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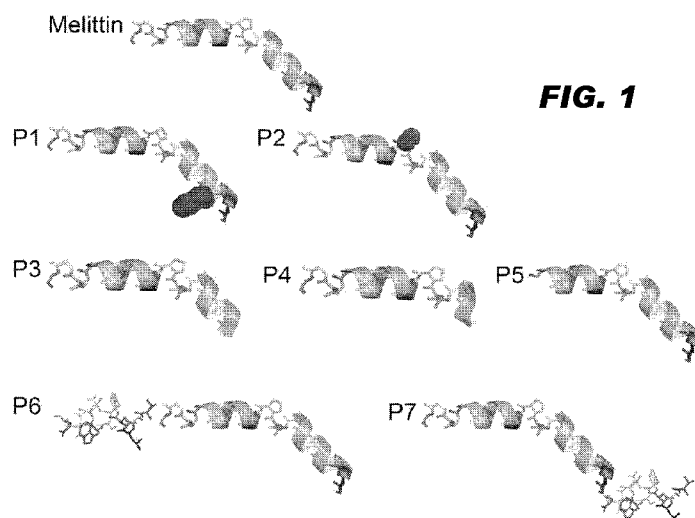
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(54) Title: UNIVERSAL ANCHOR PEPTIDE FOR NANOPARTICLES

**FIG. 1**

(57) Abstract: The present invention provides a substantially non-lytic, non-cytotoxic anchor peptide that is capable of stably inserting into lipid membranes. In particular, the invention provides nanoparticles comprising stably inserted anchor peptides, which may be conjugated to a variety of different cargo complexes.

UNIVERSAL ANCHOR PEPTIDE FOR NANOPARTICLES

GOVERNMENTAL RIGHTS

[0001] This invention was made with government support under grant number U54CA119342 awarded by the National Cancer Center and HL073646 awarded by the National Heart Lung and Blood Institute. The government has certain rights in the invention.

FIELD OF THE INVENTION

[0002] The present invention generally relates to a universal anchor peptide that is capable of stably inserting into lipid membranes. In particular, the anchor peptide is a cationic amphipathic alpha helical peptide that is capable of integrating into lipid membranes of nanoparticle or cells. Moreover, the anchor peptide may be conjugated to a variety of different cargo complexes.

BACKGROUND OF THE INVENTION

[0003] One of the vexing problems with targeted delivery of chemotherapeutic agents or other forms of therapy with the use of nanoparticle carriers is how to associate these agents with specific cells to achieve selective molecular imaging or site targeted drug therapy. This requires the incorporation of a targeting ligand that can bind to a specific molecular epitope on the cell surface, which subsequently allows detection of particle binding by imaging methods, or drug delivery to the cell of choice. Generally, this targeting ligand is formulated into the nanoparticle by a chemical reaction or by physical association, in a process that is integral to the very construction of the nanoparticle itself such that at the end of the process, a singular and highly specific targeting delivery system is produced. In order to produce an alternatively targeted delivery system, the entire formulation process must be recapitulated for another targeting ligand, typically requiring new design strategies for ligand association that could change the formulation process dramatically and affect its performance as a targeted delivery system. Consequently, there is a need in the art for a universal anchor peptide that would allow the pre-formed construction of carrier

systems, and then later allow their flexible association with a particular ligand for targeting, therapeutic, reporting, or imaging purposes.

SUMMARY OF THE INVENTION

[0004] Among the various aspects of the present invention is the provision of a nanoparticle comprising an anchor peptide that is substantially non-lytic and non-cytotoxic. The nanoparticle comprises a core encapsulated by a lipid layer, wherein the anchor peptide is stably inserted into the lipid layer.

[0005] Another aspect of the present invention encompasses a kit for preparing a nanoparticle comprising an anchor peptide. The kit comprises a first composition comprising a nanoparticle that comprises a core encapsulated by a lipid layer. The kit also comprises a second composition comprising the anchor peptide that is substantially non-lytic, non-cytotoxic, and is capable of stably inserting into the lipid layer of the nanoparticle.

[0006] A further aspect of the present invention provides a method for adding a cargo complex to a nanoparticle. The method comprises contacting the nanoparticle comprising a lipid layer with an substantially non-lytic, non-cytotoxic anchor peptide that is conjugated to the cargo complex, wherein the anchor peptide stably inserts into the lipid layer of the nanoparticle.

[0007] Yet another aspect of the invention encompasses an anchor peptide. The anchor peptide is cationic, comprises at least one amphipathic alpha helix, and is substantially non-lytic and non-cytotoxic.

[0008] Other aspects and features of the invention are detailed below.

BRIEF DESCRIPTION OF THE FIGURES

[0009] **FIG. 1** depicts structures of melittin (2mlt) and seven derivatives of melittin (P1 to P7). PDB file of 2mlt was downloaded from protein data bank, and B chain of the 2mlt was used for presenting the structures of melittin and the seven derivatives by PyMOL. The structures are presented with the C-terminal on the left and the N-terminal on the right. P1 had the 5th amino acid valine substituted by tryptophan, which is depicted in a space filling view. P2 had the 14th amino acid proline substituted

by alanine, which is also shown in a space filling model. P3 had the first 4 N-terminal amino acids deleted. P4 had first 7 N-terminal amino acids deleted. P5 had the last 4 C-terminal amino acids deleted. P6 and P7 had another small peptide added to the C- and N-terminal of melittin, respectively.

[0010] **FIG. 2** presents a graph showing the cell lytic actions of native melittin and the melittin derivatives, as defined in **FIG.1**. Plotted is the percent of live cells as a function of peptide concentration.

[0011] **FIG. 3** depicts a series of graphs showing the binding kinetics of linker peptide 1 and linker peptide 2 on perfluorocarbon and oil-based nanoparticles immobilized on Biacore Sensor chip L1. Selected sensorgrams are shown at the indicated concentrations. **(A, B)** Sensorgrams of linker peptide 1 on perfluorocarbon and oil-based nanoparticles. **(C, D)** Sensorgrams of linker peptide 2 on perfluorocarbon and oil-based nanoparticles.

[0012] **FIG. 4** illustrates dissociation of loaded linker peptide 1 from PFOB nanoparticles at different time points after the loading.

[0013] **FIG. 5** shows that insertion of the linker peptide 1 into PFOB nanoparticles forms stable peptide-nanoparticle complexes. **(A)** Presents a plot of the average hydrodynamic diameters and the zeta potentials of PFOB nanoparticles with or without insertion of linker peptide 1. **(B)** Presents a transmission electron microscopy image of linker peptide 1 inserted PFOB nanoparticles in which the lipid membrane of the PFOB nanoparticles appears intact.

[0014] **FIG. 6** illustrates that insertion of linker peptide 1 into the liposomes forms stable peptide-liposome complexes. **(A)** Presents a plot of the average hydrodynamic diameters and the zeta potentials of liposomes with or without insertion of linker peptide 1. **(B)** Presents a transmission electron microscopy image of linker peptide 1 inserted liposomes in which the lipid membrane of the liposomes appears intact..

[0015] **FIG. 7** presents a micrograph showing the cellular distributions of FITC labeled linker peptide 1-associated PFOB nanoparticles in C-32 melanoma cells as visualized by confocal microscopy. **(A)** Cells exposed to FITC labeled linker peptide 1-associated PFOB nanoparticles. **(B)** Cells exposed to plain PFOB nanoparticles.

[0016] **FIG. 8** depicts the binding kinetic of two VCAM-targeting peptides on PFOB nanoparticles immobilized on Biacore Sensor chip L1. **(A)** The sequence and structure of the VCAM-targeting peptide (TCP1), which is an anti-VCAM peptide fused on the C-terminal of linker peptide 1 with two glycines as a spacer. **(B)** The sequence and structure of the VCAM-targeting peptide (TCP2), which is an anti-VCAM peptide fused on the N-terminal of linker peptide 1 with two glycines as a spacer. **(C)** Sensorgram of the VCAM-targeting peptide (TCP1) at the indicated concentrations. **(D)** Sensorgram of the VCAM-targeting peptide (TCP2) at the indicated concentrations.

[0017] **FIG. 9** depicts dissociation of loaded TCP1 **(A)** or TCP2 **(B)** from PFOB nanoparticles at different time points after the loading.

[0018] **FIG. 10** illustrates that insertion of TCP1 or TCP2 into PFOB nanoparticles forms stable peptide-nanoparticle complexes. The average hydrodynamic diameters and zeta potentials of PFOB nanoparticles with TCP1, TCP2, or no peptide are shown.

[0019] **FIG. 11** presents micrographs showing the cellular distribution of Alexa Fluor 488 labeled nanoparticles with VCAM-targeting peptide TCP1 **(A)**, Alexa Fluor 488 labeled nanoparticles with VCAM-targeting peptide TCP2 **(B)**, and nanoparticles without the VCAM-targeting peptide **(C)** in mouse endothelial cells (2F2B cells).

[0020] **FIG. 12** depicts functional importance of specific linker-cargo conjugation site. **(A-C)** Representative ^{19}F MR spectra of 2F2B endothelial cells treated with VTNP1, VTNP2, or non-targeted PFC nanoparticles, respectively. **(D)** Quantification of specific delivery of VTNP1, VTNP2, and non-targeted nanoparticles (NP) to 2F2B cells calculated from ^{19}F MR spectra. VTNP1 or VTNP2 were generated with addition of 100 nmol of TCP1 or TCP2 on 20 μl CE nanoparticles, respectively. Data are mean $\pm$ STD (n=6). **(E)** Dose-dependent comparison between VTNP1 and VTNP2 at selected peptide loadings into 20 μl CE nanoparticles. X-axis represented the amount peptide; Y-axis represented the number of nanoparticles delivered to one 2F2B endothelial cell. A log scale was used. **(F)** Fluorine magnetic resonance image overlaid onto proton images of cell pellets in test tubes, acquired from cells treated

with VTNP2, NP, or VTNP1, from top to bottom. The ^{19}F signal (bright pellet) was apparent only in the cells treated with VTNP1.

[0021] **FIG. 13** illustrates that treatment with NBD loaded PFOB nanoparticles partially inhibit NF-kB activation. Plotted is the amount of p65 translocated into the nucleus without TNF- α stimulation (Non-Sti), after TNF- α stimulation for 4 hours (Sti), and after 1 hour pretreatment with VCAM-1 targeted NBD-loaded PFOB nanoparticles followed by TNF- α stimulation (NBD-NP-Sti).

[0022] **FIG. 14** depicts that treatment with NBD loaded PFOB nanoparticles decreases the expression of NF-kB dependent gene. **(A)** Plotted is the amount of IL-6 expressed without TNF- α stimulation (Non-Sti), after TNF- α stimulation for 4 hours (Sti), and after 1 hour pretreatment with VCAM-1 targeted NBD-loaded PFOB nanoparticles followed by TNF- α stimulation (NBD-NP-Sti). **(B)** Shown are micrographs of VCAM-1 expression on the cell membrane in the absence of TNF- α stimulation (Non-Sti), after TNF- α stimulation for 4 hours (Sti), and after 1 hour pretreatment with VCAM-1 targeted NBD-loaded PFOB nanoparticles followed by TNF- α stimulation (NBD-NP-Sti). VCAM-1 expression was detected with a FITC conjugated secondary antibody. Nuclei were stained with DAPI.

[0023] **FIG. 15** shows that the linker peptide 1 itself can be used to label cells. FITC-conjugated linker peptide 1 was incubated with human red blood cells for 1 hour, and a fluorescence image **(A)** and a bright field image **(B)** were taken after washing. The scale bars represent 100 μm .

DETAILED DESCRIPTION OF THE INVENTION

[0024] The present invention provides an anchor peptide that may be utilized in several applications to link a variety of cargo complexes to a nanoparticle and facilitate the delivery of the cargo complex to a cell. In particular, the anchor peptide is a cationic, amphipathic alpha helical peptide that is capable of stably inserting into lipid membranes. Furthermore, the anchor peptide is typically functionalized so that it may bind a variety of cargo complexes. Suitable cargo complexes may include imaging, therapeutic, cytotoxic, or targeting complexes. Accordingly, the anchor peptide, which may be linked to a cargo complex, is capable of stably inserting into the lipid membrane

of a nanoparticle. Upon delivery of the nanoparticle to a cell, the anchor peptide-cargo complex is capable of dissociating from the lipid membrane of the nanoparticle and stably associating with the lipid membrane of the cell, thereby delivering the cargo complex to the cell. In some embodiments, the anchor peptide may stably insert into the lipid bilayer of the cell, such that the associated cargo complex may be displayed on the surface of the cell. In other embodiments, the anchor peptide may penetrate the lipid bilayer of the cell, thereby delivering the associated cargo complex to the interior of the cell.

[0025] Accordingly, the present invention provides a nanoparticle comprising a stably inserted anchor peptide, a kit for preparing a nanoparticle comprising a stably inserted anchor peptide, and methods for adding cargo complexes to the nanoparticles.

(I) *Nanoparticles Comprising a Universal Anchor Peptide*

(a) anchor peptide

[0026] One aspect of the present invention encompasses an anchor peptide that is capable of stably inserting into a lipid membrane. The lipid membrane may be a monolayer, a bilayer, or a multilaminar bilayer. Accordingly, the lipid membrane may be part of a nanoparticle, a cell, or a liposome.

[0027] In general, the anchor peptide comprises at least one hydrophobic segment. More specifically, the hydrophobic segment of the anchor peptide comprises at least one amphipathic alpha helix. In some embodiments, the anchor peptide may comprise two amphipathic alpha helices. In other embodiments, the anchor peptide may comprise more than two alpha helices. The anchor peptide may also contain at least one hydrophilic segment. In some embodiments, the hydrophilic segment is positively charged. That is, the hydrophilic segment comprises a majority of positively charged amino acids (i.e., Arg, Lys, or His). Preferably, the overall charge of the anchor peptide is cationic. Thus, an exemplary anchor peptide comprises a cationic amphipathic alpha helical peptide. Accordingly, the amphipathic alpha helical segment stably inserts into a lipid membrane and the hydrophilic cationic segment remains on the surface of the lipid membrane.

[0028] As used herein, the terms “stably inserts” or “stably inserted” indicate that the hydrophobic segment of the anchor peptide integrates into the midst of a lipid monolayer or bilayer membrane. Stated another way, the amphipathic alpha helical segment of the anchor peptide interdigitates with the lipid membrane. The interactions between the hydrophobic amino acids of the anchor peptide and the lipid membrane are non-covalent hydrophobic interactions and/or van der Waals interactions. In embodiments in which the overall charge of the anchor peptide is positive, the stable integration of the anchor peptide into a lipid membrane may be assessed by changes in the zeta potential of the membrane. Specifically, the zeta potential shifts to a more positive value upon stable insertion of the positively charged anchor peptide into the lipid membrane.

[0029] In general, the anchor peptide has a high association affinity for a lipid membrane and a small dissociation constant, which allows it to rapidly and stably insert into a lipid membrane. In general, the association rate is typically greater than about $9.0 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$. In some embodiments, the association rate may be greater than about 1.0×10^6 , 2.0×10^6 , 3.0×10^6 , 4.0×10^6 , 5.0×10^6 , 6.0×10^6 , 7.0×10^6 , 8.0×10^6 , 9.0×10^6 , or $1.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$. In an exemplary embodiment, the association rate may be greater than about $4.0 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$. Methods of calculating the association rate are known in the art, and may, for instance, be calculated using the Biacore system.

[0030] The dissociation constant typically is less than about $1.0 \times 10^{-6} \text{ M}$. In some embodiments, the dissociation constant may be between about 1.0×10^{-6} and $1.0 \times 10^{-7} \text{ M}$. In other embodiments, the dissociation constant may be between about 1.0×10^{-7} and $1.0 \times 10^{-8} \text{ M}$. In still other embodiments, the dissociation constant may be between about 1.0×10^{-8} and $1.0 \times 10^{-9} \text{ M}$. In yet other embodiments, the dissociation constant may be less than $9.9 \times 10^{-10} \text{ M}$. Methods of calculating the dissociation rate are known in the art, and may, for instance, be calculated using the Biacore system, as detailed in Example 2.

[0031] Furthermore, the anchor peptide is substantially non-lytic and non-cytotoxic to cells. Although the anchor peptide is substantially non-lytic and non-cytotoxic, a cargo complex conjugated to the anchor peptide may be non-lytic or non-cytotoxic (as detailed below). The term “non-lytic” means that the lipid bilayer of the cell

typically is not compromised upon contact with the anchor peptide. The integrity of the lipid bilayer may be assessed by the improper entry or exit of cellular or extracellular components into a cell. For example, cellular proteins and/or organelles may leak out of a cell with a compromised lipid bilayer. Alternatively, extracellular components (i.e., those that normally do not enter via gap junctions, for example) may enter a cell with a compromised lipid bilayer. It should be noted, however, that the anchor peptide may penetrate the lipid bilayer of a cell and enter the interior of the cell, but in doing so the integrity of the lipid bilayer is not affected. The term “non-cytotoxic” indicates that the cell typically is not killed upon contact with the anchor peptide. For example, in one embodiment, more than 95% of the cells are viable after contact with the anchor peptide for at least 3 hours. More preferably, more than 99% of the cells are viable after contact with the anchor peptide for at least 3 hours. In embodiments in which the anchor peptide is a derivative or mutant of melittin (see below), the cytotoxicity index or IC₅₀ of the anchor peptide will generally be at least about 50-fold higher than that of melittin.

[0032] As stated above, the anchor peptide comprises at least one hydrophobic segment and preferably at least one hydrophilic segment. The hydrophobic segment comprises mainly non-polar amino acids, such as Phe (F), Met (M), Ala (A), Gly (G), Ile (I), Leu (L), Val (V), Pro (P), Cys (C), or Trp (W). In some embodiments about 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the amino acids of a hydrophobic segment of an anchor peptide are hydrophobic as defined herein. The optional hydrophilic segment of the anchor peptide comprises primarily polar amino acids, such as, Tyr (Y), Ser (S), Thr (T), Lys (K), His (H), Gln (Q), Glu (E), Arg (R), Asp (D), or Asn (N). In some embodiments about 70%, 75%, 80%, 85%, 90%, 95%, or 100% of the amino acids of a hydrophilic segment of an anchor peptide are hydrophilic as defined herein.

[0033] The length of the anchor peptide can and will vary depending upon the intended use of the anchor peptide and/or the intended cargo complex to be linked to the anchor peptide. The anchor peptide (i.e., in the absence of any conjugated cargo) may range from about 10 amino acids to about 50 amino acids in length. In general, at least one amphipathic alpha helical segment of the anchor peptide is long enough to stably interact with the lipid membrane, but short enough to avoid unwanted

molecular interactions. In some embodiments, the amphipathic alpha helical segment may be between about 5 amino acids and about 20 amino acids in length. In other embodiments, the amphipathic alpha helical segment may be between about 5 amino acids and about 18 amino acids in length. In certain embodiments, the amphipathic alpha helical segment may be between about 5 amino acids and about 13 amino acids in length. In still other embodiments, the amphipathic alpha helical segment may be between about 5 amino acids and about 10 amino acids in length. In yet other embodiments, the amphipathic alpha helical segment may be between about 5 amino acids and about 8 amino acids in length. Similarly, the optional hydrophilic segment of the anchor peptide may range from about 3 amino acids to about 10 amino acids in length. In some embodiments, however, the hydrophilic segment may represent a conjugated cargo complex. Thus, depending on the role of the hydrophobic anchoring segment, the hydrophilic segment may be a cargo peptide or protein, an antibody or antibody fragment, genomic material (i.e., DNA or RNA), or a natural or synthetic small molecule (see section (I)(c) below).

[0034] In one preferred embodiment, the at least one hydrophobic segment of the anchor peptide may comprise at least the amino acid sequence ALISWI (SEQ ID NO:1) or the amino acid sequence AWISWI (SEQ ID NO:2). In another embodiment, the hydrophobic segment may comprise SEQ ID NO:1 or SEQ ID NO:2 and additional N-terminal or C-terminal hydrophobic amino acid residues or a substantially hydrophobic amino acid sequence. The at least one hydrophilic segment of the anchor peptide may be located on the N-terminal side of the hydrophobic or amphipathic alpha helical segment, or alternatively, on the C-terminal side of the hydrophobic or amphipathic alpha helical segment. In some preferred embodiments, the hydrophilic segment may comprise at least the amino acid sequence KRKRQQ (SEQ ID NO:3) or C-terminal truncations thereof.

[0035] In other preferred embodiments, the anchor peptide may comprise the amino acid sequence $X_{aa1}ALISWIX_{aa2}$, (SEQ ID NO:4) or the amino acid sequence $X_{aa1}AWISWIX_{aa2}$ (SEQ ID NO:5), wherein X_{aa1} represents a hydrophobic amino acid as defined above, and X_{aa2} represents a hydrophilic amino acid as defined above. In another preferred embodiment, the anchor peptide may comprise the amino acid

sequence $X_{aa1}GLX_{aa3}ALISWIKRKRQQ$ (SEQ ID NO:6) or the amino acid sequence $X_{aa1}GLX_{aa3}AWISWIKRKRQQ$ (SEQ ID NO:7), wherein X_{aa1} is as defined above, and X_{aa3} may represent an amino acid selected from the group comprising Pro (P), Ala (A), Met (M), Leu (L), Ile (I), or Trp (W). In some embodiments, X_{aa3} may be located on the same face of the alpha helix as other non-polar amino acids and may be a non-polar amino acid. If X_{aa3} is a non-polar amino acid on the same face of the alpha helix as other non-polar amino acids, X_{aa3} may facilitate association with a lipid membrane.

[0036] In still yet another embodiment, the anchor peptide may comprise the amino acid sequence $X_{aa1}TTGLX_{aa3}ALISWIKRKRQQ$ (SEQ ID NO:8) or the amino acid sequence $X_{aa1}TTGLX_{aa3}AWISWIKRKRQQ$ (SEQ ID NO:9), wherein X_{aa1} and X_{aa3} are as defined above. As above, in some embodiments X_{aa3} may be located on the same face of the alpha helix as other non-polar amino acids and may be a non-polar amino acid. If X_{aa3} is a non-polar amino acid on the same face of the alpha helix as other non-polar amino acids, X_{aa3} may facilitate association with a lipid membrane.

[0037] In exemplary embodiments, the anchor peptide may consist of an amino acid sequence selected from the group consisting of SEQ ID NO:10 to SEQ ID NO:218, as detailed in Table A. In an especially exemplary embodiment, the anchor peptide may consist of the amino acid sequence of SEQ ID NO:88.

Table A. Exemplary Amino Acid Sequences of the Anchor Peptide

Amino Acid Sequence	SEQ ID NO:	Amino Acid Sequence	SEQ ID NO:
GIGAWLKVLTTGLPALISWIKRKRQQ	10	GIGAWLKVLTTGLPAWISWIKRKRQQ	114
GIGAWLKVLTTGLPALISWIKRKRQ	11	GIGAWLKVLTTGLPAWISWIKRKRQ	115
GIGAWLKVLTTGLPALISWIKRKR	12	GIGAWLKVLTTGLPAWISWIKRKR	116
IGAWLKVLTTGLPALISWIKRKRQQ	13	IGAWLKVLTTGLPAWISWIKRKRQQ	117
IGAWLKVLTTGLPALISWIKRKRQ	14	IGAWLKVLTTGLPAWISWIKRKRQ	118
IGAWLKVLTTGLPALISWIKRKR	15	IGAWLKVLTTGLPAWISWIKRKR	119
GAWLKVLTTGLPALISWIKRKRQQ	16	GAWLKVLTTGLPAWISWIKRKRQQ	120
GAWLKVLTTGLPALISWIKRKRQ	17	GAWLKVLTTGLPAWISWIKRKRQ	121
GAWLKVLTTGLPALISWIKRKR	18	GAWLKVLTTGLPAWISWIKRKR	122
AWLKVLTTGLPALISWIKRKRQQ	19	AWLKVLTTGLPAWISWIKRKRQQ	123
AWLKVLTTGLPALISWIKRKRQ	20	AWLKVLTTGLPAWISWIKRKRQ	124
AWLKVLTTGLPALISWIKRKR	21	AWLKVLTTGLPAWISWIKRKR	125
WLKVLTTGLPALISWIKRKRQQ	22	WLKVLTTGLPAWISWIKRKRQQ	126
WLKVLTTGLPALISWIKRKRQ	23	WLKVLTTGLPAWISWIKRKRQ	127
WLKVLTTGLPALISWIKRKR	24	WLKVLTTGLPAWISWIKRKR	128
GIGAVLKVLTTGLAALISWIKRKRQQ	25	GIGAVLKVLTTGLAAWISWIKRKRQQ	129
GIGAVLKVLTTGLAALISWIKRKRQ	26	GIGAVLKVLTTGLAAWISWIKRKRQ	130

Table A. Exemplary Amino Acid Sequences of the Anchor Peptide

Amino Acid Sequence	SEQ ID NO:	Amino Acid Sequence	SEQ ID NO:
GIGAVLKVLTTGLAALISWIKRKR	27	GIGAVLKVLTTGLAAWISWIKRKR	131
IGAVLKVLTTGLAALISWIKRKRQQ	28	IGAVLKVLTTGLAAWISWIKRKRQQ	132
IGAVLKVLTTGLAALISWIKRKRQ	29	IGAVLKVLTTGLAAWISWIKRKRQ	133
IGAVLKVLTTGLAALISWIKRKR	30	IGAVLKVLTTGLAAWISWIKRKR	134
GAVLKVLTTGLAALISWIKRKRQQ	31	GAVLKVLTTGLAAWISWIKRKRQQ	135
GAVLKVLTTGLAALISWIKRKRQ	32	GAVLKVLTTGLAAWISWIKRKRQ	136
GAVLKVLTTGLAALISWIKRKR	33	GAVLKVLTTGLAAWISWIKRKR	137
AVLKVLTTGLAALISWIKRKRQQ	34	AVLKVLTTGLAAWISWIKRKRQQ	138
AVLKVLTTGLAALISWIKRKRQ	35	AVLKVLTTGLAAWISWIKRKRQ	139
AVLKVLTTGLAALISWIKRKR	36	AVLKVLTTGLAAWISWIKRKR	140
VLKVLTTGLAALISWIKRKRQQ	37	VLKVLTTGLAAWISWIKRKRQQ	141
VLKVLTTGLAALISWIKRKRQ	38	VLKVLTTGLAAWISWIKRKRQ	142
VLKVLTTGLAALISWIKRKR	39	VLKVLTTGLAAWISWIKRKR	143
LKVLTTGLAALISWIKRKRQQ	40	LKVLTTGLAAWISWIKRKRQQ	144
LKVLTTGLAALISWIKRKRQ	41	LKVLTTGLAAWISWIKRKRQ	145
LKVLTTGLAALISWIKRKR	42	LKVLTTGLAAWISWIKRKR	146
KVLTTGLAALISWIKRKRQQ	43	KVLTTGLAAWISWIKRKRQQ	147
KVLTTGLAALISWIKRKRQ	44	KVLTTGLAAWISWIKRKRQ	148
KVLTTGLAALISWIKRKR	45	KVLTTGLAAWISWIKRKR	149
VLTGLAALISWIKRKRQQ	46	VLTGLAAWISWIKRKRQQ	150
VLTGLAALISWIKRKRQ	47	VLTGLAAWISWIKRKRQ	151
VLTGLAALISWIKRKR	48	VLTGLAAWISWIKRKR	152
LTTGLAALISWIKRKRQQ	49	LTTGLAAWISWIKRKRQQ	153
LTTGLAALISWIKRKRQ	50	LTTGLAAWISWIKRKRQ	154
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TTGLAALISWIKRKRQQ	52	TTGLAAWISWIKRKRQQ	156
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TGLAALISWIKRKRQQ	55	TGLAAWISWIKRKRQQ	159
TGLAALISWIKRKRQ	56	TGLAAWISWIKRKRQ	160
TGLAALISWIKRKR	57	TGLAAWISWIKRKR	161
GLAALISWIKRKRQQ	58	GLAAWISWIKRKRQQ	162
GLAALISWIKRKRQ	59	GLAAWISWIKRKRQ	163
GLAALISWIKRKR	60	GLAAWISWIKRKR	164
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LAALISWIKRKR	63	LAAWISWIKRKR	167
AALISWIKRKRQQ	64	AAWISWIKRKRQQ	168
AALISWIKRKRQ	65	AAWISWIKRKRQ	169
AALISWIKRKR	66	AAWISWIKRKR	170
ALISWIKRKRQQ	67	AWISWIKRKRQQ	171
ALISWIKRKRQ	68	AWISWIKRKRQ	172
ALISWIKRKR	69	AWISWIKRKR	173
IGAVLKVLTTGLPALISWIKRKRQQ	70	IGAVLKVLTTGLPAWISWIKRKRQQ	174
IGAVLKVLTTGLPALISWIKRKRQ	71	IGAVLKVLTTGLPAWISWIKRKRQ	175
IGAVLKVLTTGLPALISWIKRKR	72	IGAVLKVLTTGLPAWISWIKRKR	176
GAVLKVLTTGLPALISWIKRKRQQ	73	GAVLKVLTTGLPAWISWIKRKRQQ	177

Table A. Exemplary Amino Acid Sequences of the Anchor Peptide

Amino Acid Sequence	SEQ ID NO:	Amino Acid Sequence	SEQ ID NO:
GAVLKVLTTGLPALISWIKRKRQ	74	GAVLKVLTTGLPAWISWIKRKRQ	178
GAVLKVLTTGLPALISWIKRKR	75	GAVLKVLTTGLPAWISWIKRKR	179
AVLKVLTTGLPALISWIKRKRQQ	76	AVLKVLTTGLPAWISWIKRKRQQ	180
AVLKVLTTGLPALISWIKRKRQ	77	AVLKVLTTGLPAWISWIKRKRQ	181
AVLKVLTTGLPALISWIKRKR	78	AVLKVLTTGLPAWISWIKRKR	182
VLKVLTTGLPALISWIKRKRQQ	79	VLKVLTTGLPAWISWIKRKRQQ	183
VLKVLTTGLPALISWIKRKRQ	80	VLKVLTTGLPAWISWIKRKRQ	184
VLKVLTTGLPALISWIKRKR	81	VLKVLTTGLPAWISWIKRKR	185
LKVLTTGLPALISWIKRKRQQ	82	LKVLTTGLPAWISWIKRKRQQ	186
LKVLTTGLPALISWIKRKRQ	83	LKVLTTGLPAWISWIKRKRQ	187
LKVLTTGLPALISWIKRKR	84	LKVLTTGLPAWISWIKRKR	188
KVLTTGLPALISWIKRKRQQ	85	KVLTTGLPAWISWIKRKRQQ	189
KVLTTGLPALISWIKRKRQ	86	KVLTTGLPAWISWIKRKRQ	190
KVLTTGLPALISWIKRKR	87	KVLTTGLPAWISWIKRKR	191
VLTGLPALISWIKRKRQQ	88	VLTGLPAWISWIKRKRQQ	192
VLTGLPALISWIKRKRQ	89	VLTGLPAWISWIKRKRQ	193
VLTGLPALISWIKRKR	90	VLTGLPAWISWIKRKR	194
LTGLPALISWIKRKRQQ	91	LTGLPAWISWIKRKRQQ	195
LTGLPALISWIKRKRQ	92	LTGLPAWISWIKRKRQ	196
LTGLPALISWIKRKR	93	LTGLPAWISWIKRKR	197
TTGLPALISWIKRKRQQ	94	TTGLPAWISWIKRKRQQ	198
TTGLPALISWIKRKRQ	95	TTGLPAWISWIKRKRQ	199
TTGLPALISWIKRKR	96	TTGLPAWISWIKRKR	200
TGLPALISWIKRKRQQ	97	TGLPAWISWIKRKRQQ	201
TGLPALISWIKRKRQ	98	TGLPAWISWIKRKRQ	202
TGLPALISWIKRKR	99	TGLPAWISWIKRKR	203
GLPALISWIKRKRQQ	100	GLPAWISWIKRKRQQ	204
GLPALISWIKRKRQ	101	GLPAWISWIKRKRQ	205
GLPALISWIKRKR	102	GLPAWISWIKRKR	206
LPALISWIKRKRQQ	103	LPAWISWIKRKRQQ	207
LPALISWIKRKRQ	104	LPAWISWIKRKRQ	208
LPALISWIKRKR	105	LPAWISWIKRKR	209
PALISWIKRKRQQ	106	PAWISWIKRKRQQ	210
PALISWIKRKRQ	107	PAWISWIKRKRQ	211
PALISWIKRKR	108	PAWISWIKRKR	212
GIGAVLKVLTTGLPALISWIKRKRQ	109	GIGAVLKVLTTGLPAWISWIKRKRQQ	213
GIGAVLKVLTTGLPALISWIKRKR	110	GIGAVLKVLTTGLPAWISWIKRKRQ	214
GIGAVLKVLTTGLPALISWIKRK	111	GIGAVLKVLTTGLPAWISWIKRKR	215
GIGAVLKVLTTGLPALISWIKR	112	GIGAVLKVLTTGLPAWISWIKRK	216
GIGAVLKVLTTGLPALISWIK	113	GIGAVLKVLTTGLPAWISWIKR	217
		GIGAVLKVLTTGLPAWISWIK	218

[0038] The anchor peptide of the invention may be produced using means commonly known in the art.

(b) functionalization

[0039] As stated above, the anchor peptide may be functionalized so as to form a bond with a cargo complex. Generally speaking, the anchor peptide may be functionalized with any of a variety of active groups known in the art so as to facilitate bond formation with a cargo complex. The bond may be a covalent bond or a non-covalent bond. For instance, the bond may be a covalent bond, a hydrogen bond, an ionic bond, a bond based on van der Waals, or a hydrophobic bond. One of skill in the art would recognize that the choice of functional group can and will vary depending on the cargo complex.

[0040] By way of non-limiting example, the anchor peptide may be functionalized with an active group, such as a photo-reactive group, that when contacted with light may become activated, and capable of covalently attaching to the cargo complex. Exemplary reactive groups include, but are not limited to, reactive groups typically used in the preparation of chromatography media which include: epoxides, oxiranes, esters of N-hydroxysuccinimide, aldehydes, hydrazines, maleimides, mercaptans, amino groups, alkylhalides, isothiocyanates, carbodiimides, diazo compounds, trisyl chloride, tosyl chloride, and trichloro S-triazine. Exemplary photo-reactive groups include aryl azides, diazaarenes, beta-carbonyldiazo, and benzophenones. The reactive species are nitrenes, carbenes, and radicals. These reactive species are generally capable of covalent bond formation.

[0041] In some embodiments, the anchor peptide may be functionalized with a bi-functional peptide that would connect the anchor peptide to the cargo complex. The bi-functional peptide may be homo-bi-functional or hetero-bi-functional.

[0042] Methods of functionalizing the anchor peptide are known in the art.

(c) cargo complex

[0043] The anchor peptide may be bound to a cargo complex. The means by which the complex is associated with the anchor peptide can and will vary depending on the embodiment. The cargo complex may be covalently or non-covalently bound to the anchor peptide, as detailed above. The cargo complex may be any molecule or agent that may be carried by or bound to the anchor peptide of a nanoparticle, and in

some instances, may even be a complex of micron size. For instance, the cargo complex may be an imaging cargo, a therapeutic cargo, a cytotoxic cargo, or a targeting cargo.

(i) imaging cargo

[0044] Non-limiting examples of imaging cargo molecules and agents may include any molecule, agent, or material having a detectable physical or chemical property. Such imaging cargos have been well-developed in the field of fluorescent imaging, magnetic resonance imaging, positron emission tomography, Raman imaging, optical coherence tomography, photoacoustic imaging, Fourier transform infrared imaging, or immunoassays and, in general, most any label useful in such methods may be applied to the present invention. Thus, an imaging cargo may be any molecule or agent detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical, physical (e.g. atomic force microscopy) or chemical means. Useful imaging molecules and agents in the present invention may include visible or infrared fluorescent dyes (e.g., fluorescein isothiocyanate, AlexaFluor555, Texas red, rhodamine, and the like), radiolabels (e.g., ^3H , ^{14}C , ^{35}S , ^{125}I , ^{121}I , ^{112}In , $^{99\text{m}}\text{Tc}$), other imaging agents such as microbubbles or nanobubbles (for ultrasound imaging), ^{18}F , ^{11}C , ^{15}O , (for positron emission tomography), $^{99\text{m}}\text{Tc}$, ^{111}In (for single photon emission tomography), gadolinium chelate or iron (for magnetic resonance imaging), enzymes (e.g., horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and calorimetric labels such as colloidal gold or colored glass or plastic (e.g. polystyrene, polypropylene, latex, and the like) beads. Patents that describe the use of such imaging molecules include U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241, each incorporated herein by reference in their entirety. See also Handbook of Fluorescent Probes and Research Chemicals, 6th Ed., Molecular Probes, Inc., Eugene Oreg.

[0045] The anchor peptide may also be conjugated directly to signal generating compounds, e.g., by conjugation with an enzyme or fluorophore. Enzymes of interest may be hydrolases, particularly phosphatases, esterases and glycosidases, or oxidoreductases, particularly peroxidases. Fluorescent compounds may include

fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, and the like. Chemiluminescent compounds may include luciferin, and 2,3-dihydrophthalazinediones, e.g., luminol. For a review of various labeling or signal producing systems that may be used, see U.S. Pat. No. 4,391,904, incorporated herein by reference in its entirety.

(ii) therapeutic cargo

[0046] Non-limiting examples of therapeutic cargo may include any substance that has a biological activity, such as pharmacological agents. Such therapeutic cargo may include analgesics, antipyretics, antiasthmatics, antibiotics, antidepressants, antidiabetics, antifungal agents, antihypertensive agents, anti-inflammatories including non-steroidal and steroidal, antineoplastics, antianxiety agents, immunosuppressive agents, antimigraine agents, sedatives, hypnotics, antianginal agents, antipsychotic agents, antimanic agents, antiarrhythmics, antiarthritic agents, antigout agents, anticoagulants, thrombolytic agents, antifibrinolytic agents, hemorheologic agents, antiplatelet agents, anticonvulsants, antiparkinson agents, antihistamines, anti-restenosis agents, antipruritics, agents useful for calcium regulation, antibacterial agents, antiviral agents, antimicrobials, anti-infectives, bronchodilators, steroidal compounds and hormones, and combinations thereof. Alternatively, the cargo complex could be in the form of components of molecular complexes or pharmacologically acceptable salts.

[0047] Suitable therapeutic cargos include, without limit, immune-related agents such as immune serums, antitoxins, antivenoms bacterial vaccines, viral vaccines, rabies prophylaxis products; thyroid agents such as iodine products and anti-thyroid agents; respiratory products such as xanthine derivatives theophylline and aminophylline; antineoplastic agents such as platinum compounds (e.g., spiroplatin, cisplatin, and carboplatin), methotrexate, fluorouracil, adriamycin, mitomycin, ansamitocin, bleomycin, cytosine arabinoside, arabinosyl adenine, mercaptopolylysine, vincristine, busulfan, chlorambucil, melphalan (e.g., PAM, L-PAM or phenylalanine mustard), mercaptopurine, mitotane, procarbazine hydrochloride dactinomycin (actinomycin D), daunorubicin hydrochloride, doxorubicin hydrochloride, paclitaxel and

other taxenes, rapamycin, manumycin A, TNP-470, plicamycin (mithramycin), aminoglutethimide, estramustine phosphate sodium, flutamide, leuprolide acetate, megestrol acetate, tamoxifen citrate, testolactone, trilostane, amsacrine (m-AMSA), asparaginase (L-asparaginase) Erwinia asparaginase, interferon α -2a, interferon α -2b, teniposide (VM-26), vinblastine sulfate (VLB), vincristine sulfate, bleomycin sulfate, hydroxyurea, procarbazine, and dacarbazine; anti-helminthics such as pyrantel pamoate, piperazine, tetrachloroethylene, thiabendazole, and niclosamide; antimalarials such as chloroquine, amodiaquine, antifolate drugs, proguanil (chloroguanide), mefloquine, quinine, halofantrine, artemesinin and derivatives, primaquine, doxycycline, tetracycline, and clindamycin; mitotic inhibitors such as tauromustine, bofumustine, fotemustine, etoposide, colchicine, methotrexate, fluorouracil, 5-bromodeoxyuridine, 6-azacytidine, cytarabine and the vinca alkaloids, such as vincristine, paclitaxel, etoposide, nocodazole, indirubin, anthracycline derivatives, daunorubicin, daunomycin, plicamycin, and the like; hormones such as androgens, progestins, estrogens and antiestrogens, growth hormone, melanocyte stimulating hormone, estradiol, beclomethasone dipropionate, betamethasone, betamethasone acetate and betamethasone sodium phosphate, betamethasone disodium phosphate, betamethasone sodium phosphate, cortisone acetate, dexamethasone, dexamethasone acetate, dexamethasone sodium phosphate, flunisolide, hydrocortisone, hydrocortisone acetate, hydrocortisone cypionate, hydrocortisone sodium phosphate, hydrocortisone sodium succinate, methylprednisolone, methylprednisolone acetate, methylprednisolone sodium succinate, paramethasone acetate, prednisolone, prednisolone acetate, prednisolone sodium phosphate, prednisolone tebutate, prednisone, triamcinolone, triamcinolone acetonide, triamcinolone diacetate, triamcinolone hexacetonide, fludrocortisone acetate, oxytocin, vasopressin, glucagon and their derivatives; antiprotozoans such as chloroquine, hydroxychloroquine, metronidazole, quinine, and meglumine antimonite; antituberculars such as para-aminosalicylic acid, isoniazid, capreomycin sulfate cycloserine, ethambutol hydrochloride ethionamide, pyrazinamide, rifampin, and streptomycin sulfate; cardiovascular products such as chelating agents and mercurial diuretics and cardiac glycosides; blood products such as parenteral iron, hemin, hematoporphyrins and their derivatives; biological response modifiers such as muramyl dipeptide,

muramyltripeptide, microbial cell wall components, lymphokines (e.g., bacterial endotoxin such as lipopolysaccharide, macrophage activation factor), sub-units of bacteria (such as Mycobacteria, Corynebacteria), the synthetic dipeptide N-acetylmuramyl-L-alanyl-D-isoglutamine, and the like; anti-fungal agents such as ketoconazole, nystatin, griseofulvin, flucytosine (5-fc), miconazole, amphotericin B, ricin, cyclosporins, and β -lactam antibiotics (e.g., sulfazecin); vitamins such as cyanocobalamin neinoic acid, retinoids and derivatives such as retinol palmitate, and α -tocopherol; peptides such as manganese super oxide dismutase; enzymes such as alkaline phosphatase; anti-allergic agents such as amalexanox; anti-coagulation agents such as phenprocoumon, and heparin; circulatory drugs such as propranolol and other beta blockers; metabolic potentiators such as glutathione; antivirals such as acyclovir, amantadine azidothymidine (AZT, DDI, Foscarnet, or Zidovudine), ribavirin and vidarabine monohydrate (adenine arabinoside, ara-A); antianginals such as diltiazem, nifedipine, verapamil, erythritol tetranitrate, isosorbide dinitrate, nitroglycerin (glyceryl trinitrate) and pentaerythritol tetranitrate; antibiotics such as dapsone, chloramphenicol, neomycin, cefaclor, cefadroxil, cephalixin, cephradine erythromycin, clindamycin, lincomycin, amoxicillin, ampicillin, bacampicillin, carbenicillin, dicloxacillin, cyclacillin, picloxacillin, hetacillin, methicillin, nafcillin, oxacillin, penicillin including penicillin G and penicillin V, ticarcillin rifampin, aminoglycosides, and tetracycline; antiinflammatories such as diflunisal, ibuprofen, indomethacin, meclofenamate, mefenamic acid, naproxen, oxyphenbutazone, phenylbutazone, piroxicam, sulindac, tolmetin, aspirin, and salicylates; antirheumatics such as adalimumab, azathioprine, chloroquine and hydroxychloroquine (antimalarials), cyclosporine (Cyclosporin A), D-penicillamine, etanercept, gold salts (sodium aurothiomalate, auranofin), infliximab, leflunomide, methotrexate, minocycline (a tetracycline antibiotic), and sulfasalazine; narcotics such as paregoric, opiates, codeine, heroin, methadone, morphine, and opium; cardiac glycosides such as deslanoside, digitoxin, digoxin, digitalin, and digitalis; neuromuscular blockers such as atracurium mesylate, gallamine triethiodide, hexafluorenum bromide, metocurine iodide, pancuronium bromide, succinylcholine chloride (suxamethonium chloride), tubocurarine chloride, and vecuronium bromide; sedatives (hypnotics) such as amobarbital, amobarbital sodium, aprobarbital, butabarbital sodium, chloral hydrate,

ethchlorvynol, ethinamate, flurazepam hydrochloride, glutethimide, methotrimeprazine hydrochloride, methypylon, midazolam hydrochloride, paraldehyde, pentobarbital, pentobarbital sodium, phenobarbital sodium, secobarbital sodium, talbutal, temazepam, and triazolam; local anesthetics such as bupivacaine hydrochloride, chloroprocaine hydrochloride, etidocaine hydrochloride, lidocaine hydrochloride, mepivacaine hydrochloride, procaine hydrochloride, and tetracaine hydrochloride; general anesthetics such as droperidol, etomidate, fentanyl citrate with droperidol, ketamine hydrochloride, methohexital sodium, and thiopental sodium; radioactive particles or ions such as strontium, iodide rhenium, yttrium; and radiopharmaceuticals such as radioactive iodine and phosphorus products.

(iii) cytotoxic cargo

[0048] Cytotoxic cargo refers to a molecule or agent that is detrimental to (e.g., kills or damages) a cell. Examples may include anti-microtubule drugs such as the taxols (paclitaxel, docetaxel) and vinca alkaloids (vincristine, vinblastine). For instance, examples may include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin didne, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

[0049] Non-limiting examples of cytotoxic cargo may also include radionuclides suitable for pharmacological administration. Such radionuclides may include ^{123}I , ^{125}I , ^{131}I , ^{90}Y , ^{211}At , ^{67}Cu , ^{186}Re , ^{188}Re , ^{212}Pb , and ^{212}Bi . Additionally, cytotoxic agents may include chemotoxic agents or toxins. Examples of chemotoxic agents may include small-molecule drugs such as methotrexate, and pyrimidine and purine analogs.

[0050] Proteins that may be used as cytotoxic agents may include ricin, abrin, diphtheria toxin, cholera toxin, gelonin, Pseudomonas exotoxin, Shigella toxin, pokeweed antiviral protein, and other toxin proteins known in the medicinal biochemistry arts.

(iv) targeting cargo

[0051] Targeting cargo may be any molecule or agent that directs a nanoparticle to a specific location. Non-limiting examples of targeting agents may include an antibody or antibody fragment, receptor ligand, small molecule, peptide, polypeptide, lipid, carbohydrate, nucleic acid, siRNA, shRNA, antisense RNA, dendrimer, or aptamer. A targeting cargo may be directed to a eukaryotic target cell or a prokaryotic target cell.

(d) nanoparticles

[0052] Generally speaking, the nanoparticle comprises a core encapsulated by a lipid layer. The lipid membrane of the nanoparticle may comprise a single lipid layer, two lipid layers (a bilayer), more than two lipid layers, a multilaminar lipid layer, and the like. As used herein, the term nanoparticle encompasses particle carriers that may be nano scale, micro scale, or macro scale. Suitable particle carriers include nanoparticles, nanospheres, nanostructures, liposomes, micelles, microbubbles, gas-filled microbubbles, dendrimers, polymeric structures, or any such carrier (naturally occurring or synthetic) that has an exterior lipid layer into which the anchor peptide may stably insert. Bacteria, viruses, prions, red blood cells, white blood cells, isolated tissue cells, platelets, and other such biologically derived, lipid-encased structures also may be carriers of the anchor peptide. Structures comprising a glycolipid membrane surrounding a cell (animal or vegetable) or particle or synthetic nanostructure also are potential hosts for the anchor peptide. In exemplary embodiments, the particle carrier may be a lipid-encapsulated nanoparticle.

[0053] In some embodiments, the anchor peptide may be bound to a cargo complex, as described above. The anchor peptide itself is generally non-lytic and non-cytotoxic, but the cargo complex may be lytic or cytotoxic. In some embodiments, a single nanoparticle may be associated with more than one type of cargo complex. For instance, a nanoparticle may be associated with both an anchor peptide bound to a targeting cargo and an anchor peptide bound to a therapeutic cargo. In one embodiment, a nanoparticle may be associated with at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 different cargo complexes. In another embodiment, a nanoparticle may be associated

with at least one therapeutic cargo, at least one imaging cargo, at least one cytotoxic cargo, at least one targeting cargo, or any combination thereof.

[0054] Methods of making lipid-encapsulated nanoparticles are well known in the art. For instance, see US Patent Nos. 6,676,963, 7,255,875 and 7,186,399, herein incorporated by reference in their entirety. As described below, a nanoparticle may be initially produced with an anchor peptide, or, alternatively, a nanoparticle may be pre-formed, and the anchor peptide subsequently added to the lipid membrane. Methods of adding an anchor peptide to a pre-formed nanoparticle are described in more detail below.

(e) administration

[0055] Nanoparticles comprising an anchor peptide, which may be linked to a cargo complex, may be administered to a subject. Suitable subjects include, but are not limited to, mammals, amphibians, reptiles, birds, fish, insects, and plants. In certain embodiments, nanoparticles of the invention may be used to image a tissue from a subject. Tissue, as used herein, may refer to cells, organs, tumors, or material associated with cells, organs, or tumors, such as blood clots. Suitable tissues may include, but are not limited to, heart, lungs, brain, eye, stomach, spleen, bones, pancreas, kidneys, liver, intestines, skin, uterus, bladder, eyes, lymph nodes, blood vessels, and blood and lymph components.

[0056] The nanoparticles may be formulated and administered to a subject by several different means. For instance, nanoparticles may generally be administered parenterally, intraperitoneally, intravascularly, topically, or intrapulmonarily in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intrathecal, or intrasternal injection, or infusion techniques. In one embodiment, the composition may be administered in a bolus. In a preferred embodiment, the composition may be administered intravenously. Formulation of pharmaceutical compositions is discussed in, for example, Hoover, John E., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa. (1975),

and Liberman, H. A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y. (1980).

[0057] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions, may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed, including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are useful in the preparation of injectables. Dimethyl acetamide, surfactants including ionic and non-ionic detergents, and polyethylene glycols can be used. Mixtures of solvents and wetting agents such as those discussed above are also useful.

[0058] For imaging purposes, formulations for parenteral administration may be in the form of aqueous or non-aqueous isotonic or hypotonic sterile injection solutions or suspensions. These solutions and suspensions may be prepared from sterile powders or granules having one or more carriers or diluents. The compounds may be dissolved in water, polyethylene glycol, propylene glycol, ethanol, corn oil, cottonseed oil, peanut oil, sesame oil, benzyl alcohol, sodium chloride, and/or various buffers. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art.

[0059] One of skill in the art will recognize that the amount and concentration of the composition administered to a subject will depend in part on the subject and the reason for the administration (i.e. imaging, drug delivery, etc.). Methods for determining optimal amounts are known in the art. In one embodiment, the amount administered may be between about 0.1 cc/kg to about 5 cc/kg. In another embodiment, the amount administered may be about 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, or 5.0 cc/kg. In yet another embodiment, the amount may be 1 cc/kg.

[0060] Following administration, the nanoparticle comprising the anchor peptide contacts a cell. As detailed above and demonstrated in Example 5, the

nanoparticle may be targeted to a particular cell by linking a specific targeting cargo to the anchor peptide of the nanoparticle. Upon contact with the cell, the anchor peptide of the nanoparticle may be transferred or move from the lipid monolayer of the nanoparticle and interact with and stably insert into the lipid bilayer of the cell. Depending upon the type of cargo complex that is linked to the anchor peptide, the anchor peptide may remain tethered to the lipid membrane of the cell. In such an embodiment, the cargo complex is on the surface of the cell via its attachment to the hydrophilic segment of the anchor peptide, whereas the hydrophobic segment of the anchor peptide is stably integrated into the lipid bilayer of the cell. In other embodiments, the anchor peptide and linked cargo complex may penetrate the lipid bilayer and enter the cell cytoplasm (as demonstrated in Examples 3 and 5, and depicted in **FIG. 7** and **FIG. 11**). In such an embodiment, the cargo that is linked to the anchor peptide may be an imaging agent or a therapeutic agent, for example. Those of skill in the art will appreciate that a number of scenarios are possible, depending upon the properties of the anchor peptide and the conjugated cargo complex.

(II) Kits

[0061] In some embodiments, the invention encompasses a kit. The kit generally comprises a first composition comprising a nanoparticle, and a second composition comprising an anchor peptide. In exemplary embodiments, the nanoparticle of the kit may be pre-formed. By following directions provided by the kit, a user of the kit may bind the anchor peptide to a cargo complex of interest, and then mix the conjugated anchor peptide/cargo complex with the pre-formed nanoparticles, so as to incorporate the anchor peptide into the lipid membrane of the pre-formed nanoparticle. In some embodiments, the pre-formed nanoparticles may be sterile.

(III) Methods

[0062] Yet another aspect of the present invention encompasses methods for adding a cargo complex to a nanoparticle. A first method generally comprises contacting the nanoparticle with an anchor peptide that is bound to a cargo complex. The anchor peptide stably inserts into the lipid membrane of the nanoparticle, thereby

linking the cargo complex with the nanoparticle. This method advantageously allows the nanoparticle to be pre-formed and then loaded with one or more cargo complexes at a later time.

[0063] In one embodiment, a nanoparticle may be contacted with an anchor peptide by combining a composition comprising a nanoparticle with a composition comprising an anchor peptide under conditions suitable for the anchor peptide to stably insert into the lipid membrane of the nanoparticle. Typically, such conditions may comprise a temperature of about 20°C to about 30°C, and incubation times of between about 30 sec and 30 min. In some embodiments, a nanoparticle may be incubated with the anchor peptide for about 5, 10, 15, 20, or 25 minutes at a temperature between about 20°C and 30°C. One skilled in the art will appreciate that the length and temperature of incubation can and will vary depending on the composition of the nanoparticle, the anchor peptide, and the cargo complex.

[0064] The ratio of the anchor peptide to the nanoparticle will also vary depending on the composition of the nanoparticle, the anchor peptide, and the cargo complex. Molar membrane lipid to anchor peptide ratios may range from about 1500 to about 20. In some embodiments, the ratio may range from about 1000 to about 40. In other embodiments, the ratio may be about 900, 800, 700, 600, 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30 or 20.

[0065] In one example, nanoparticles may be formulated by mixing known amounts of anchor peptides to perfluorocarbon nanoparticles. An anchor peptide may be dissolved in 100 mM KCl (pH 7, 10 mM HEPES) at 0.1 mM and 2x 20 mL may be added to 50 ml of nanoparticle suspension with mixing. After incubation at room temperature for 10 min, the nanoparticles may be washed twice by centrifugation (100 g, 10 min) to remove unbound anchor peptide. In some embodiments in which the anchor peptide contains a tryptophan residue, the anchor peptide in the supernatant may be quantified by measuring the tryptophan fluorescence using methods known in the art.

[0066] A second method comprises adding a cargo complex to a nanoparticle comprising a stably inserted (functionalized) anchor peptide. The starting nanoparticle may already comprise at least one cargo complex that was previously

conjugated with the stably inserted anchor peptide. The method comprises contacting the nanoparticle with the cargo complex, wherein the cargo complex forms a bond with the functionalized anchor peptide. The conditions under which the nanoparticle comprising the stably inserted anchor peptide is contacted with the cargo complex can and will vary depending upon, for example, the type of cargo complex and the type of functionalization of the anchor peptide. Those of skill in the art are familiar with suitable reaction conditions.

(IV) Anchor peptides

[0067] A further aspect of the invention comprises an anchor peptide as detailed above in section (I)(a). In particular, an anchor peptide comprises a substantially non-lytic, non-cytotoxic, amphipathic alpha helical peptide that is capable of stably inserting into a lipid membrane. In general, the anchor peptide is cationic and comprises at least one amphipathic alpha helix.

[0068] The anchor peptide may be associated with nanoparticles to facilitate the delivery of cargo complexes to cells, as detailed above in section (I). Alternatively, the anchor peptide itself may associate with and insert into the cell membranes of cells. Thus, the anchor peptide itself may be used to label cells, target cells, or deliver cargos to cells. As an example, the anchor peptide may be conjugated to a fluorescent molecule, wherein the anchor peptide may stably insert into the lipid bilayer of a cell and "label" the cell (as demonstrated in Example 7 and **FIG. 15**). In other embodiments, the anchor peptide may be conjugated to a specific targeting agent and/or therapeutic agent, such that conjugated anchor peptide is targeted to a specific cell or a therapeutic agent is delivered to the cell. As detailed above, the anchor peptide may stably integrate into the lipid membrane of the cell such that the conjugated agent is displayed on the cell surface. Alternatively, the anchor peptide may penetrate the cell membrane and deliver the conjugated agent to the interior of the cell. In general, the nature of the anchor peptide and the conjugated agent will determine the eventual fate of the conjugated anchor peptide. The anchor peptides of the invention, therefore, may be used as research tools or as medical tools for imaging and/or therapeutics.

[0069] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventors to function well in the practice of the invention. Those of skill in the art should, however, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention, therefore all matter set forth or shown in the accompanying drawings is to be interpreted as illustrative and not in a limiting sense.

EXAMPLES

[0070] The following examples illustrate various iterations of the invention.

Example 1: Lytic activity of anchor peptide

[0071] Typically, cargos such as targeting ligands, imaging agents, and/or drugs are incorporated into nanocarriers during the formulation process, which requires a dedicated particle design and preformulation for each individual application. In contrast, a strategy designed to accommodate swapping and/or combining multiple cargos in generic base nanocarriers would enable flexible and personalized decision making for application to any disease. Despite the myriad regulatory hurdles to the adoption of such a paradigm, the design of flexible nanocarriers with polyvalent and rapidly swappable targeting and drug delivery cargos represents a necessary first step.

[0072] The first goal was to design an appropriate anchoring agent that could be used with generic nanoparticle carriers, and for this purpose the model peptide, melittin (MMLT), was selected. Melittin is a 26 amino acid peptide that comprises more than half of the dry weight of the venom of the honeybee *Apis mellifera*. Residues 1-20 of melittin form two amphipathic α -helixes with an intervening proline hinge, while residues 21-26 of melittin are highly positively charged. Both hydrophobic and hydrophilic segments of melittin are essential for its lytic activity. It was hypothesized that the structure of melittin could be modified to attenuate its lytic activity while retaining the property of stable insertion into perfluorocarbon (PFC) nanoparticles.

Accordingly, the native melittin sequence was altered by selective point mutations and truncations. Table 1 presents the amino acid sequences of the various peptides and **FIG. 1** presents schematics of their secondary structures.

Table 1. Melittin and Derivatives of Melittin

Name(s)	Peptide Sequence	SEQ ID NO:
MMLT	GIGAVLKVLTTGLPALISWIKRKRQQ	219
P1	GIGAWLKVLTTGLPALISWIKRKRQQ	10
P2 (LP2)	GIGAVLKVLTTGLAALISWIKRKRQQ	25
P3	VLKVLTTGLPALISWIKRKRQQ	79
P4 (LP1)	VLTTGLPALISWIKRKRQQ	88
P5	GIGAVLKVLTTGLPALISWIKR	112
P6	GIGAVLKVLTTGLPALISWIKRKRQQTALDWSWLQTE	220
P7	TALDWSWLQTEGIGAVLKVLTTGLPALISWIKRKRQQ	221

[0073] The peptides were synthesized automatically by Fmoc solid-phase peptide synthesis. The product identities were assessed by mass spectrometry (Washington University Proteomics Center, St. Louis, MO) and the purity (>99%) was determined by analytical HPLC. All the peptides contained N-terminal acetylation and C-terminal amidation to mimic the situation of covalent cargo attachment that would apply when these peptides would be used as a linker on the nanocarriers.

[0074] For each peptide, cytotoxicity was assessed in 2F2B mouse endothelial cells by the XTT cell viability assay (Biotium, Hayward, CA). Cells were seeded in 96-well plates at 10,000 cells/well 24 hours before treatment with melittin and mutated melittins at selected concentrations. After 3 hours of treatment at 37°C, 25 µl of activated XTT solution was added to each well and incubated at 37°C for 5 hours. After 5 hours of incubation, plates were gently shaken to achieve even dye distribution. The absorbances of the samples were measured by a microplate reader (Biorad, Model 550, Hercules, CA) at a wavelength of 450 nm. Background was subtracted and the results were normalized to untreated control cells.

[0075] **FIG. 2** presents the cytotoxicity results. Among the modified peptides, P2 and P4 exhibited the least cytotoxicity. In particular, P4 had essentially no cell toxicity up to a concentration of 90 µM. Table 2 presents the IC₅₀ (µM) values of some of the tested peptides. The cytotoxicity index of P4 (114.4 ± 10.2) was reduced

most compared to that of MMLT (2.36 ± 0.23), representing about a 50-fold decrease in cytotoxicity. From this point forward, P4 is referred to as linker peptide 1 (LP1) and P2 is referred to as linker peptide 2 (LP2).

Table 2. Cytotoxicity of Peptides	
Peptide	IC ₅₀ (μ M)
MMLT	2.36 ± 0.23
P1	0.75 ± 0.04
P2	26.56 ± 0.56
P3	14.44 ± 0.92
P4	114.6 ± 10.2
P5	6.41 ± 0.64

Example 2: Anchor peptide affinity for lipid membranes

[0076] In order to evaluate whether linker peptide 1 (LP1) and linker peptide 2 (LP2) still retained high affinity on a lipid monolayer of the nanoparticles, surface plasmon resonance (SPR) studies were performed by using Biacore X 100 (Biacore Inc, Piscataway, NJ). The binding kinetics and affinity of LP1 and LP2 on two type of nanoparticles were studied. One type was perfluorocarbon nanoparticles (e.g., perfluorooctylbromide, PFOB), the other type was oil-based (safflower core) nanoparticles. PBS (Ca^{2+} and Mg^{2+} free) was used as the running buffer. Nanoparticles were immobilized on Biacore sensor chip L1 by injecting nanoparticles at a flow rate of 1 μ l/min for 30 minutes, followed by a running buffer wash at a flow rate of 100 μ l/min for 50 seconds. Peptides in selected concentrations, prepared in running buffer, were then injected at a flow rate of 30 μ l/min for 1 minute. At the end of each experiment, the sensor chip surface was regenerated by injection of 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) at a flow rate of 100 μ l/min for 24 seconds.

[0077] Selected sensorgrams are shown in **FIG. 3**. The dissociation constants of LP1 and LP2 on perfluorocarbon nanoparticles were 2.25×10^{-7} M and 5.48×10^{-9} M, respectively. The dissociation constants of linker peptides 1 and 2 on oil-based nanoparticles were 2.29×10^{-8} and 6.4×10^{-10} M, respectively. These results indicated that both anchor peptides retained high affinity with both perfluorocarbon and

oil-based nanoparticles. These results compare with a previously measured dissociation constant for melittin on perfluorocarbon nanoparticles of 1.5×10^{-9} M.

[0078] The dissociation of the peptide from linker peptide 1-loaded PFOB nanoparticles is depicted in **FIG. 4**. After being loaded with the linker peptide 1, the loaded PFOB nanoparticles were stored at 4°C for 30 minutes, 1 hour, 2 hours, 4 hours, 22 hours, 46 hours, and 70 hours. The loaded nanoparticles were centrifuged at 100g for 10 minutes, and the dissociated linker peptide 1 was measured by using tryptophan fluorescence after excitation at 280 nm in a fluorescent spectrofluorometer (Varian Inc, Palo Alto, CA). The results showed that less 10% of the loaded linker peptide 1 was dissociated from the PFOB nanoparticles for up 70 hours after being loaded. These data suggest that the association between linker peptide 1 and the PFOB nanoparticles was stable.

[0079] The size distributions of the nanoparticles with or without the linker peptide were analyzed by dynamic light scattering (Brookhaven Instruments Corp., Holtsville, NY). Zeta potential (ζ) values were determined with a Brookhaven Instruments PALS Zeta Potential Analyzer (Brookhaven Instruments Corp., Holtsville, NY). Data were acquired in the phase-analysis light scattering (PALS) mode following solution equilibration at 25°C. **FIG. 5A** shows that the average hydrodynamic diameter of the PFOB nanoparticles was not affected by linker peptide 1 insertion, and after incorporating the positively charged linker peptide 1, the PFOB nanoparticles exhibited a zeta potential shift from -17.62 mV to +23.33 mV. This change further confirms the insertion of the linker peptide 1 into the PFOB nanoparticles. Furthermore, the structural integrity of linker peptide 1 inserted PFOB nanoparticles was confirmed by transmission electron microscopy. As shown in **FIG. 5B**, the lipid membrane of the PFOB nanoparticles appears to remain intact.

[0080] The interactions between linker peptide 1 and liposomes are depicted in **FIG. 6**. **FIG. 6A** shows that the average hydrodynamic diameter of the liposomes was not affected by the insertion of linker peptide 1, and after incorporating the positively charged linker peptide 1, the liposomes exhibited a zeta potential shift from -36.73 mV to -29.843 mV. This change further confirms that the linker peptide 1 inserted into the liposomes. Furthermore, transmission electron microscopy of linker

peptide 1-loaded liposomes revealed that the lipid membrane of the liposomes appeared intact (**FIG. 6B**).

Example 3: Cellular delivery of an anchor peptide

[0081] The cellular delivery of FITC-labeled peptide 1, incorporated onto perfluorocarbon nanoparticles, was visualized in C-32 melanoma cells by confocal microscopy. PFOB nanoparticles incorporated with FITC labeled linker peptide 1 or plain PFOB nanoparticles were incubated with C-32 melanoma cells in Delta T Culture dish (0.17 mm, Biotech's Inc., Butler, PA) at 37°C for 1 hour, followed by 5 PBS washes. The cells were fixed by incubating in 1 ml of 4% paraformaldehyde at 37°C for 5 minutes. After the fixation, cells were washed with PBS for 5 times and visualized by using a Zeiss 510 confocal microscope.

[0082] As shown in **FIG. 7A**, the FITC signal was detected both on the cell surface and in the cytoplasm of cells incubated with the linker-loaded nanoparticles. In contrast, cells exposed to plain PFOB nanoparticles displayed no FITC signal (**FIG. 7B**). These results demonstrate that linker peptide 1 retained the sufficient stability on the lipid monolayer of the nanoparticles for cellular delivery.

Example 4: VCAM-targeting anchor peptide affinity for lipid membranes

[0083] To evaluate the function of a cargo complex, two VCAM-targeting peptides were synthesized. For this, an anti-VCAM peptide, VHPKQHR (SEQ ID NO:222), was fused on the N-terminal side or C-terminal side of linker peptide 1 with two glycines as a spacer, as depicted in Table 3.

Table 3. VCAM-targeting peptides		
Name	Sequence	SEQ ID NO:
TCP1	VLTTGLPALISWIKRggVHPKQHR	223
TCP2	VHPKQHRggVLTTGLPALISWIKR	224

[0084] The binding kinetics of the resulting VCAM-targeting peptides (i.e., TCP1 and TCP2) on perfluorocarbon nanoparticles were examined using Biacore X 100. PBS (Ca²⁺ and Mg²⁺ free) was used as the running buffer. Nanoparticles were

immobilized on Biacore sensor chip L1 by injecting nanoparticles at a flow rate of 1 $\mu\text{l}/\text{min}$ for 30 minutes, followed by a running buffer wash at a flow rate of 100 $\mu\text{l}/\text{min}$ for 50 seconds. Targeting peptide prepared at selected concentrations in running buffer was sequentially injected at a flow rate of 30 $\mu\text{l}/\text{min}$ for 1 minute. At the end of each experiment, the sensor chip surface was regenerated by injection of 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) at a flow rate of 100 $\mu\text{l}/\text{min}$ for 24 seconds. **FIG. 8** presents selected sensorgrams at several different concentrations of the two VCAM-targeting peptides. The dissociation constant of TCP1 and TCP2 were 0.13 μM and 0.04 μM , respectively. These data suggest that the insertion of linker peptide 1 into PFC nanoparticles was not affected by addition of a targeting ligand cargo.

[0085] **FIG. 9** presents the dissociation of TCP1 or TCP2 from loaded PFOB nanoparticles as a function of time after loading. After loaded with TCP1 or TCP2, the PFOB nanoparticles were stored at 4°C for 30 minutes, 1 hour, 2 hours, 4 hours, 22 hours, 46 hours, and 70 hours. The stored nanoparticles were then centrifuged at 100g for 10 minutes and dissociated TCP1 or TCP2 was measured using tryptophan fluorescence after excitation at 280 nm in a fluorescent spectrofluorometer (Varian Inc, Palo Alto, CA). The results showed that up to 70 hours after being loaded into the PFOB nanoparticles, less 15% of the loaded TCP1 or TCP2 had dissociated. Furthermore, these data suggest that targeting peptide-loaded PFOB nanoparticles are stable.

[0086] The average hydrodynamic diameter of the PFOB nanoparticles was not affected by the insertion of TCP1 and TCP2 (see **FIG. 10**). Additionally, after incorporation of the positively charged TCP1 or TCP2, the PFOB nanoparticles exhibited a zeta potential shift from -17.62 mV to 13.47 mV or 12.43 mV, respectively. These data further confirm the stable insertion of TCP1 and TCP2 into the PFOB nanoparticles.

Example 5: Specific targeting nanoparticles

[0087] The specific binding of VCAM targeted nanoparticles was evaluated by confocal microscopy in 2F2B cells, which express VCAM-1 on the cell surface after

TNF- α stimulation. Alexa Fluor 488 labeled PFOB nanoparticles comprising the VCAM-targeting peptide (TCP1 or TCP2) or nanoparticles themselves were incubated with TNF- α stimulated 2F2B cells in Delta T Culture dish at 37°C for 1 hour, followed by five PBS washes. Cells were then fixed by incubation in 1 ml of 4% paraformaldehyde at 37°C for 5 minutes. After fixation, the cells were washed with PBS for 5 times and the cells were visualized using a confocal microscope (Zeiss Meta 510, Thornwood, NY) with standard filter sets. Concomitant differential interference contrast (DIC) imaging was used to determine the location of nanoparticles in the cells.

[0088] Manifestly stronger fluorescent signals emanated from the cells treated with VCAM-targeted nanoparticles (**FIG. 11A** and **11B**) than from cells treated with nontargeted nanoparticles (**FIG. 11C**), which demonstrates the specific binding of targeted nanoparticles to cellular VCAM-1. It is also clear that the exposure of VCAM-1 targeting peptides fused on the C-terminus of the linker (i.e., TCP1) exhibited better binding (**FIG. 11A**) than when VCAM-1 targeting peptide is attached to the N-terminus (i.e., TCP2) of the linker (**FIG. 11B**). These findings conform to the known orientation of melittin in membranes where the N-terminus situates more deeply into the lipid membrane and the C-terminus remains exposed on the surface.

[0089] Two formulations of VCAM-1-Targeted NanoParticles (VTNP) were prepared by using the anchor peptide strategy. The first formulation of VCAM-1-Targeted NanoParticles (VTNP1) was produced by mixing pre-formulated PFC nanoparticles with TCP1. The second formulation (VTNP2) was produced by mixing pre-formulated PFC nanoparticles with TCP2. All nanoparticles had a perfluoro-15-crown 5-ether (CE) core. VTNP1 or VTNP2 were generated by addition of 100 nmol of TCP1 or TCP2 on 20 μ l CE nanoparticles, respectively.

[0090] The ^{19}F perfluorocarbon core of PFC nanoparticles enabled these nanoparticles to serve as unique magnetic resonance spectroscopic and imaging agents with no background signal in vivo. Furthermore, the ^{19}F spectroscopic signature offered the potential for quantification of VCAM-1 biomarkers by providing a rough estimate of the number of delivered nanoparticles per cell. Using this approach, ^{19}F MR spectra of TNF- α stimulated 2F2B endothelial cells treated with VTNP1 (**FIG. 12A**), VTNP2 (**FIG. 12B**), and non-targeted (**FIG. 12C**) CE nanoparticles were acquired.

Because of linear relation between ^{19}F signal intensity and the concentration of ^{19}F molecules, the amount of nanoparticles delivered to the cell could be estimated. The number of bound nanoparticles was normalized by the total number of measured cells to calculate the number of the bound nanoparticles per cell (**FIG. 12D**). These data further support the specific binding of targeted nanoparticles, and also confirmed that VTNP1 manifests better targeting efficiency than does VTNP2. Essentially then, the VTNP2 construct serves as a control scrambled peptide against the more specific VTNP1 sequence.

[0091] The dose dependency of targeting for VTNP1 and VTNP2 in selected concentrations was also evaluated in vitro. **FIG. 12E** again confirms the better targeting efficiency of VTNP1, while also demonstrating that the binding of VCAM-targeted nanoparticles was dependent upon the amount of targeting peptide incorporated onto the nanoparticle. Therefore, optimization of specific targeting might be achieved by adjusting the volume ratio of peptides and PFC nanoparticles. As expected the ^{19}F image of the cells treated with VTNP1, VTNP2, or non-targeted nanoparticles showed the same preference for C-terminus addition of the targeting sequence (**FIG. 12F**). A stronger ^{19}F signal was detected from the cells treated with VTNP1 than with VTNP2, whereas no ^{19}F signal was detected from the cells treated with non-targeted nanoparticles.

Example 6: Cellular response to VCAM-1-targeted nanoparticles

[0092] Downstream signaling events were examined in cells exposed to VCAM-1-targeted nanoparticles. In particular, the inhibition of NF-kB activation was examined (see **FIG. 13**). Without TNF- α (10 ng/ml) stimulation, 2F2B cells had a baseline level of p65 translocated into the nuclear (Non-Sti). After the TNF- α (10 ng/ml) stimulation for 4 hours, NF-kB signaling pathway was upregulated, therefore, and more p65 translocated into the nucleus (Sti). After 1 hour pretreatment with VCAM-1 targeted NBD-loaded PFOB nanoparticles, 2F2B cells had statistically significant less nuclear translocation of p65 (NBD-NP-Sti) than 2F2B cells not pretreated with the VCAM-1 targeted nanoparticles.

[0093] Cells treated with VCAM-1 targeted nanoparticles had decreased expression of a NF- κ B dependent gene (see **FIG. 14A**). 2F2B cells produced a basal level of IL-6 in the absence of TNF- α stimulation (Non-stim). After TNF- α stimulation, the level of IL-6 increased (Sti). After 1 hour pretreatment with VCAM-1 targeted NBD-loaded PFOB nanoparticles, 2F2B cells produced statistically significant less IL-6 (NBD-NP-Sti) than the 2F2B cells that were not exposed to the nanoparticles. As shown in **FIG. 14B**, VCAM-1 expression on the cell membrane was also down regulated in the 2F2B cells after 1 hour pretreatment with VCAM-1 targeted NBD-loaded nanoparticles (NBD-NP-Sti), as compared to the 2F2B cells that were not pretreated with the nanoparticles (Sti).

Example 7: Cell labeling

[0094] The linker peptide 1 not only can serve as an anchoring agent for synthesized nanoscale particles, but also it can be used for labeling cells. FITC-conjugated linker peptide 1 was incubated with human red blood cells for 1 hour, and a fluorescence image (**FIG 15A**) and light image (**FIG. 15B**) were taken after washing. The green signal in fluorescence image indicates that linker peptide 1 may be used for cell labeling.

CLAIMS

What is claimed is:

1. A nanoparticle, the nanoparticle comprising a core encapsulated by a lipid layer, the lipid layer comprising a stably inserted anchor peptide, wherein the anchor peptide is substantially non-lytic and non-cytotoxic.
2. The nanoparticle as in claim 1, wherein the anchor peptide comprises at least one hydrophobic segment.
3. The nanoparticle as in claim 1 or claim 2, wherein the anchor peptide further comprises at least one hydrophilic segment.
4. The nanoparticle as in any of claims 1-3, wherein the anchor peptide comprises at least one amphipathic alpha helix.
5. The nanoparticle as in any of claims 1-4, wherein the anchor peptide is cationic.
6. The nanoparticle as in any of claims 1-5, wherein the stably inserted anchor peptide has an association rate of at least about $9 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and a dissociation constant of less than about $1 \times 10^{-6} \text{ M}$.
7. The nanoparticle as in any of claims 1-6, wherein the anchor peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs:4-218.
8. The nanoparticle as in claim 7, wherein the anchor peptide consists of SEQ ID NO:88.
9. The nanoparticle as in any of claims 1-8, wherein the anchor peptide is conjugated to at least one cargo complex selected from the group consisting of an imaging cargo, a therapeutic cargo, a cytotoxic cargo, and a targeting cargo.

10. A kit for preparing a nanoparticle comprising an anchor peptide, the kit comprising a first composition and a second composition, the first composition comprising a nanoparticle comprising a core encapsulated by a lipid layer, the second composition comprising the anchor peptide which is substantially non-lytic and non-cytotoxic, wherein the anchor peptide is capable of stably inserting into the lipid layer of the nanoparticle.
11. The kit as in claim 10, wherein the anchor peptide comprises at least one hydrophobic segment.
12. The kit as in claim 10 or claim 11, wherein the anchor peptide further comprises at least one hydrophilic segment.
13. The kit as in any of claims 10-12, wherein the anchor peptide comprises at least one amphipathic alpha helix.
14. The kit as in any of claims 10-13, wherein the anchor peptide is cationic.
15. The kit as in any of claims 10-14, wherein the anchor peptide has an association rate of at least about $9 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and a dissociation constant of less than about $1 \times 10^{-6} \text{ M}$.
16. The kit as in any of claims 10-15, wherein the anchor peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs:4-218.
17. The kit as in claim 16, wherein the anchor peptide consists of SEQ ID NO:88.
18. The kit as in any of claims 10-17, wherein the anchor peptide is conjugated to a cargo complex selected from the group consisting of an imaging cargo, a therapeutic cargo, a cytotoxic cargo, and a targeting cargo.
19. A method for adding a cargo complex to a nanoparticle comprising a lipid layer, the method comprising contacting the nanoparticle with an anchor peptide that is conjugated to the cargo complex, the anchor peptide being

substantially non-lytic and non-cytotoxic, wherein the anchor peptide stably inserts into the lipid layer of the nanoparticle.

20. The method as in claim 19, wherein the anchor peptide comprises at least one hydrophobic segment.
21. The method as in claim 19 or claim 20, wherein the anchor peptide further comprises at least one hydrophilic segment.
22. The method as in any of claims 19-21, wherein the anchor peptide comprises at least one amphipathic alpha helix.
23. The method as in any of claims 19-22, wherein the anchor peptide is cationic.
24. The method as in any of claims 19-23, wherein the anchor peptide has an association rate of at least about $9 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and a dissociation constant of less than about $1 \times 10^{-6} \text{ M}$.
25. The method as in any of claims 19-24, wherein the anchor peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs:4-218.
26. The method as in any of claims 19-25, wherein the cargo complex is selected from the group consisting of an imaging cargo, a therapeutic cargo, a cytotoxic cargo, and a targeting cargo.
27. An anchor peptide that is cationic and comprises at least one amphipathic alpha helix, wherein the anchor peptide is substantially non-lytic and non-cytotoxic.
28. The anchor peptide as in claim 27, wherein the anchor peptide consists of an amino acid sequence selected from the group consisting of SEQ ID NOs:4-218.

29. The anchor peptide as in claim 27 or claim 28, wherein the anchor peptide is capable of stably inserting into a lipid membrane, the lipid membrane being that of a nanoparticle, a liposome, or a cell.

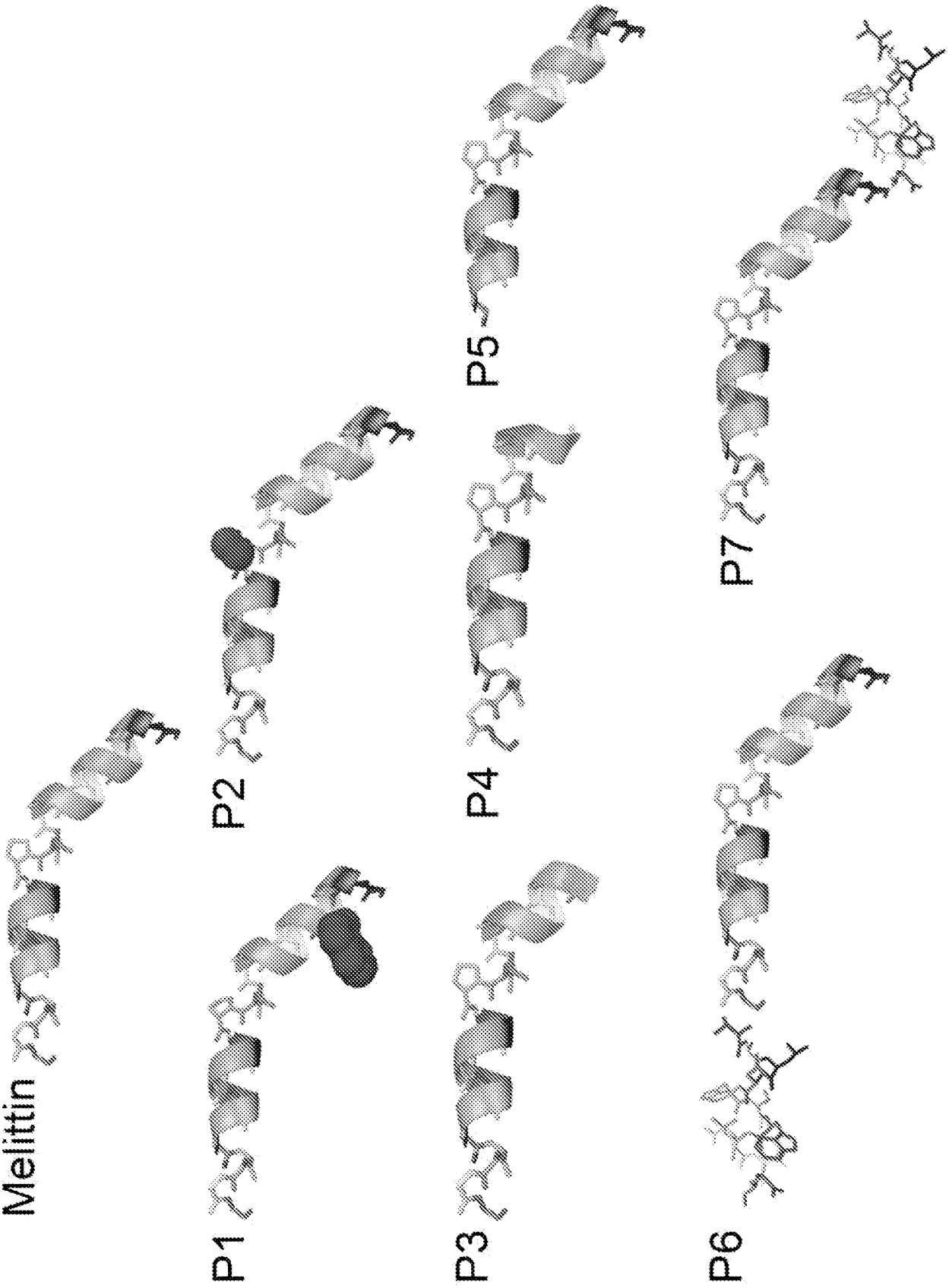
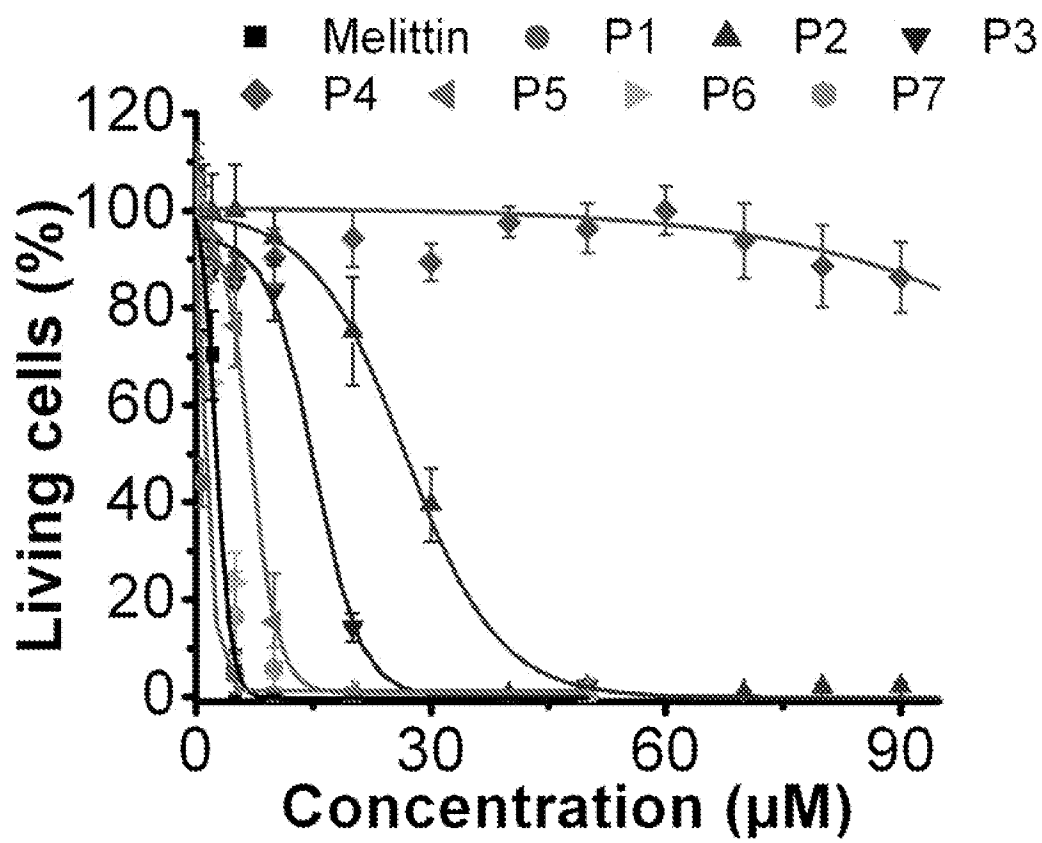
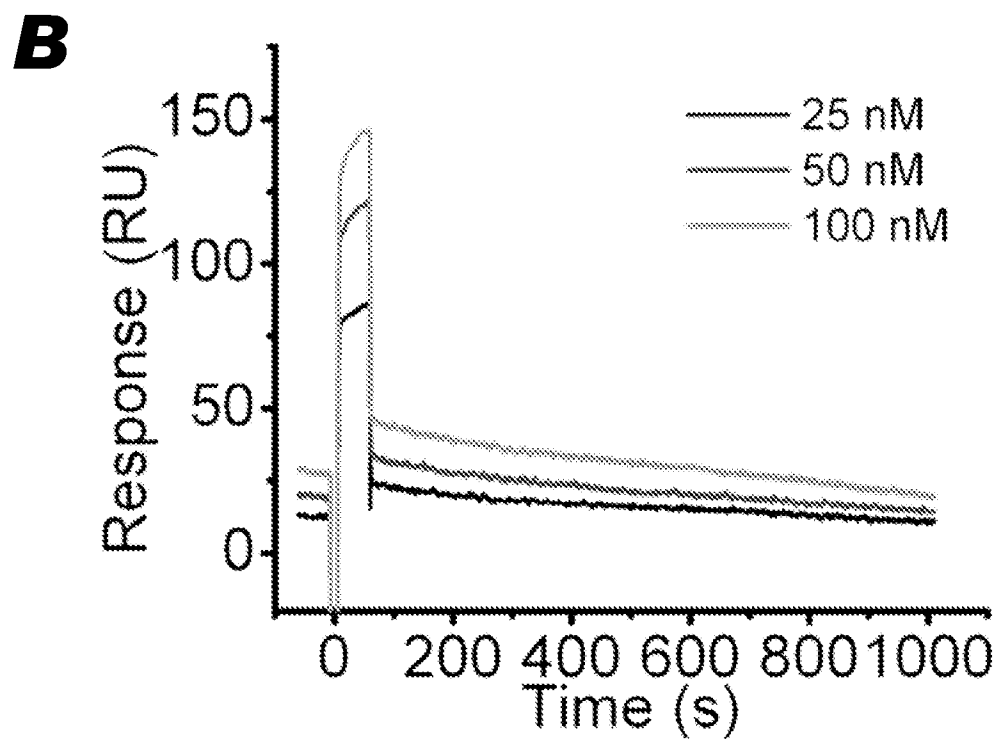
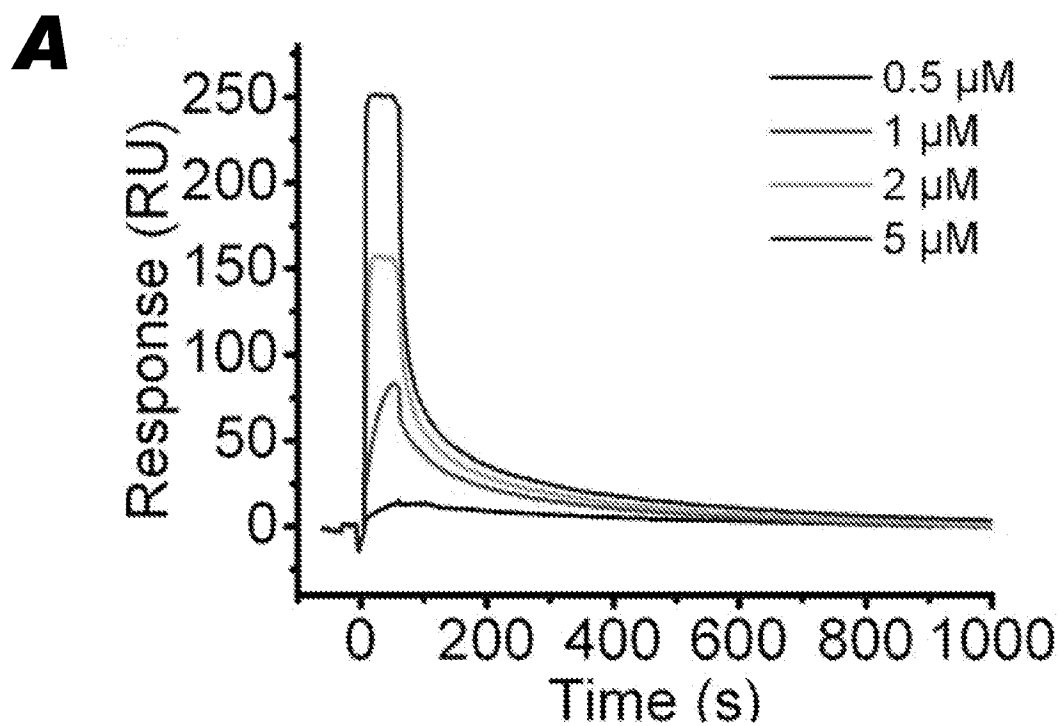


FIG. 1

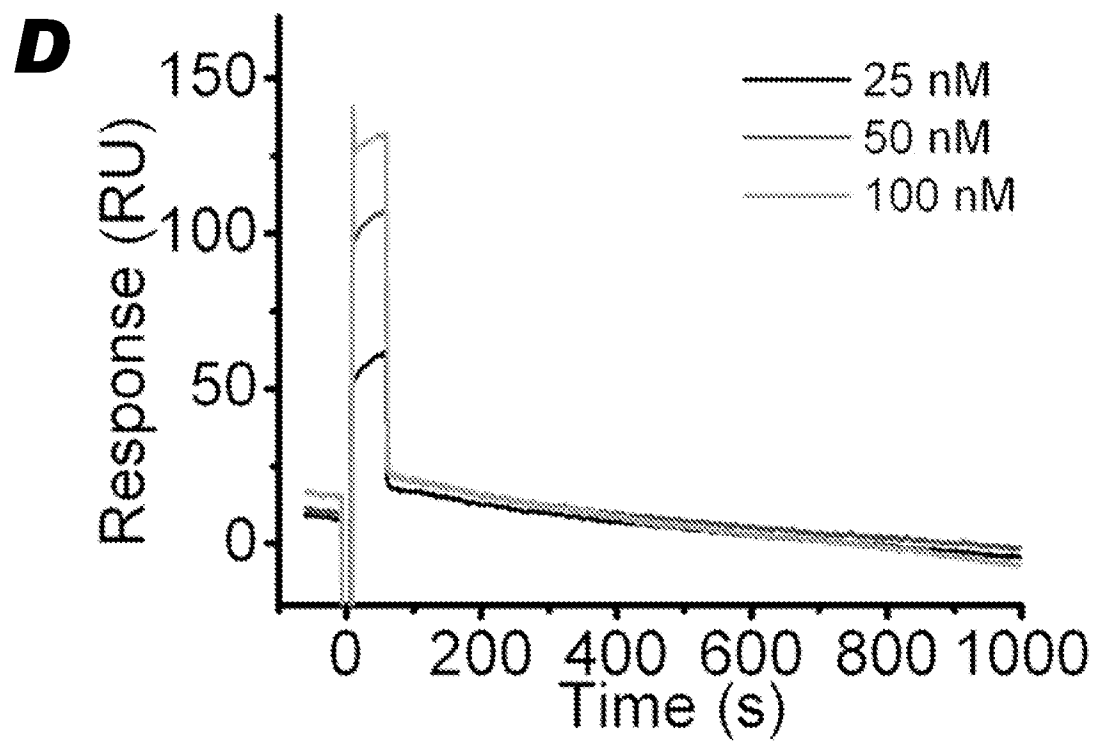
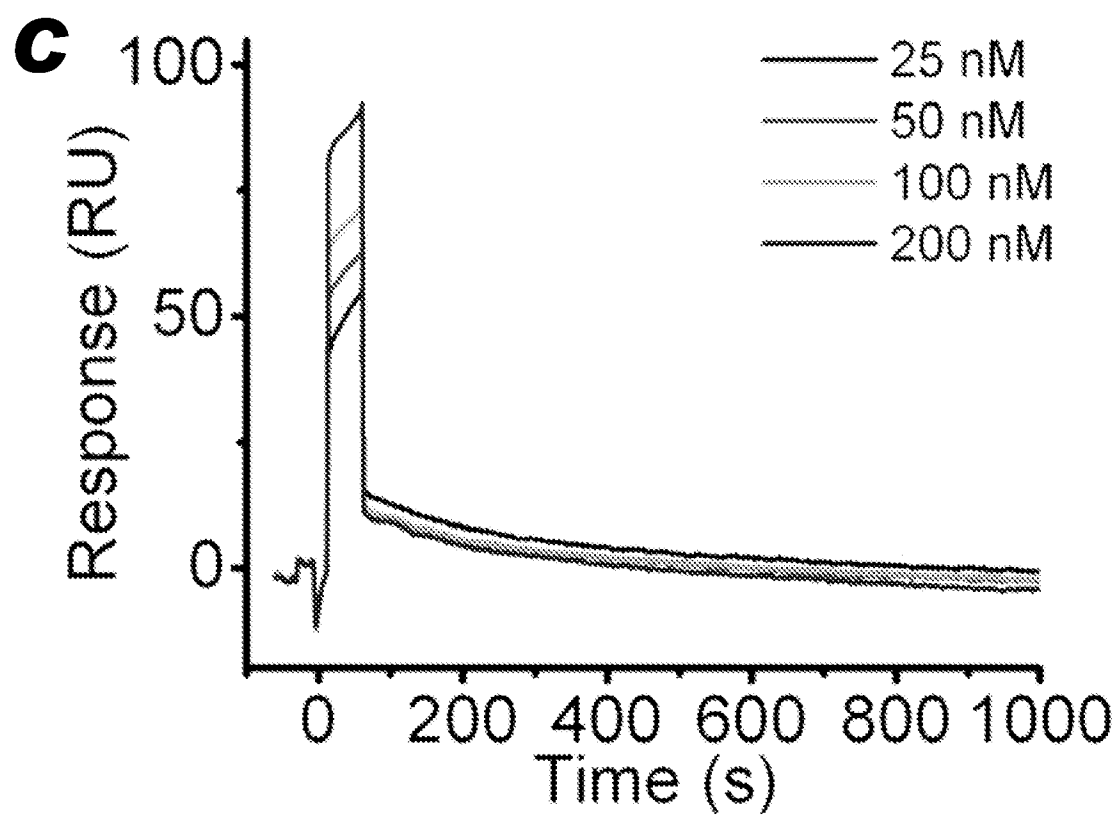
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**FIG. 2**

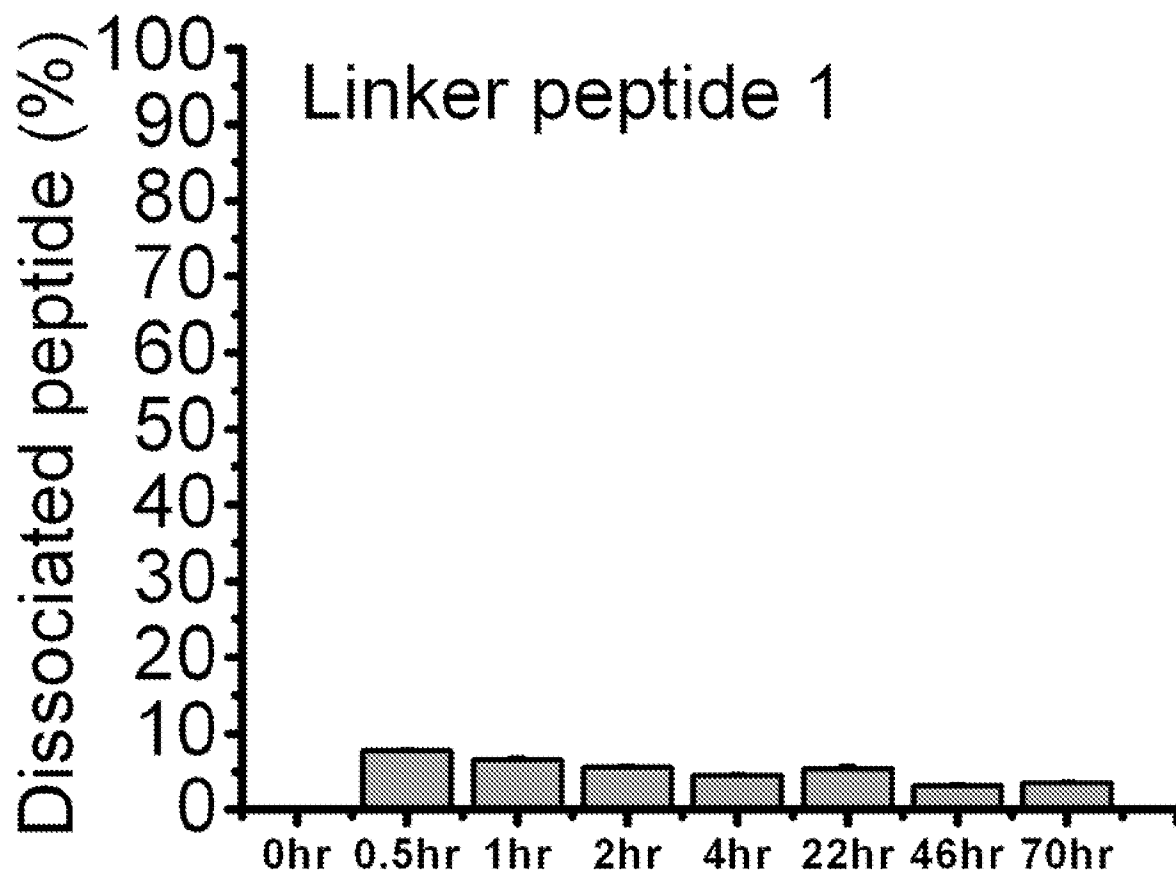
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**FIG. 3**

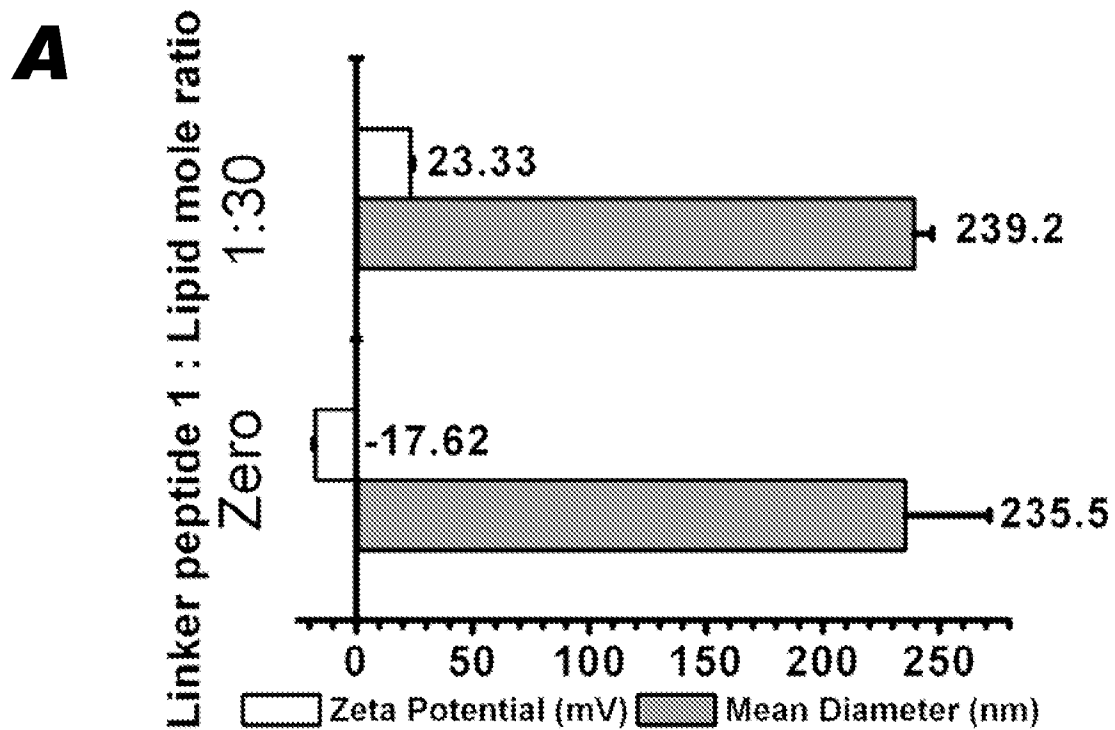
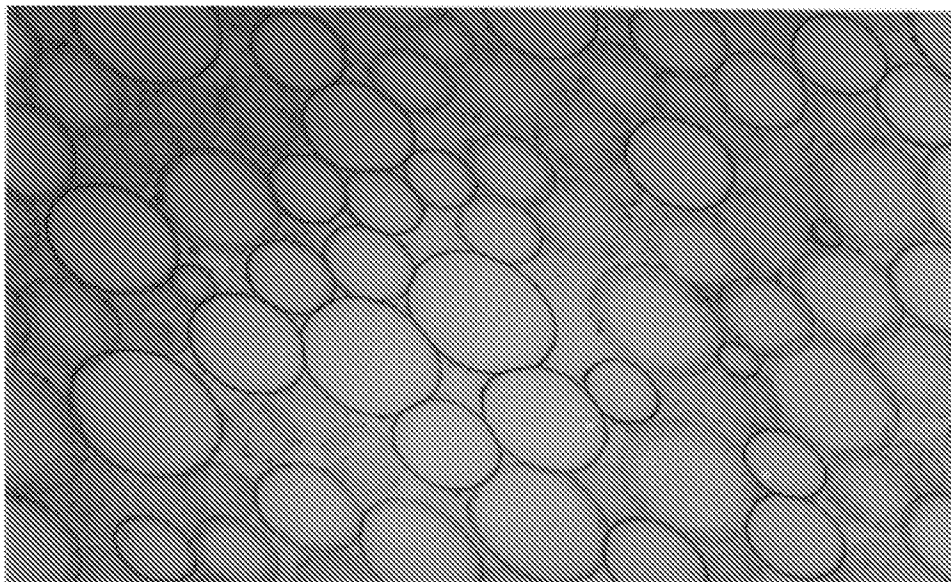
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**FIG. 3**

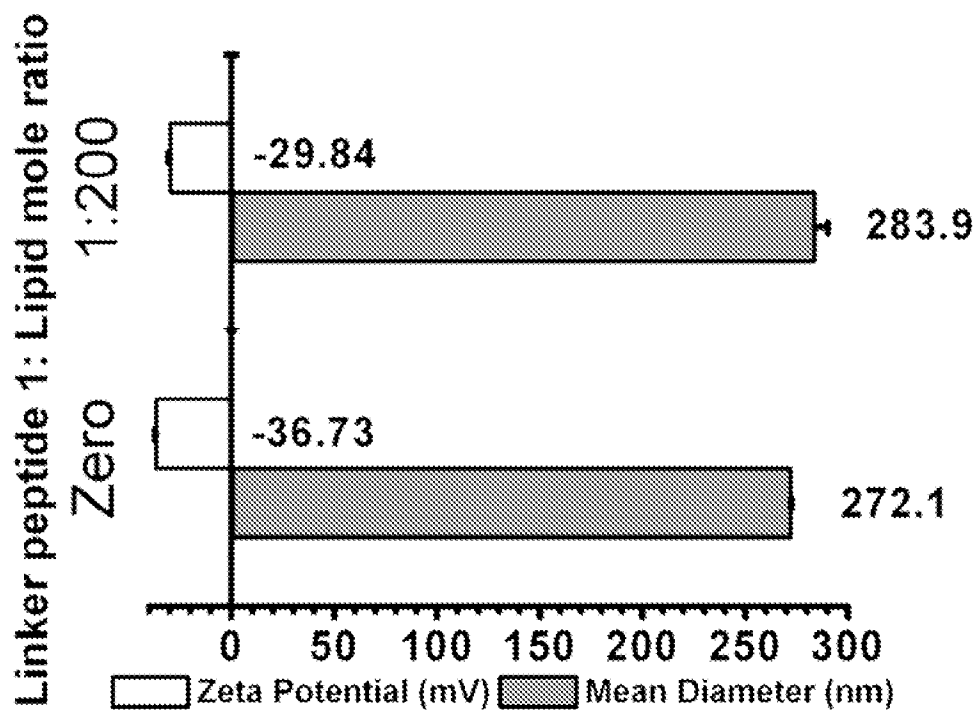
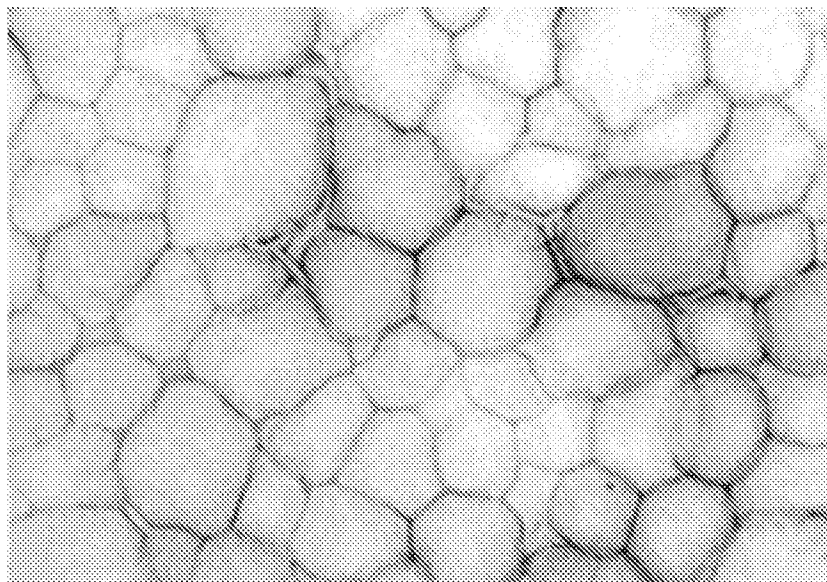
5/20

**FIG. 4**

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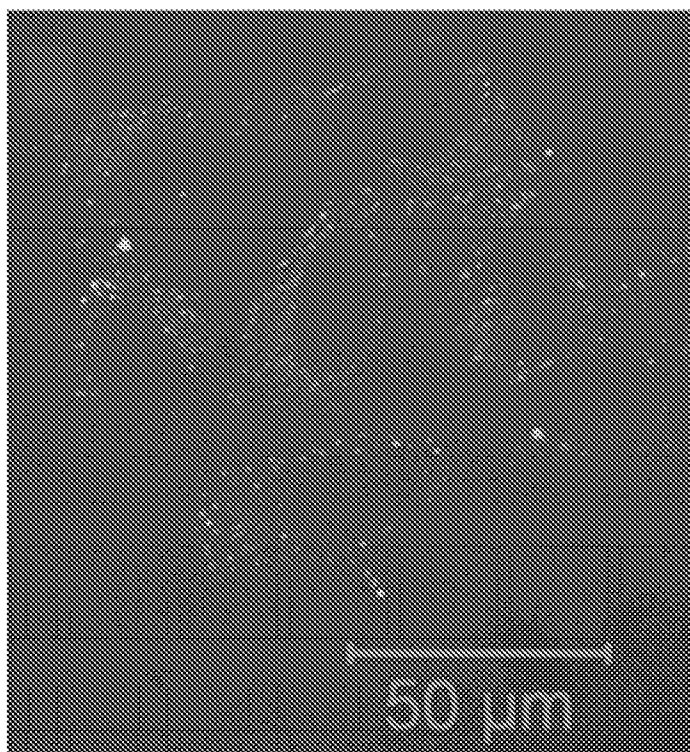
**B****FIG. 5**

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A**B****FIG. 6**

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A



B

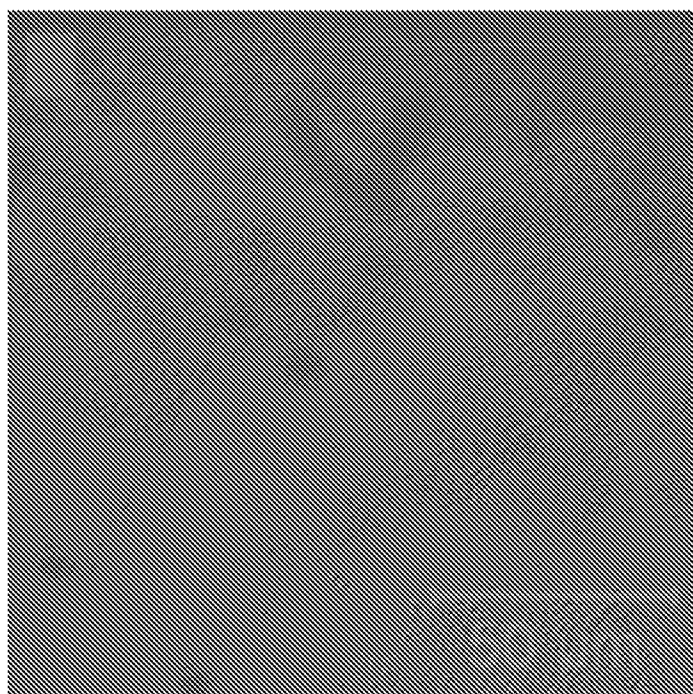


FIG. 7

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A

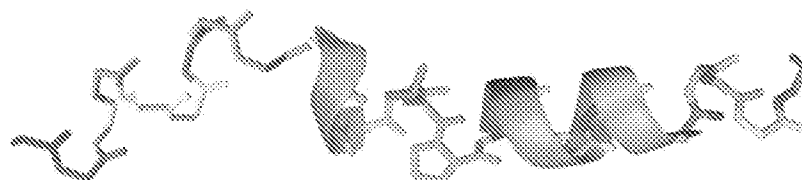
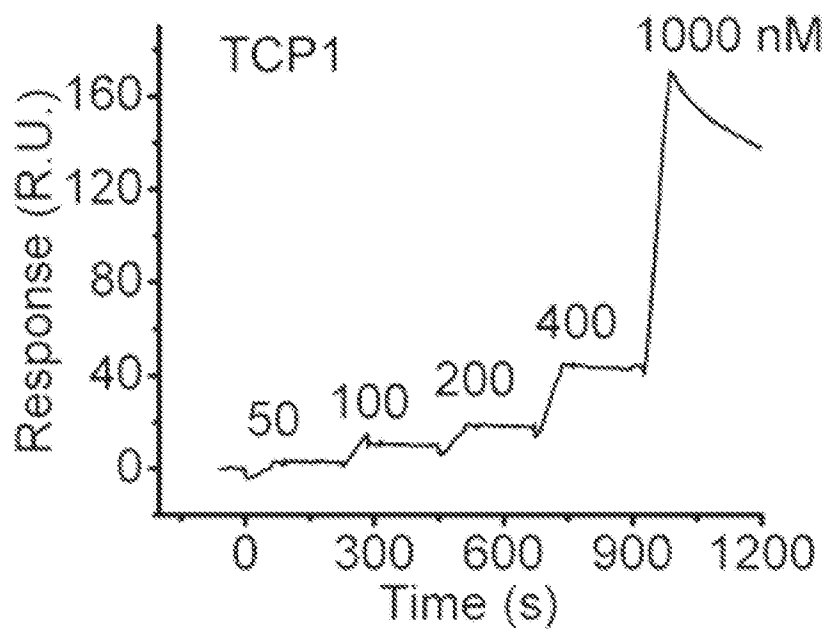
VLTTGLPALISWIKRKRQQggVHPKQHR

TCP1

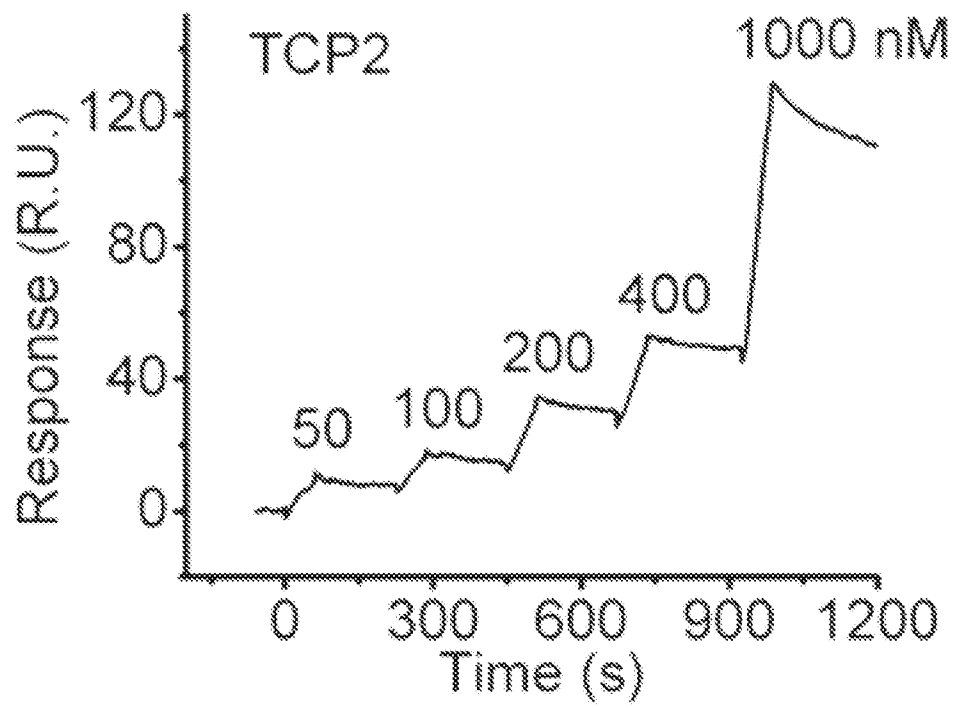
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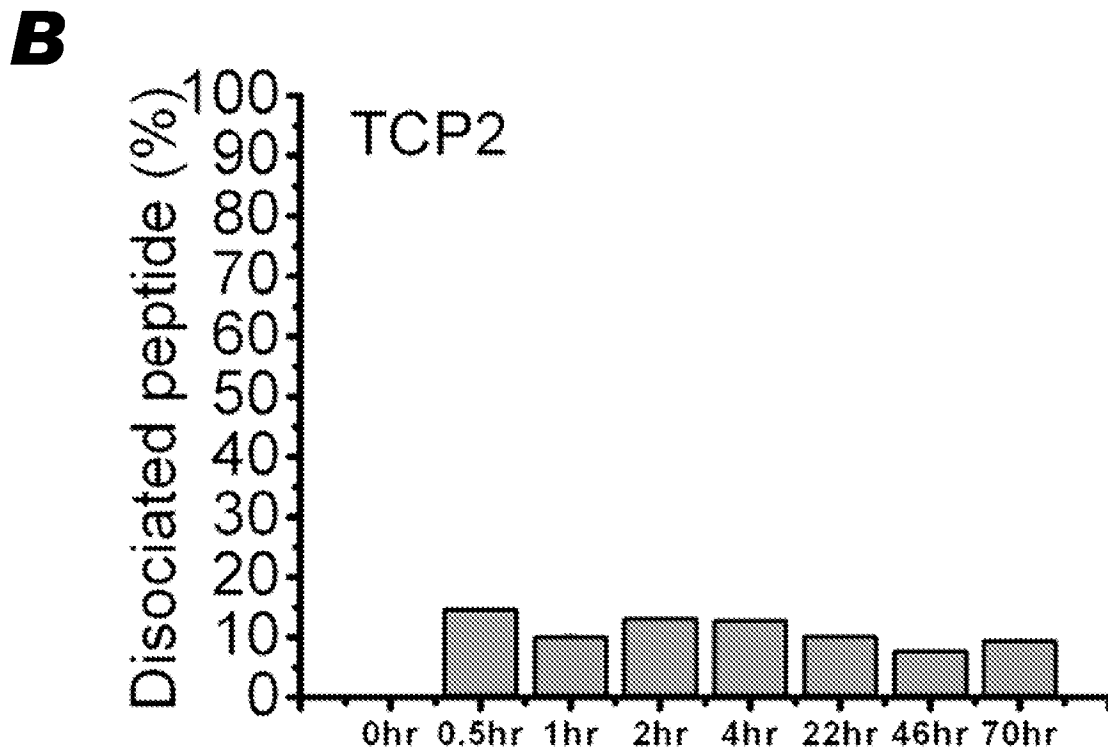
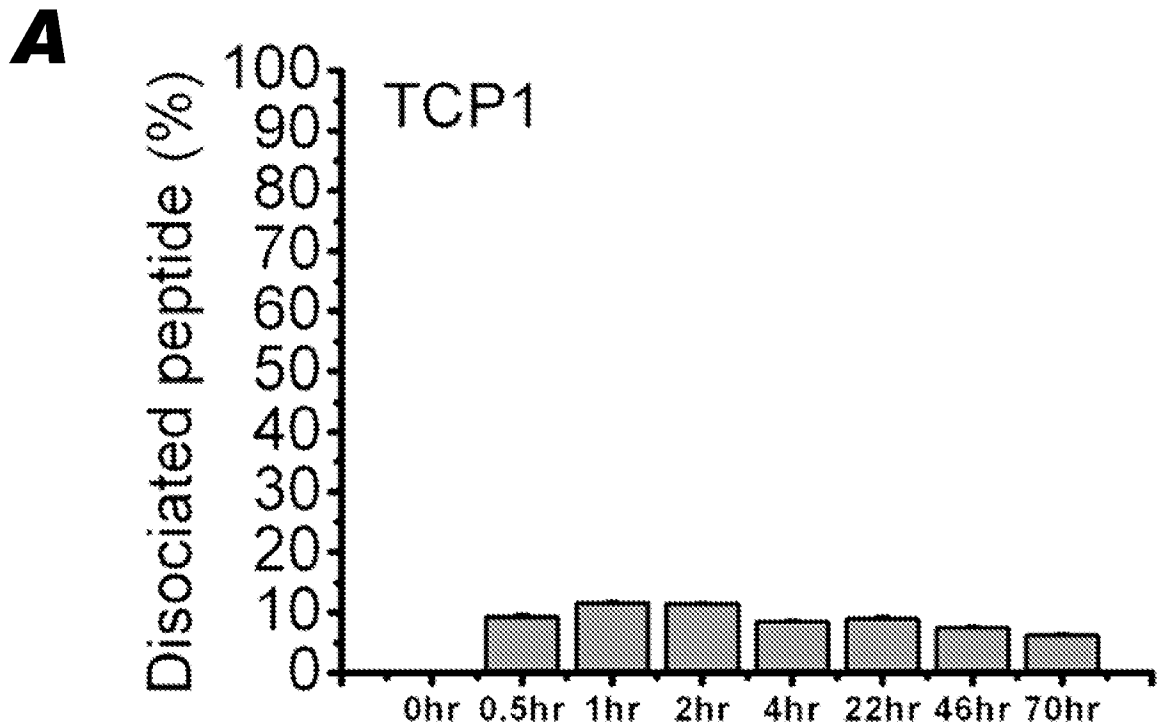
TCP2

**C****FIG. 8**

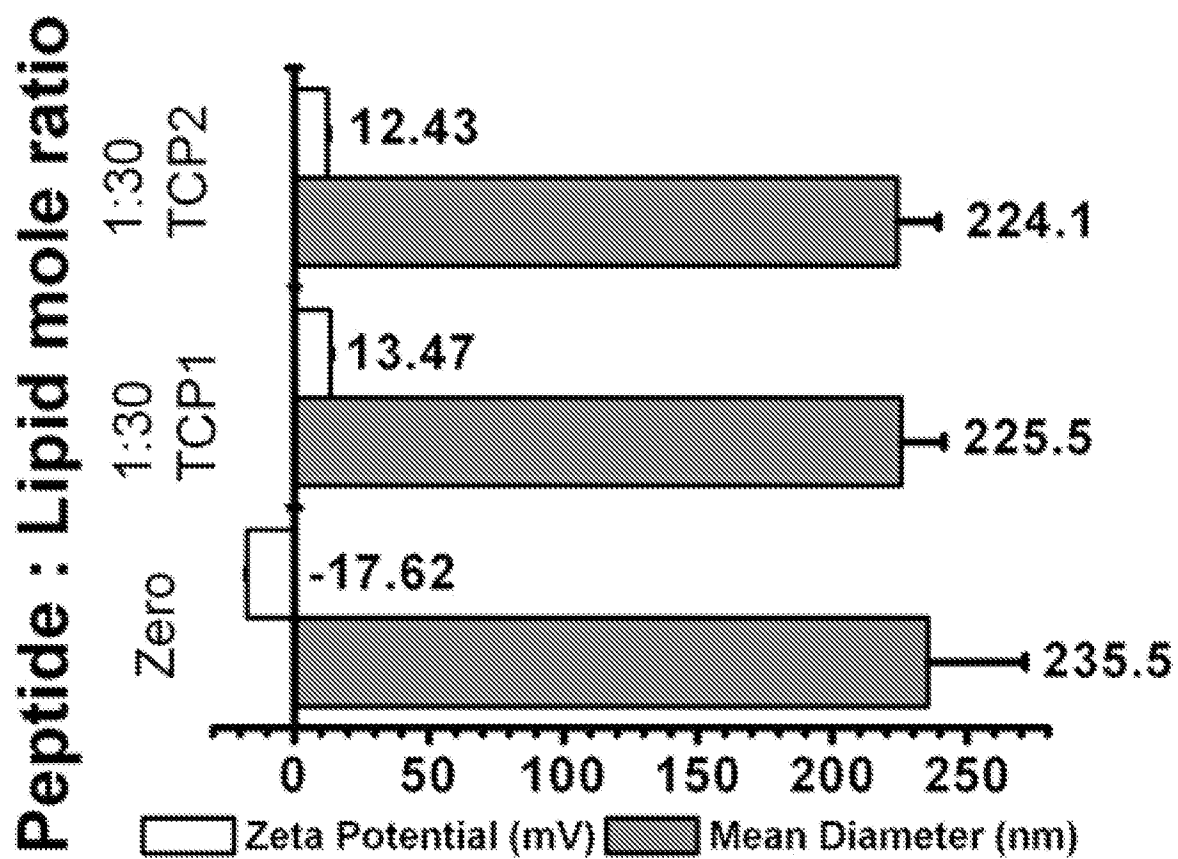
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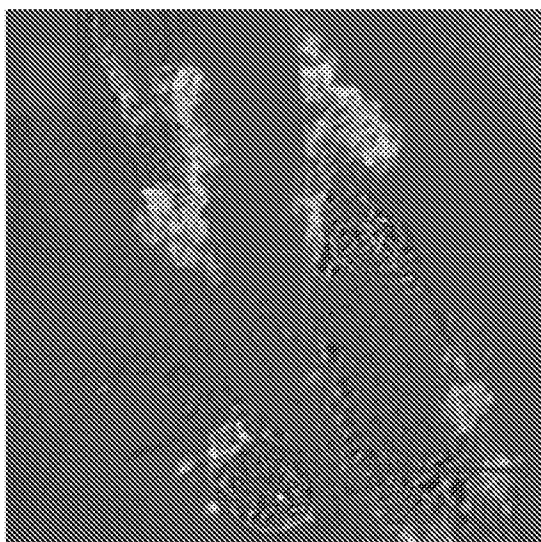
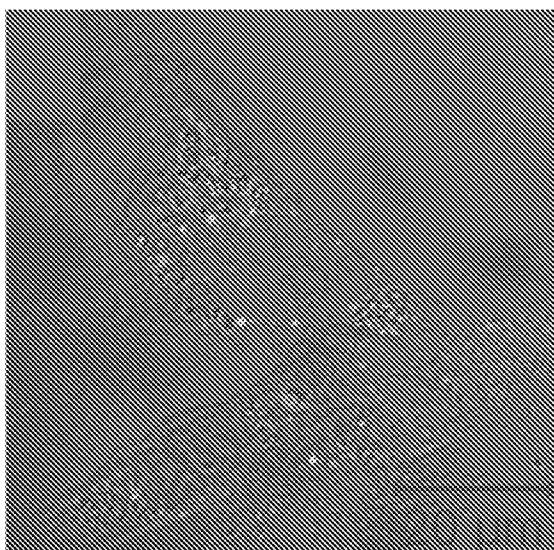
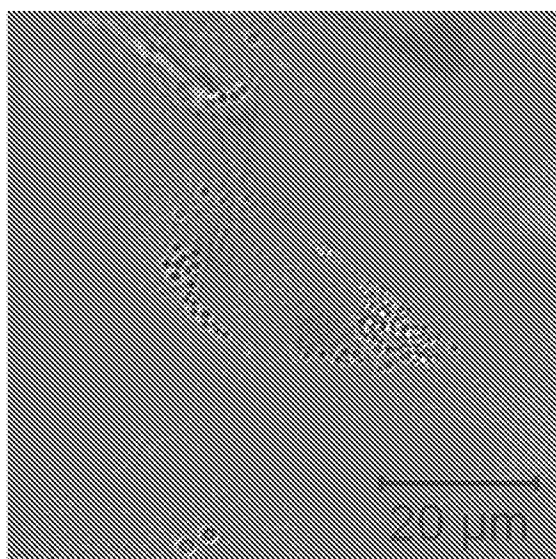
**FIG. 8D**

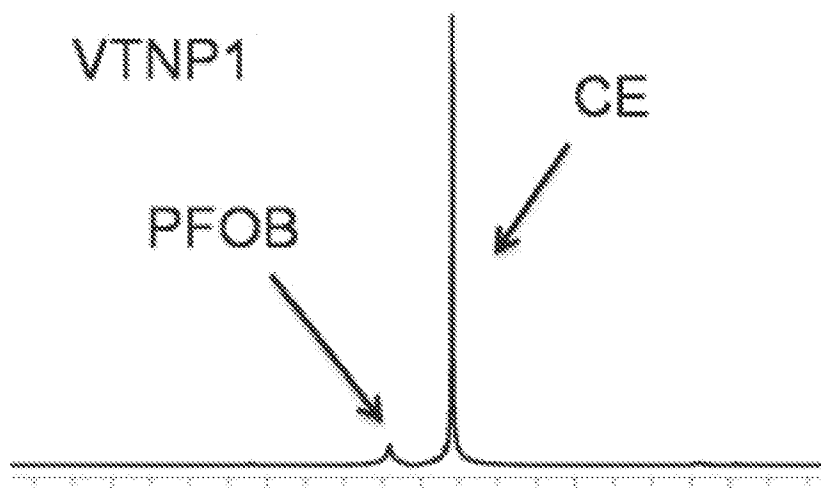
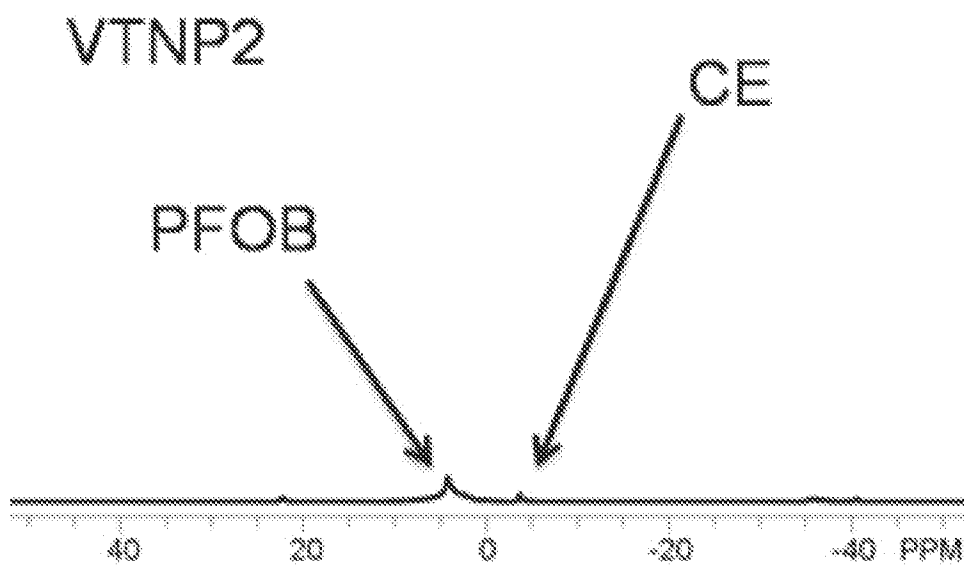
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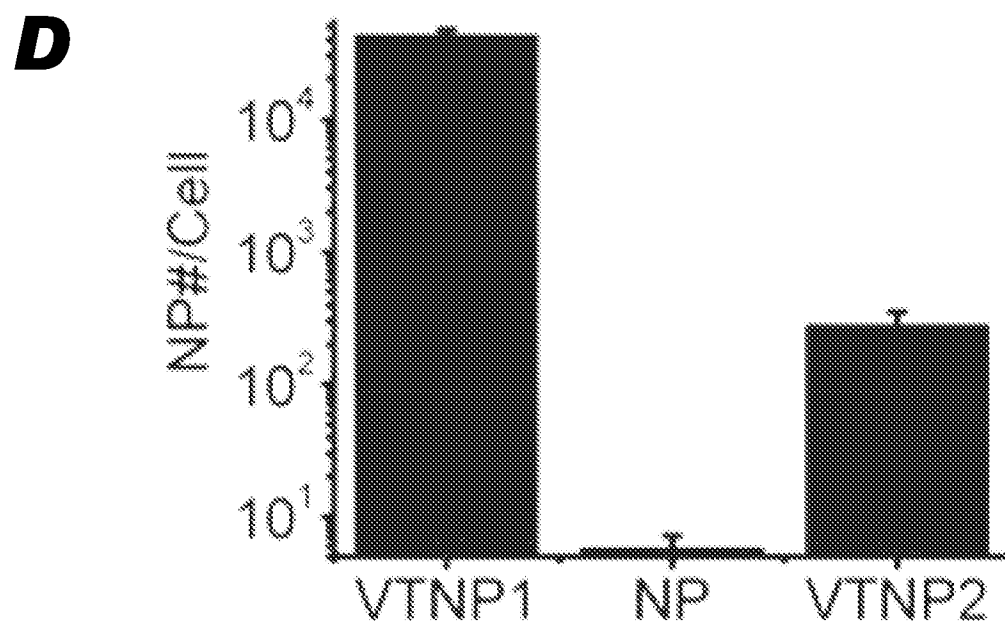
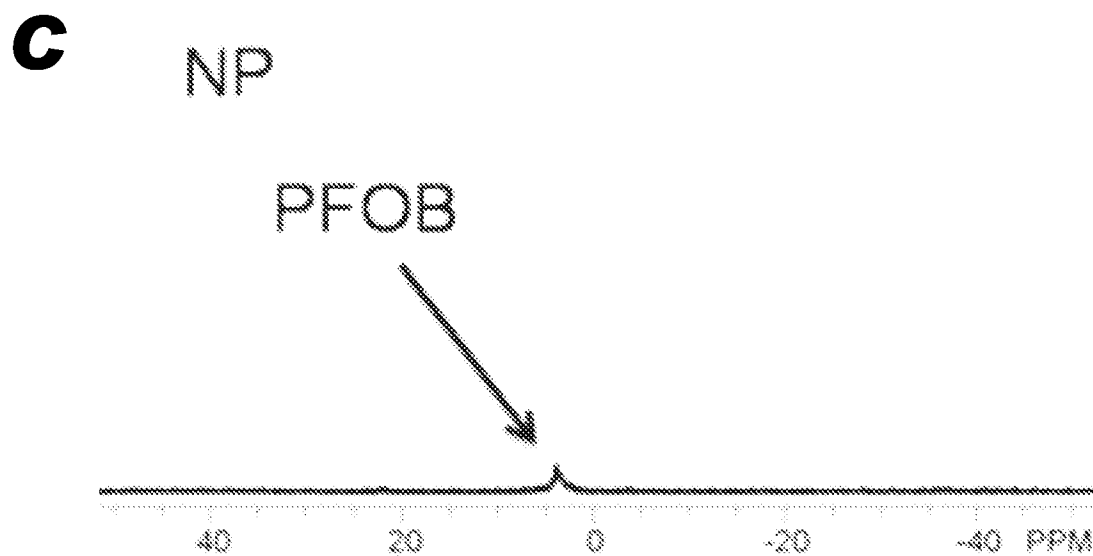
**FIG. 9**

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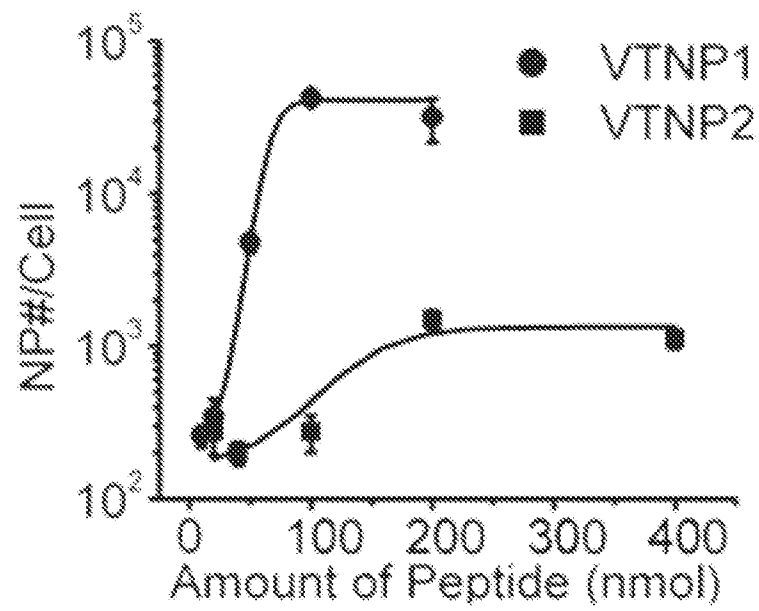
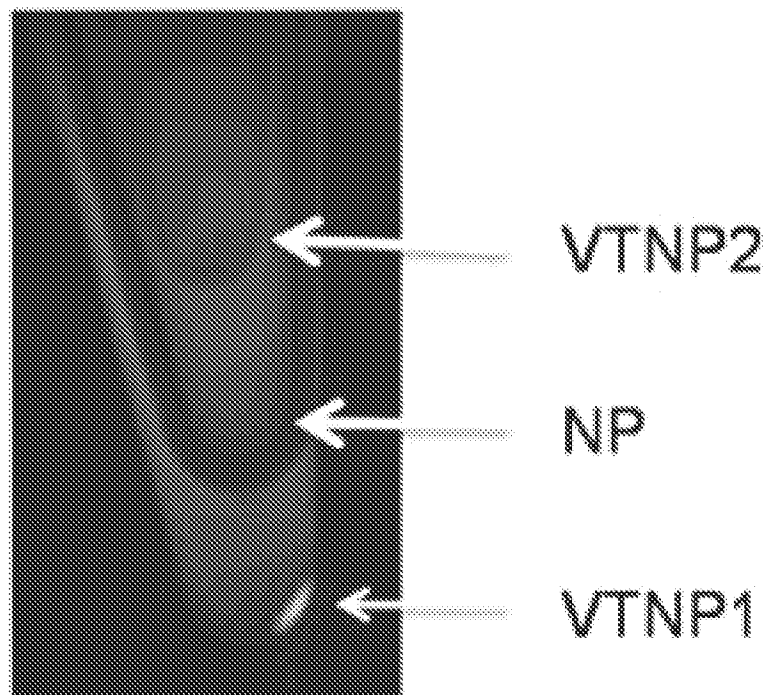
**FIG. 10**

A**B****C****FIG. 11**

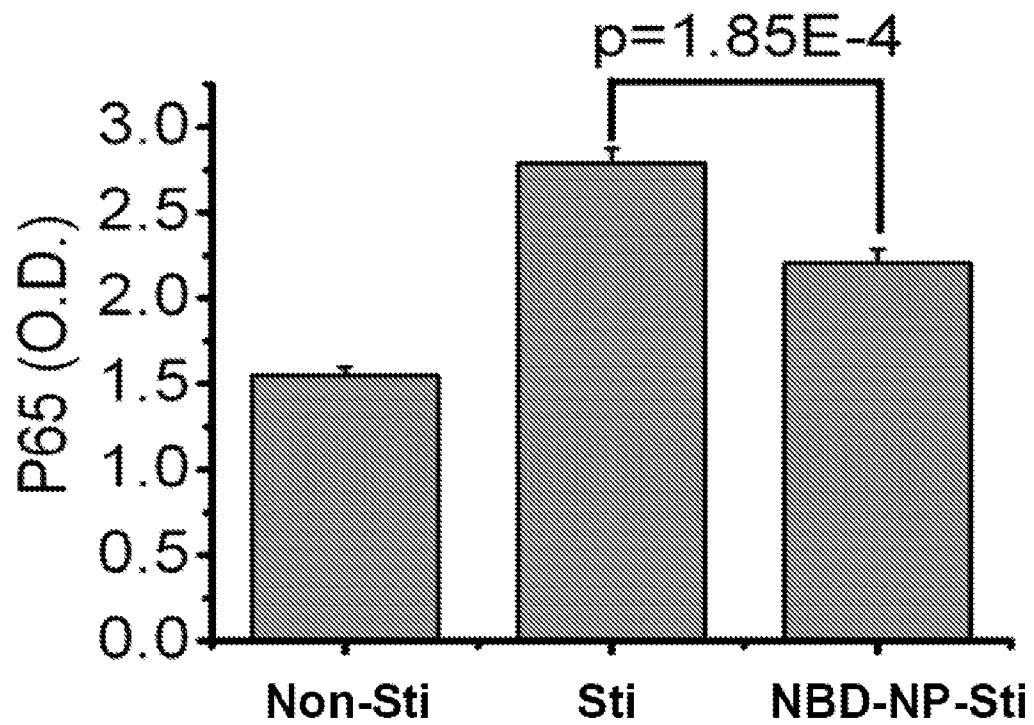
A**B****FIG. 12**

**FIG. 12**

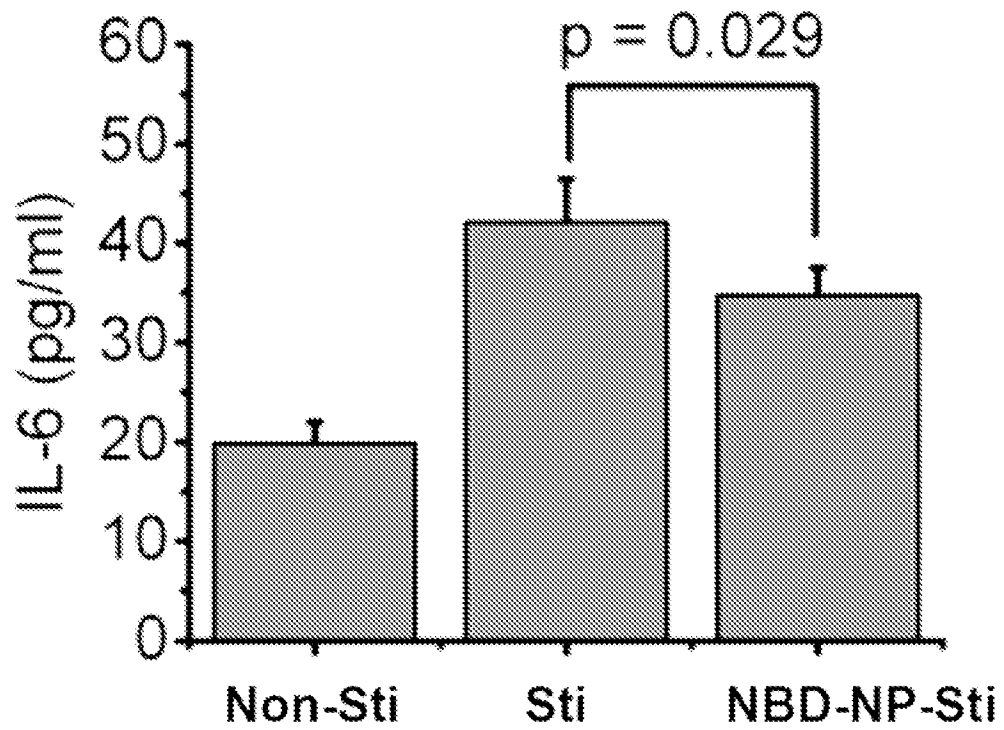
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E**F****FIG. 12**

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**FIG. 13**

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**FIG. 14A**

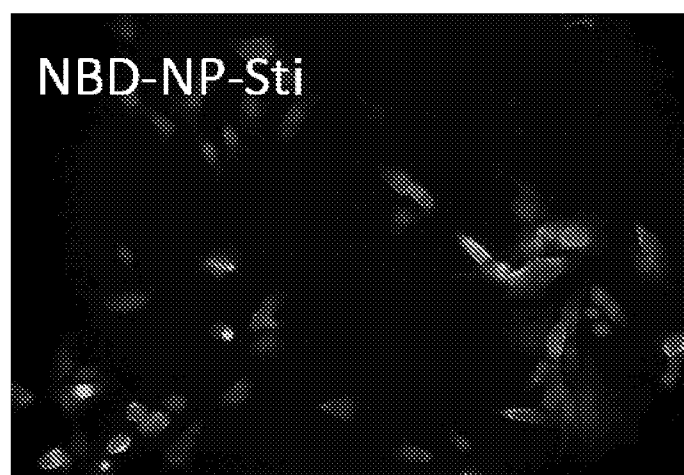
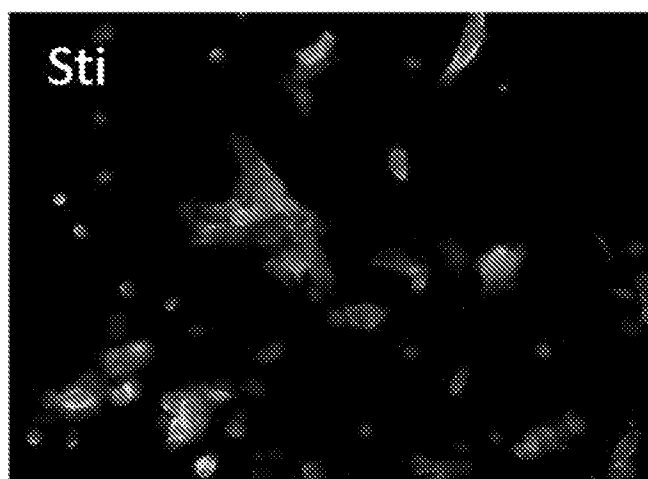
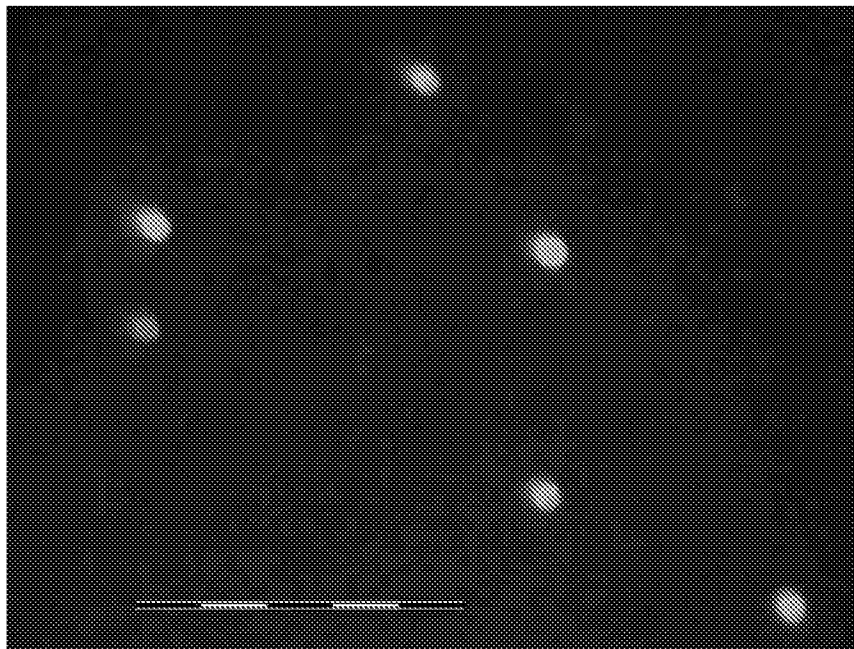


FIG. 14B

A



B

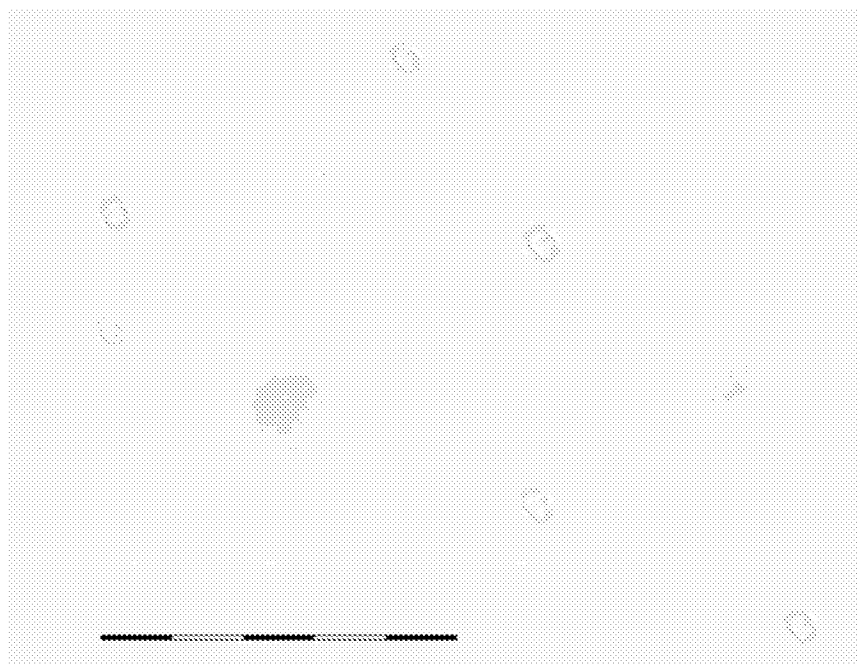


FIG. 15