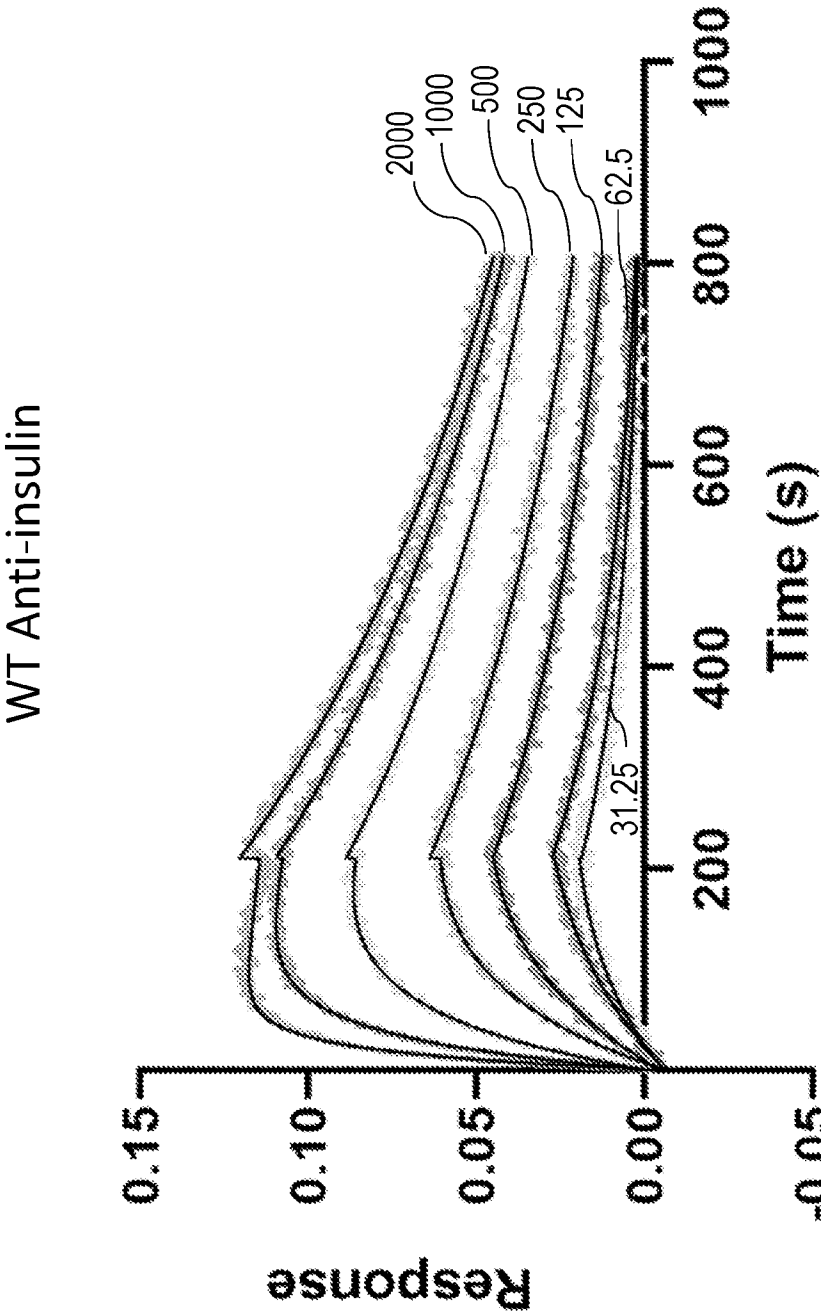


Fig. 21B

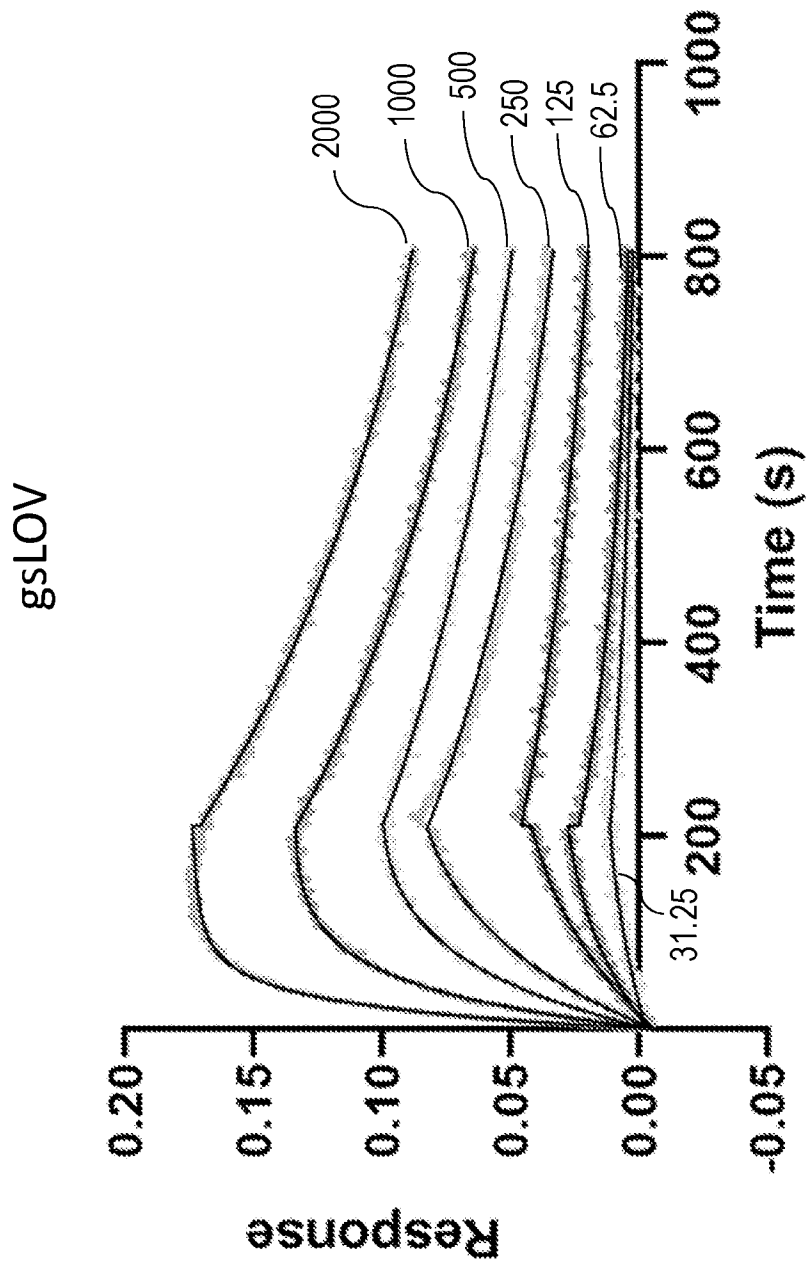


Fig. 21C

gsLOV I272E, A276E “light”

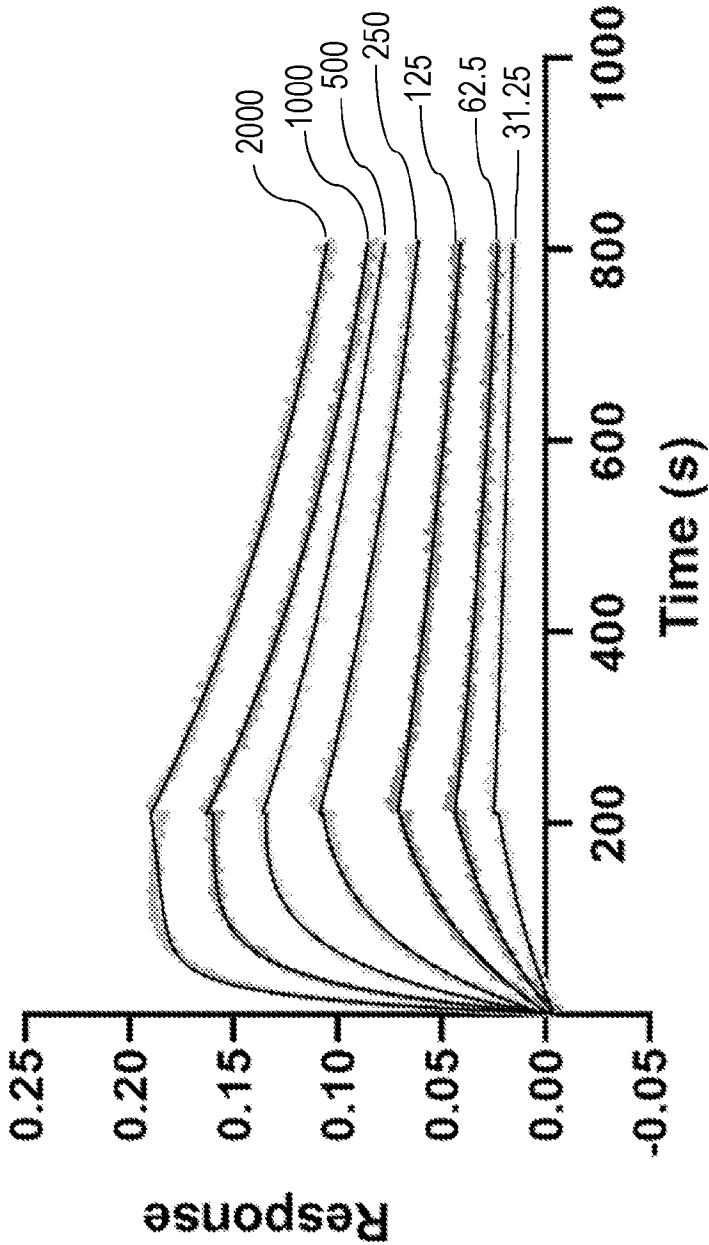


Fig. 21D

gsLOV C190A “dark”

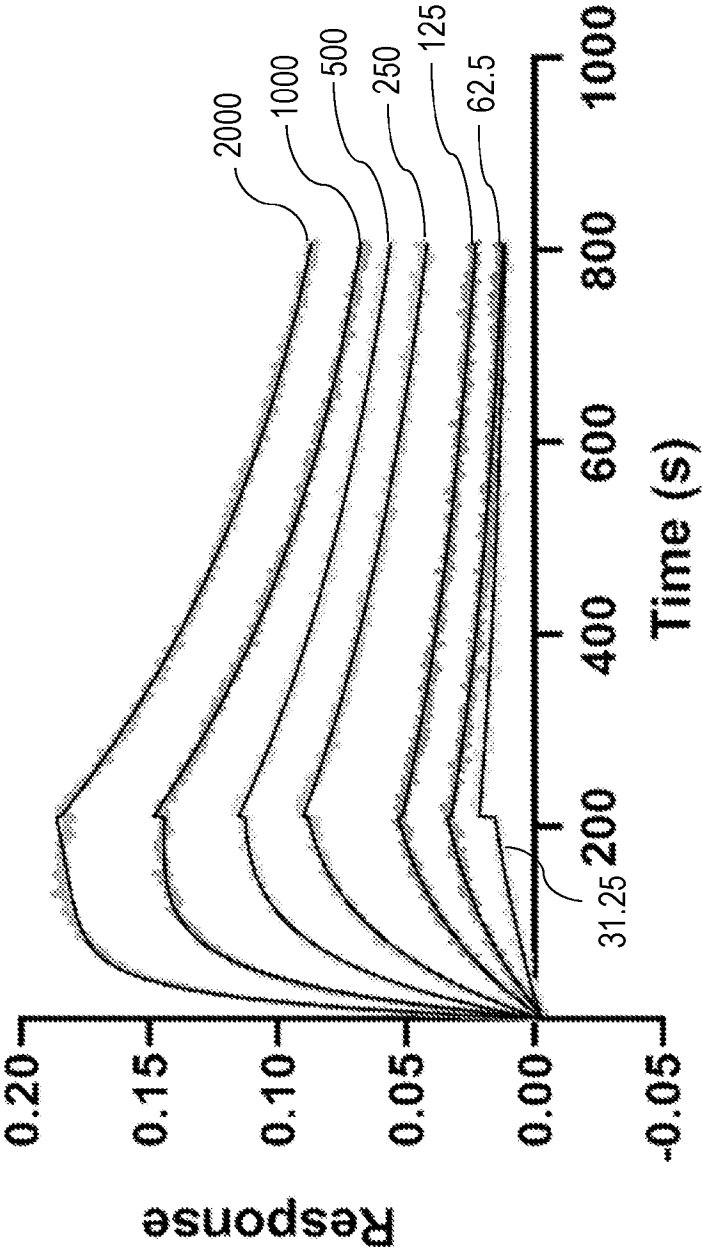


Fig. 21E

nolinkLOV

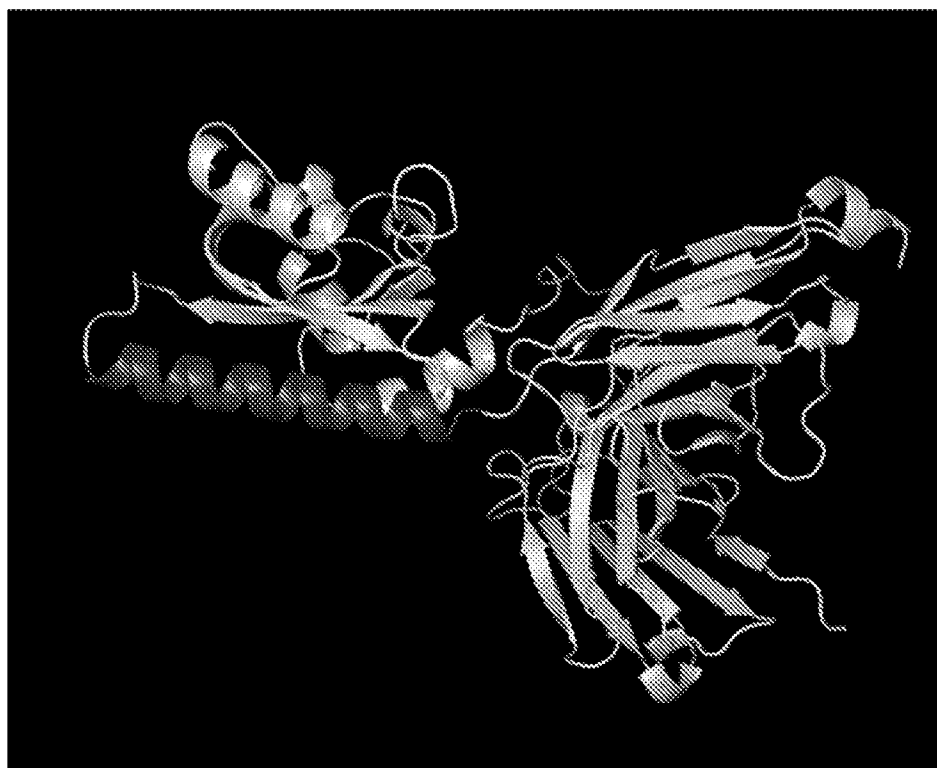


Fig. 22

nolinkLOV

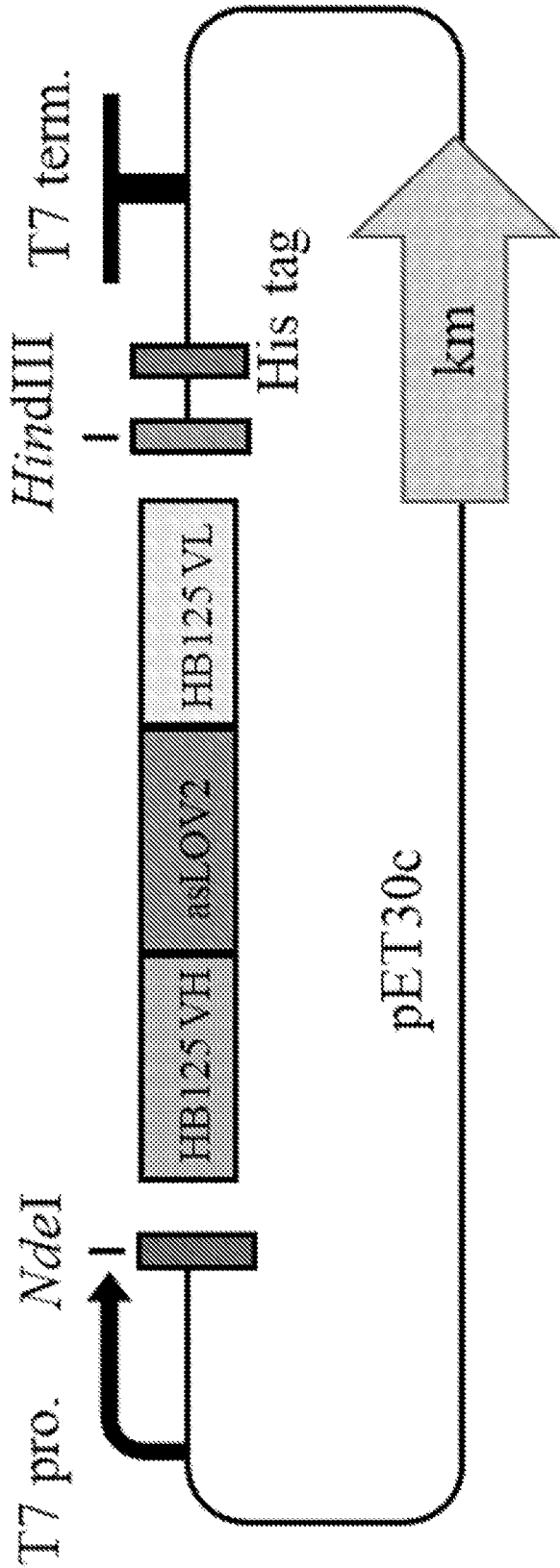


Fig. 23

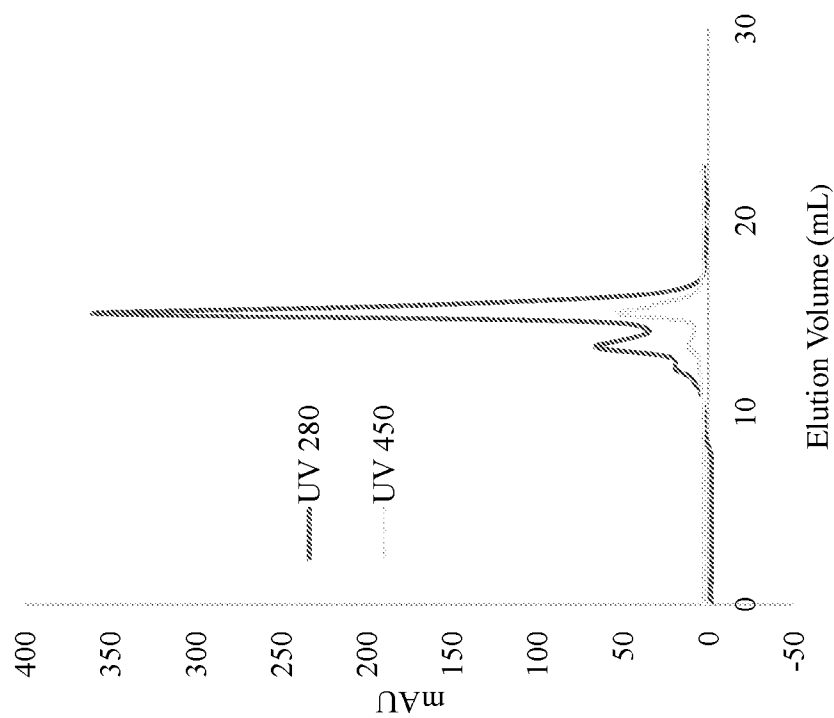


Fig. 24B

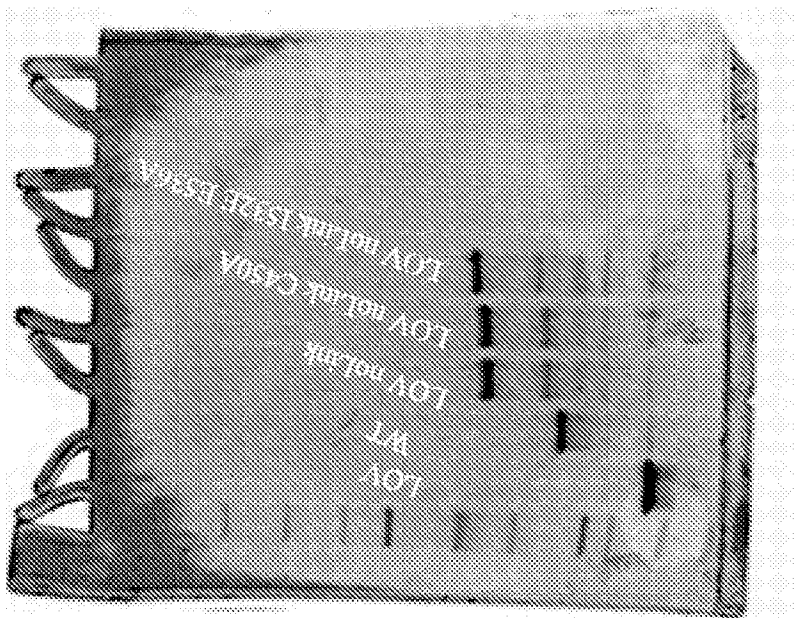


Fig. 24A

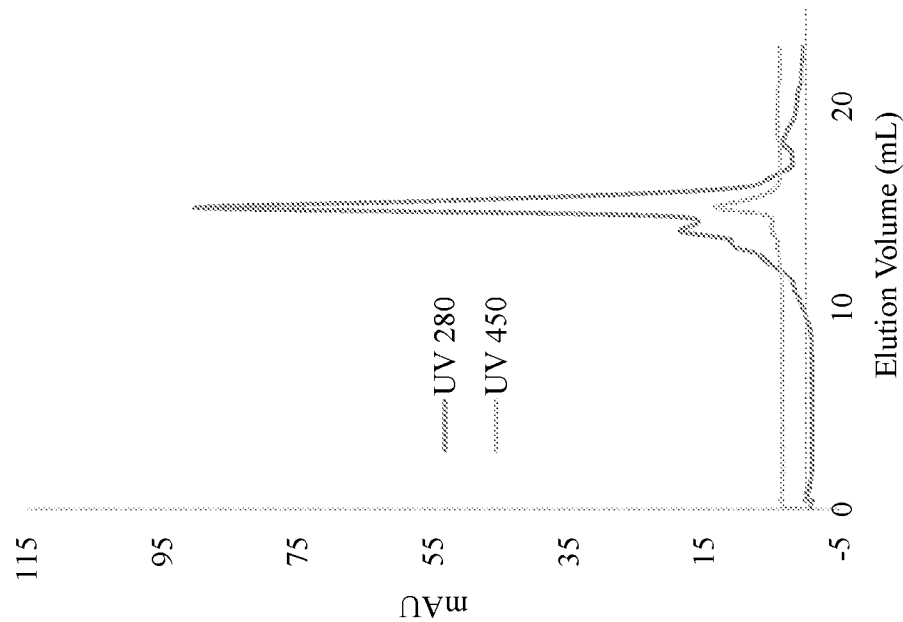


Fig. 24D

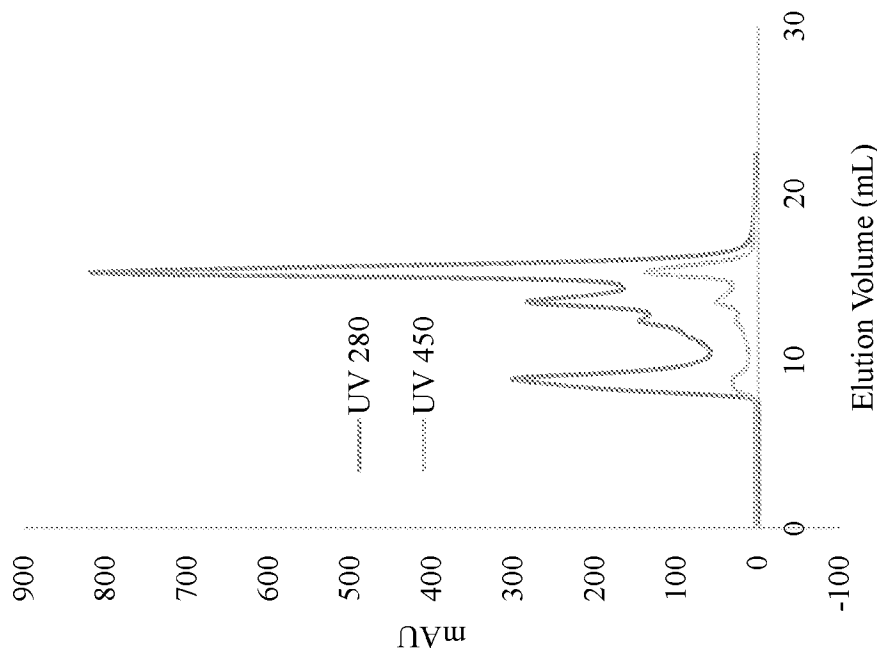


Fig. 24C

WT anti-insulin

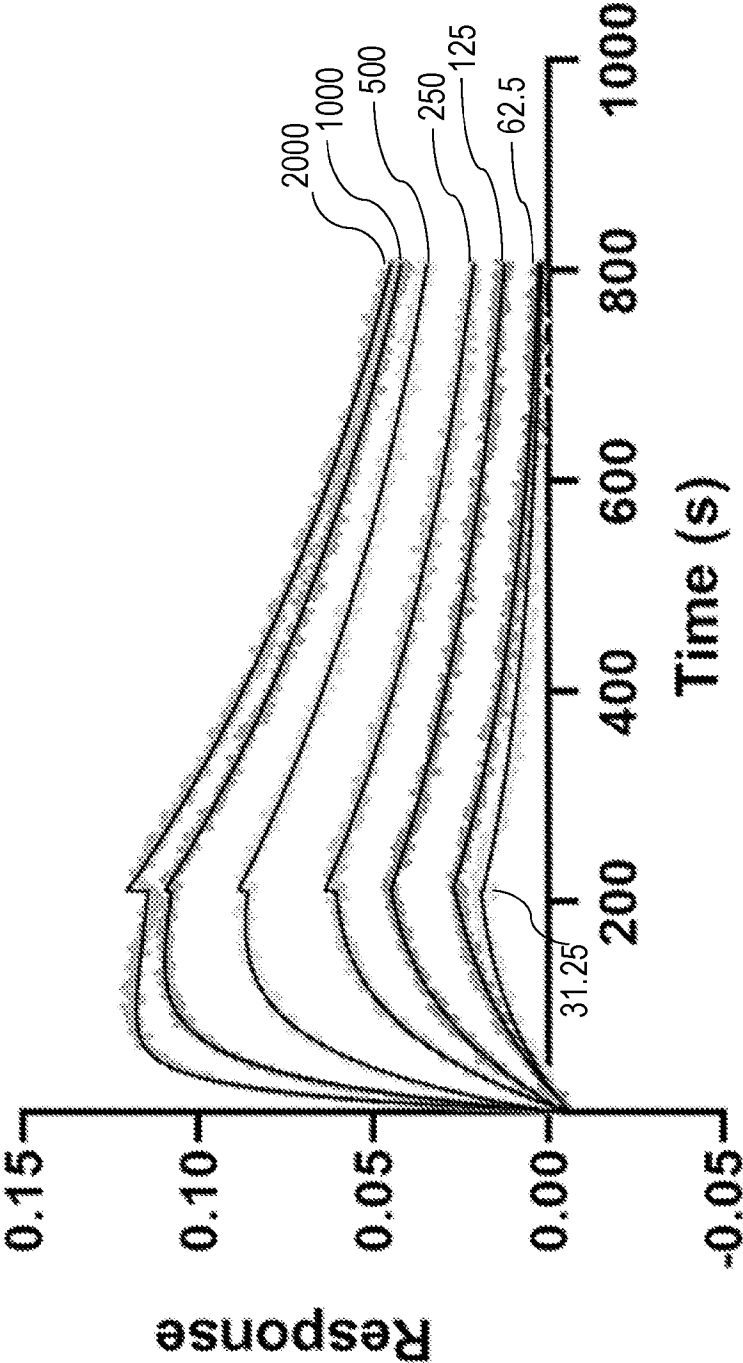


Fig. 25A

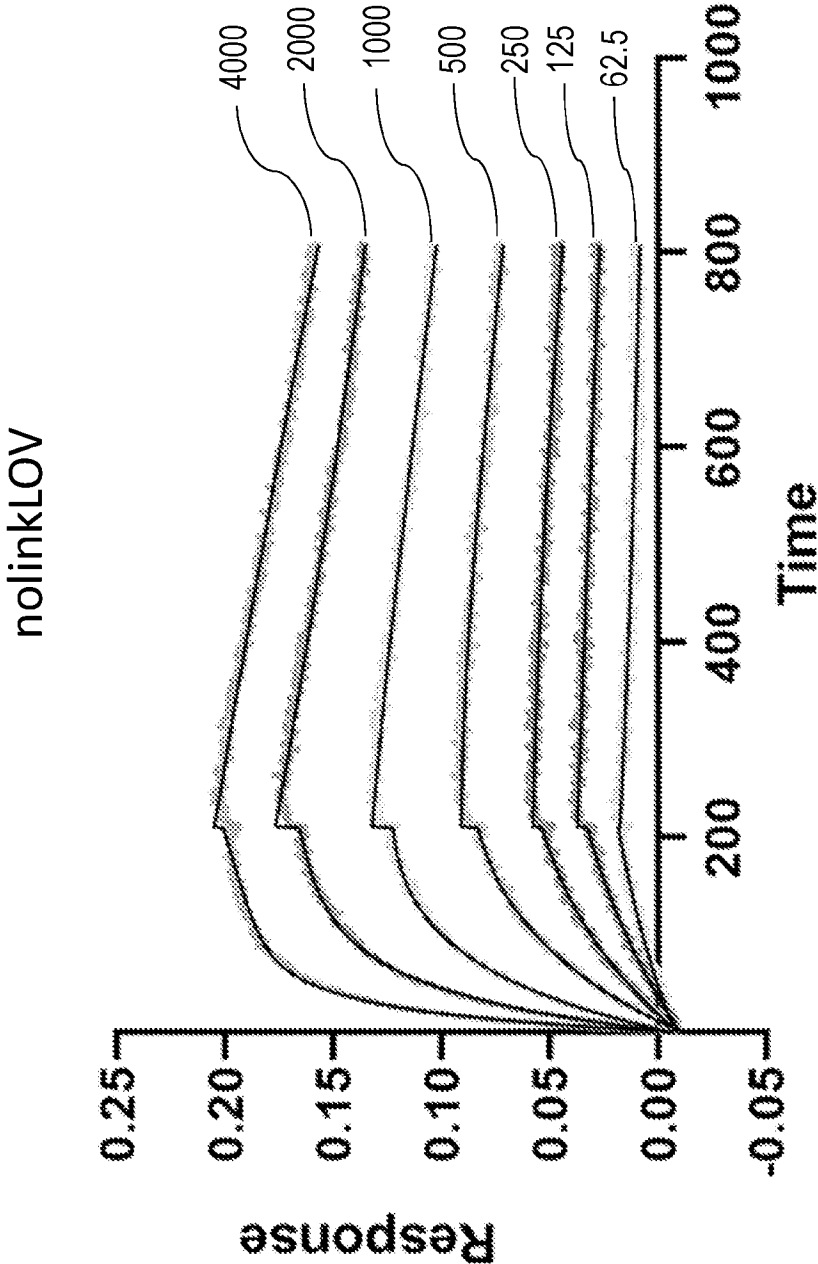


Fig. 25B

nolinkLOV C185A “dark”

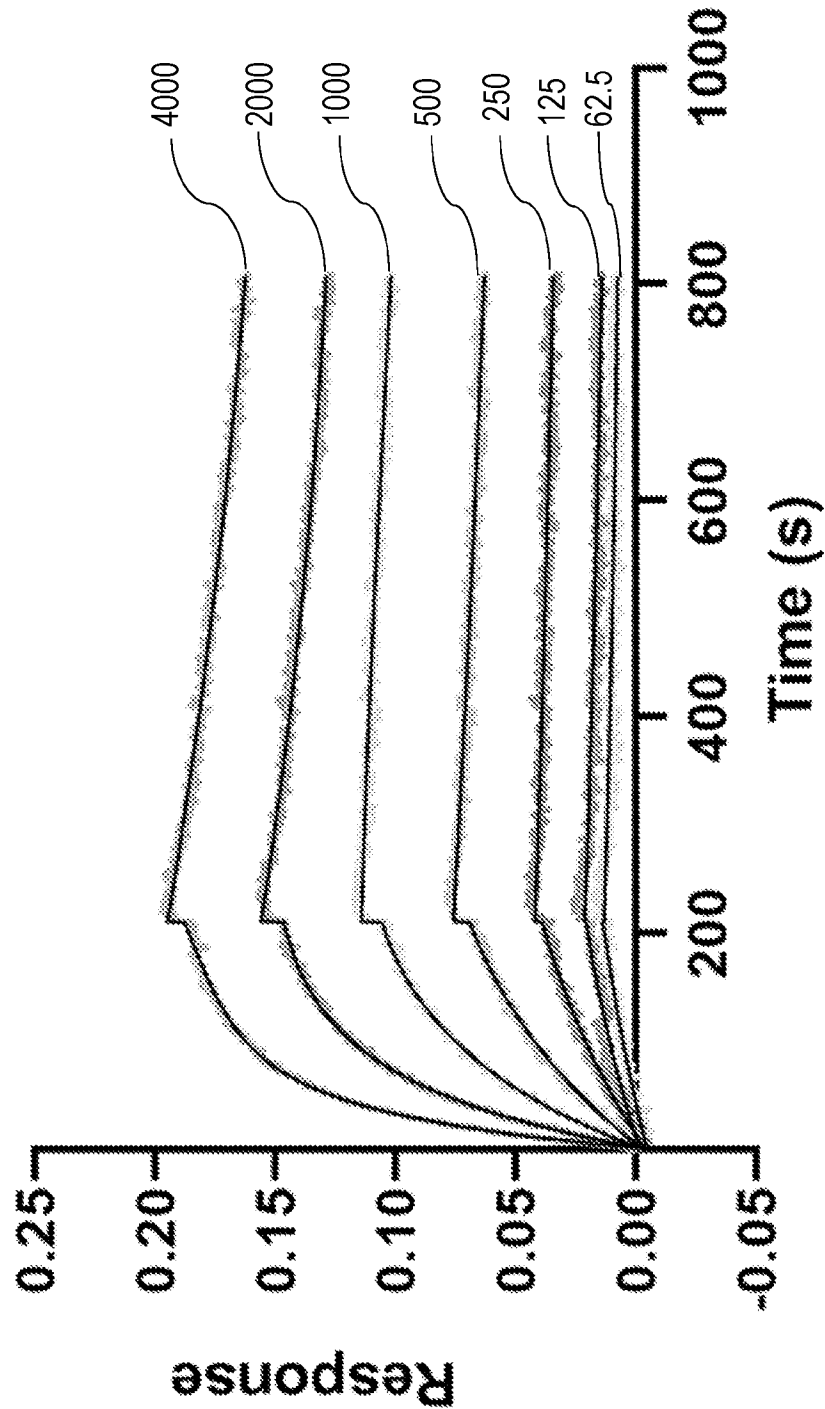


Fig. 25C

nolinkLOV I267E, A271E “light”

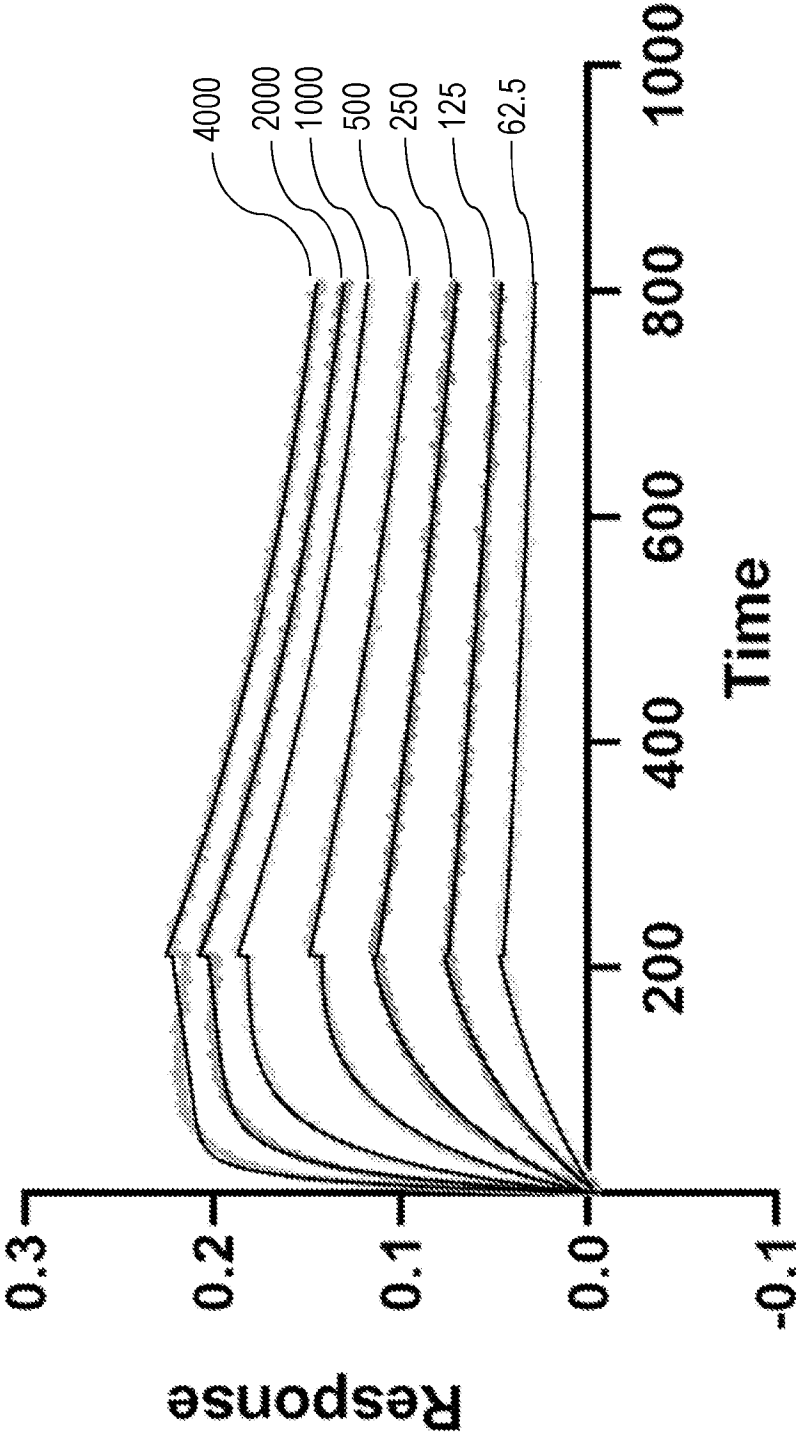


Fig. 25D

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/033094

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/00(2006.01); C07K 14/415(2006.01);

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K 16/00(2006.01); A61K 38/18(2006.01); A61K 38/19(2006.01); A61K 39/00(2006.01); A61K 47/69(2017.01);
C07K 14/47(2006.01); C07K 19/00(2006.01); C12N 9/10(2006.01); C12P 21/00(2006.01)

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

Korean utility models and applications for utility models
Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & Keywords: fusion protein, antibody, immunoglobulin, heavy chain, light chain, redox, domain,
external, amino acid, sequence**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 114867754 A (HOSPITAL FOR SICK CHILDREN et al.) 05 August 2022 (2022-08-05) abstract; claims 1, 2, 9; paragraphs [0237], [0337], [0397]	1-7
A	LUKYANOV, K. A. et al. "Genetically encoded fluorescent redox sensors", Biochimica et Biophysica Acta, 2014, Vol. 1840, pages 745-756 abstract; pages 745, 746	1-7
A	WO 2019-010224 A1 (TORQUE THERAPEUTICS, INC.) 10 January 2019 (2019-01-10) abstract; claims 1, 14-16, 40-45	1-7
A	US 10738338 B2 (THE RESEARCH FOUNDATION FOR THE STATE UNIVERSITY OF NEW YORK) 11 August 2020 (2020-08-11) abstract; claims 9, 11, 12, 15-17, 21	1-7
A	CN 110114369 A (VETOQUINOL SA) 09 August 2019 (2019-08-09) abstract; claims 1, 8-11; paragraph [0026]	1-7



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

07 October 2024

Date of mailing of the international search report

07 October 2024

Name and mailing address of the ISA/KR

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Telephone No. +82-42-481-5373

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/033094

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KR 10-2010-0099249 A (UNIVERSITY OF MEDICINE AND DENTISTRY OF NEW JERSEY) 10 September 2010 (2010-09-10) the whole document	1-7

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/033094

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)),
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/033094

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: **9-13, 16, 17, 19, 21, 23, 25, 27, 30, 32, 34, 35, 38, 42, 43, 45, 47**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims 9-13, 16, 17, 19, 21, 23, 25, 27, 30, 32, 34, 35, 38, 42, 43, 45, 47 are unclear since they are referring to the multiple dependent claims which do not comply with PCT Rule 6.4(a).
3. ☒ Claims Nos.: **8, 14, 15, 18, 20, 22, 24, 26, 28, 29, 31, 33, 36, 37, 39-41, 44, 46**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/US2024/033094

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
CN	114867754	A	05 August 2022	EP	4007779	A1	08 June 2022
				EP	4007779	A4	06 September 2023
				JP	2022-543070	A	07 October 2022
				KR	10-2022-0107151	A	02 August 2022
				US	2023-0145060	A1	11 May 2023
				WO	2021-016724	A1	04 February 2021
WO	2019-010224	A1	10 January 2019	CN	111093689	A	01 May 2020
				CN	111094330	A	01 May 2020
				EP	3648786	A1	13 May 2020
				EP	3648786	A4	15 December 2021
				EP	3649147	A2	13 May 2020
				EP	3649147	A4	14 April 2021
				EP	3675892	A1	08 July 2020
				EP	3675892	A4	06 October 2021
				JP	2020-530280	A	22 October 2020
				JP	2020-530440	A	22 October 2020
				KR	10-2020-0037781	A	09 April 2020
				US	11471490	B2	18 October 2022
				US	2020-0306301	A1	01 October 2020
				US	2020-0330514	A1	22 October 2020
				US	2021-0017247	A1	21 January 2021
				WO	2019-010219	A1	10 January 2019
				WO	2019-010222	A2	10 January 2019
				WO	2019-010222	A3	07 March 2019
US	10738338	B2	11 August 2020	US	2018-0112245	A1	26 April 2018
				US	2020-0377920	A1	03 December 2020
CN	110114369	A	09 August 2019	EP	3526246	A1	21 August 2019
				US	2020-0181258	A1	11 June 2020
				WO	2018-073185	A1	26 April 2018
KR	10-2010-0099249	A	10 September 2010	CN	101511181	A	19 August 2009
				CN	101511181	B	21 August 2013
				CN	101932609	A	29 December 2010
				CN	101932609	B	23 March 2016
				CN	103275980	A	04 September 2013
				CN	103275980	B	09 September 2015
				CN	104066452	A	24 September 2014
				EP	2037737	A2	25 March 2009
				EP	2037737	B1	02 April 2014
				EP	2215126	A2	11 August 2010
				EP	2471809	A1	04 July 2012
				EP	2471809	B1	02 September 2015
				EP	2471815	A1	04 July 2012
				EP	2471815	B1	30 March 2016
				EP	2753363	A2	16 July 2014
				JP	2009-543551	A	10 December 2009
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				JP	5248494	B2	31 July 2013
				KR	10-1413005	B1	02 July 2014
				KR	10-1573009	B1	02 December 2015

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/US2024/033094

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)
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		KR 10-2014-0057283 A	12 May 2014
		US 2009-0208473 A1	20 August 2009
		US 2009-0318348 A1	24 December 2009
		US 2011-0202033 A1	18 August 2011
		US 2011-0287004 A1	24 November 2011
		US 2011-0287015 A1	24 November 2011
		US 2012-0309051 A1	06 December 2012
		US 2015-0110778 A1	23 April 2015
		US 7981866 B2	19 July 2011
		US 9139630 B2	22 September 2015
		US 9458465 B2	04 October 2016
		WO 2008-054561 A2	08 May 2008
		WO 2009-073808 A2	11 June 2009
		WO 2013-036610 A2	14 March 2013