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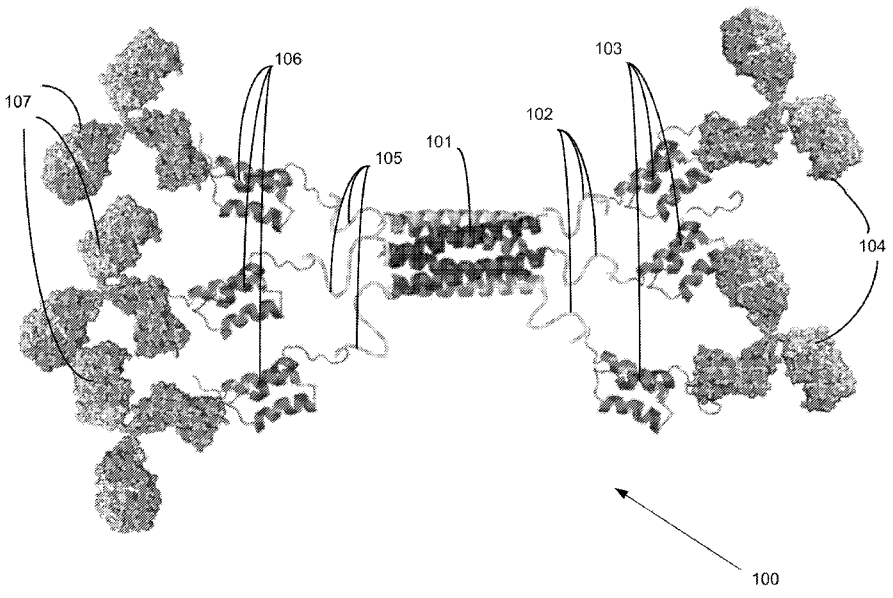


FIG. 1

(57) Abstract: An embodiment of the present disclosure can include a nanocarrier for delivering a cargo to a cell comprising: a bundle domain and a binding domain, the binding domain configured to bind to the cargo. An embodiment of the present disclosure can include a method for delivering a cargo to a cell comprising: introducing an amino-acid based nanocarrier to the cell, the nanocarrier can comprise an alpha-helical protein bundle domain and a binding domain, the cargo can bound to the binding domain and the cargo can be a therapeutic cargo.

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NANOCARRIERS FOR INTRACELLULAR DELIVERY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application Ser. No 62/346,702, filed June 7, 2016, entitled "Engineered Protein Nanocarriers For Intracellular Antibody Delivery," and U.S. Provisional Patent Application Ser. No 62/380,509, filed August 29, 2016, entitled "Multimeric Extracellular Antibody Carriers," the entire contents and substance of these applications are hereby incorporated by reference as if fully set forth below.

BACKGROUND

[0002] Protein engineering and recombinant technologies have created new classes of protein therapeutics with bioactivities not possible from small molecules. A particular success is antibodies, which have been engineered to bind a number of extracellular targets and are a significant and growing fraction of the protein therapeutics portfolio. These have shown incredible promise in the clinic for a variety of applications including inflammation, regenerative medicine, cancer, and autoimmune disease. Despite the successes of antibodies, the most notable exception from the current list is those with intracellular targets or function. The reason is not a lack of medical need. There is a significant list of "undruggable" targets, disease related protein-protein interactions that small molecules cannot block. The issue is not that antibodies cannot be found or engineered to bind these targets, as antibody engineering is a powerful tool and already many antibodies exist for intracellular targets but they are only used on fixed cells in laboratory immunostaining assays. The intrabody, an intracellularly expressed antibody, has been developed to target cellular components in live cells, but requires in vivo genetic manipulation and delivery for therapeutic applications. Arguably, the primary reason there are no intracellular antibody therapeutics is the significant, unmet challenge in delivering sufficient amounts of functional protein inside cells. Successful design and implementation of a functional intracellular antibody delivery system that is generally applicable to all antibodies would have significant impact on human health as it would open the door for a host of new antibody drug therapies to be realized for a wide variety of diseases.

BRIEF SUMMARY

[0003] An embodiment of the present disclosure can include a nanocarrier for delivering a cargo to a cell comprising: a bundle domain and a binding domain, the binding domain configured to bind to the cargo. In an embodiment, the bundle domain can be an alpha-helical protein. In an embodiment, the alpha-helical protein can comprise from about 2 to about 14 coils. In an embodiment, the alpha-helical protein can comprise 6 coils. In an embodiment, the binding domain can comprise a peptide. In an embodiment, the peptide can be selected from the group consisting of domain B of staphylococcal protein A (SPAB), domain C2 of streptococcal protein G (SPGC2), domain D of staphylococcal protein A (SPAD), domain C4 of peptostreptococcal protein L (PpLC4), and double-stranded RNA binding domain (DRBD). In am embodiment, the binding domain can be domain B of staphylococcal protein A (SPAB).

[0004] In an embodiment, the cargo can be bound to the binding domain. In an embodiment, the cargo can comprise a protein. In am embodiment, the cargo can be selected from the group consisting of an antibody, a siRNA molecule, and a DNA molecule. In an embodiment, the cargo can be an antibody. In an embodiment, the cargo can be immunoglobulin G (IgG). In an embodiment, the binding domain can be SPAB and the cargo can be IgG. In an embodiment, the binding domain can be DRBD and the cargo can be a siRNA molecule.

[0005] In an embodiment, the nanocarrier can comprise a linker. In am embodiment, an N-terminus of the linker can be bound to a C-terminus of the bundle domain, and a C-terminus of the linker can be bound to an N-terminus the binding domain. In an embodiment, an N-terminus of the linker can be bound to a C-terminus of the binding domain, and C-terminus of the linker can be bound to an N-terminus of the bundle domain.

[0006] In an embodiment, the linker can comprise an amino acid residue. In an embodiment, the linker can comprise a glycine residue, a serine residue, a proline residue, or mixtures thereof. In an embodiment, the linker can comprise from about 2 to about 30 amino acid residues. In an embodiment, the linker can comprise about 16 amino acid residues.

[0007] In an embodiment, the nanocarrier can comprise a delivery enhancer, the delivery enhancer can be configured to increase uptake of the cargo in the cell. In an embodiment, the

delivery enhancer can be a peptide. In an embodiment, the delivery enhancer can be aurein or hexa-histadine.

[0008] In an embodiment, the nanocarrier can comprise a tag. In an embodiment, the tag can be selected from the group consisting of his-tag, glutathione-S-transferase tag, FLAG tag, and myc-tag. In an embodiment, the tag can be his-tag.

[0009] An embodiment of the present disclosure can include a method for delivering a cargo to a cell comprising: introducing an amino-acid based nanocarrier to the cell, the nanocarrier can comprise an alpha-helical protein bundle domain and a binding domain, the cargo can bound to the binding domain and the cargo can be a therapeutic cargo. In an embodiment, method can further comprise releasing the cargo from the binding domain into the cell.

[0010] An embodiment of the present disclosure can include a method making an assembled nanocarrier comprising: mixing a first nanocarrier monomer and a second nanocarrier monomer, the first nanocarrier monomer can comprise a first alpha-helical protein bundle domain and a first binding domain, the second nanocarrier monomer can comprise a second alpha-helical protein bundle domain and a second binding domain; unfolding the first and second alpha-helical protein bundle domains; coupling the first nanocarrier monomer and the second nanocarrier monomer to form an assembled nanocarrier.

[0011] In an embodiment, the first bundle domain and the second bundle domain can be the same. In an embodiment, the first bundle domain and the second bundle domain can be different. In an embodiment, the first binding domain and the second binding domain can be the same. In an embodiment, the first binding domain and the second binding domain can be different.

[0012] In an embodiment, the coupling can comprise self-assembly of the first and second alpha-helical protein bundle domains. In an embodiment, the unfolding can comprise introducing an unfolding agent selected from the group consisting of detergents, chaotropic salts, and alcohols. In an embodiment, the unfolding agent can be sodium dodecyl sulfate (SDS). In an embodiment, the method can further comprise refolding the first and second alpha-helical protein bundle domains. In an embodiment,, the refolding can comprise removing the unfolding agent.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. 1 illustrates a nanocarrier in accordance with one or more embodiments of the present disclosure.

[0014] FIG. 2 illustrates a method of producing a nanocarrier in accordance with one or more embodiments of the disclosure.

[0015] FIG. 3 illustrates an SDS page gel of protein expression for fusion proteins in accordance with one or more exemplary embodiments of the disclosure.

[0016] FIG. 4 illustrates a graph of fluorescence intensity at different time points using antibodies labeled with fluorescent TAM (IgG-TAM) or antibodies labeled with TAM which are bound to a nanocarrier (IgG-TAM-HEX) in accordance with an exemplary embodiment of the disclosure.

[0017] FIG. 5 illustrates a graph of cellular fluorescence using antibodies labeled with TAM which are bound to a nanocarrier at different ratios of antibody (IgG) to nanocarrier (HEX) in accordance with an exemplary embodiment of the disclosure.

[0018] FIG. 6 illustrates microscopy fluorescence images demonstrating delivery of rabbit or mouse antibodies labeled with TAM bound to HEX nanocarrier at varying ratios of labeled antibody (IgG-TAM) to HEX nanocarrier.

[0019] FIG. 7 illustrates a graph of cell viability under varying conditions. AntiTub-AuHEX and AntiTub-HEX demonstrate cell viability under conditions using an anti-tubulin antibody (AntiTub) which is bound to a nanocarrier (AuHEX or HEX) in accordance with one or more exemplary embodiments of the disclosure.

[0020] FIG. 8a illustrates microscopy fluorescence images demonstrating delivery of nuclear pore complex antibody. FIG. 8b illustrates microscopy fluorescence visualizing delivery of nuclear pore complex antibody and the cell nucleus.

[0021] FIG. 9 illustrates microscopy fluorescence images demonstrating delivery of mouse antibodies labeled with TAM (IgG_M-TAM) bound to a nanocarrier (HEX or AuHEX) after 1 h, 3 h, and 6 h.

[0022] FIG. 10 illustrates a graph of cell viability under varying conditions in accordance with one or more embodiments of the disclosure.

[0023] FIG. 11 illustrates a graph of size determined by Dynamic Light Scattering (DLS) against molecular weight (M_w) indicating a nanocarrier in accordance with an exemplary embodiment of the disclosure.

[0024] FIGs. 12a and 12b illustrate graphs displaying size of the antibody loaded nanocarrier at a ratio of mouse antibody (IgG_M) or rabbit antibody (IgG_R) to nanocarrier (HEX) of 0, 3, and 6 in accordance with an exemplary embodiment of the disclosure. The black bar shows size for antibody labeled with TAM.

DETAILED DESCRIPTION

[0025] Although preferred embodiments of the disclosure are explained in detail, it is to be understood that other embodiments are contemplated. Accordingly, it is not intended that the disclosure is limited in its scope to the details of construction and arrangement of components set forth in the following description or illustrated in the drawings. The disclosure is capable of other embodiments and of being practiced or carried out in various ways. Also, in describing the preferred embodiments, specific terminology will be resorted to for the sake of clarity.

[0026] It must also be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

[0027] Also, in describing the preferred embodiments, terminology will be resorted to for the sake of clarity. It is intended that each term contemplates its broadest meaning as understood by those skilled in the art and includes all technical equivalents which operate in a similar manner to accomplish a similar purpose.

[0028] Ranges can be expressed herein as from "about" or "approximately" one particular value and/or to "about" or "approximately" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value.

[0029] By "comprising" or "including" is meant that at least the named compound, element, particle, or method step is present in the composition or article or method, but does not exclude the presence of other compounds, materials, particles, method steps, even if the other such compounds, material, particles, method steps have the same function as what is named.

[0030] It is also to be understood that the mention of one or more method steps does not preclude the presence of additional method steps or intervening method steps between those

steps expressly identified. Similarly, it is also to be understood that the mention of one or more components in a device or system does not preclude the presence of additional components or intervening components between those components expressly identified.

[0031] The disclosed nanocarriers represent a unique drug delivery platform for carrying and delivering a therapeutic cargo to a cell. In an embodiment, the nanocarriers can be fabricated completely from amino acids, peptides and proteins without the need for synthetic or other small molecules. One of ordinary skill in the art would understand that amino acids are the building blocks of peptides, proteins, and other biological molecules and thus, the nanocarriers may consist of amino acids, peptides, proteins, other biological molecules, or combinations thereof.

[0032] In an embodiment, the cargo can also consist of amino acids. In an embodiment, the cargo can be a biological molecule, such as a DNA or RNA. In an embodiment, the nanocarriers can be fabricated through mixing, self-assembly and other biological techniques without the need for chemistry or chemical modification. The nanocarriers can be capable of carrying any therapeutic cargo without any modification to the cargo itself. That is, the nanocarriers can have a binding domain with a specific binding affinity for a particular cargo. The nanocarriers can comprise binding domains that bind to the same cargo or can comprise multiple binding domains that are each specific to a different cargo, thus a single nanocarrier can be capable of delivering various types of cargo to the same site. The disclosed nanocarriers can be made up entirely of amino acids, proteins, and peptides and thus can advantageously be completely biocompatible (e.g., low to no cytotoxicity), easy-to-fabricate (e.g., modular, mix-and-go self-assembly), can achieve a very high cargo loading, and can be tumor-penetrable due to size.

[0033] FIG. 1 illustrates an exemplary embodiment of nanocarrier structure **100** comprising six separate nanocarrier monomers, each nanocarrier monomer comprising a bundle domain **101**, a linker **102**, **105** and binding domain **103**, **106**. FIG. 1 shows a non-limiting example of nanocarrier **100** wherein the nanocarrier **100** comprises six bundle monomers and five cargo **104**, **107**. However, nanocarriers comprising varying number of nanocarrier monomers and cargo are contemplated. That is, the number of bundle domains **101**, linkers **102**, **105**, binding domains **103**, **106**, and cargo **104**, **107** present in a single nanocarrier can vary. In an embodiment, the described nanocarrier comprises a bundle domain and a binding domain. Additionally nanocarrier can also comprise additional components such as a linker, a tag, delivery enhancer, and the like.

[0034] In an embodiment, an N-terminus end of the linker **102** can be bound to a C-terminus of the bundle domain **101**, and a C-terminus of the linker **102** can be bound to an N-terminus the binding domain **103**. In an embodiment, an N-terminus of the linker **105** can be bound to a C-terminus of the binding domain **106**, and C-terminus of the linker **105** can be bound to an N-terminus of the bundle domain **101**.

[0035] In an embodiment, the nanocarrier can be a nanocarrier for delivering a cargo, e.g., a therapeutic cargo, to a cell. The nanocarrier can comprise a bundle domain and a binding domain.

[0036] In an embodiment, the bundle domain can be a protein, such as an alpha-helical protein. The alpha-helical protein can comprise any number of coils. For example, the alpha-helical protein can comprise from about 2 to about 14 coils. The alpha-helical protein can be a HEX protein, or a protein comprising 6 coils. The coils in a bundle may be the same, or the coils in the bundle may be different. The bundle can comprise long coils or short coils such that the aspect ratio and dimensions of the bundle can depend on the number of coils that make it and the length of those coils. One of ordinary skill in the art would understand what is considered to be short coils or long coils. The bundles may be responsive to stimuli, for example disassembling at lower pH (such as that of an intracellular endosome). The coils may also be responsive to other stimuli such as temperature and salt concentration. The bundle domain can be heat stable, e.g., stable up to about 60 °C, and acid-stable, e.g., stable down to a pH of about 4. While not wishing to be bound by theory, it is thought that the bundle domain serves as a stable core from which to recombinantly attach cargo binding domains.

[0037] In an embodiment, the binding domain can be configured to bind to the cargo. As such, the binding domain can exhibit a particular binding affinity for certain cargo, e.g., an antibody, an siRNA, or the like. The binding domain can bind to the cargo without the need for covalent bonds. In an embodiment, the binding domain can be configured to exhibit a binding affinity to the cargo such that the cargo can stay bound to the nanocarrier in the blood stream. In an embodiment, the binding domain can be configured to exhibit a binding affinity to the cargo such that the cargo can stay bound to the nanocarrier in the blood stream but can be released from the nanocarrier in the cell. In an embodiment, the binding domain can consist of amino acids, e.g., peptides, proteins, and the like. In an embodiment, the binding domain comprises a peptide.

[0038] In an embodiment, the binding domain can be a binding domain configured to bind to an antibody, e.g., IgG. In an embodiment, the binding domain can be a naturally occurring IgG binding protein found in bacteria. In an embodiment, the Fc domain of IgG can be bound to the binding domain because it is away from the antigen binding site. In an embodiment, the binding domain can be domain B of staphylococcal protein A (SPAB), domain C2 of streptococcal protein G (SPGC2), domain D of staphylococcal protein A (SPAD), domain C4 of peptostreptococcal protein L (PpLC4), or combinations thereof. The binding domain can bind the top of the heavy chain or the light chain. The binding domain can also be a human Fc receptor that naturally binds the Fc domain of IgG. In an embodiment, the binding domain can be an Fc binding domain of Fc receptors, including, but not limited to, all of the Fc gamma receptors and FcRN.

[0039] In an embodiment, the binding domain can be configured to bind to siRNA. For instance, the binding domain can be double stranded RNA binding domain (DRBD). In an embodiment, the binding domain can be a peptide that is configured to bind to DNA. The peptide can be either non-sequence specific or sequence specific.

[0040] In an embodiment, the nanocarrier does not include a binding domain. For instance, the cargo can be direct fused to the linker, or to the bundle domain.

[0041] In an embodiment, the linker can comprise amino acids. In an embodiment, the linker can consist of only amino acids. In an embodiment, the linker can comprise repeat units of amino acids selected from the group consisting of glycine, serine, and proline. In an embodiment, the linker can comprise unstructured or unstructured repeats of glycine and serine. In an embodiment, the linker can comprise repeats of glycine, serine, and proline. One of ordinary skill in the art would appreciate that various combinations of repeat units would yield more flexible or stiffer linkers. Thus, the linker can be a flexible linker or a stiff linker. The linkers can comprise about 2 to about 30 amino acids, about 2 to about 20 amino acids, about 2 to about 12 amino acids, about 5 to about 30 amino acids, about 5 to about 20 amino acids, about 5 to about 15 amine acids, about 5 to about 10 amino acids, or about 10 to about 16 amino acids. In an embodiment, the linker can comprise about 16 amino acids.

[0042] In an embodiment, the linker can be structured, including, but not limited to, alpha-helical linkers. While not wishing to be bound by theory, it is believed that the linkers generally serve to spatially separate different nanocarrier components or domains, for example, the bundle

domain and the binding domain. However, the linker can increase the size and flexibility of the protein, which can affect its function in vivo and in cells.

[0043] In an embodiment, the linker can be "functional". For example, it can be made to be cleaved by proteases. For instance, in a non-limiting example, an intracellular protease cleavage site can be imbedded in the linker, and the antibody can be released from the bundle domain upon exposure to the protease. Linkers can also affect protein expression.

[0044] In an embodiment, the nanocarrier can comprise a delivery enhancer configured to increase uptake of cargo in the cell. For instance, the delivery enhancer can increase cell uptake or escape for the intracellular endosome (e.g., to get to the cytosol). In an embodiment, the binding domain can be modified to comprise a delivery enhancer, e.g., a cell penetrating or endosomal escape peptide or protein configured increase delivery of cargo to the cytosol. The delivery enhancer can be located on or between various components of the nanocarrier. For example, the delivery enhancer can be bound to the binding domain and to a linker, or the delivery enhancer can be bound to the bundle domain and to a linker, and the like. In an embodiment, the delivery enhancer can comprise aurein. In an embodiment, the delivery enhancer can comprise hexa-histadine (e.g, 6 histadines, for a total of 36 on the nanocarrier). Other non-limiting examples of nanocarriers can be cationic peptides, e.g., TAT from HIV, or octa-arginine or octa-lysine, and amphipathic peptides including, but not limited to primary amphipathic peptides like Pep-1 from simian virus and HIV, beta-sheet amphipathic peptides like MPG from simian virus and HIV, and alpha-helical amphipathic peptides, e.g., INF from influenza. A wide range of delivery enhancers are contemplated for use in the disclosed nanocarriers. For instance, the delivery enhancer can comprise peptides that can be derived from or based on designs from viral pathogens, including, but not limited to, the sweet arrow peptide and derivatives. In an embodiment, the delivery enhancer can comprise an amphibian microbial peptide. In an embodiment, the delivery enhancer can comprise aurein, magainin 2, buforin 2, or combinations thereof.

[0045] In an embodiment, the nanocarrier can comprise a tag configured to increase solubility of the nanocarrier. In an embodiment, the tag can be used for purification. In an embodiment, the tag can be a his-tag. In an embodiment, the tag can be a his-tag, glutathione-S-transferase tag, FLAG tag, myc-tag, or combinations thereof.

[0046] In an embodiment, the nanocarrier can be configured to deliver a cargo to a cell. For instance the nanocarrier can be configured to deliver a therapeutic cargo to a cell. In an embodiment, the cargo can be a biological molecule. In an embodiment, the cargo can be an antibody, including, but not limited to immunoglobulin G (IgG). Without wishing to be bound by theory, it is believed that the Fc domain provides a particular advantage for binding to the carrier because it is spatially located away from the antigen binding site. In an embodiment, the cargo can be an RNA molecule, such as siRNA. In a embodiment, the cargo can be a DNA molecule. In an embodiment, the cargo can be any other therapeutic peptide or protein. In an embodiment, the cargo can be a small molecule drug.

[0047] FIG. 2 illustrates an exemplary method of making the disclosed nanocarrier structure **220**. The method can comprise mixing a first nanocarrier monomer **200** and a second nanocarrier monomer **210**. The first nanocarrier monomer **200** can comprise a first bundle domain **201** and a first binding domain **203**. The first nanocarrier monomer **200** can further comprise first linker **202**. The first nanocarrier monomer **210** can comprise a second bundle domain **211** and a second binding domain **213**. The second nanocarrier monomer **210** can further comprise second linker **212**. The method can further comprise an unfolding step **204**. The unfolding **204** can comprise unfolding the first bundle domain **201** and the second bundle domain **210**. The unfolding can comprise introducing an unfolding agent. The unfolding agent can be any agent that promotes unfolding of the bundle domain, including, but not limited to detergents, chaotropic salts, and alcohols. In an embodiment, the unfolding agent comprises sodium dodecyl sulfate (SDS). The unfolding agent can be a detergent, including, but not limited to, SDS, Triton X-100, and sodium deoxycholate. The unfolding agent can be a chaotropic salt, including, but not limited to urea, guanidinium hydrochloride, lithium perchlorate, guanidine thiocyanate. The unfolding agent can be an alcohol, including but not limited to short-chain alcohols, e.g., methanol, ethanol, and the like. The unfolding step **204** can also comprise heating, raising pH, e.g., with sodium bicarbonate, or lowering pH, e.g., with acetic acid.

[0048] The method can further comprise a coupling step **205**. The coupling **205** can comprise coupling the first nanocarrier monomer **200** and the second nanocarrier monomer **210** to form assembled nanocarrier **220**. Coupling **205** can comprise refolding the first bundle domain **201** and the second bundle domain **210**. Coupling **205** can comprise self-assembly of the first bundle

domain **201** and the second bundle domain **210** to form assembled nanocarrier **220**. The refolding step can comprise removing or reversing the unfolding agent.

[0049] The method can further comprise loading the cargo into or onto the nanocarrier. The loading step can comprise mixing the cargo with the assembled nanocarrier such that the cargo binds to the binding domain.

[0050] Each component of the nanocarrier (e.g., each bundle domain, binding domain, linker, delivery enhancer, cargo, tag, etc.) can be the same, or different. For instance, in an embodiment, the bundle domains of the nanocarrier can be the same, or homo-bundle domains. In an embodiment, the bundle domains of the nanocarriers can be different, or hetero-bundle domains. Likewise, in an embodiment the binding domains of the nanocarrier can be the same. In an embodiment, the binding domains of the nanocarriers can be different.

[0051] In an embodiment, the linkers of the nanocarrier can be the same. In an embodiment, the linkers of the nanocarriers can be different. Likewise, in an embodiment, the delivery enhancers of the nanocarrier can be the same. In an embodiment, the delivery enhancers of the nanocarriers can be different.

[0052] The type of cargo can be based on the type of binding domain present in the nanocarrier. That is, the binding domain can be configured to bind to a specific type of cargo. For instance, if the nanocarrier comprises various binding domains, then various types of cargo can be bound to the nanocarrier at those binding domains.

[0053] An embodiment of the invention can be a method for delivering a cargo, such as a therapeutic cargo, to a cell. The method can comprise introducing an amino-acid based nanocarrier to the cell. The nanocarriers contemplated for *in vivo* and *in vitro* applications. That is, the nanocarrier can be introduced to a subject, such as a human. The nanocarrier can comprise a bundle domain and a binding domain, wherein the cargo can be bound to the binding domain and wherein the cargo can be a therapeutic cargo. The method can further comprise releasing the cargo from the binding domain into the cell. In an embodiment, the method can comprise delivering the cargo to the cell by introducing the entire nanocarrier to the cell, wherein the cargo is not released from the binding domain or the nanocarrier.

EXAMPLES

Example 1 ***Cloning and Expression of Fusion Peptides***

[0054] The gene construct encoding components of an exemplary nanocarrier: either H6-SPAB-Hex or Hex-SPAB-H6 was subcloned into an IPTG-inducible bacterial expression vector pQE80, yielding pQE80- SPAB-Hex and pQE80-Hex-SPAB, respectively. The bacterial expression host, Escherichia coli TOP10, was transformed with the pQE80- SPAB-Hex or pQE80-Hex-SPAB, and was grown in 2 × YT medium at 37 °C. Protein expression was induced by 1 mM IPTG when the OD reached 0.6, and continued for 5 h. After collecting cells by centrifugation, proteins were purified by Ni-NTA affinity chromatography under native conditions according to the manufacturer's instruction. For expression of the Au-Hex nanocarrier, the restriction-free cloning method was used to insert the Aurein 1.2 domain N-terminally and C-terminally to pQE-Hex-SPAB and pQE-SPAB-Hex, respectively. A vector encoding H6-SPAB-Hex- TAT-HA2 was similarly constructed. Transformation, expression and protein purification were performed in the same way as described above.

[0055] As shown in **FIG. 3** a stained SDS-PAGE gel was used to visualize expression of H6-SPAB-Hex (lane 2) or Hex-SPAB-H6 (lane 4) and purification of the respective fusion proteins (lanes 6 and 7). An example protein, lysozyme, was used in lane 5.

Example 2 ***Generation of nanocarrier***

[0056] Sodium dodecyl sulfate (SDS) was added to a final concentration of 0.2% (w/v) to a 1:1 M ratio mixture of purified H6-SPAB-Hex (1.5 mg/mL) and Hex-SPAB-H6 (1.5 mg/mL) in PBS. After 10 min, the mixture was buffer-exchanged to PBS using a PD-10 desalting column. The resulting Hex nanocarrier can be concentrated using Vivaspin centrifugal filters.

Example 3 ***Cell Culture***

[0057] HeLa cells were seeded onto a 96-well plate at a density of 5000 cells per well in 100 µL DMEM supplemented with 10% (v/v) fetal bovine serum (FBS) and antibiotics (100 µg/mL penicillin and 50 µg/mL streptomycin), and incubated overnight for attachment at 37 °C under humidified air containing 5% (v/v) CO2. The medium was replaced with 100 µL fresh medium containing a protein(s) of interest at appropriate concentrations and/or molar ratios.

Example 4 ***Binding affinity of Hex nanocarrier towards IgG***

[0058] Biotinylated IgG was immobilized onto optical biosensors to enable high throughput kinetic analyses in microplate format. The IgG-binding behavior to the Hex nanocarrier in comparison to soluble SPAB at neutral and acidic pH was assessed. Soluble SPAB displayed nanomolar affinities towards mouse IgG (IgG_M), with a 17-fold stronger binding at neutral pH compared to pH 5.0 (Table 1). On the other hand, the binding between the Hex nanocarrier and IgG_M at neutral pH was so strong that KD was below picomolar concentration, which is the lower limit detectable by the system. At pH 5.0, the binding became weaker with K_D of 5.14 nM, but was still stronger by >91-fold in comparison to the interaction between SPAB and IgG_M. A similar trend was observed in the binding assay using rabbit IgG (IgG_R).

Table 1

IgG subtype	Antigen	pH 7.4			pH 5.0		
		$k_{on}\times10^5$ (s^2)	$k_{off}\times10^5$ (M^2s^2)	K_D (nM)	kon$\times10^5$ (s^2)	$k_{off}\times10^5$ (M^2s^2)	K_D (nM)
IgG_M	SPAB	2.06 ± 0.20	5.82 ± 0.07	28.3 ± 2.8	0.084 ± 0.003	3.94 ± 0.06	470 ± 19
	Hex	1.51 ± 0.01	$<10^{-4}$	$<10^{-3}$	2.20 ± 0.04	1.13 ± 0.01	5.14 ± 0.12
IgG_R	SPAB	1.59 ± 0.06	8.42 ± 0.06	53 ± 2	0.14 ± 0.09	3.57 ± 0.04	255 ± 17
	Hex	1.95 ± 0.02	$<10^{-4}$	$<10^{-3}$	3.11 ± 0.06	0.43 ± 0.00	1.39 ± 0.03

Example 5 ***Intracellular delivery of fluorescently labeled antibody by a nanocarrier***

[0059] Mouse or rabbit IgG dissolved in PBS (pH 8.5) at the concentration of 7 mg/mL (47 μM) was mixed with a 10-fold molar excess of TAMRA- NHS at RT for 1 h, and then desalted using a PD-10 column to remove residual dye. HeLa cells were plated at on a glass bottom dish at a density of 5000 cells per cm^2 for microscopy or a 48-well microplate at a density of 4 × 104 cells per well for flow cytometry in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS)

and antibiotics (100 μg/mL penicillin and 50 μg/mL streptomycin), and incubated overnight for attachment at 37 °C under humidified air containing 5% (v/v) CO_2. The Hex nanocarrier and the labeled IgG were mixed at appropriate molar ratios and concentrations in 100 μL of fresh supplemented medium, left to stand for 10 min at RT, and then used to replace the old medium. At appropriate time points, cells were washed three times with PBS, and then imaged by an Axio Observer Z1 fluorescence microscope or a LSM 700 confocal laser scanning microscope (Carl Zeiss, Germany). For flow cytometry, cells were harvested with 0.2% trypsin-EDTA, and then counted with an Accuri C6 flow cytometer (BD Biosciences, San Jose, CA) using the FL2 channel (λex = 488 nm, λem = 540/20 nm). Accuri CFlow software was used for analysis.

[0060] IgG_M-TAM or IgG_R-TAM at the fixed concentration of 0.6 μM was mixed with the Hex nanocarrier at various molar ratios of 1:0, 1:1, 3:1, 6:1 IgG- TAM:Hex. The cellular fluorescence was increasingly pronounced with higher IgG_R loading density while the inverse correlation was observed with IgG_M.

[0061] As shown in **FIG. 4** fluorescence intensity measured by flow cytometry demonstrates intracellular delivery of either mouse IgG (IgGM) or rabbit IgG (IgGRb) when mixed with HEX nanocarrier. In the absence of HEX nanocarrier delivery is negligible.

[0062] As shown in **FIG. 5** fluorescence intensity measured by flow cytometry demonstrates intracellular delivery of either IgGM or IgGRb at various molar ratios 1:0 (IgG only), 6:1, 3:1, and 1:1. In the absence of HEX nanocarrier delivery is negligible.

[0063] As shown in **FIG. 6** TAMRA-labeled IgG_M or IgG_R at 0.6 μM was mixed with HEX nanocarrier at various molar ratios of 1:0, 1:1, 3:1, and 6:1 IgG-TAM:Hex, and then incubated with HeLa cells for 24 h. Cellular fluorescence was imaged by fluorescence microscopy.

Example 6 ***Intracellular delivery of anti-beta tubulin r anti-NPC antibody by the Hex nanocarrier***

[0064] HeLa cells were plated and cultured as described above. Anti-tubulin antibody or anti-nuclear pore complex antibody was mixed with the Hex nanocarrier, and then incubated with cells in the same way. At 24 h post-incubation, cells were washed three times with PBS, and then fixed with 4% (v/v) paraformaldehyde at RT for 10 min. After washing cells three times with PBS, cells were permeabilized in a solution consisting of 0.25% (v/v) Triton X-100 and 2% (w/v) bovine serum albumin (BSA) in PBS at RT for 5 min. After washing, cells were incubated

in blocking solution consisting of 2% (w/v) BSA in PBS at RT for 1 h to inhibit non-specific immunostaining, and then treated with goat anti-mouse IgG Alexa Fluor 647 and Hoechst 33,342 at RT for 1 h to visualize intracellularly delivered antibodies and the nucleus, respectively. Microscopy images were obtained by a LSM 700 confocal microscope or an Axio Observer Z1 microscope.

[0065] As shown in **FIG. 7** Cell viability of HeLa cells incubated for 48 h with 0.2 or 0.4 μM of IgGM or anti-beta tubulin antibody mixed with the Hex nanocarrier at a fixed molar ratio of 3:1 IgG:Hex was normalized to that of untreated HeLa cells (Control). Nocodazole and paclitaxel are cytotoxic agents that interfere with microtubule polymerization and depolymerization, respectively, and are positive controls for delivery of anti-beta tubulin antibody. Error bars indicate mean ± s.d. (n = 3); *p b 0.01 (t-test: two-sample assuming unequal variances).

[0066] In a different experiment, HeLa cells were incubated with 0.6 μM of mouse anti-NPC antibody with 0.2 μM of the Hex nanocarrier. After 24 h, cells were washed, fixed, permeabilized, and stained with goat anti-mouse IgG Alexa Fluor 647 conjugate as shown in **FIG. 8a** to visualize intracellular distribution of antibodies. Nuclei were then stained with Hoechst 33342 as shown in **Fig. 8b**.

Example 7 ***Intracellular delivery of antibody by Hex nanocarrier fused to Aurein peptide***

[0067] The Aurein 1.2 sequence was appended to the C-terminus of H6-SPAB-Hex and to the N-terminus of Hex-SPAB-H6 such that the re-assembled Hex nanocarrier could have evenly distributed Aurein motifs opposite the SPAB domains. Fluorescently labeled IgG_M was mixed with the Hex nanocarrier or the Au-Hex nanocarrier prior to incubation with HeLa cells. Cellular fluorescence was measured by flow cytometry to compare IgG uptake efficiency. Notably, IgG_M carried by the Au-Hex nanocarrier showed internalization 4- fold higher than IgG_M bound to the Hex nanocarrier at 5 h post-incubation, which was even greater than the maximum uptake of IgG_M delivered by the Hex nanocarrier upon saturation at 24 h. HeLa cells were incubated for 24 h with 0.6 μM of mouse anti-NPC antibody and mixed with 0.2 μM of the Au-Hex nanocarrier or the Hex nanocarrier. Faster and greater uptake of IgG_M carried by the Au-Hex nanocarrier was demonstrated in time-course fluorescence microscopy. Cellular fluorescence started to appear as early as 1 h after incubation for the Au-Hex nanocarrier. In contrast, no fluorescence was visible until 5 h incubation for the Hex nanocarrier.

[0068] Fluorescence images were taken during a time course to demonstrate uptake of TAMRA-labeled IgG_M as shown in **FIG. 9**. The delivery of IgG_M was conducted at a fixed molar ratio of 3:1 IgG_M-TAM:Hex or Au-Hex, 0.4μM IgG_M-TAM was mixed with the Hex or Au-Hex nanocarrier, and then incubated with HeLa cells at 37°C. Cellular fluorescence was imaged at various time points by fluorescence microscopy. Using the Au-Hex nanocarrier produced detectable fluorescence in about 1 h, while using the Hex nanocarrier produces detectable fluorescence after about 6 h as indicated in **FIG. 9**.

CLAIMS

We claim:

1. A nanocarrier for delivering a cargo to a cell comprising:

   a bundle domain; and

   a binding domain, the binding domain configured to bind to the cargo.

2. The nanocarrier of claim 1, wherein the bundle domain is an alpha-helical protein.

3. The nanocarrier of claim 2, the alpha-helical protein comprising from about 2 to about 14 coils.

4. The nanocarrier of claim 3, the alpha-helical protein comprising 6 coils.

5. The nanocarrier of claim 1, the binding domain comprising a peptide.

6. The nanocarrier of claim 5, wherein the peptide is selected from the group consisting of domain B of staphylococcal protein A (SPAB), domain C2 of streptococcal protein G (SPGC2), domain D of staphylococcal protein A (SPAD), domain C4 of peptostreptococcal protein L (PpLC4), and double-stranded RNA binding domain (DRBD).

7. The nanocarrier of claim 5, wherein the binding domain is domain B of staphylococcal protein A (SPAB).

8. The nanocarrier of claim 1, wherein the cargo is bound to the binding domain.

9. The nanocarrier of claim 1, the cargo comprising a protein.

10. The nanocarrier of claim 1, wherein the cargo is selected from the group consisting of an antibody, a siRNA molecule, and a DNA molecule.

11. The nanocarrier of claim 9, wherein the cargo is an antibody.

12. The nanocarrier of claim 11, wherein the cargo is immunoglobulin G (IgG).

13. The nanocarrier of claim 8, wherein the binding domain is SPAB and the cargo is IgG.

14. The nanocarrier of claim 8, wherein the binding domain is DRBD and the cargo is a siRNA molecule.

15. The nanocarrier of claim 1, further comprising a linker.

16. The nanocarrier of claim 15, an N-terminus of the linker bound to a C-terminus of the bundle domain, and a C-terminus of the linker bound to an N-terminus the binding domain.

17. The nanocarrier of claim 15, an N-terminus of the linker bound to a C-terminus of the binding domain, and C-terminus of the linker bound to an N-terminus of the bundle domain.

18. The nanocarrier of claim 15, the linker comprising an amino acid residue.

19. The nanocarrier of claim 18, the linker comprising a glycine residue, a serine residue, a proline residue, or mixtures thereof.

20. The nanocarrier of claim 18, the linker comprising from about 2 to about 30 amino acid residues.

21. The nanocarrier of claim 20, the linker comprising about 16 amino acid residues.

22. The nanocarrier of claim 1, further comprising a delivery enhancer, the delivery enhancer configured to increase uptake of the cargo in the cell.

23. The nanocarrier of claim 22, wherein the delivery enhancer is a peptide.

24. The nanocarrier of claim 22, wherein the delivery enhancer is aurein or hexa-histadine.

25. The nanocarrier of claim 1, further comprising a tag.

26. The nanocarrier of claim 25, wherein the tag is selected from the group consisting of his-tag, glutathione-S-transferase tag, FLAG tag, and myc-tag.

27. The nanocarrier of claim 25, wherein the tag is his-tag.

28. A method for delivering a cargo to a cell comprising:

introducing an amino-acid based nanocarrier to the cell, the nanocarrier comprising an alpha-helical protein bundle domain and a binding domain, wherein the cargo is bound to the binding domain and wherein the cargo is a therapeutic cargo.

29. The method of claim 28, further comprising releasing the cargo from the binding domain into the cell.

30. The nanocarrier of claim 28, the alpha-helical protein comprising from about 2 to about 12 coils.

31. The method of claim 28, the alpha-helical protein comprising 6 coils.

32. The method of claim 28, wherein the binding domain is selected from the group consisting of domain B of staphylococcal protein A (SPAB), domain C2 of streptococcal protein G (SPGC2), domain D of staphylococcal protein A (SPAD), domain C4 of peptostreptococcal protein L (PpLC4), and double-stranded RNA binding domain (DRBD).

33. The method of claim 28, wherein the cargo is selected from the group consisting of an antibody, a siRNA molecule, and a DNA molecule.

34. The method of claim 33, wherein the cargo is immunoglobulin G (IgG).

35. The method of claim 33, wherein the binding domain is SPAB and the cargo is IgG.

36. The method of claim 33, wherein the binding domain is DRBD and the cargo is a siRNA molecule.

37. The method of claim 28, further comprising a linker.

38. The method of claim 37, an N-terminus end of the linker bound to a C-terminus of the bundle domain, and a C-terminus of the linker bound to an N-terminus the binding domain.

39. The method of claim 37, an N-terminus of the linker bound to a C-terminus of the binding domain, and C-terminus of the linker bound to an N-terminus of the bundle domain.

40. The method of claim 37, the linker comprising a glycine residue, a serine residue, a proline residue, or mixtures thereof.

41. The method of claim 37, the linker comprising from about 2 to about 30 amino acid residues.

42. The method of claim 28, further comprising a delivery enhancer, the delivery enhancer configured to increase uptake of the cargo in the cell.

43. The method of claim 42, wherein the delivery enhancer is aurein or hexa-histadine.

44. The method of claim 28, further comprising a tag.

45. The method of claim 44, wherein the tag is selected from the group consisting of his-tag, glutathione-S-transferase tag, FLAG tag, and myc-tag.

46. A method of making an assembled nanocarrier comprising:

mixing a first nanocarrier monomer and a second nanocarrier monomer, the first nanocarrier monomer comprising a first alpha-helical protein bundle domain and a first binding domain, the second nanocarrier monomer comprising a second alpha-helical protein bundle domain and a second binding domain;

unfolding the first and second alpha-helical protein bundle domains;

coupling the first nanocarrier monomer and the second nanocarrier monomer to form an assembled nanocarrier.

47. The method of claim 46, wherein the first bundle domain and the second bundle domain are the same.

48. The method of claim 46, wherein the first bundle domain and the second bundle domain are different.

49. The method of claim 46, wherein the first binding domain and the second binding domain are the same.

50. The method of claim 46, wherein the first binding domain and the second binding domain are different.

51. The method of claim 46, wherein the coupling comprises self-assembly of the first and second alpha-helical protein bundle domains.

52. The method of claim 46, wherein the unfolding comprises introducing an unfolding agent selected from the group consisting of detergents, chaotropic salts, and alcohols.

53. The method of claim 52, wherein the unfolding agent is sodium dodecyl sulfate (SDS).

54. The method of claim 31, further comprising refolding the first and second alpha-helical protein bundle domains.

55. The method of claim 52, wherein the refolding comprises removing the unfolding agent.

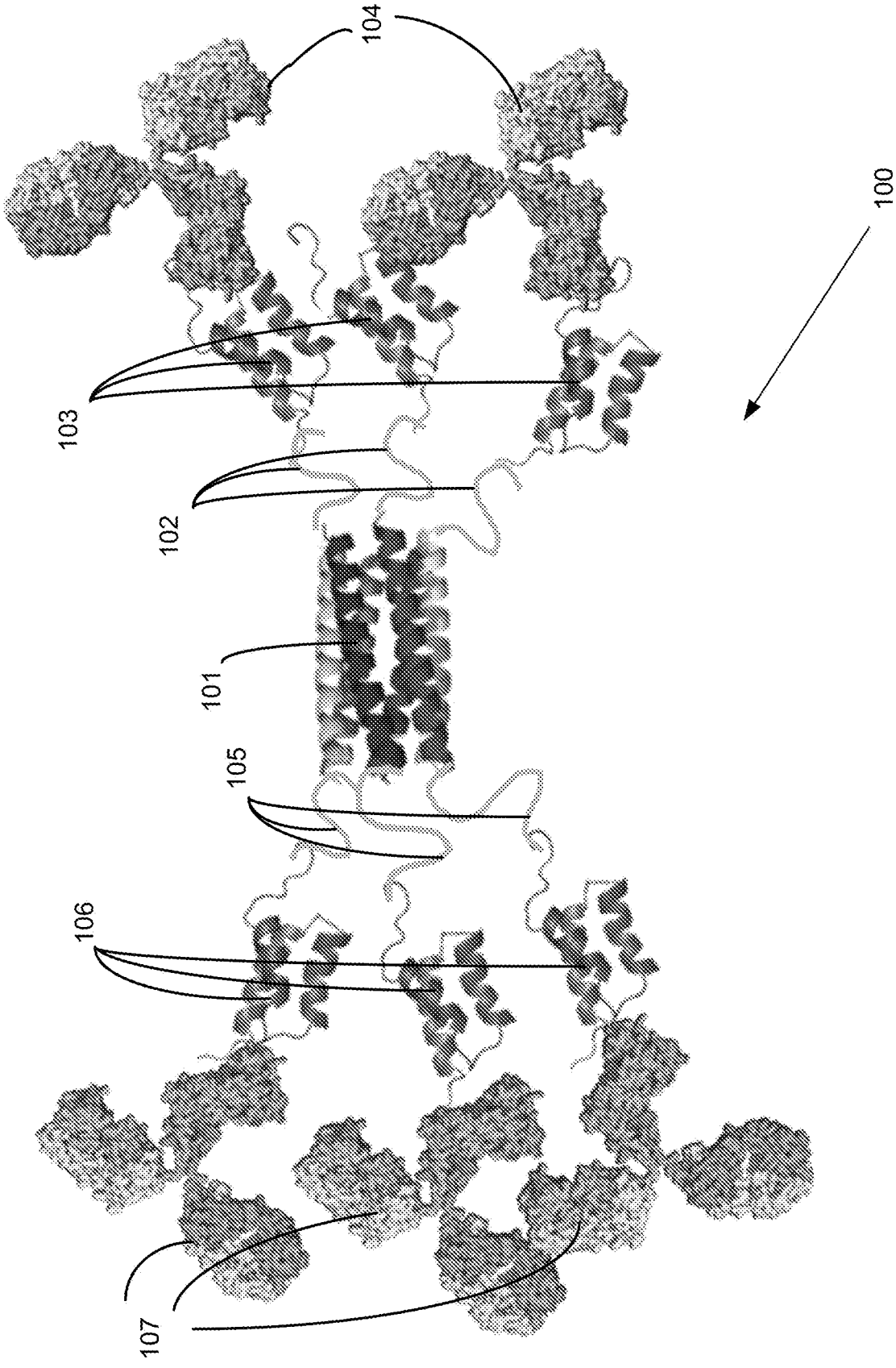


FIG. 1

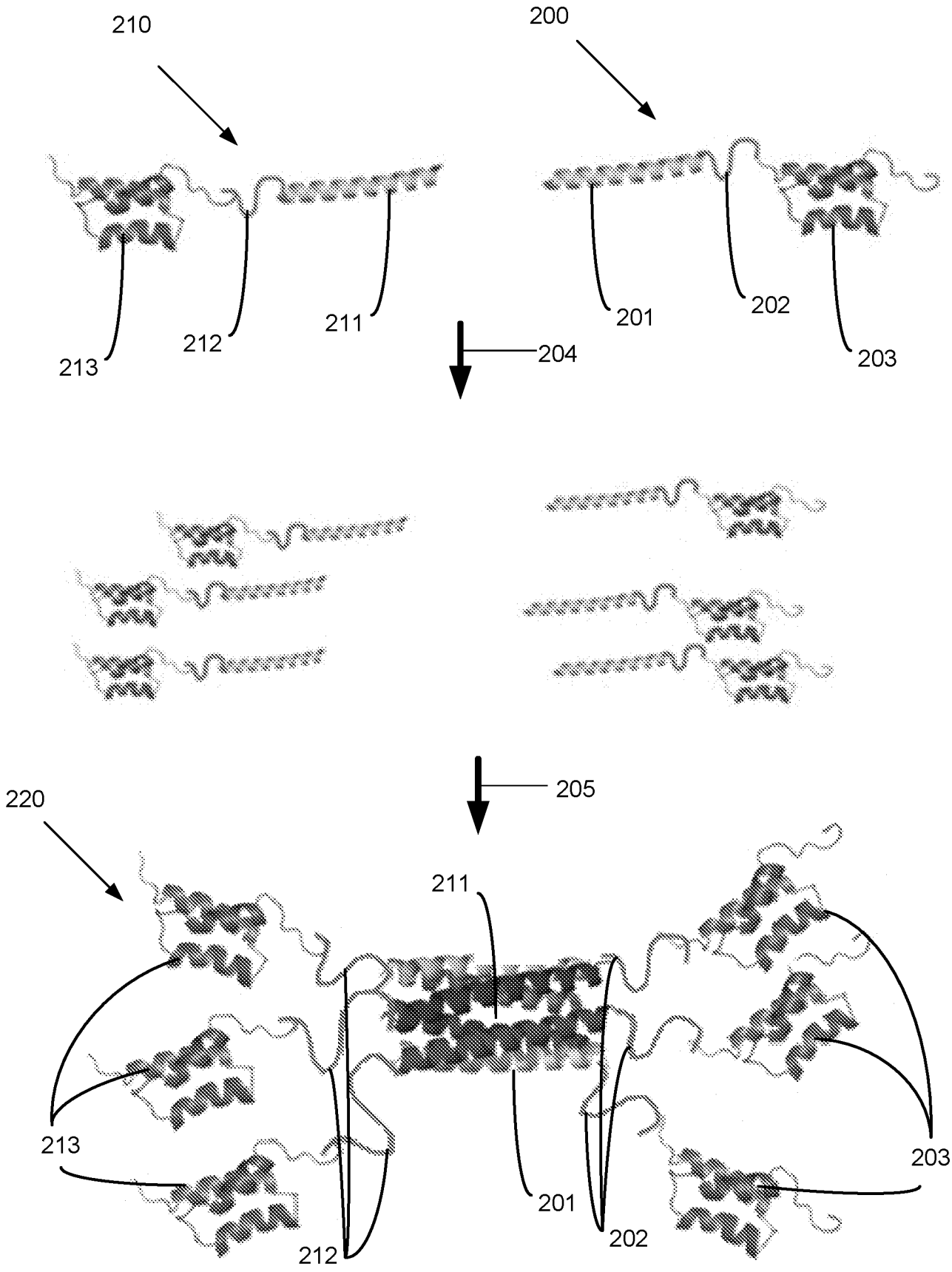


FIG. 2

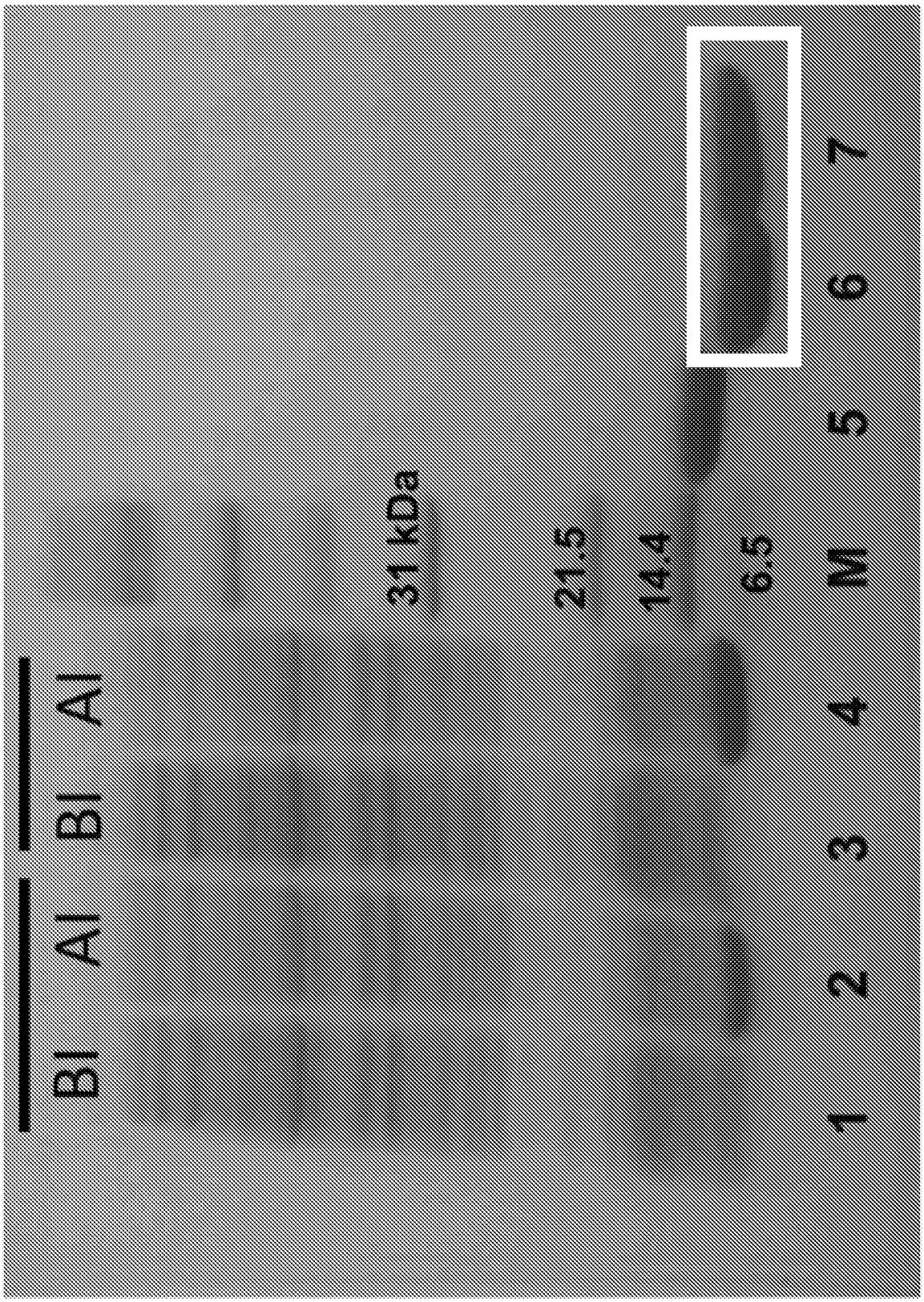


FIG. 3

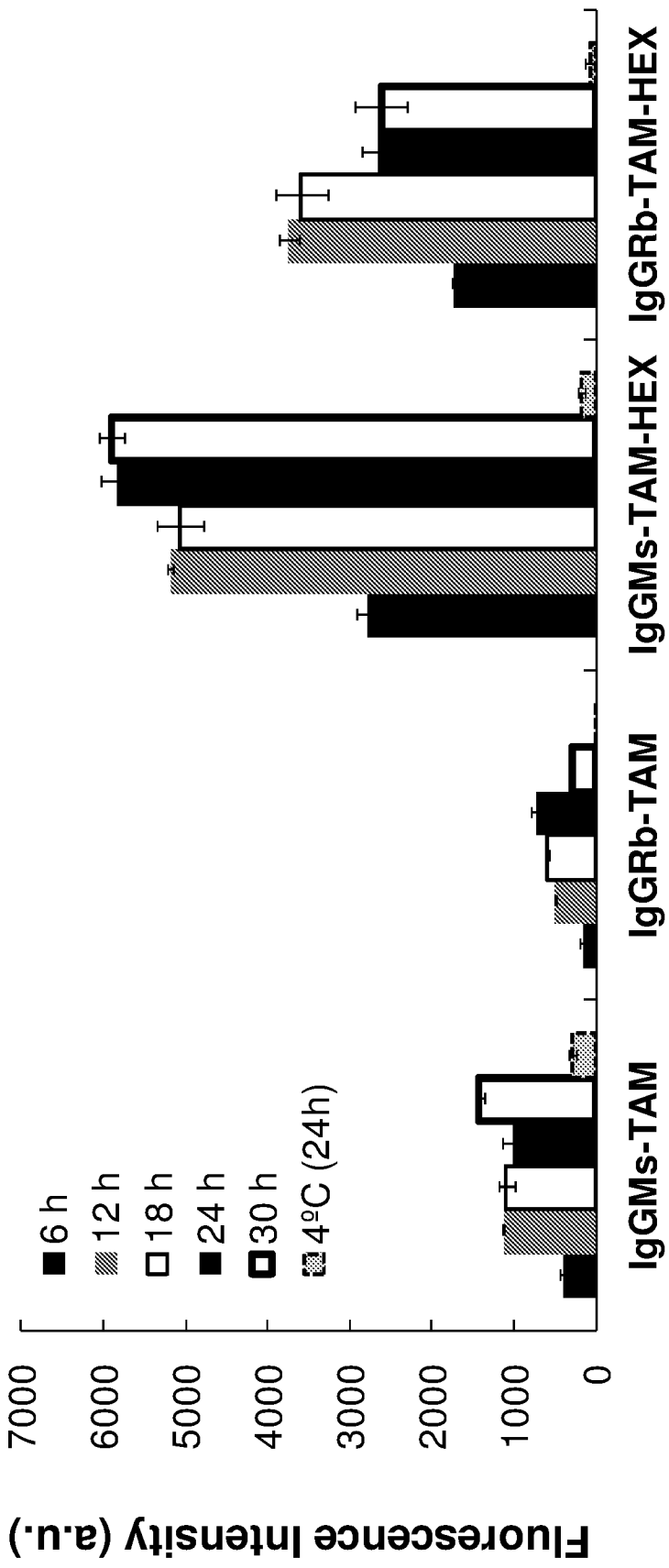

Fluorescence Intensity (a.u.)
7000
6000
5000
4000
3000
2000
1000
0
6 h
12 h
18 h
24 h
30 h
4ºC (24h)
IgGMs-TAM
IgGRb-TAM
IgGMs-TAM-HEX
IgGRb-TAM-HEX

FIG. 4

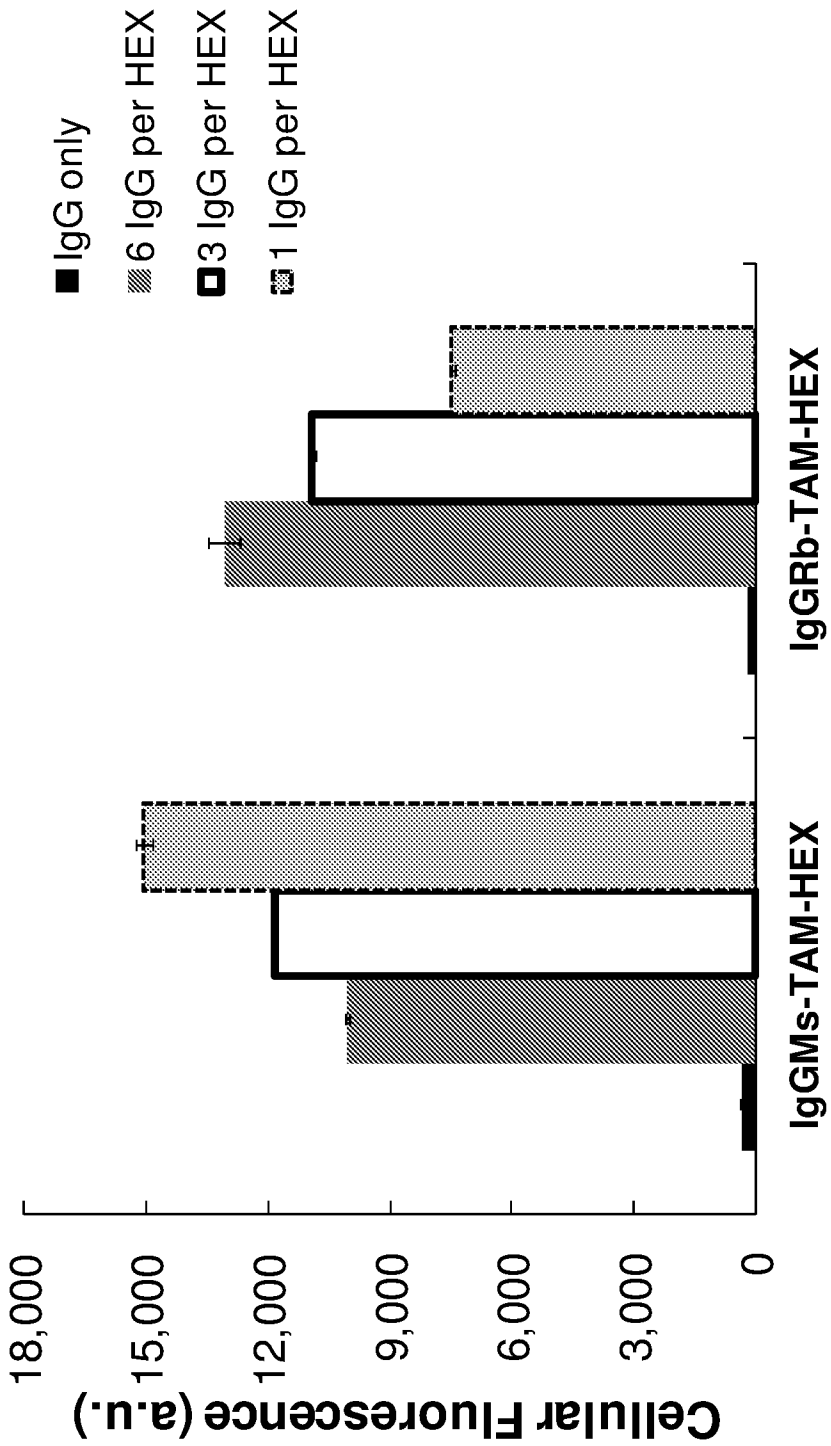

IgG only
6 IgG per HEX
3 IgG per HEX
1 IgG per HEX
Cellular Fluorescence (a.u.)
18,000
15,000
12,000
9,000
6,000
3,000
0
IgGMs-TAM-HEX
IgGRb-TAM-HEX

FIG. 5

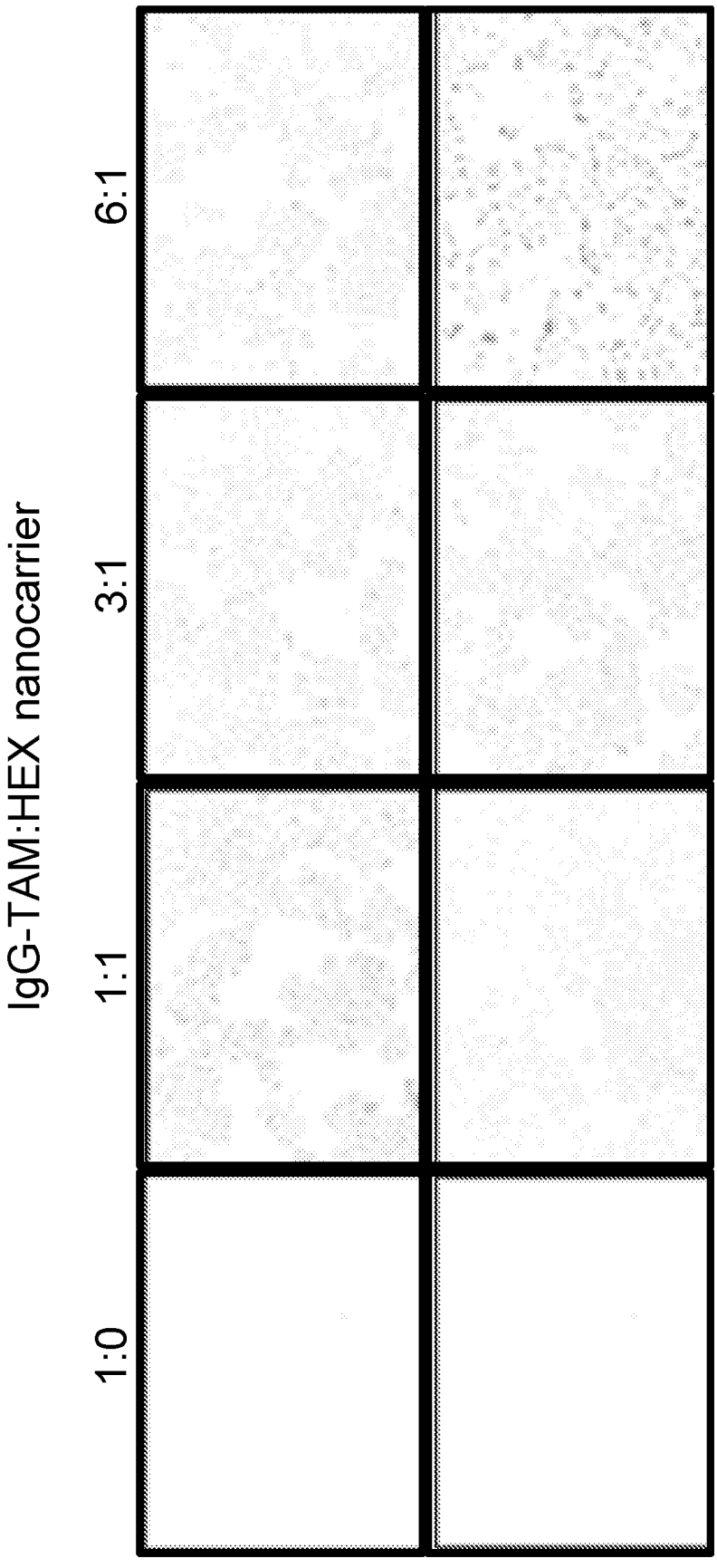


FIG. 6

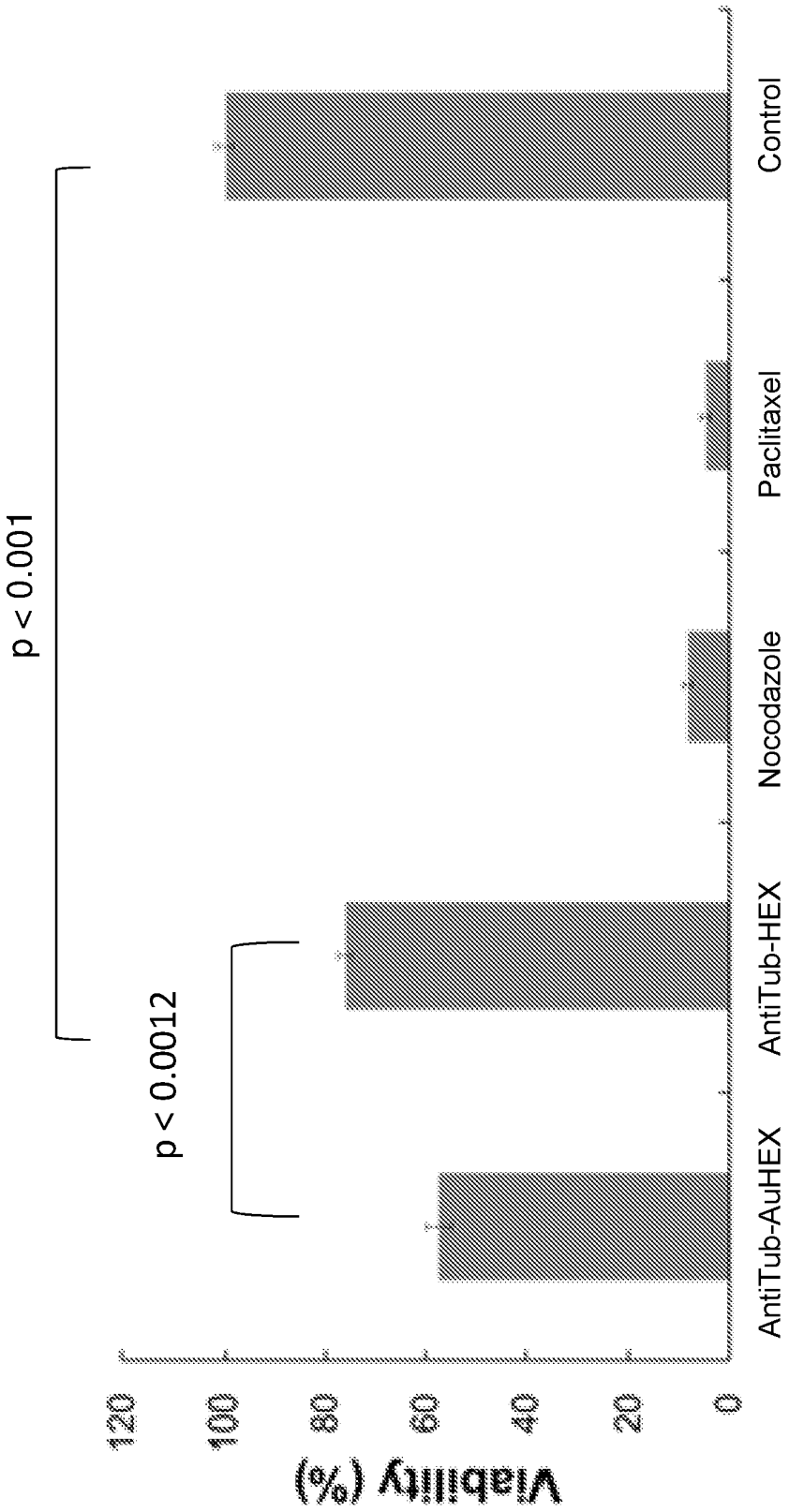

Viability (%)
120
100
80
60
40
20
0
AntiTub-AuHEX
AntiTub-HEX
Nocodazole
Paclitaxel
Control
p < 0.0012
p < 0.001

FIG. 7

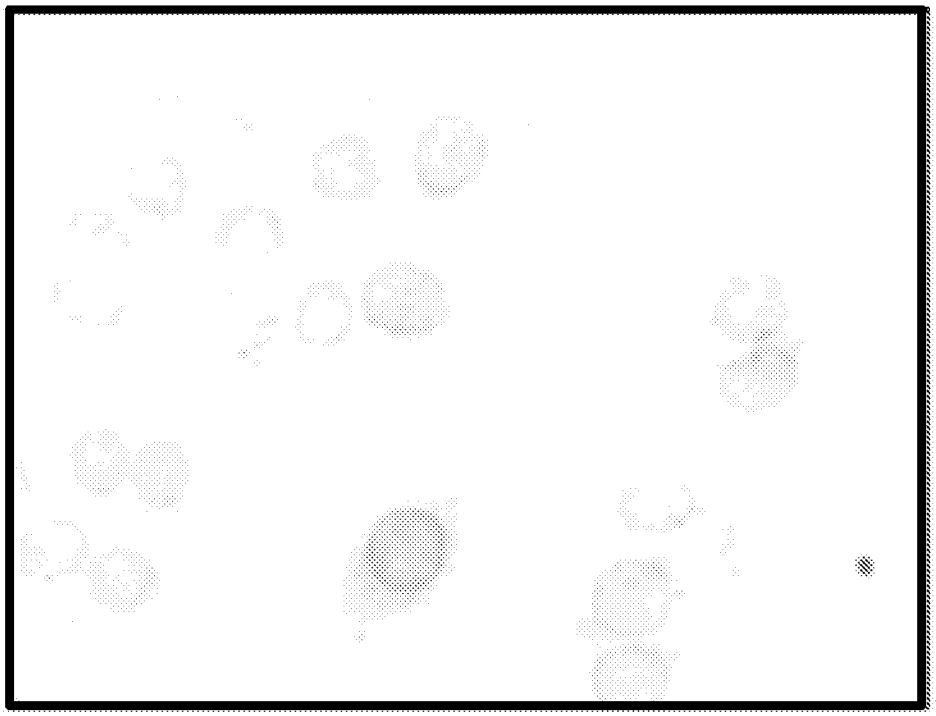


FIG. 8a

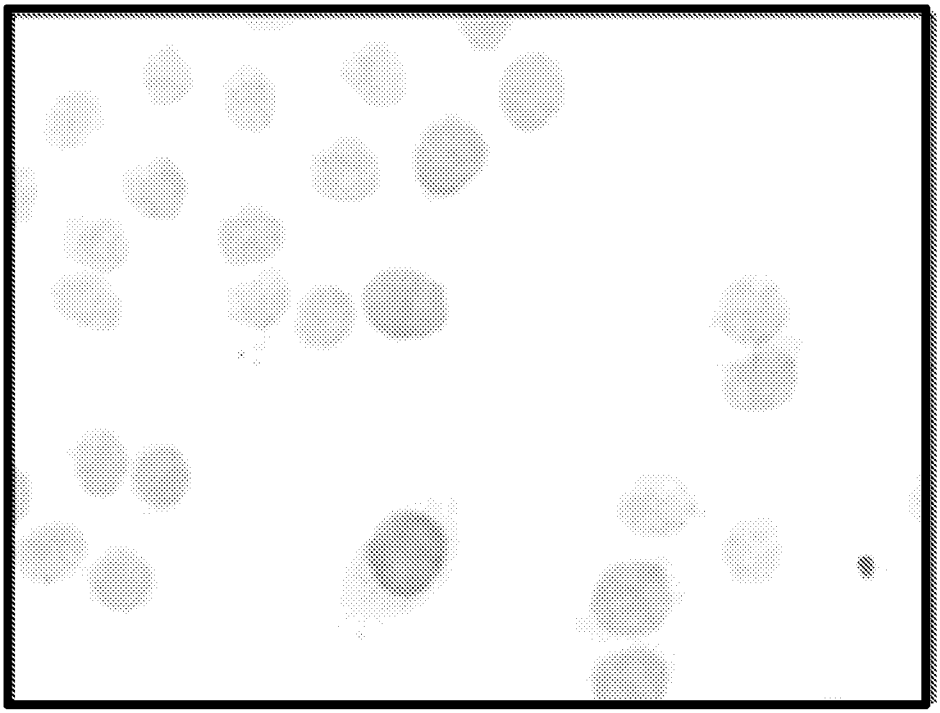


FIG. 8b

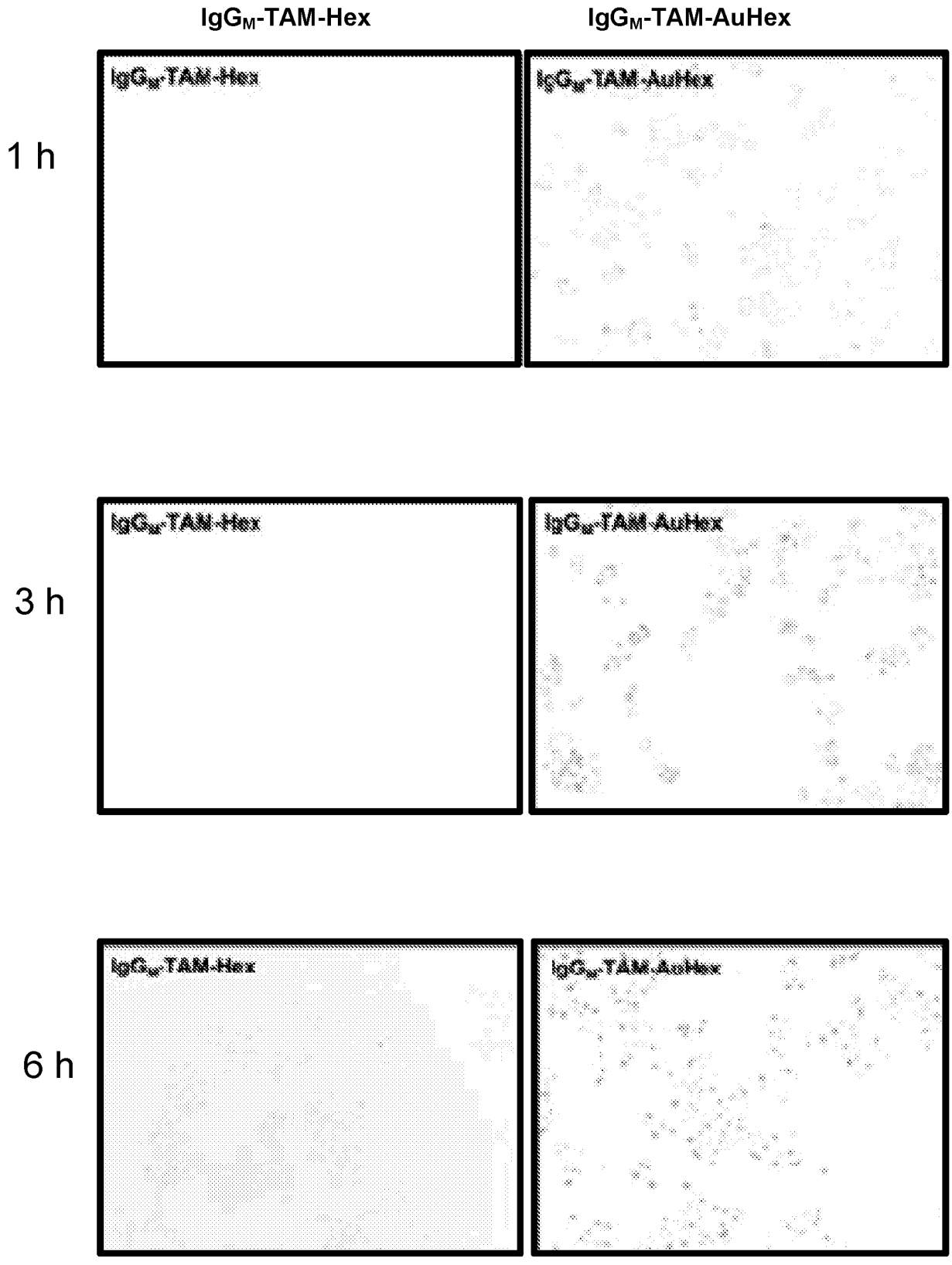


FIG. 9

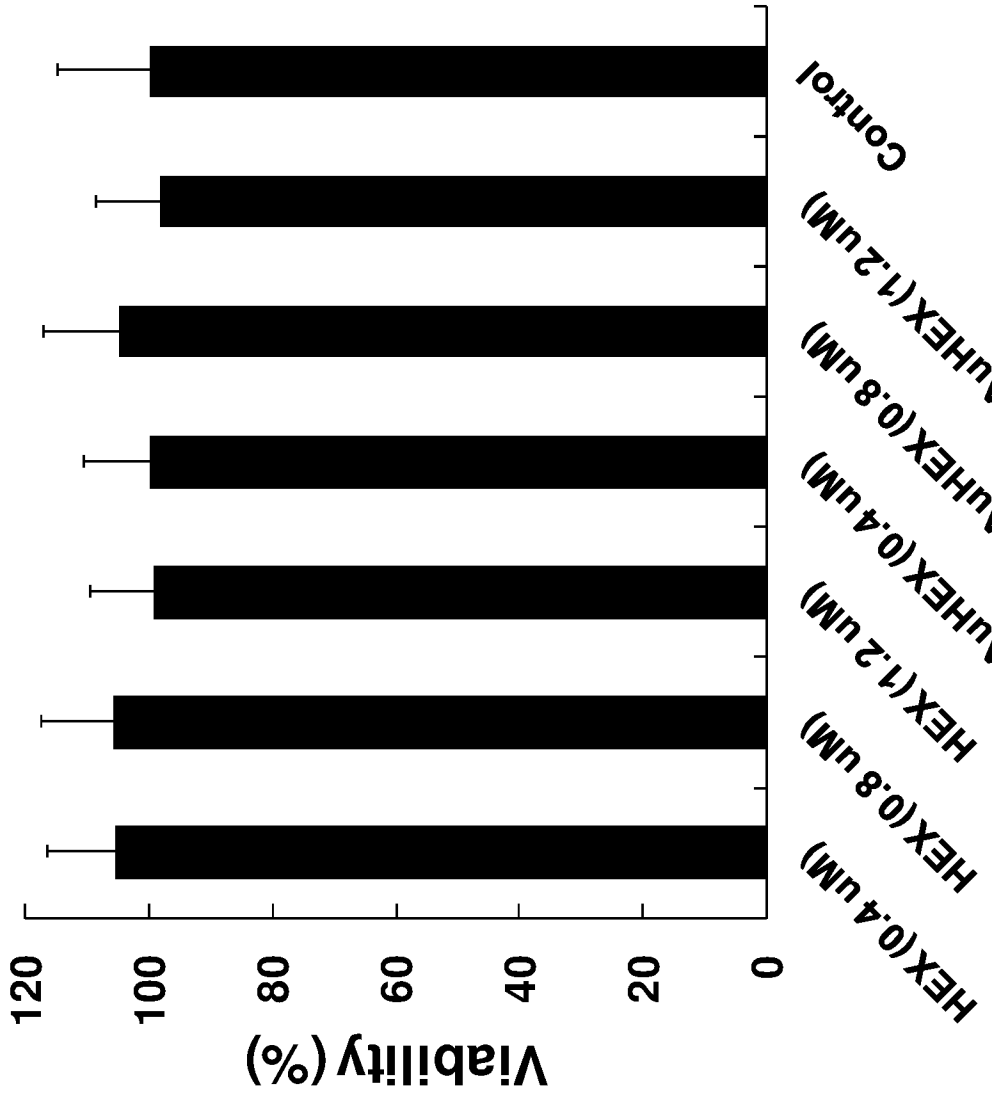

Viability (%)
120
100
80
60
40
20
0
HEX(0.4uM)
HEX(0.8uM)
HEX(1.2uM)
AuHEX(0.4uM)
AuHEX(0.8uM)
AuHEX(1.2uM)
Control

FIG. 10

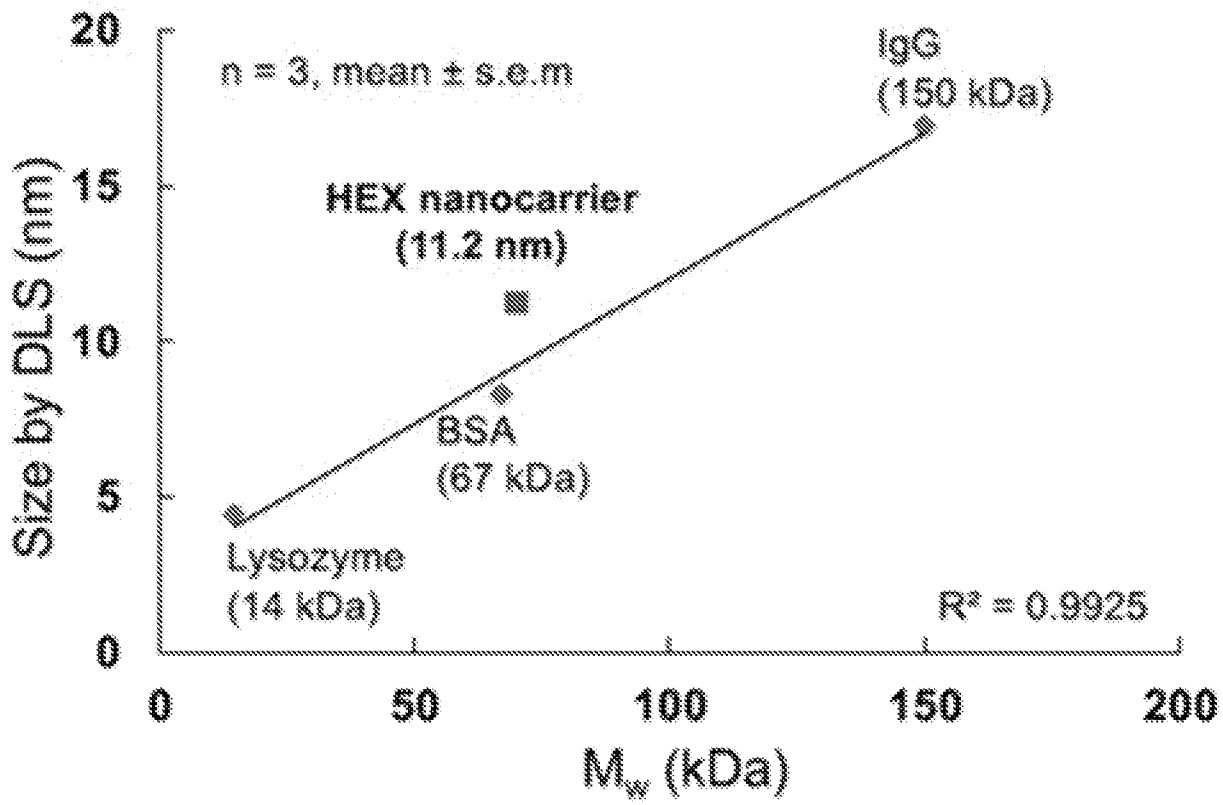

20
15
10
5
0
Size by DLS (nm)
n = 3, mean ± s.e.m
IgG
(150 kDa)
HEX nanocarrier
(11.2 nm)
BSA
(67 kDa)
Lysozyme
(14 kDa)
R² = 0.9925
0
50
100
150
200
Mw (kDa)

FIG. 11

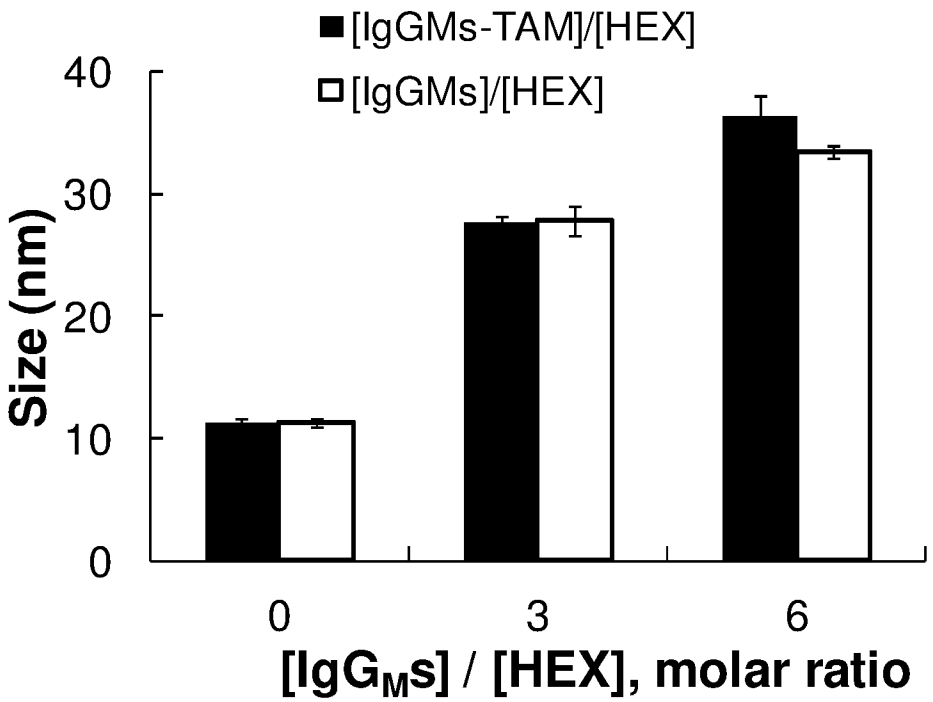


FIG. 12a

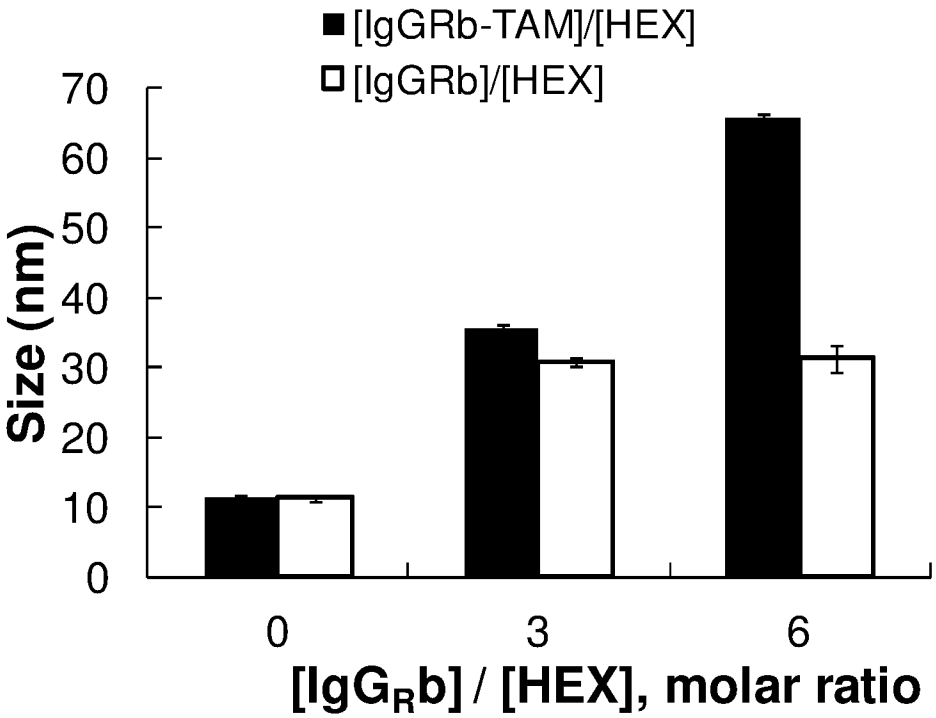


FIG. 12b

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/36331

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/385, A61K 39/00, A61K 39/395, A61K 9/127, A61K 49/00, C12P 21/08 (2017.01)
CPC - A61K 9/5169, A61K 39/015, A61K 39/385, A61K 39/39, A61K 47/646, A61K 47/6415, A61K 2039/6037, C07K 2319/73, A61K 2039/55505, A61K 2039/55544, A61K 2039/55566, A61K 2039/6031, A61K 2039/542

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 8,580,274 B2 (Arakawa, et al.) 12 November 2013 (12.11.2013) claim 1, col 7, ln 65-67; col 8, ln 57-67; col 9, ln 9-20; col 10, ln 45-67; col 12, ln 27 to col 13, ln 40;	1-3, 5-9, 15-30, 32, 37-45 ---------- 10-13, 33-35
A	Efimov, et al. Crystallization and preliminary crystallographic study of the pentamerizing domain from cartilage oligomeric matrix protein: a five-stranded alpha-helical bundle. Proteins 1996, 24(2):259-62; Abstract	1-13, 15-35, 37-45
Y	Kato, et al. Model for the complex between protein G and an antibody Fc fragment in solution. Structure 1995, 3(1):79-85; Abstract, pg 79, col 2 to pg 80, col 1	10-13, 33-35
Y	Abraham, et al. Intracellular delivery of antibodies by chimeric Sesbania mosaic virus (SeMV) virus like particles. Sci Rep. 24 February 2016, 6:21803; Abstract; pg 2, Fig 1, and its legend	1, 3, 4, 28, 31
Y	Thomas, et al. Controlling the Assembly of Coiled-Coil Peptide Nanotubes. Angew Chem Int Ed Engl. Epub 14 December 2015, 55(3):987-91; Abstract, pg 987; pg 988, col 1	1, 3, 4, 28, 31

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 August 2017	12 OCT 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/36331

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

********** See Supplemental Sheet to continue **********

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-13, 15-35, 37-45, restricted to domain B of Staphylococcal Protein A and an antibody

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/36331

In Continuation of Box III. Observations where unity of invention is lacking:

Group I+: claims 1-45, directed to a nanocarrier for delivering a cargo to a cell, and a method of using thereof for delivering a cargo to a cell. The nanocarrier and the method will be searched to the extent that the binding domain encompasses domain B of Staphylococcal Protein A and the cargo encompasses an antibody. It is believed that claims 1-13, 15-35, 37-45 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass a nanocarrier for delivering a cargo to a cell, said nanocarrier comprising domain B of Staphylococcal Protein A and an antibody, and a method of using said nanocarrier for delivering said antibody to a cell. Additional binding domain(s) and/or cargo will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected binding domain(s) and/or cargo. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a nanocarrier comprising DRBD domain as the binding domain and siRNA as a cargo, and a method of using said nanocarrier for delivering the siRNA cargo to a cell, i.e., claims 1-6, 8, 10, 14-33, 36-45.

Group II: claims 46-55, directed to a method of making an assembled nanocarrier.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features
The special technical feature of some inventions of Group I+ is a specific binding domain and/or a specific cargo recited therein. The inventions of Group I+ do not include the shared or common technical feature of a method of making an assembled nanocarrier, as required by Group II.

The inventions of Group II do not include the shared or common technical feature of a nanocarrier comprising a bundle domain; and a binding domain configured to bind to the cargo, or a method of using said nanocarrier, as required by Group I+.

Common Technical Features
The inventions of Group I+ share the technical feature of a nanocarrier of claim 1. Some inventions of Group I+ share the technical feature of a method for delivering a cargo of claim 28. However, these shared technical features do not represent a contribution over prior art as being obvious over a paper titled "Intracellular delivery of antibodies by chimeric Sesbania mosaic virus (SeMV) virus like particles" by Abraham, et al. (Sci Rep. 24 February 2016, 6:21803) (hereinafter "Abraham") in view of a paper titled "Controlling the Assembly of Coiled-Coil Peptide Nanotubes" by Thomas, et al. (Angew Chem Int Ed Engl. Epub 14 December 2015, 55(3):987-91 (hereinafter "Thomas").

Abraham discloses a nanocarrier and a method for delivering a cargo to a cell (pg 2, Figure 1, and its legend, "Design of SeMV CP [Sesbania mosaic virus coat protein] based chimera. ... (B) Schematic representation of SLB VLP showing the 8 residue [beta]H-[beta]I loops (dark grey) and Ser 242 (red spheres). (C) Ribbon representation of the SeMV CP C subunit showing the position of insertion of the B-domain ... at the Ser242 site (red). (D) Schematic representation of intracellular delivery of antibodies using SLB nanocarriers") comprising:
a nanocarrier domain (Abstract, plant virus based nanoparticles, VLP) ; and a binding domain configured to bind to the cargo (Abstract, B domain of Staphylococcus aureus protein A, "Sesbania mosaic virus coat protein (CP) was genetically engineered with the B domain of Staphylococcus aureus protein A (SpA) at the [beta]H-[beta]I loop, to generate SeMV loop B (SLB), which self-assembled to virus like particles (VLPs) with 43 times higher affinity towards antibodies. CP and SLB could internalize into various types of mammalian cells and SLB could efficiently deliver three different monoclonal antibodies-D6F10 (targeting abrin), anti-alpha-tubulin (targeting intracellular tubulin) and Herclon (against HER2 receptor) inside the cells. Such a mode of delivery was much more effective than antibodies alone treatment. These results highlight the potential of SLB as a universal nanocarrier for intracellular delivery of antibodies").

As the VLP disclosed by Abraham, is not a bundle domain, Abraham does not specifically disclose a nanocarrier comprising a bundle domain, wherein said bundle domain comprises an alpha-helical protein bundle domain. Thomas discloses a nanocarrier comprising such a bundle domain (pg 987, col 2, "PNTs [peptide nanotubes] from alpha-helical barrels (aHBs), Figure 1"). It would have been obvious to one of ordinary skill in the art to combine, in the course of routine experimentation and with a reasonable expectation of success, Abraham and Thomas by substituting the SeMV CP VLP in the nanocarrier of Abraham by a bundle domain of Thomas, to achieve an easily tunable nanocarrier (Thomas, Abstract, "we describe strategies for controlling this lateral association, and also for the longitudinal assembly. We show that PNT [peptide nanotubes] thickening is pH sensitive, and can be reversed under acidic conditions. Based on this, repulsive charge interactions are engineered into the building blocks leading to the assembly of single PNTs at neutral pH. The building blocks are modified further to produce covalently linked PNTs via native chemical ligation, rendering ca. 100 nm-long nanotubes"), thereby improving efficiency of cargo delivery in the method of Abraham. As said technical features would have been obvious to one of ordinary skill in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the inventions.

The inventions of Groups I+ and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.