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(71) Applicant *(for all designated States except US)*: **ST. LOUIS UNIVERSITY** [US/US]; 221 North Grand Boulevard, St. Louis, MO 63103 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **KAMINSKI, Henry** [US/US]; c/o St. Louis University, 221 North Grand Boulevard, St. Louis, MO 63103 (US). **KUSNER, Linda** [US/US]; c/o St. Louis University, 221 North Grand Boulevard, St. Louis, MO 63103 (US). **SATIJA, Namita** [US/US]; c/o St. Louis University, 221 North Grand Boulevard, St. Louis, MO 63103 (US).

(74) Agent: **KAZMIERSKI, Steven, T.**; Armstrong Teasdale LLP, 7700 Forsyth Blvd., Suite 1800, St. Louis, MO 63105 (US).

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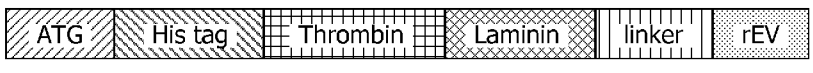
(54) Title: TARGETING THE NEUROMUSCULAR JUNCTION FOR TREATMENT

FIG. 1



(57) Abstract: Compositions and methods for targeting therapeutic agents to neuromuscular junctions are disclosed. Also disclosed are methods for treating diseases and conditions affecting the neuromuscular junction. Compositions include a neuromuscular junction targeting peptide coupled to a therapeutic agent. Compositions may further include a linker peptide. Methods for targeting therapeutic agents to neuromuscular junctions and treating diseases and conditions affecting the neuromuscular junction include administering a composition including a neuromuscular junction targeting peptide coupled to a therapeutic agent.

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TARGETING THE NEUROMUSCULAR JUNCTION FOR TREATMENT

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to provisional patent application no. 61/498,707, filed on June 20, 2011, which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under Grant Award No. EY14837 awarded by the National Eye Institute of the National Institutes of Health. The government may have certain rights in the invention.

INCORPORATION OF SEQUENCE LISTING

[0003] A paper copy of the Sequence Listing and a computer readable form of the sequence containing the file named "31065-30_ST25.txt", which is 32,205 bytes in size (as measured in MS-DOS), are provided herein and are herein incorporated by reference. This Sequence Listing consists of SEQ ID NOs: 1 - 35.

BACKGROUND OF THE DISCLOSURE

[0004] The present disclosure relates generally to compositions and methods for targeting therapeutic agents. More particularly, the present disclosure relates to compositions and methods useful for targeting therapeutic agents to the neuromuscular junction using neuromuscular junction targeting peptides.

[0005] The neuromuscular junction is the point at which nerve signals the muscle to contract. More particularly, the neuromuscular junction is the synapse or junction of the axon terminal of a neuron with a muscle fiber plasma membrane.

[0006] Several diseases involve the neuromuscular junction as the primary site of injury. For example, myasthenia gravis is an autoimmune disorder that is caused by autoantibodies directed primarily toward the skeletal muscle acetylcholine receptor (AChR) at the neuromuscular junction. The antibodies bind to the post-synaptic surface of the neuromuscular junction and produce a reduction in AChR number and damage the muscle endplate, which leads to a failure of neuromuscular transmission that results in muscle weakness.

Another category of gravis is caused by antibodies against muscle specific kinase ("MuSK") at the neuromuscular junction. Lambert-Eaton syndrome is yet another disorder characterized by the attack of voltage-gated calcium channels at the neuromuscular junction by antibodies. Miller Fischer syndrome is another disorder involving the attack of nerve terminals by antibodies.

[0007] The complement system may underlie one effector mechanism for antibody-mediated immunity, which begins with antibody binding to a cell surface antigen and the formation of a membrane attack complex. The membrane attack complex is a multimeric protein complex that produces cell lysis and, in the case of myasthenia gravis, destruction of the neuromuscular junction. In antibody-initiated activation of the complement cascade, nascent C4b and C3b fragments condense with free hydroxyl and amino groups on biological membranes. Once bound, these fragments serve as sites for assembly of C4b2a and C3bBb, the central amplification enzymes of the cascade. Control of their activities to protect host tissues from autologous complement-mediated injury is through a system of cell-associated and serum regulatory proteins.

[0008] Complement inhibitors are a class of drugs that show promise for treating neuromuscular diseases. Complement inhibitors may stop the body's immune response system from attacking itself. Eculizumab, for example, is an anti-C5 antibody that is approved for use in paroxysmal nocturnal hemoglobinuria and in Phase 2 trials as a treatment for myasthenia gravis. Eculizumab functions by inhibiting complement. Because administration occurs by infusion, the agent may inhibit complement throughout the body.

[0009] Another complement inhibitor is rEV576 (OmCI or Conversin). rEV576 is an 18.5 kDa recombinantly produced protein derived from tick (*Ornithodoros moubata*) saliva that specifically inhibits C5 complement. rEV576 appears to directly bind C5 to prevent interaction with C5 convertase. Administration of rEV576 has been shown to reduce serum complement activity, diminish C9 deposition at the neuromuscular junction, and reduce cytotoxicity of serum from treated animals.

[0010] Therapies for myasthenia gravis generally focus on enhancing neuromuscular transmission by inhibition of cholinesterase using agents such as pyridostigmine. Other treatments such as corticosteroids, azathioprine, tacrolimus, and mycophenolate, are directed to suppressing or modulating the immune system. Acute exacerbations of weakness may be treated

by plasmapheresis or intravenous immunoglobulins. While effective, these treatments can be expensive and may entail side affects that affect organ systems beyond the neuromuscular junction. These treatments may additionally result in systemic side effects because administration occurs throughout the body. The immunotherapies are not specifically focused on myasthenia gravis, but rather generally moderate immune response through reduction of autoantibody levels directly or indirectly through suppression of B and T cell activity.

[0011] Although treatments are available for conditions resulting from neuromuscular junction injury, there remains a concern over their efficacy, side-effects, and/or costs. Moreover, complement inhibitor strategies rely on systemic inhibition of complement. Accordingly, there exists a continued need to develop alternative treatments and methods for treating conditions resulting from neuromuscular junction injury.

SUMMARY OF THE DISCLOSURE

[0012] The present disclosure relates generally to compositions and methods for targeting therapeutic agents. More particularly, the present disclosure relates to compositions and methods useful for targeting therapeutic agents to the neuromuscular junctions using neuromuscular junction targeting peptides.

[0013] In one aspect, the present disclosure is directed to compositions including a neuromuscular junction targeting peptide coupled to a therapeutic agent.

[0014] In another aspect, the present disclosure is directed to a method of delivering a therapeutic agent to a neuromuscular junction. The method includes administering a composition that includes a neuromuscular junction targeting peptide coupled to a therapeutic agent.

[0015] In another aspect, the present disclosure is directed to methods for treating a neuromuscular junction-related disease or condition. The method includes administering to a subject in need thereof a composition that includes a neuromuscular junction targeting peptide coupled to a therapeutic agent.

[0016] In another aspect, the present disclosure is directed to a recombinant nucleic acid construct encoding a neuromuscular junction targeting peptide operably linked to a heterologous nucleic acid sequence encoding a therapeutic polypeptide.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] The disclosure will be better understood, and features, aspects and advantages other than those set forth above will become apparent when consideration is given to the following detailed description thereof. Such detailed description makes reference to the following drawings, wherein:

[0018] FIG. 1 is a schematic showing the domain structure of the laminin-rEV polypeptide as described in Example 1.

[0019] FIG. 2 is a map of the pET28a expression vector.

[0020] FIG. 3 is an SDS-gel showing the expression of the laminin-rEV polypeptide in BL21 as described in Example 1.

[0021] FIG. 4 is a Western blot of purified rEV, expressed rEV from induced cells, expressed laminin-rEV (LrEV) from induced cells, and uninduced cells.

[0022] FIG. 5 is a schematic showing the domain structure of the HIV-rEV polypeptide as described in Example 2.

[0023] FIG. 6 is a schematic showing the domain structure of the RVG-rEV polypeptide as described in Example 3.

[0024] FIG. 7 is a schematic showing the construction of the pET16b-scFv-DAF expression vector as described in Example 4.

[0025] FIG. 8 is a photomicrograph showing GFP expression in BHK-21 cells transfected with the IgGsp-V_H-V_L as described in Example 4.

[0026] FIG. 9 is an SDS-gel showing the expression and purification of scFv-DAF as described in Example 4.

[0027] FIG. 10 is a graph showing the specificity binding of scFv-DAF and scFv1956 to hAChRα1-210 polypeptides as described in Example 5.

[0028] FIG. 11 is a graph showing complement-mediated haemolysis of sheep erythrocytes incubated with scFv-DAF as described in Example 6.

[0029] FIG. 12 are photomicrographs showing localization of the scFv-35-DAF to the neuromuscular junction in diaphragms of mice as described in Example 7.

[0030] FIG. 13 shows immunofluorescence micrographs showing scFv-DAF reduction of C3 deposits on TE671 cells as described in Example 8.

[0031] FIG. 14 is a schematic showing the domain structures of various neuromuscular junction targeting constructs according to the present disclosure.

[0032] FIG. 15 is a graph depicting the therapeutic effect of scFv and scFv-DAF in EAMG mice as described in Example 9.

[0033] FIG. 16 is a graph is a graph depicting the therapeutic effect of scFv and scFv-DAF in EAMG Lewis rats as described in Example 9.

[0034] While the disclosure is susceptible to various modifications and alternative forms, specific embodiments thereof have been shown by way of example in the drawings and are herein described below in detail. It should be understood, however, that the description of specific embodiments is not intended to limit the disclosure to cover all modifications, equivalents and alternatives falling within the spirit and scope of the disclosure as defined by the appended claims.

DETAILED DESCRIPTION

[0035] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure belongs. Although any methods and materials similar to or equivalent to those described herein can be used in the practice or testing of the present disclosure, the preferred methods and materials are described below.

[0036] In accordance with the present disclosure, compositions and methods have been discovered that allow for targeting neuromuscular junctions. The compositions and methods have significant impact as they allow for the targeted delivery of therapeutic agents to the neuromuscular junction. The compositions and methods further allow for the treatment of neuromuscular junction-related diseases and conditions such as, for example, myasthenia gravis,

experimentally acquired myasthenia gravis, Lambert-Eaton syndrome, and Miller Fischer syndrome in which neuromuscular junctions may be affected.

Compositions

[0037] In one aspect, the present disclosure is directed to a composition including a neuromuscular junction targeting peptide ("NMJTP") coupled to a therapeutic agent ("TA") (see e.g., FIG. 14). The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues of any length, unless indicated otherwise.

[0038] As used herein, "coupled to" refers to a composition wherein the neuromuscular junction targeting peptide is directly or indirectly attached to, fused with, joined to, and/or linked to the therapeutic agent. For example, if the composition is prepared using known recombinant protein expression methods, a nucleic acid sequence encoding the neuromuscular junction targeting peptide may be joined to a nucleic acid sequence encoding the therapeutic agent. In such an example, the neuromuscular junction targeting peptide would be directly coupled to the therapeutic agent. In another example, the nucleic acid sequence encoding the neuromuscular junction targeting peptide may be indirectly joined to a nucleic acid sequence encoding the therapeutic agent by including at least one linker between the neuromuscular junction targeting peptide and the therapeutic agent.

[0039] The compositions of the present disclosure may be prepared as part of a larger construct that is subjected to further processing to produce the final composition having the neuromuscular junction targeting peptide coupled to the therapeutic agent. Domain structures of larger constructs are illustrated in FIG. 14. In one embodiment, for example, the construct may include an ATG start site coupled to an affinity tag coupled to a protease cleavage site ("Protease Site") coupled to a neuromuscular junction targeting peptide ("NMJTP") coupled to a linker coupled to a therapeutic agent ("TA") (see, FIG. 14). In another embodiment, the domain structure may include an ATG start site coupled to an affinity tag coupled to a protease cleavage site coupled to a NMJTP coupled to a linker coupled to a NMJTP coupled to a linker coupled to a TA (see, FIG. 14). Table 1 summarizes the neuromuscular junction targeting peptide indicated in FIG. 14 and their specificities (binding target).

Table 1. Neuromuscular Junction Targeting Peptides.

NMJTP	Specificity
McAb35	Muscle nicotinic acetylcholine receptor
AE-2	Acetylcholinesterase
AE-3	Acetylcholinesterase
C1B7	Acetylcholinesterase
HB-189	Neuronal acetylcholine receptor
HB8987	Neuronal acetylcholine receptor alpha subunit

[0040] The therapeutic agent may be coupled to either the N-terminus or C-terminus of the neuromuscular junction targeting peptide. After further processing as further described herein, for example, the composition may have the structure NMJTP-TA, in which the therapeutic agent is coupled to the C-terminus of the neuromuscular junction targeting peptide. In another example, the composition may have the structure TA-NMJTP after further processing, in which the therapeutic agent is coupled to the N-terminus of the neuromuscular junction targeting peptide. Similarly, the neuromuscular junction targeting peptide may be coupled to a linker at either the N-terminus, the C-terminus, or both the N-terminus and C-terminus of the neuromuscular junction targeting peptide (see, FIG. 14). When a linker is included in the composition, the linker is positioned between the neuromuscular junction targeting peptide and the therapeutic agent. In one embodiment, the composition may have the structure: NMJTP-linker-TA after further processing. In another embodiment, the composition may have the structure: TA-linker-NMJTP after further processing. In yet another embodiment, the composition may have the structure: NMJTP-linker-NMJTP-linker-TA after further processing. In still another embodiment, the composition may have the structure: TA-linker-NMJTP-linker-NMJTP after further processing.

[0041] As described herein, the composition may be subjected to further processing. For example, the presence of an affinity tag allows for purification via interaction between the affinity tag and an affinity substrate as known by those skilled in the art and described herein. The composition may also be subjected to treatment with a protease that cleaves the composition at a position between the affinity tag and the neuromuscular junction targeting peptide. Protease treatment allows for the preparation of a composition including a neuromuscular junction targeting peptide and a therapeutic agent, which may further include at least one linker.

Neuromuscular Junction Targeting Peptides

[0042] The composition of the present disclosure includes a neuromuscular junction targeting peptide ("NMJTP"). The neuromuscular junction targeting peptide may be a peptide molecule that binds to or interacts with a molecule located at or near the neuromuscular junction. For example, the molecule may be located at the presynaptic or postsynaptic side of the neuromuscular junction. Suitable neuromuscular junction targeting peptides may bind to or interact with a molecule present on a neuron at or near the neuromuscular junction. Other suitable neuromuscular junction targeting peptides may bind to or interact with a molecule present on a muscle cell at or near the neuromuscular junction. The neuromuscular junction targeting peptides have a binding affinity from about 0.5 nM to about 50 μM. A particularly suitable binding affinity may be, for example, from about 1 nM to about 40 μM. A even more suitable binding affinity may be, for example, from about 0.1 μM to about 0.75 μM. Binding of a neuromuscular targeting peptide may be determined by methods known by those skilled in the art. Suitable methods for determining binding affinity may be, for example, enzyme-linked immunosorbent assay (ELISA), Western blot analysis, and immunostaining. Neuromuscular junction targeting peptides alone or coupled to therapeutic agents may also be prepared using, for example, recombinant protein expression methods to further include a detectable label such as, for example, a fluorescent tag, such that binding may be directly detected. Analysis of immunostained or directly labeled compositions may be further analyzed by fluorescent pixel analysis, for example, to determine binding affinity.

[0043] Suitable neuromuscular junction targeting peptides may be, for example, peptides obtained from larger protein molecules. Other suitable neuromuscular junction targeting peptides may be, for example, antibody molecules. Particularly suitable antibody molecules may be, for example, antibody variable regions, antibody heavy chains, antibody light chains, Fab, $F(ab')_2$, F(ab') and single chain antibodies (scFv).

[0044] Suitable neuromuscular junction targeting peptides may be obtained from proteins such as, for example, laminin, a zinc finger domain of the human immunodeficiency virus (HIV) nucleocapsid protein, rabies virus glycoprotein (RVG), α-bungarotoxin, agrin, antibodies to the acetylcholine receptor, antibodies to acetylcholinesterase, muscle specific kinase, calcium channels, and sodium channels. Particularly suitable neuromuscular junction targeting peptides may be, for example, a laminin peptide, a human immunodeficiency virus

peptide such as, for example, a zinc finger domain of the HIV nucleocapsid protein, a rabies virus glycoprotein peptide, an α-bungarotoxin peptide, an agrin peptide, a single chain antibody peptide that specifically binds to acetylcholinesterase and a single chain antibody peptide that specifically binds to acetylcholine receptor. Suitable neuromuscular junction targeting peptides may also be non-peptide chemicals, such as nicotine. Particularly suitable neuromuscular junction targeting peptides may be, for example, those having an amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9.

Therapeutic Agents

[0045] The composition of the present disclosure includes a therapeutic agent that is coupled to the neuromuscular junction targeting peptide. As used herein, the term "therapeutic agent" refers to any type of drug, medicine, pharmaceutical, hormone, antibiotic, protein, gene, growth factor, bioactive material, etc., used for treating, controlling, or preventing diseases or medical conditions. As used herein, the term "derivative" refers to a compound or peptide obtained from another compound (e.g., a lead or parent compound). The therapeutic agent may be a protein that may be recombinantly expressed such that a nucleic acid sequence encoding an amino acid sequence corresponding to a therapeutic polypeptide may be operably linked to a nucleic acid sequence encoding an amino acid sequence corresponding to a neuromuscular junction targeting peptide and/or a nucleic acid sequence encoding an amino acid sequence corresponding to a linker.

[0046] Suitable therapeutic agents may be, for example, complement inhibitors, acetylcholinesterase inhibitors, trophic agents, and paralytic agents. Suitable complement inhibitors may be, for example, decay accelerating factor ("DAF"; "CD55")), rEV576, complement receptor 1 ("CR1"; "CD35"), membrane cofactor protein ("MCP"; "CD46"), compstatin, compstatin derivatives, POT-4, C1 inhibitor, C4b-binding protein ("C4BP"), factor H ("FH"), complement receptor Ig ("CRIg"), CD59, clusterin, C3-inhibitors, peptide 2J, human beta-defensin 2, CRIT-H17, Ac-SHLGLAR-H, Ac-RLLLAR-H, C1s-INH-248, S-protein, and Crry. Other suitable therapeutic agents may be, for example, peptides obtained from full length complement inhibitors. For example, control protein repeats of DAF may be used as the therapeutic agent. Other suitable therapeutic agents such ass chemicals and peptidomimetics, that are not peptides, but inhibit complement may be, for example, circumin, W-54011, NGD

2000-1, NDT9520492, CP-447,697, NDT 9513727, SB290157, SB290157(A), SB290157(B), BCX1470, a C1s inhibitor, BCX1470, PMX53, PMX205, C089, JPE1375.

[0047] Particularly suitable therapeutic agents may be, for example, those having an amino acid sequence of SEQ ID NO: 11 and SEQ ID NO: 12.

Linkers

[0048] Additionally or alternatively, the composition may include at least one linker such that the neuromuscular junction targeting peptide is indirectly coupled to the therapeutic agent (see, FIG. 14). In one embodiment, the composition may include a linker positioned between the neuromuscular junction targeting peptide ("NMJTP") and the therapeutic agent ("TA") (see, FIG. 14). In another embodiment, the composition may include a linker positioned between two domains of the neuromuscular junction targeting peptide such as, for example, when the composition uses a single chain antibody wherein the first linker is positioned between a V_H and a V_L of the single chain antibody (see, FIG. 14). In embodiments using a single chain antibody as the neuromuscular junction targeting peptide, the two variable regions of the antibody are separated by a linker to allow proper folding so binding to the targeted protein may occur. For example, if the composition is prepared using known recombinant protein expression methods, a nucleic acid sequence encoding the neuromuscular junction targeting peptide may be coupled to a nucleic acid sequence encoding the linker that may be coupled to a nucleic acid sequence encoding the therapeutic agent.

[0049] Without intending to be bound by theory, it is believed that a linker provides additional distance or separation between the neuromuscular targeting peptide and the therapeutic agent, or also, for example, to limit possible steric hindrance that may otherwise interfere with the activity of the therapeutic agent or targeting by the neuromuscular junction targeting peptide. Linkers may further allow for may further allow for proper folding of the neuromuscular junction targeting peptides and/or therapeutic agents. Nucleotide sequences encoding particularly suitable linkers may be, for example, those shown in Table 2.

TABLE 2. Linker Sequences.

SEQ ID NO:	Description	Nucleotide Length	Sequence
-	2aa GS linker	6	ggcagc
19	6aa [GS]x linker	18	ggtagcggcagcggtagc
20	10aa [GS]x linker	30	ggtagcggcagcggtagcggtagcggcagc
21	10 aa flexible protein domain linker	30	ggtgaaaatttgtattttcaatctggtggt
22	8 aa protein domain linker	24	tccgcttgttactgtgagctttcc
23	Split fluorophore linker; Freiburg standard	51	cgaccagcctgtaagattccaaatgacctgaagcagaaagttatgaatcac
24	15 aa flexible GS linker; Freiburg standard	45	ggtggaggaggttctggaggcggtggaagtggtggcggaggtagc
25	Short Linker (Gly-Gly-Ser-Gly)	12	ggtggttctggt
26	Middle Linker (Gly-Gly-Ser-Gly)x2	24	ggtggttctggtggtggttctggt
27	Long Linker (Gly-Gly-Ser-Gly)x3	36	ggtggttctggtggtggttctggtggtggttctggt
28	GSAT Linker	108	ggtggttctgccggtggctccggttctggctccagcggtggcagctctggtgcgtccggcacgggtactgcgggtggcactggcagcggttccggtactggctctggc
29	SEG-Linker	108	ggtggttctggcggcggttctgaaggtggcggctccgaaggcggcggcagcgagggcggtggtagcgaaggtggtggctccgagggtggcggttccggcggcggtagc
30	Z-EGFR-1907_Short-Linker	192	gtggataacaaatttaacaaagaaatgtgggcggcgtgggaagaaattcgtaacctgccgaacctgaacggctggcagatgaccgcgtttattgcgagcctggtggatgatccgagccagagcgcgaacctgctggcggaagcgaaaaaactgaacgatgcgcaggcgccgaaaaccggcggtggttctggt
31	Z-EGFR-1907_Middle-Linker	204	gtggataacaaatttaacaaagaaatgtgggcggcgtgggaagaaattcgtaacctgccgaacctgaacggctggcagatgaccgcgtttattgcgagcctggtggatgatccgagccagagcgcgaacctgctggcggaagcgaaaaaactgaacgatgcgcaggcgccgaaaaccggcggtggttctggtgtggtggttctggt

32	Z-EGFR-1907_Long-Linker	216	gtggataacaaatttaacaaagaaatgtgggcggcgtgggaagaaattc gtaacctgccgaacctgaacggctggcagatgaccgcgtttattgcgag cctggtggatgatccgagccagagcgcgaacctgctggcggaagcga aaaaactgaacgatgcgcaggcgccgaaaaccggcggtggttctggt ggtggttctggtggtggttctggt
33	(Gly4Ser)3 Flexible Peptide Linker	45	ggtggaggaggctctggtggaggcggtagcggaggcggagggtcg

Pharmaceutical Formulations

[0050] The composition of the present disclosure may also include pharmaceutical formulations. Pharmaceutical formulations may include, for example, pharmaceutically acceptable salts, carriers, adjuvants, vehicles, oils, and lipids as known by those skilled in the art. The pharmaceutical formulations may also be, for example, tablets, capsules, ingestible liquids, powders, liposomes, nanoparticles and controlled release formulations as known by those skilled in the art.

Recombinant Proteins, Vectors, Host Cells and Expression

[0051] The compositions of the present disclosure may be prepared as recombinant proteins using recombinant protein expression methods. Suitable polynucleotides may be, for example, SEQ ID NO: 13 (laminin-rEV), SEQ ID NO: 15 (HIV-rEV), and SEQ ID NO: 17 (RVG-rEV). These too permit a degree of variability in their sequence, as for example due to degeneracy of the genetic code, codon bias in favor of the host cell expressing the polypeptide, and conservative amino acid substitutions in the resulting protein. Consequently, the polypeptides and constructs of the invention include not only those which are identical in sequence to the above sequence but also those variant polypeptides with the structural and functional characteristics that remain substantially the same.

[0052] Such variants (or "analogs") may have a sequence homology ("identity") of 80%, 90%, 95%, 96%, 97%, 98%, 99%, or more with the reference sequence. In this sense, techniques for determining amino acid sequence "similarity" are well known in the art. In general, "similarity" means the exact amino acid to amino acid comparison of two or more polypeptides at the appropriate place, where amino acids are identical or possess similar chemical and/or physical properties such as charge or hydrophobicity. A so-termed "percent

similarity" may then be determined between the compared polypeptide sequences. Techniques for determining nucleic acid and amino acid sequence identity also are well known in the art and include determining the nucleotide sequence of the mRNA for that gene (usually via a cDNA intermediate) and determining the amino acid sequence encoded therein, and comparing this to a second amino acid sequence. In general, "identity" refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of two polynucleotides or polypeptide sequences, respectively. Two or more polynucleotide sequences can be compared by determining their "percent identity", as can two or more amino acid sequences. Programs available such as, for example, BLAST, are capable of calculating both the identity between two polynucleotides and the identity and similarity between two polypeptide sequences, respectively. Other programs for calculating identity or similarity between sequences are known in the art.

[0053] The present disclosure is further directed to vectors including nucleic acid sequences encoding the compositions including a neuromuscular junction targeting peptide. The term "vector", as used herein, refers to any recombinant polynucleotide construct that may be used to introduce heterologous DNA into a host cell. Vectors of the present disclosure may further include nucleic acid sequences encoding a neuromuscular junction targeting peptide operably linked to a nucleic acid sequence encoding a therapeutic agent. Vectors of the present disclosure may further include nucleic acid sequences encoding a neuromuscular junction targeting peptide operably linked to a nucleic acid sequence encoding a linker that is further operably linked to a nucleic acid sequence encoding a therapeutic agent.

[0054] The compositions of the present disclosure may be produced in prokaryotic and eukaryotic cells using expression vectors suitable for the particular host cell. Particularly suitable prokaryotic cells may be, for example, *Escherichia coli* and *Salmonella* sp. Particularly suitable eukaryotic cells may be, for example, mammalian cells, insect cells, and yeast cells.

[0055] The term "construct", as used herein, refers to any recombinant polynucleotide molecule. Examples of constructs may be a plasmid, a cosmid, a virus, an autonomously replicating polynucleotide molecule, a phage, or a linear or circular single-stranded or double-stranded DNA or RNA polynucleotide molecule, derived from any source, capable of genomic integration or autonomous replication, comprising a polynucleotide molecule where one or more polynucleotide molecule(s) has been linked in a functionally operative manner, i.e., operably linked.

[0056] As used herein, "operably linked" refers to the joining of nucleic acid sequences such that one sequence can provide a required function to a linked sequence. In the context of a promoter, "operably linked" means that the promoter is connected to a sequence of interest such that the transcription of that sequence of interest is controlled and regulated by that promoter. When the sequence of interest encodes a protein and when expression of that protein is desired, "operably linked" means that the promoter is linked to the sequence in such a way that the resulting transcript will be efficiently translated. If the linkage of the promoter to the coding sequence is a transcriptional fusion and expression of the encoded protein is desired, the linkage is made so that the first translational initiation codon in the resulting transcript is the initiation codon of the coding sequence. Alternatively, if the linkage of the promoter to the coding sequence is a translational fusion and expression of the encoded protein is desired, the linkage is made so that the first translational initiation codon contained in the 5' untranslated sequence associated with the promoter and is linked such that the resulting translation product is in frame with the translational open reading frame that encodes the desired protein. Nucleic acid sequences that can be operably linked may be, for example, sequences that provide gene expression functions (i.e., gene expression elements such as promoters, 5' untranslated regions, introns, protein coding regions, 3' untranslated regions, polyadenylation sites, and/or transcriptional terminators), sequences that provide DNA transfer and/or integration functions, sequences that provide for selective functions (i.e., antibiotic resistance markers, biosynthetic genes), sequences that provide scoreable marker functions (i.e., reporter genes), sequences that facilitate in vitro or in vivo manipulations of the sequences (i.e., polylinker sequences, site specific recombination sequences) and sequences that provide replication functions (i.e., bacterial origins of replication, autonomous replication sequences, centromeric sequences). Additional sequences that may be operably linked may be, for example, sequences that facilitate purification of the recombinantly expressed protein such as, for example, affinity tags. Other additional sequences that may be operably linked may be, for example, sequences that encode protease cleavage sites.

[0057] An "isolated" polynucleotide (e.g., an "isolated DNA" or an "isolated RNA") refers to a polynucleotide at least partially separated from at least some of the other components of the naturally occurring organism such as, for example, the cell structural components or other polypeptides or nucleic acids commonly found associated with the polynucleotide.

[0058] An "isolated" polypeptide refers to a polypeptide that is at least partially separated from at least some of the other components of the naturally occurring organism such as, for example, the cell or structural components or other polypeptides or nucleic acids commonly found associated with the polypeptide.

[0059] Once the vector has been constructed by operably linking the components, it may be introduced into a host cell. Operably linking the polynucleotide sequence encoding a neuromuscular junction targeting peptide to a polynucleotide sequence encoding a therapeutic agent and/or a linker, as provided by the present disclosure, results in the production of an expressed polypeptide from the host cell. The vector may be introduced into the host cell by methods known by those skilled in the art. Suitable methods may be, for example, transfection, transformation, and electroporation. The host cell is then cultured under suitable conditions to permit expression of the composition, which is then recovered by isolation and/or purification.

[0060] Recombinantly expressed compositions according to the present disclosure may be further isolated and purified according to methods known by those skilled in the art. For example, protein purification may be performed by affinity chromatography, precipitation, chromatography (e.g., affinity, ion exchange, HPLC, gel filtration), extraction, ultrafiltration, electrophoresis, and combinations thereof. Protein isolation and purification may be facilitated by including sequences and amino acid motifs or use of protein expression vectors having sequences and amino acid motifs for affinity purification. Suitable sequences and motifs may be, for example, histidine tags, antigen peptide tags, chitin binding protein, maltose binding protein, glutathione-S-transferase and other suitable tags known by those skilled in the art. Particularly suitable affinity tags may be, for example, histidine tags ("His tags") as illustrated in FIG. 14. As known by those skilled in the art, affinity tags aid in the purification of the protein containing the affinity tag.

[0061] Recombinantly expressed compositions according to the present disclosure may further include cleavage sites for proteases to further isolate the composition from other domains. Suitable protease cleavage sites may be any protease cleavage site known by those skilled in the art. Suitable proteases may be, for example, Factor Xa, enterokinase, thrombin, TEV protease (Invitrogen, Carlsbad, CA), PRESCISSION (GE Healthcare Life Sciences, Piscataway, NJ), pepsin cleavage sites, trypsin cleavage sites, chymotrypsin cleavage sites,

thermolysin cleavage sites, and other protease cleavage sites known by those skilled in the art. Particularly suitable protease cleavage sites may be, for example, a thrombin cleavage site.

[0062] Additionally or alternatively, the compositions of the present disclosure may be prepared using chemical synthesis methods. Suitable chemical synthesis methods are well-known in the art and may include, for example, liquid-phase peptide synthesis, solid-phase peptide synthesis, fragment condensation, and chemical ligation.

Methods for Delivering a Therapeutic Agent to the Neuromuscular Junction

[0063] In another aspect, the present disclosure is directed to a method for delivering a therapeutic agent to the neuromuscular junction. The method includes administering a composition including a neuromuscular junction targeting peptide coupled to a therapeutic agent.

[0064] The neuromuscular junction targeting peptide may be any neuromuscular junction targeting peptide as described herein. The therapeutic agent may be any therapeutic agent as described herein. The composition may further include at least one linker as described herein.

[0065] Administration may be to a subject. Suitable methods for administration to a subject may be, for example, by intravenous injection, intravenous infusion, intraperitoneal injection, intradermal injection, intramuscular injection, subcutaneous injection, intranasal, oral, and other methods known by those skilled in the art. Administration may also be to a cell in culture. Suitable methods for administration to a cell in culture may also, for example, by pipetting, pouring a solution containing the composition, and other methods know in the art.

[0066] Dosage of the composition to be administered may be determined by those skilled in the art. Dosage may depend on various factors such as, for example, the condition or disease, weight of the subject, age of the subject, method of administration, route of administration, whether administered for an *in vivo* purpose, whether administered for an *in vitro* purpose, and other factors. The dosage to a subject will generally include an amount that is sufficient to provide some improvement or benefit to the subject so as to provide some alleviation, mitigation, or decrease in at least one clinical symptom in the subject. The dosage may also include an amount that is sufficient to provide an *in vitro* complement inhibitory effect or prevention of complement-dependent cell lysis, for example.

[0067] Suitable dosages may be from about 0.001 μg/ml to about 4 μg/ml. Particularly suitable dosages may be from about 0.001 μg/ml to about 0.02 μg/ml. Suitable dosages may be determined *in vitro*, for example, by investigating the inhibition of complement hemolytic activity of antibody sensitized erythrocytes, by luminescent bioassay of antibody-initiated, complement mediated injury of cell lines (for example, the toxilight bioassay commercially available from Cambrex, Rockland, ME). Suitable dosages may also be determined *in vivo*, for example, by analyzing targeting to the neuromuscular junction, analyzing the production of weakness in the subject, and determining alterations in systemic complement activity. Those skilled in the art will appreciate that the therapeutic effects need not be complete or curative, as long as some benefit is provided to the subject.

Methods for Treating Neuromuscular Junction-Related Diseases or Conditions

[0068] In another aspect, the present disclosure is directed to a method for treating a neuromuscular junction-related disease or condition. The method includes administering to a subject in need thereof a composition including a neuromuscular junction targeting peptide coupled to a therapeutic agent.

[0069] As used herein "neuromuscular junction-related diseases or conditions" refer to diseases or conditions resulting from injury at and/or to the neuromuscular junction. A neuromuscular junction-related disease or condition may be, for example, myasthenia gravis, experimentally acquired myasthenia gravis, Lambert-Eaton syndrome, Miller Fischer syndrome, congenital myasthenic syndromes, botulism, organophosphate poisoning, and other toxins that compromise the neuromuscular junction.

[0070] The methods include the administration of the compositions to a subject in need thereof including individuals afflicted with neuromuscular junction-related diseases or conditions resulting from injury at and/or to the neuromuscular junction as described herein. Additionally, a subject in need thereof includes laboratory animals experimentally induced to mimic diseases or conditions resulting from injury at and/or to the neuromuscular junction, thus serving as animal models of these diseases and conditions. As such, in some embodiments of the present disclosure, the methods disclosed herein are directed to a subset of the general population such that not all of the general population may benefit from these methods.

[0071] Suitable subjects may be mammals. Suitable mammals may be, for example, humans, mice, rats, rabbits, guinea pigs, and monkeys.

[0072] The neuromuscular junction targeting peptide may be any neuromuscular junction targeting peptide described herein. The therapeutic agent may be any therapeutic agent as described herein. The composition may further include at least one linker as described herein.

[0073] Suitable methods for administration may be, for example, by intravenous injection, intravenous infusion, intraperitoneal injection, intradermal injection, intramuscular injection, subcutaneous injection, intranasal, oral, and other methods known by those skilled in the art.

[0074] Suitable therapeutically effective amounts may be, for example, from about 1 ng/kg to about 0.1 mg/kg. More particularly, the therapeutically effective amount may be about 5 mg/kg. Suitable therapeutically effective amounts may further be described as having a half maximal inhibitory concentration (IC_{50}) of from about 0.001 μg/ml to about 40 μg/ml. A particularly suitable IC_{50} may be from about 10 ng/ml to about 20 μg/ml. The therapeutically effective amount may be characterized, for example, by observing a prolonged biological effect without observation of the production of weakness in the subject. The therapeutically effective amount of the composition to be administered to the subject may be determined by those skilled in the art. The therapeutically effective amount may depend on various factors such as, for example, the condition or disease, weight of the subject, age of the subject, method of administration, route of administration, and other factors. Generally, the therapeutically effective amount will include an amount that is sufficient to provide some improvement or benefit to the subject so as to provide some alleviation, mitigation, or decrease in at least one clinical symptom in the subject. Those skilled in the art will appreciate that the effect need not be complete or curative, as long as some benefit is provided to the subject.

[0075] The disclosure will be more fully understood upon consideration of the following non-limiting Examples.

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EXAMPLES

EXAMPLE 1

L-rEV Cloning and Expression

[0076] In this Example, a composition including the neuromuscular junction targeting peptide obtained from laminin (encoded by SEQ ID NO: 1) coupled to rEV (encoded by SEQ ID NO: 10) was prepared by recombinant protein expression.

[0077] Specifically, the laminin neuromuscular junction targeting peptide was coupled to rEV. As shown in FIG. 1, the construct further included a linker, a His tag and a thrombin cleavage site. Codon optimization was performed using the codon optimization program available from Integrated DNA Technologies.

[0078] The cDNA encoding laminin-linker-rEV was subcloned into the prokaryotic expression vector plasmid pET-28-a(+) via *NheI* and *EcoRI* sites within multi-cloning sites. The plasmid was verified by restriction enzyme analysis and direct sequencing. A schematic of the plasmid construct is shown in FIG. 2.

[0079] For recombinant protein expression of the laminin-linker-rEV, BL21 *E. coli* were transformed with the pET28a-laminin-linker-rEV plasmid prepared above and induced with 1 mM 1mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 4 hrs. Cells were lysed by sonication in lysis buffer containing 3.2 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 1.3 mM KCl, 135 mM NaCl, pH 7.4, 5 mM imidazole, lysozyme and protease inhibitor tablet (Roche). After centrifugation at 13,000 rpm at 4°C for 20 minutes, the soluble fraction was applied to a Talon metal affinity column (Clontech), washed with buffer A (3.2 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 1.3 mM KCl, 135 mM NaCl, pH 7.4) and eluted with buffer A containing different concentrations of Imidazole (20mM to 500mM). The fractions containing the protein of interest were pooled and concentrated using Amicon ultra 10K MWCO filters (Millipore). The concentrated protein was then applied onto a Superdex200 column (GE healthcare) for gel filtration chromatography. The eluted fractions were analyzed for the presence of protein and concentration of positive fractions was undertaken. The resultant protein was stored at -80°C or used for further experimentation.

[0080] Concentration of the purified protein was determined by the Bradford assay. As shown in FIG. 3, induced BL21 cells began expressing laminin-rEV within 1 hour after induction with maximal expression at 4 hours. Western blot analysis using an anti-rEV antibody confirmed expression in induced cells. See, FIG. 4.

EXAMPLE 2

HIV-rEV

[0081] In this Example, a composition including the neuromuscular junction targeting peptide from HIV (encoded by SEQ ID NO: 3) coupled to rEV (encoded by SEQ ID NO: 10) was prepared as set forth in Example 1.

[0082] Specifically, the HIV neuromuscular junction targeting peptide was coupled to rEV. As shown in FIG. 5, the construct further included a linker, a His tag and a thrombin cleavage site. Codon optimization was performed using the codon optimization program available from Integrated DNA Technologies.

[0083] BL21 *E. coli* were transformed with the pET28a-HIV-linker-rEV plasmid and induced with IPTG for 4 hours. Cells were lysed and the protein was purified as set forth in Example 1 above. The resultant protein was stored at -80°C or used for further experimentation.

EXAMPLE 3

RVG-rEV

[0084] In this Example, a composition including the neuromuscular junction targeting peptide obtained from RVG (encoded by SEQ ID NO: 5) coupled to rEV (encoded by SEQ ID NO: 10) was prepared as set forth in Example 1.

[0085] Specifically, the RVG neuromuscular junction targeting peptide was coupled to rEV. As shown in FIG. 6, the construct further included a linker, a His tag and a thrombin cleavage site. Codon optimization was performed using the codon optimization program available from Integrated DNA Technologies.

[0086] BL21 *E. coli* were transformed with the pET28a-RVG-linker-rEV plasmid and induced with IPTG for 4 hours. Cells were lysed and the protein was purified as set forth in Example 1 above. The resultant protein was stored at -80°C or used for further experimentation.

EXAMPLE 4

[0087] In this Example, a composition including the neuromuscular junction targeting peptide using the single chain antibody fragment (scFv) obtained from Mab 35 coupled to decay accelerating factor (DAF) was prepared as set forth in Example 1.

[0088] Specifically, the scFv was fused to DAF and inserted into the pET16b expression vector as outlined in FIG. 7. To generate the V_H-V_L fragment of the scFv, RNA was obtained from the TIB-175 hybridoma cell line (obtained from ATCC), which secretes the Mab 35 antibody. The variable heavy chain (V_H) and/or variable light chain (V_L) were the amplified by RT-PCR. Overlapping PCR was used to generate the V_H-V_L fragment with the $(GGGGS)_3$ (SEQ ID NO: 34) linker to produce the single chain AChR antibody scFv. Sequencing confirmed the identity of the cloned fragments as V_H and V_L regions. Two signaling peptide constructs were then produced using the CD59 fragment or the IgG signal peptide. The CD59sp- or IgGsp-signaling peptide was then fused to the V_H-V_L fragment and cloned into the pIRES2-AcGFP1 vector (Clonetech, Mountain View, CA). BHK-21 cells were transfected with these constructs. FIG. 8 shows expression of GFP by transfected BHK-21 cells transfected with the IgG V_H-V_L fragment.

[0089] DAF was then fused to either the CD59sp-V_H-V_L or IgGsp-V_H-V_L. The CD59sp-V_H-V_L-DAF is a 537 amino acid peptide (SEQ ID NO: 34). The IgGsp-V_H-V_L-DAF is a 534 amino acid peptide (SEQ ID NO: 35).

[0090] BL21 cells were transformed with the pET16b expression vector containing the scFv-DAF construct as previously described. Expressed protein was purified using a Hitrap chelating HP column (manufacturer) and refolded by urea gradient dialysis. Samples taken during the expression, purification, and refolding steps were analyzed by SDS-PAGE. See, FIG. 9. Lane 1 is a sample of thepET16b-scFv-DAF/BL21 before induction; lane 2 is a sample of thepET16b-scFv-DAF/BL21 after induction; lanes 3 and 4 are eluted peaks of the fusion protein from Hitrap chelating HP columns; lanes 5 and 6 are refolded fusion protein after urea gradient dialysis; lane M is molecular weight markers.

EXAMPLE 5

[0091] The scFv-DAF fusion protein was analyzed for specificity binding to hAChRα1-210 peptides by ELISA.

[0092] hAChRα1-210 peptides (2 μg/ml) were coated on plates and incubated with serially diluted scFv-DAF or scFv1956. Results were expressed in ODs. Results are presented in FIG. 10. Values represented the mean ±SD. *P<0.05.

EXAMPLE 6

[0093] The scFv-DAF fusion protein was analyzed for *in vitro* complement regulatory function.

[0094] Antibody-sensitized sheep erythrocytes were used as target. The degree of complement-mediated haemolysis was quantified by the release of haemoglobin to the supernatant and plotted as molar concentration of inhibitor present in the assay. Results represent the man value±SD of experiments carried out in triplicate. Results are presented in FIG. 11. *P0.05.

EXAMPLE 7

[0095] In this Example, targeting of scFv-35-DAF to the neuromuscular junction was analyzed.

[0096] The scFv-35-DAF prepared in Example 6 was injected into C57/Black 6 and CD59-/-, DAF-/- mice, which are highly susceptible to complement injury. After 24 hours weight and weakness were assessed. The animals were found to gain weight and exhibited no loss of strength indicating the scFv-35-DAF did not inhibit neuromuscular junction transmission. Diaphragms were used to visualize localization of the scFv-35-DAF to the neuromuscular junction by the anti-Rat IgG and Bungarotoxin to identify junctions. As shown in FIG. 12, the scFv-35-DAF was localized to the neuromuscular junction in both animals and there was no evidence of tissue destruction, even in the complement regulator deficient mouse. These results confirmed that the scFv-35-DAF construct is safe and specifically targets to the neuromuscular junction.

[0097] The scFv-35-DAF was also administered to Lewis rats. The acetylcholine receptor antibody was administered to the Lewis rats 24 hours later to induce experimental myasthenia gravis. At 48 hours, the severity of weakness was maximal in control rats. Of five animals treated with scFv-35-DAF, all survived and showed mild-to-moderate weakness. In the rats treated with scFv-35, 3 animals died and 2 had severe weakness. These results indicated that scFv-35-DAF was safe and has a robust protective effect.

EXAMPLE 8

[0098] In this Example, the affect of scFv-35-DAF on the deposit of C3 on TE671 cells was analyzed.

[0099] TE671 cells were treated with normal basal medium with scFv-DAF, scFv1956, and DAF (100 nM). Negative controls were treated with PBS in place of mAb35. Cells were stained with FITC-conjugated anti-C3 antibody and stained with Eosin staining solution as contrast stain. Images were obtained at 200X (Panels A-E), 400X (Panels F-J), and 1000X (Panels K-O). Cells were also analyzed by flow cytometry.

[00100] Results are presented in FIG. 13. Staining of C3 deposited on cells treated with scFv-DAF and scFv1956 was patchy and moderate (see, Panels L and M). The staining of C3 deposits around TE671 cells was diffuse (see, Panels M, N, and O). Scale bar = 10 μm.

[00101] Results demonstrated that scFv-DAF fusion protein inhibits C3 deposition on the TE671 cell surface.

EXAMPLE 9

[00102] In this Example, the therapeutic effect of scFv-35-DAF in EAMG mice and rats was analyzed.

[00103] Specifically, EAMG was induced in DAF-/-, CD59ab-/- mice and Lewis rats followed by treatment with scFv, scFv-DAF, and PBS (as a control). All mice showed no evidence of disease (see FIG. 15), while rats treated with scFv-DAF showed significant protection from EAMG (FIG. 16). Quantitative analysis of complement deposition demonstrated significantly less MAC deposition at endplates of scFv-Daf-treated animals compared to both vehicle- and scFv-treated rats. In comparing the scFv and the vehicle, the scFv had a marginally

significant increase in complement deposition. Consistent with the better clinical outcome, AChR density was significantly better in the scFv-DAF-treated rats than scFv and vehicle treated rats (not shown).

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[00104] In view of the above, it will be seen that the several advantages of the disclosure are achieved and other advantageous results attained. As various changes could be made in the above processes and composites without departing from the scope of the disclosure, it is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

[00105] When introducing elements of the present disclosure or the various versions, embodiment(s) or aspects thereof, the articles "a", "an", "the" and "said" are intended to mean that there are one or more of the elements. The terms "comprising", "including" and "having" are intended to be inclusive and mean that there may be additional elements other than the listed elements.

What is claimed is:

1. A composition comprising a neuromuscular junction targeting peptide coupled to a therapeutic agent.

2. The composition of claim 1, wherein the neuromuscular junction targeting peptide comprises a laminin peptide, a human immunodeficiency virus nucleocapsid zinc finger domain, a rabies virus glycoprotein peptide, an α-bungarotoxin peptide, an agrin peptide, a single chain antibody peptide that specifically binds to acetylcholinesterase and a single chain antibody peptide that specifically binds to acetylcholine receptor.

3. The composition of claim 1, wherein the neuromuscular junction targeting peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9.

4. The composition of claim 1, wherein the therapeutic agent is selected from the group consisting of a complement inhibitor, an acetylcholinesterase inhibitor, a trophic agent, and a paralytic agent.

5. The composition of claim 4, wherein the complement inhibitor is decay accelerating factor, rEV, rEV576, membrane cofactor protein, compstatin, a compstatin derivative, POT-4, a C1 inhibitor, C4b-binding protein, factor H, complement receptor Ig, CD59, clusterin, a C3-inhibitor, peptide 2J, human beta-defensin 2, CRIT-H17, Ac-SHLGLAR-H, Ac-RLLLAR-H, C1s-INH-248, S-protein, Crry, circumin, W-54011, NDT9520492, NGD 2000-1, CP-447,697, NDT 9513727, SB290157, SB290157(A), SB290157(B), BCX1470, PMX53, PMX205, C089, and JPE1375.

6. The composition of claim 1, further comprising a linker.

7. The composition of claim 6, wherein the linker is selected from the group consisting of a glutamine-serine linker, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33.

8. A method of delivering a therapeutic agent to the neuromuscular junction, the method comprising:

administering a composition comprising a neuromuscular junction targeting peptide coupled to a therapeutic agent.

9. The method of claim 8, wherein the neuromuscular junction targeting peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9.

10. The method of claim 8, wherein the therapeutic agent is selected from the group consisting of a complement inhibitor, an acetylcholinesterase inhibitor, a trophic agent, and a paralytic agent.

11. The method of claim 8, wherein the composition further comprises a linker.

12. The method of claim 11, wherein the linker is selected from the group consisting of a glutamine-serine linker, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33.

13. A method for treating a neuromuscular junction-related disease or condition in a subject in need thereof, the method comprising:

administering to the subject a therapeutically effective amount of a composition comprising a neuromuscular junction targeting peptide coupled to a therapeutic agent.

14. The method of claim 13, wherein the neuromuscular junction-related disease or condition is selected from the group consisting of myasthenia gravis, experimentally acquired myasthenia gravis, Lambert-Eaton syndrome, Miller Fischer syndrome, congenital myasthenic syndromes, botulism, and organophosphate poisoning.

15. The method of claim 13, wherein the neuromuscular junction targeting peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9.

16. The method of claim 13, wherein the therapeutic agent is selected from the group consisting of a complement inhibitor, an acetylcholinesterase inhibitor, a trophic agent, and a paralytic agent.

17. The method of claim 16, wherein the complement inhibitor is selected from the group consisting of DAF, rEV, rEV576, membrane cofactor protein, compstatin, a compstatin derivative, POT-4, a C1 inhibitor, C4b-binding protein, factor H, complement receptor Ig, CD59, clusterin, a C3-inhibitor, peptide 2J, human beta-defensin 2, CRIT-H17, Ac-SHLGLAR-H, Ac-RLLLAR-H, C1s-INH-248, S-protein, Crry, circumin, W-54011, NDT9520492, NGD 2000-1, CP-447,697, NDT 9513727, SB290157, SB290157(A), SB290157(B), BCX1470, PMX53, PMX205, C089, and JPE1375.

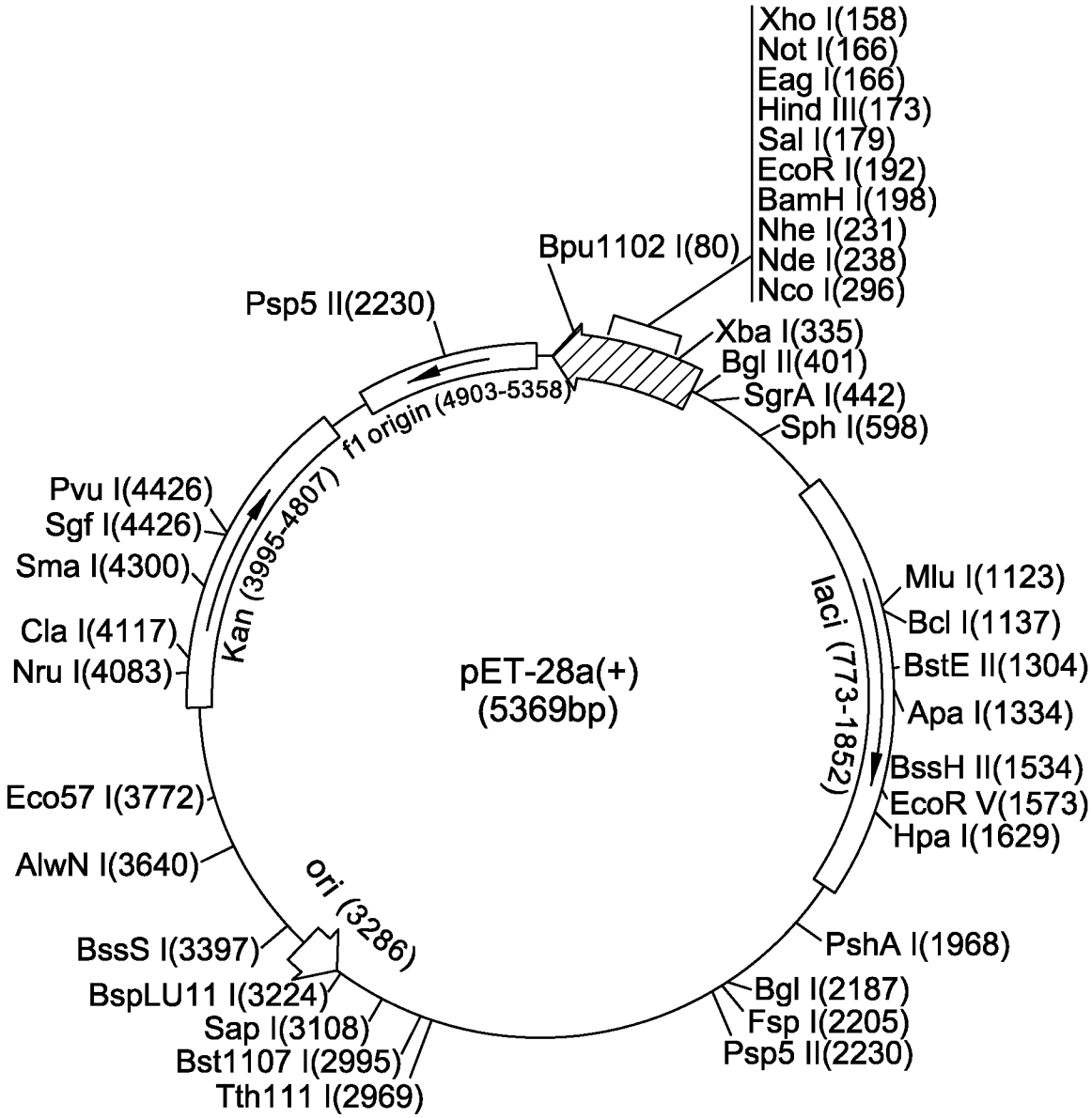
18. The method of claim 13, wherein the administering step is by infusion, injection, orally, nasally, topically, and subcutaneously.

19. The method of claim 13, wherein the therapeutically effective amount has a half maximal inhibitory concentration of from about 0.001 μg/ml to about 40 μg/ml.

20. The method of claim 13, wherein the composition has a binding affinity of from about 0.5 nM to about 50 μM.

FIG. 1

FIG. 2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Not I(166)
Eag I(166)
Hind III(173)
Sal I(179)
EcoR I(192)
BamH I(198)
Nhe I(231)
Nde I(238)
Nco I(296)
Bpu1102 I(80)
Psp5 II(2230)
Xba I(335)
Bgl II(401)
SgrA I(442)
Sph I(598)
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Pvu I(4426)
Sgf I(4426)
Sma I(4300)
Kan (3995-4807)
Cla I(4117)
Nru I(4083)
pET-28a(+)
(5369bp)
lacI (773-1852)
Mlu I(1123)
Bcl I(1137)
BstE II(1304)
Apa I(1334)
BssH II(1534)
EcoR V(1573)
Hpa I(1629)
Eco57 I(3772)
AlwN I(3640)
ori (3286)
BssS I(3397)
BspLU11 I(3224)
Sap I(3108)
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Fsp I(2205)
Psp5 II(2230)

FIG. 3

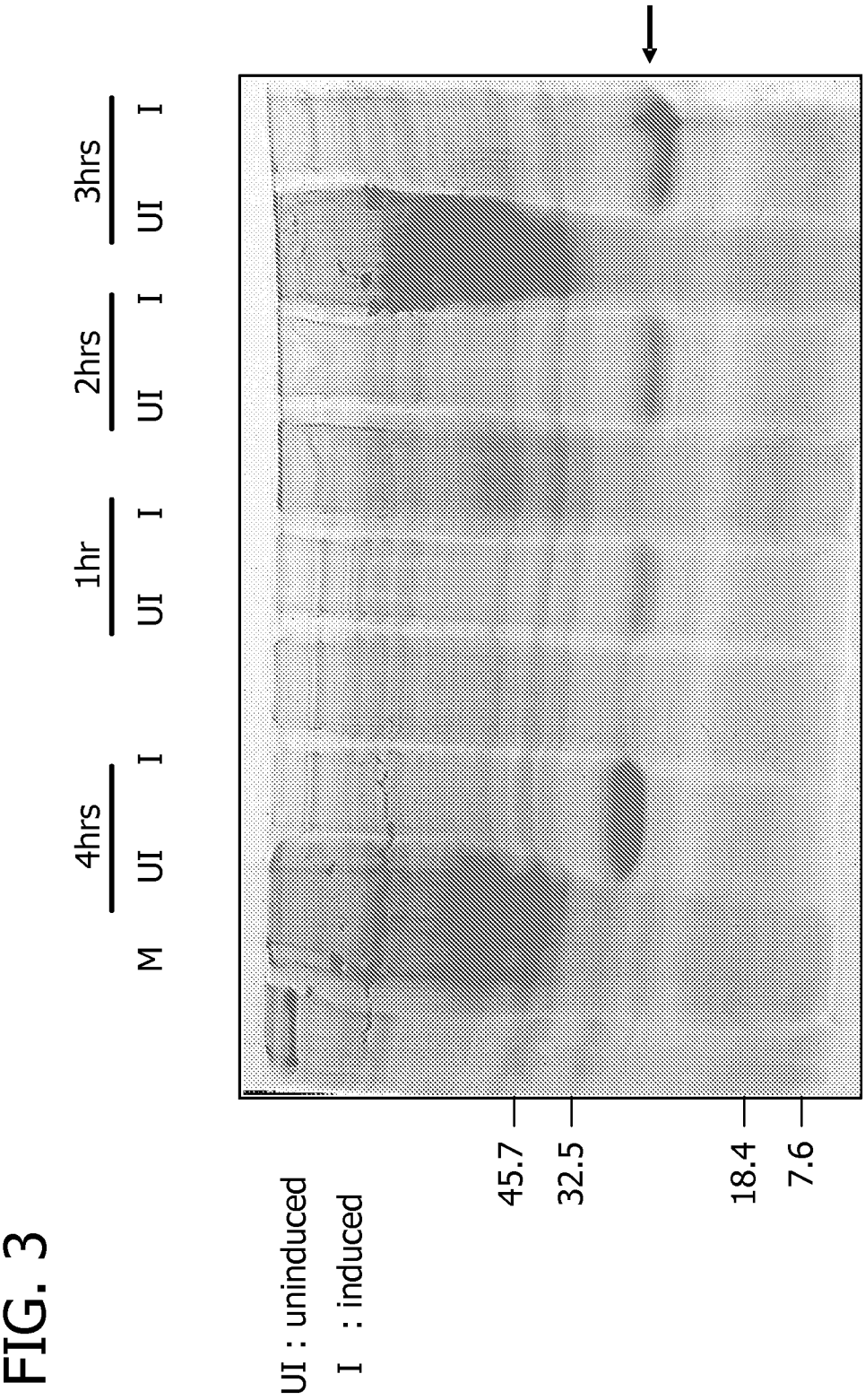
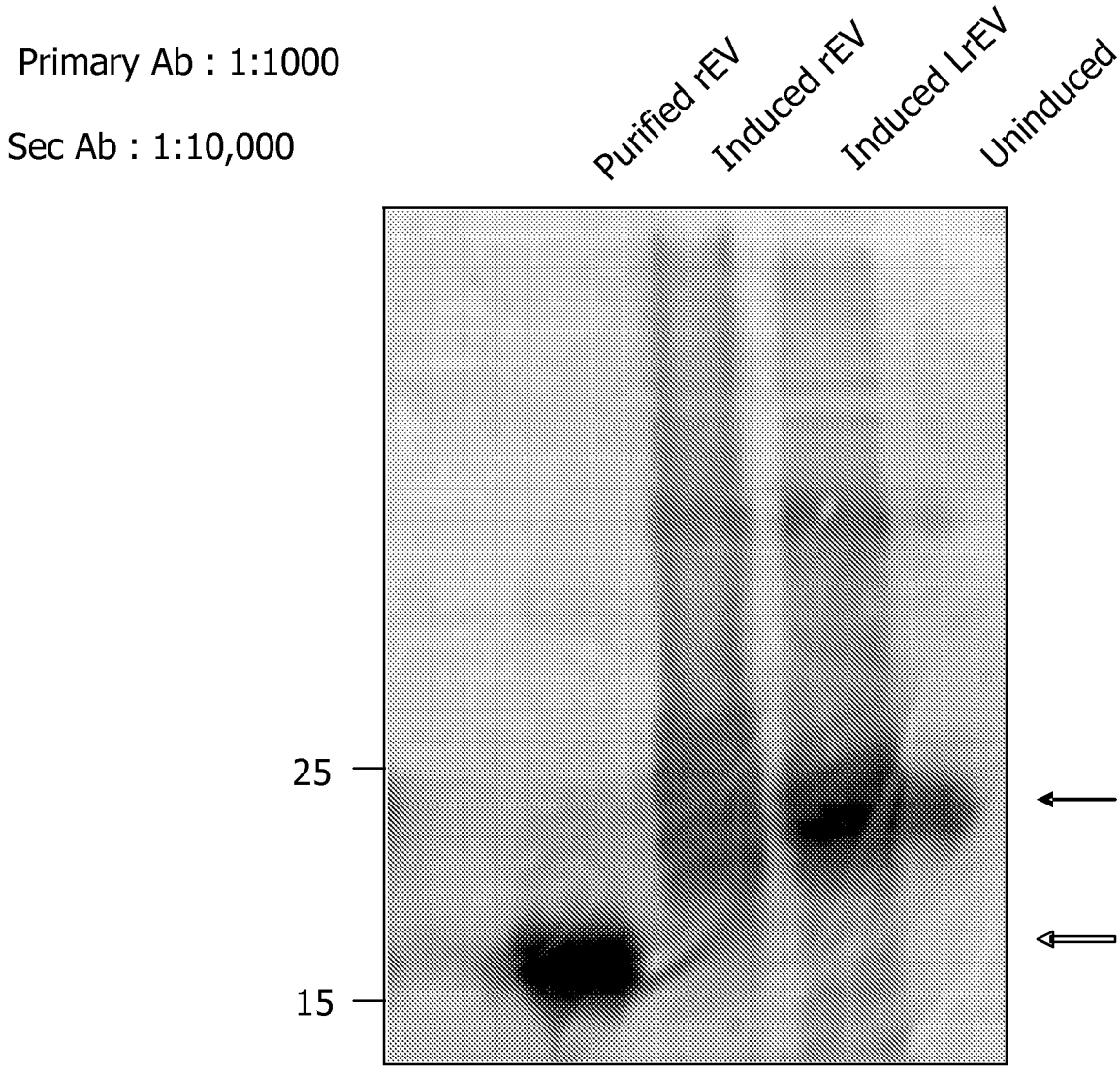

M
4hrs
UI
I
1hr
UI
I
2hrs
UI
I
3hrs
UI
I
45.7
32.5
18.4
7.6
UI : uninduced
I : induced

FIG. 4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Sec Ab : 1:10,000
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Induced rEV
Induced LrEV
Uninduced
25
15

FIG. 5

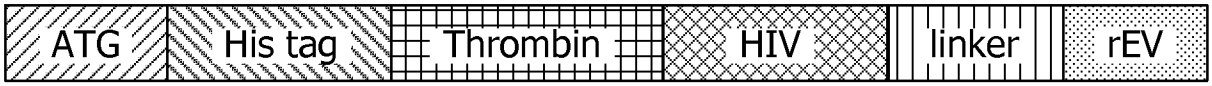


FIG. 6

FIG. 7

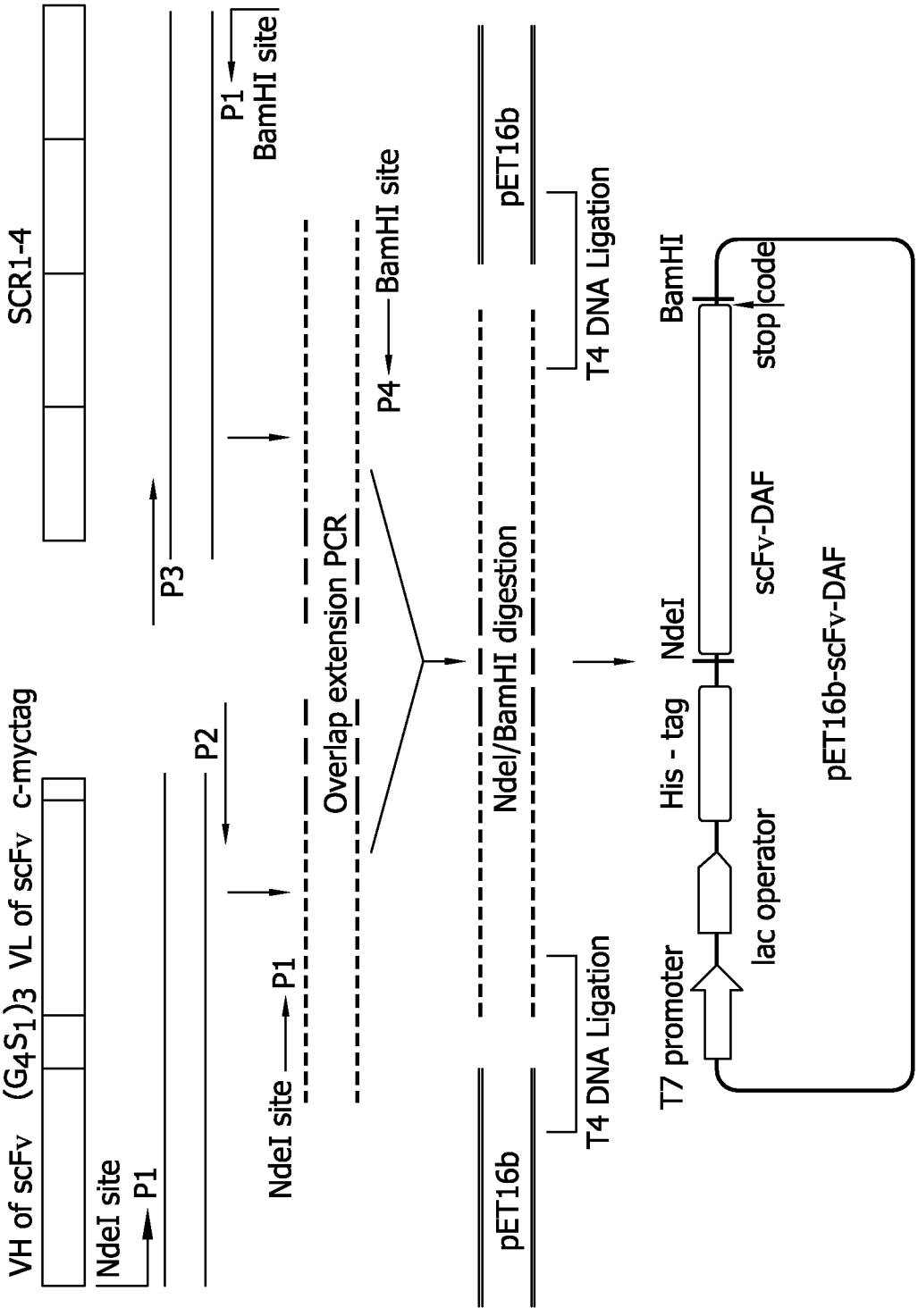

SCR1-4
VH of scFv
(G4S1)3
VL of scFv
c-myctag
NdeI site
P1
P2
P3
P1
BamHI site
NdeI site
P1
Overlap extension PCR
P4
BamHI site
pET16b
NdeI/BamHI digestion
pET16b
T4 DNA Ligation
T4 DNA Ligation
BamHI
NdeI
scFv-DAF
stop code
His - tag
T7 promoter
lac operator
pET16b-scFv-DAF

FIG. 8

IgGsp_Vh_V_L in BHK-21 cell

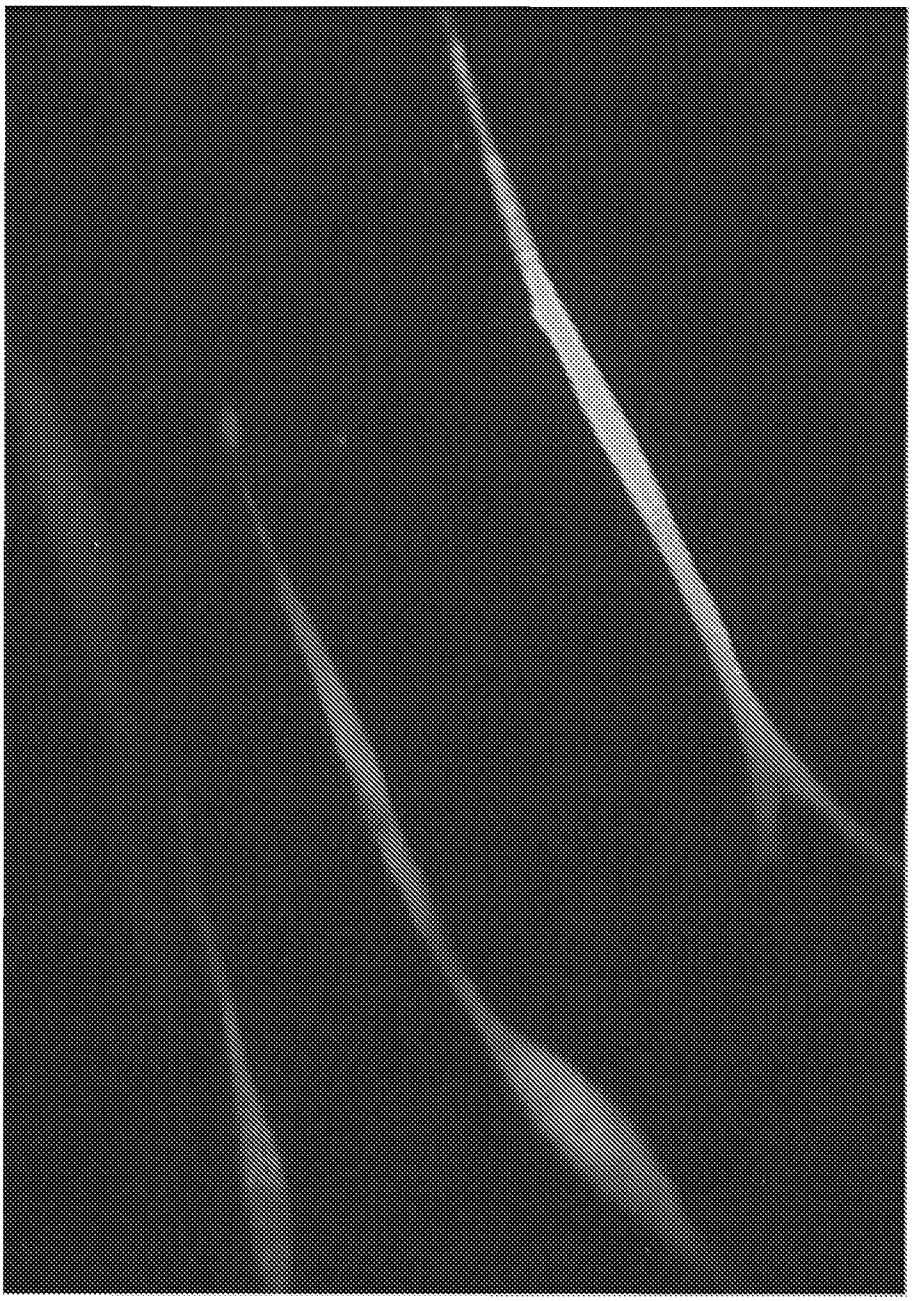


FIG. 9

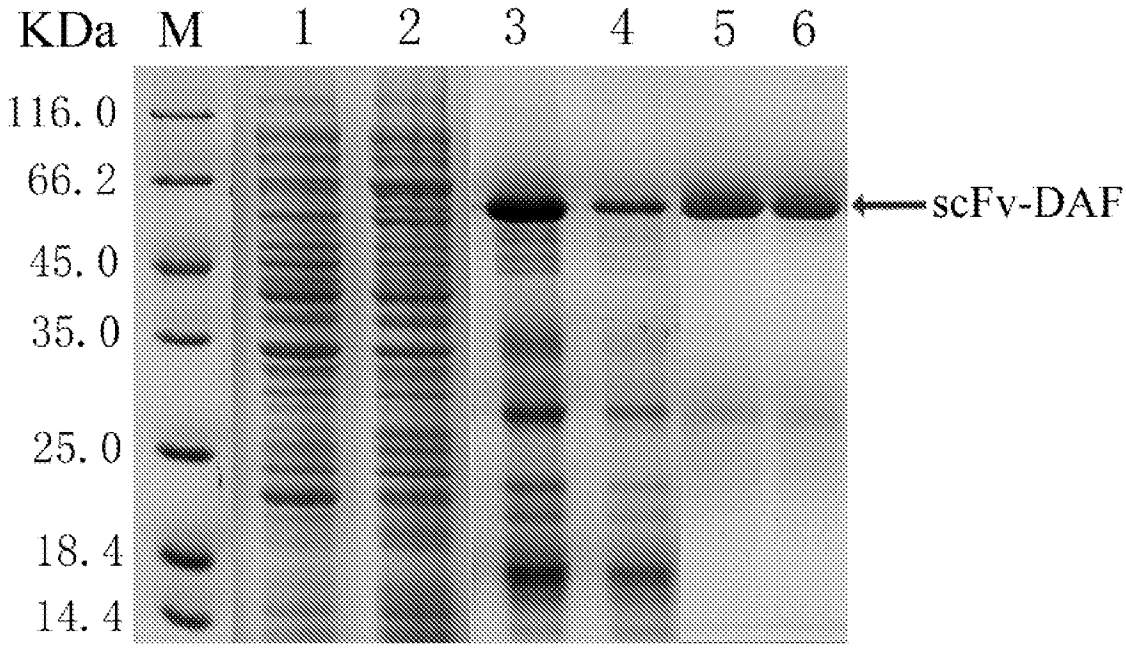


FIG. 10

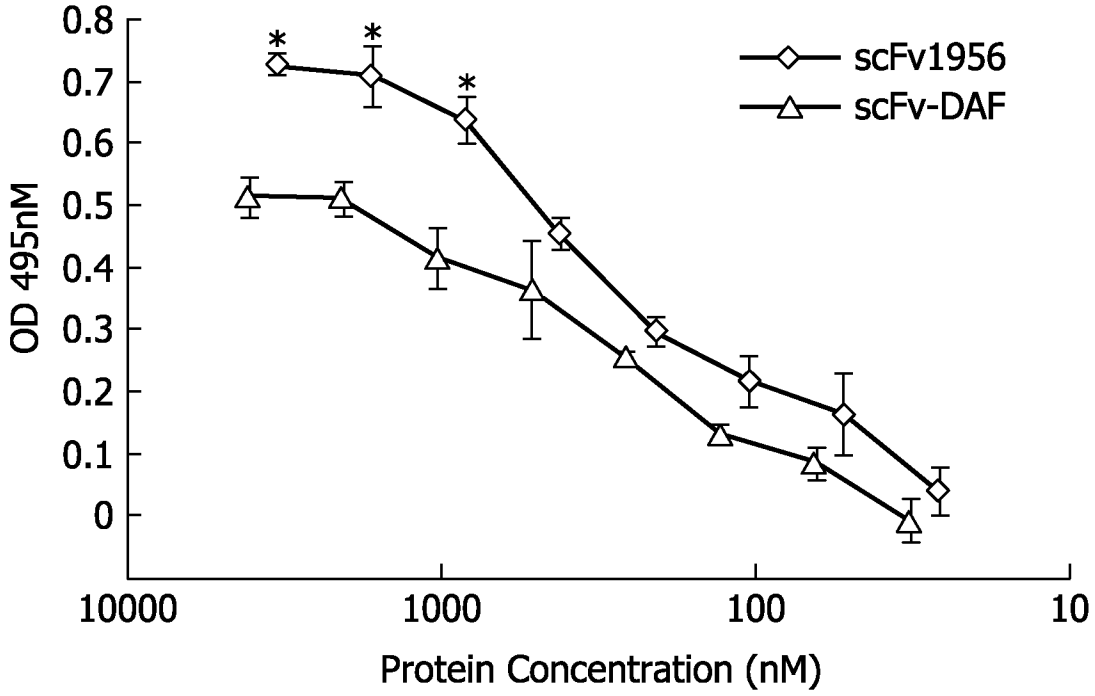

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
OD 495nM
*
*
*
scFv1956
scFv-DAF
10000
1000
100
10
Protein Concentration (nM)

FIG. 11

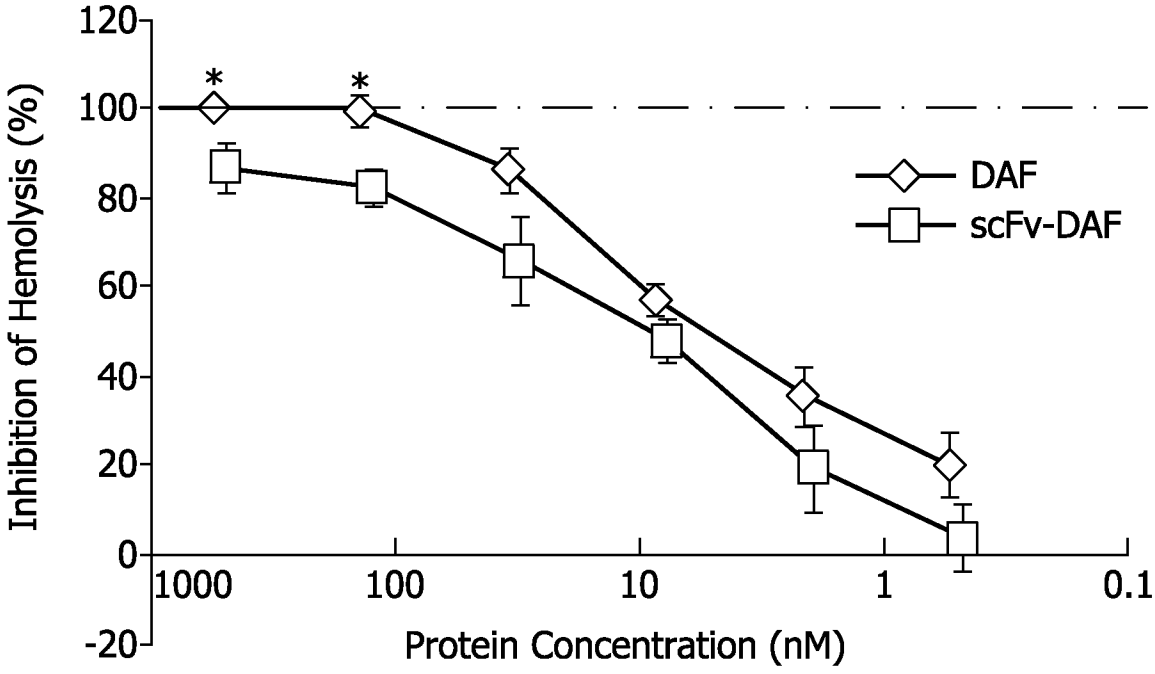

120
100
80
60
40
20
0
-20
Inhibition of Hemolysis (%)
*
*
DAF
scFv-DAF
1000
100
10
1
0.1
Protein Concentration (nM)

FIG. 12

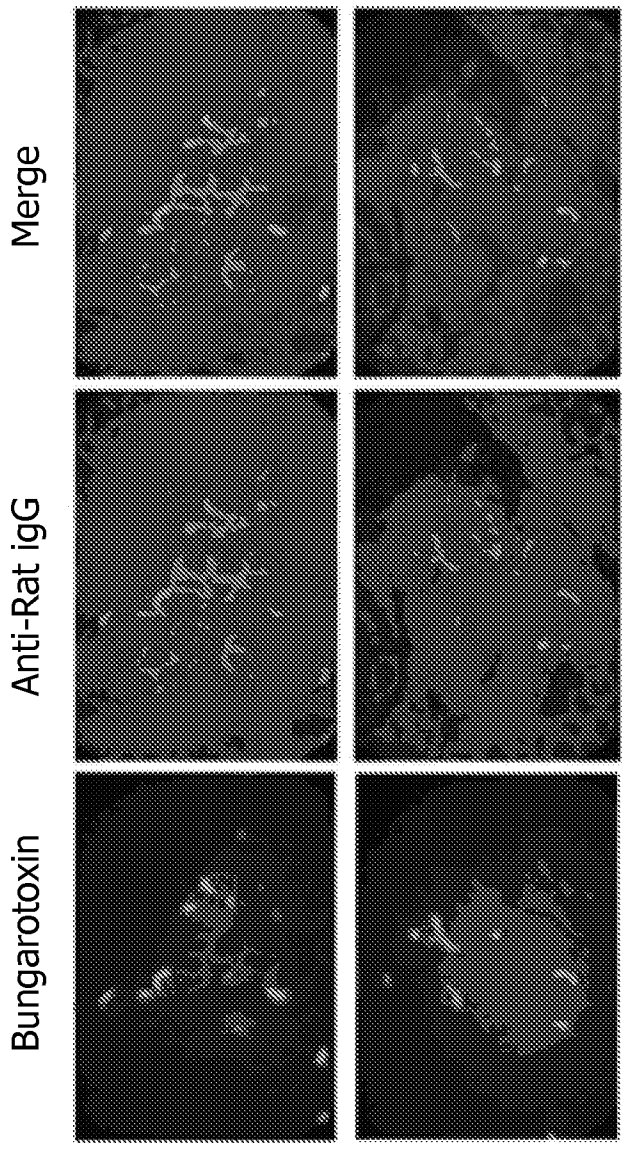


FIG. 13

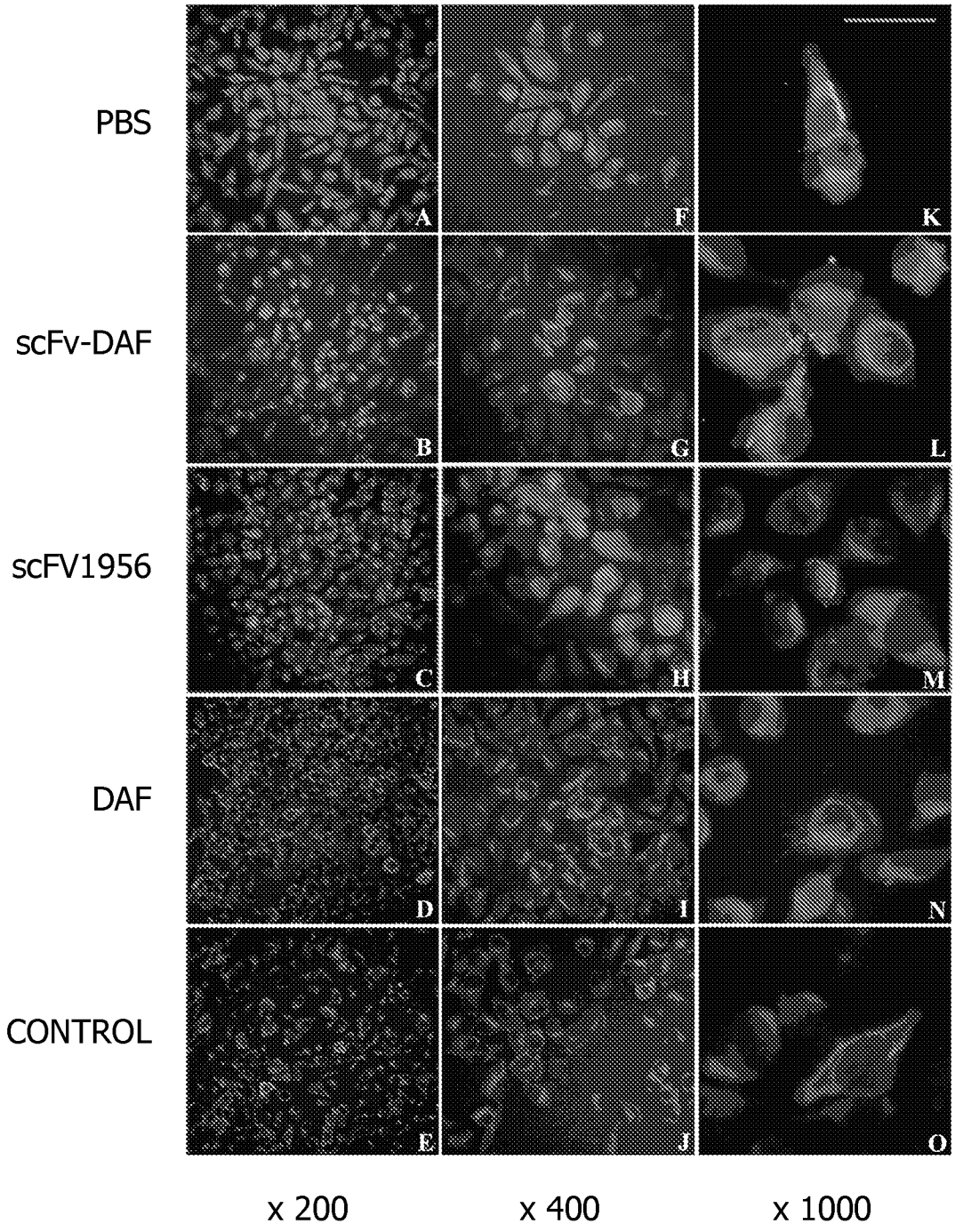

PBS
scFv-DAF
scFV1956
DAF
CONTROL
A
F
K
B
G
L
C
H
M
D
I
N
E
J
O
x 200
x 400
x 1000

FIG. 14

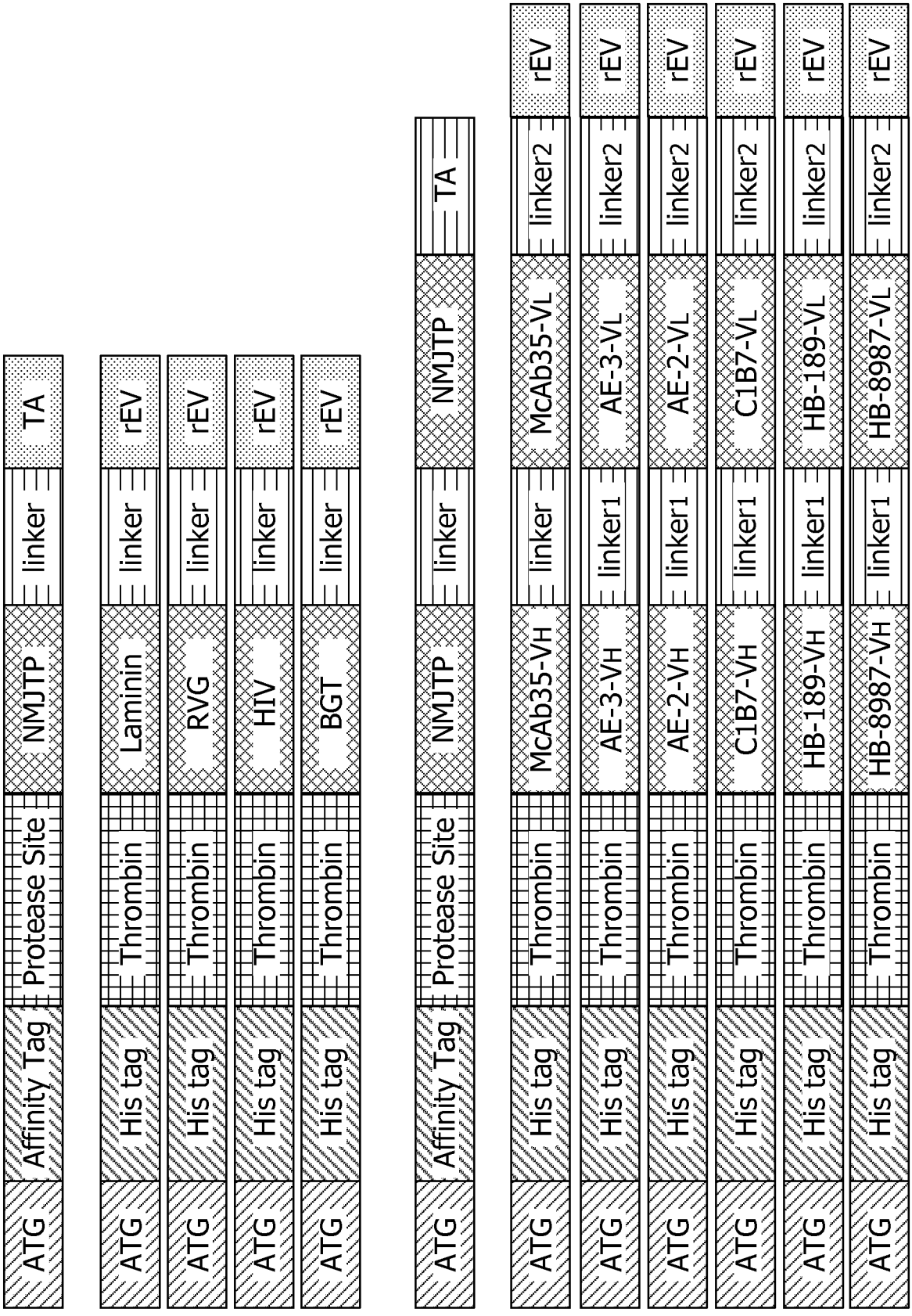

ATG
Affinity Tag
Protease Site
NMJTP
linker
TA
ATG
His tag
Thrombin
Laminin
linker
rEV
ATG
His tag
Thrombin
RVG
linker
rEV
ATG
His tag
Thrombin
HIV
linker
rEV
ATG
His tag
Thrombin
BGT
linker
rEV
ATG
Affinity Tag
Protease Site
NMJTP
linker
TA
ATG
His tag
Thrombin
McAb35-VH
linker
McAb35-VL
linker2
rEV
ATG
His tag
Thrombin
AE-3-VH
linker1
AE-3-VL
linker2
rEV
ATG
His tag
Thrombin
AE-2-VH
linker1
AE-2-VL
linker2
rEV
ATG
His tag
Thrombin
C1B7-VH
linker1
C1B7-VL
linker2
rEV
ATG
His tag
Thrombin
HB-189-VH
linker1
HB-189-VL
linker2
rEV
ATG
His tag
Thrombin
HB-8987-VH
linker1
HB-8987-VL
linker2
rEV

FIG. 15

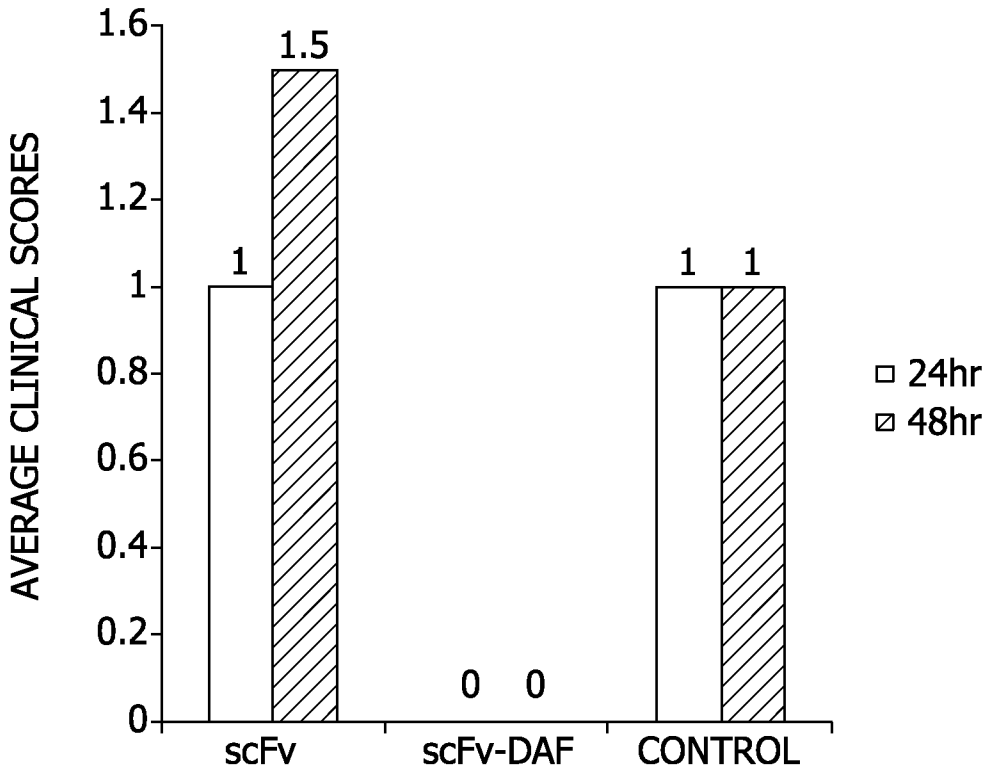
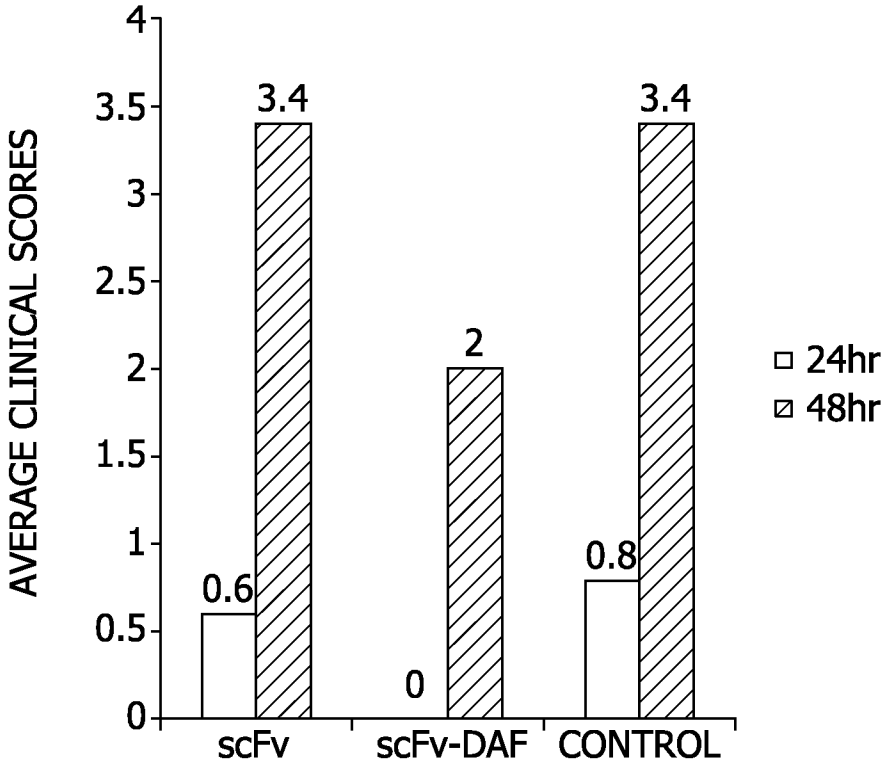

AVERAGE CLINICAL SCORES
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1.5
1
1 1
0 0
scFv
scFv-DAF
CONTROL
24hr
48hr

FIG. 16


AVERAGE CLINICAL SCORES
4
3.5
3
2.5
2
1.5
1
0.5
0
3.4
3.4
2
0.6
0
0.8
scFv
scFv-DAF
CONTROL
24hr
48hr