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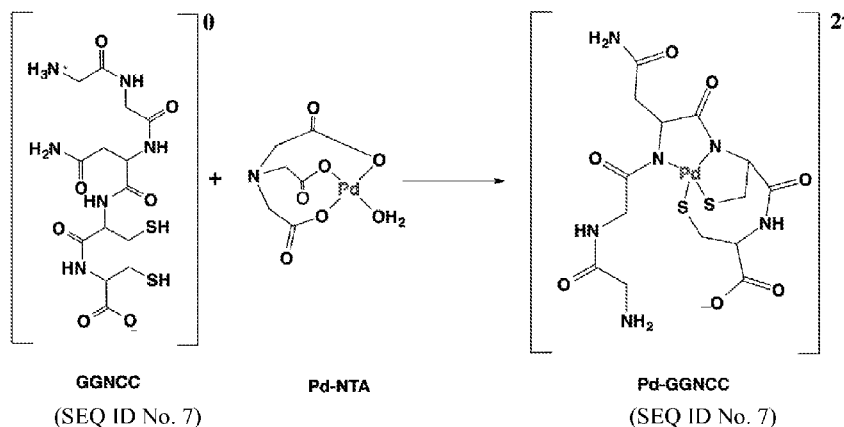
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(54) Title: PEPTIDE COMPLEXES AND THERAPEUTIC USES

**FIGURE 1**

(57) **Abstract:** Compositions comprising peptides with a chemical tag are disclosed. Such compositions can be used to modulate formulation properties of a biomolecule. The peptides are useful as cancer therapeutics and for modulating the physiochemical properties including the rheology of compositions by altering the viscosity and density of the compositions.

PEPTIDE COMPLEXES AND THERAPEUTIC USES

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 61/810,604, filed on April 10, 2013, and U.S. Provisional Patent Application No. 61/881,892, filed on September 24, 2013, each of which is incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] Tags that can be encoded in the genetic material of an organism for recombinant expression of proteins have been utilized for purification and identification of protein products. The advantage of a peptide tag is that the tag is covalently attached to the protein of interest without the need for additional chemical steps to label the protein. One example of such a tag is a poly His-tag, which is used for isolating the tagged protein from whole cells using immobilized metal affinity chromatography (IMAC). Other peptide-based tags have been developed to allow for detecting a tagged protein in cell culture assays or cell lysates using antibodies that recognize the peptide tag. Peptide-based tags have also been used for *in vivo* imaging (lanthanides).

[0003] Other types of tags exist that cannot be encoded genetically because the tags rely on incorporation of something other than an amino acid residue into a peptide. Such tags are used, for example, to improve a property of a peptide, or to modulate a formulation of a peptide.

SUMMARY OF THE INVENTION

[0004] In some embodiments, the invention provides a composition comprising a concentration of a synthetic complex, or a pharmaceutically-acceptable salt thereof, wherein the synthetic complex comprises a therapeutic molecule covalently bound to an additional group, wherein the composition has a viscosity less than that of a second composition, wherein the second composition comprises the therapeutic molecule at the concentration in absence of the covalently-bound additional group.

[0005] In some embodiments, the invention provides a synthetic complex, or a pharmaceutically-acceptable salt thereof, comprising a therapeutic molecule covalently bound to an additional group, wherein a composition comprising the complex at a concentration has a viscosity less than that of a second composition, wherein the second composition comprises the therapeutic molecule at the concentration in absence of the covalently-bound additional group.

[0006] In some embodiments, the invention provides a method of treating a cancer, the method

comprising administering to a subject in need or want of relief thereof a composition comprising a therapeutically-effective amount of a synthetic complex, wherein the synthetic complex comprises a therapeutic molecule and an additional group, and wherein the composition has a viscosity less than that of a second composition, wherein the second composition comprises the therapeutic molecule at the concentration in absence of the covalently-bound additional group.

INCORPORATION BY REFERENCE

[0007] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

- [0008] **FIGURE 1** illustrates the synthesis of a MAP/Pd(II) complex.
- [0009] **FIGURE 2** tabulates the results of the syntheses of a MAP/Pd(II) complexes.
- [0010] **FIGURE 3** illustrates the chemical structure of KCC-am.
- [0011] **FIGURE 4** illustrates the chemical structure of ac-KCC-am.
- [0012] **FIGURE 5** illustrates the chemical structure of KGNCC (SEQ ID No. 1).
- [0013] **FIGURE 6** illustrates the chemical structure of ac-KGNCC (SEQ ID No. 2).
- [0014] **FIGURE 7** tabulates the aqueous solubility of MAPs over time.
- [0015] **FIGURE 8** tabulates the incorporation of Pd(II) into MAPs over time.
- [0016] **FIGURE 9** illustrates the chemical structure of ac-WKCCW-am (SEQ ID No. 3).
- [0017] **FIGURE 10** illustrates the chemical structure of ac-KCCLW-am (SEQ ID No. 4).
- [0018] **FIGURE 11** illustrates the chemical structure of NCCGW (SEQ ID No. 5).
- [0019] **FIGURE 12** illustrates the chemical structure of NCCGG (SEQ ID No. 6).
- [0020] **FIGURE 13** tabulates the aqueous solubility of MAPs over time based, in part, on the presence of hydrophobic side chains.
- [0021] **FIGURE 14** tabulates the incorporation of Pd(II) into MAPs over time based, in part, on the presence of hydrophobic side chains.
- [0022] **FIGURE 15** illustrates the chemical structure of GGNCC (SEQ ID No. 7).
- [0023] **FIGURE 16** illustrates the chemical structure of WGNCC (SEQ ID No. 8).
- [0024] **FIGURE 17** tabulates the aqueous solubility of GGNCC (SEQ ID No. 7) with various cosolvents over time.
- [0025] **FIGURE 18** tabulates the aqueous solubility of WGNCC (SEQ ID No. 8) with various cosolvents over time.

[0026] **FIGURE 19** tabulates the incorporation of Pd(II) into GGNCC (SEQ ID No. 7) over time in the presence of various cosolvents.

[0027] **FIGURE 20** tabulates the incorporation of Pd(II) into WGNCC (SEQ ID No. 8) over time in the presence of various cosolvents.

[0028] **FIGURE 21** illustrates the reaction and product of the experiment of Example 8.

[0029] **FIGURE 22** illustrates the reaction and product of the experiment of Example 9.

DESCRIPTION

[0030] Peptides and proteins are often poorly soluble, particularly at the concentrations necessary for practical application. Large solubility tags, such as maltose binding protein (MBP), have been used to increase yield of such proteins in recombinant expression systems. Peptide- or protein-based therapies suffer from aggregation, which can reduce efficacy and/or induce mild to severe side effects, as well as poor syringability due to high viscosity in high-concentration formulations. Charged moieties on proteins contribute to solubility and self-association. The outcomes of self-association, such as aggregation and viscosity, depend on the specific interactions between individual protein molecules. Solution conditions and modification and/or mutation of the protein can mitigate undesirable behavior. Modulation of electrostatic interaction can be accomplished by changing the solution conditions, such as pH or ionic strength. A favorable outcome of charge manipulation can be the formation of stable associated states, such as colloids or suspensions, which reduce viscosity and improve the performance characteristics of a product. The peptides and complexes of the invention solve these problems.

[0031] The present disclosure generally relates to complexes of peptides, proteins, and antibodies with a chemical group capable of modulating the physical or formulation properties of the peptide, protein, or antibody. The chemical group can be a small organic molecule, or a peptide sequence that binds a metal, such as a metal abstraction peptide (MAP). The invention also provides methods of preparing and using such complexes for a variety of uses, including cancer therapeutics.

[0032] Small molecule tags of the invention can be chosen from chemicals that are generally regarded as safe. Such chemicals can have low molecular weights, positive, negative, or neutral charges, and can be zwitterionic. Such tags can be incorporated into a biomolecule, such as a peptide, protein, or antibody, and can modify or improve a property, such as solubility, solution viscosity, aggregation, density, or homogeneity.

[0033] MAPs can provide similar benefits. Metal-based chemotherapeutics, such as *cis*-platin, suffer from non-specific toxicity. Incorporation of the metal into a MAP spares the cancer

subject from the unwanted side effects arising from non-specific metal binding. Moreover, benign metals can be incorporated in place of more aggressive, toxic metals, such as platinum. The MAP tag creates a tool for reversibly or irreversibly manipulating charge to control such behaviors.

[0034] MAP-metal complexes and small, charged organic groups can also be used to modulate the solubility of larger biomolecules. For example, by incorporating such a tag into a peptide, protein, or antibody, the solubility of the resultant complex can be improved. The improved solubility can aid in delivery of biologicals and pharmaceuticals.

Small Organic Groups.

[0035] The present disclosure provides small organic groups that can be attached to biomolecules, such as peptides, proteins, and antibodies to modify or improve properties. The improvements can lead to improved formulations. A small organic group can possess one or more functional groups that impart a desired property to a biomolecule. Non-limiting examples of the functional group include carboxyl groups, amino groups, hydroxyl groups, sulfhydryl groups, phosphate groups, phosphonate groups, sulfate groups, sulfonate groups, sulfite groups, amides, such as primary amides, carbamates, such as primary carbamates, and heterocycles, such as a pyridine group, a pyrazine group, a triazine group, an imidazole group, a pyrazole group, an oxazole group, an isoxazole group, a thiazole group, or an isothiazole group.

[0036] An organic group can have a net charge, for example, -10, -9, -8, -7, -6, -5, -4, -3, -2, -1, 0, +1, +2, +3, +4, +5, +6, +7, +8, +9, or +10. A zwitterionic group can have a net charge of zero, but still have a number of positive and negative charges that sum to zero.

[0037] An organic group can have a molecular weight that is small in comparison to the biomolecule to which it is attached. The molecular weight can be, for example, no greater than 2,000 Daltons, no greater than 1,900 Daltons, no greater than 1,800 Daltons, no greater than 1,700 Daltons, no greater than 1,600 Daltons, no greater than 1,500 Daltons, no greater than 1,400 Daltons, no greater than 1,300 Daltons, no greater than 1,200 Daltons, no greater than 1,100 Daltons, no greater than 1,000 Daltons, no greater than 950 Daltons, no greater than 900 Daltons, no greater than 850 Daltons, no greater than 800 Daltons, no greater than 775 Daltons, no greater than 750 Daltons, no greater than 725 Daltons, no greater than 700 Daltons, no greater than 675 Daltons, no greater than 650 Daltons, no greater than 625 Daltons, no greater than 600 Daltons, no greater than 590 Daltons, no greater than 580 Daltons, no greater than 570 Daltons, no greater than 560 Daltons, no greater than 550 Daltons, no greater than 540 Daltons, no greater than 530 Daltons, no greater than 520 Daltons, no greater than 510 Daltons, no greater than 500

Daltons, no greater than 490 Daltons, no greater than 480 Daltons, no greater than 470 Daltons, no greater than 460 Daltons, no greater than 450 Daltons, no greater than 440 Daltons, no greater than 430 Daltons, no greater than 420 Daltons, no greater than 410 Daltons, no greater than 400 Daltons, no greater than 390 Daltons, no greater than 380 Daltons, no greater than 370 Daltons, no greater than 360 Daltons, no greater than 350 Daltons, no greater than 340 Daltons, no greater than 330 Daltons, no greater than 320 Daltons, no greater than 310 Daltons, no greater than 300 Daltons, no greater than 290 Daltons, no greater than 280 Daltons, no greater than 270 Daltons, no greater than 260 Daltons, no greater than 250 Daltons, no greater than 240 Daltons, no greater than 230 Daltons, no greater than 220 Daltons, no greater than 210 Daltons, no greater than 200 Daltons, no greater than 190 Daltons, no greater than 180 Daltons, no greater than 170 Daltons, no greater than 160 Daltons, no greater than 150 Daltons, no greater than 140 Daltons, no greater than 130 Daltons, no greater than 120 Daltons, no greater than 110 Daltons, no greater than 100 Daltons, no greater than 90 Daltons, no greater than 80 Daltons, no greater than 70 Daltons, no greater than 60 Daltons, or no greater than 50 Daltons. The molecular weight can be, for example, about 2,000 Daltons, about 1,900 Daltons, about 1,800 Daltons, about 1,700 Daltons, about 1,600 Daltons, about 1,500 Daltons, about 1,400 Daltons, about 1,300 Daltons, about 1,200 Daltons, about 1,100 Daltons, about 1,000 Daltons, about 950 Daltons, about 900 Daltons, about 850 Daltons, about 800 Daltons, about 775 Daltons, about 750 Daltons, about 725 Daltons, about 700 Daltons, about 675 Daltons, about 650 Daltons, about 625 Daltons, about 600 Daltons, about 590 Daltons, about 580 Daltons, about 570 Daltons, about 560 Daltons, about 550 Daltons, about 540 Daltons, about 530 Daltons, about 520 Daltons, about 510 Daltons, about 500 Daltons, about 490 Daltons, about 480 Daltons, about 470 Daltons, about 460 Daltons, about 450 Daltons, about 440 Daltons, about 430 Daltons, about 420 Daltons, about 410 Daltons, about 400 Daltons, about 390 Daltons, about 380 Daltons, about 370 Daltons, about 360 Daltons, about 350 Daltons, about 340 Daltons, about 330 Daltons, about 320 Daltons, about 310 Daltons, about 300 Daltons, about 290 Daltons, about 280 Daltons, about 270 Daltons, about 260 Daltons, about 250 Daltons, about 240 Daltons, about 230 Daltons, about 220 Daltons, about 210 Daltons, about 200 Daltons, about 190 Daltons, about 180 Daltons, about 170 Daltons, about 160 Daltons, about 150 Daltons, about 140 Daltons, about 130 Daltons, about 120 Daltons, about 110 Daltons, about 100 Daltons, about 90 Daltons, about 80 Daltons, about 70 Daltons, about 60 Daltons, or about 50 Daltons.

[0038] Non-limiting examples of organic groups suitable for use in the invention include groups derived from: citric acid, tartric acid, lactic acid, glutamic acid, ascorbic acid, malic acid, maleic acid, nicotinic acid, sorbic acid, propylene glycol, glycerine, succinic acid, tannic acid, histidine, inositol, lysine, manitol, pantothenic acid, gluconic acid, 2-amino butyric acid, 3-amino butyric

acid, 4-amino butyric acid, hyaluronic acid, or polymers thereof, and carboxymethylcellulose. A small organic group can be a carbohydrate residue or sequence thereof, or an amino acid residue or sequence thereof.

[0039] An organic group can be incorporated into a molecule having therapeutic or diagnostic use or potential by the methods described above. In some embodiments, a therapeutic or diagnostic molecule is a biomolecule. A therapeutic or diagnostic molecule can be natural, synthetic, or semi-synthetic.

Metal Abstraction Peptide (MAP).

[0040] The present disclosure provides peptide motifs and methods of using such motifs. These peptides have the ability to bind to metals, which makes the resultant complexes useful for a variety of applications. Peptides of the present disclosure have applications in cancer therapy and modulation of formulation rheology.

[0041] Rheology modulation can affect the physical properties of a composition containing a metal-peptide complex. Non-limiting examples of physical properties include density, viscosity, flow rate, tackiness, and homogeneity. The level of a complex in a composition can be modulated to increase or decrease the value of a desired physical property.

[0042] The present disclosure provides a peptide comprising the sequence XC_1C_2 ; wherein X is any natural or non-natural amino acid or amino acid analogue such that XC_1C_2 is capable of binding a metal. In some embodiments, the peptide is capable of binding metal in a square planar orientation, a square pyramidal orientation, or both. In some embodiments, C_1 and C_2 are the same or different; and C_1 and C_2 individually are sulfur containing amino acid residues such as cysteine and a cysteine-like non-natural amino acid or amino acid analogue. In some embodiments, C_1 and C_2 are each individually chosen from sulfur-containing alpha or beta amino acids.

[0043] The present disclosure also provides a peptide comprising the sequence XC_1C_2 and a bound metal, wherein the metal is complexed with or bound to the tripeptide. In some embodiments, X is any natural or non-natural amino acid or amino acid analogue such that XC_1C_2 and the bound metal are in a square planar orientation, a square pyramidal orientation, or both, and wherein C_1 and C_2 are the same or different, and wherein C_1 and C_2 individually are sulfur containing amino acid residues such as cysteine and a cysteine-like non-natural amino acid or amino acid analogue. In some embodiments, C_1 and C_2 are each individually chosen from sulfur-containing alpha or beta amino acids.

[0044] The present disclosure provides methods comprising complexing a metal together with a

tripeptide having the sequence XC_1C_2 to form a metal- XC_1C_2 complex, wherein X is any natural or non-natural amino acid or amino acid analogue such that metal- XC_1C_2 complex has a square planar orientation, a square pyramidal orientation, or both, and wherein C_1 and C_2 are the same or different, and wherein C_1 and C_2 individually are sulfur containing amino acid residues such as cysteine and a cysteine-like non-natural amino acid or amino acid analogue. In some embodiments, C_1 and C_2 are each individually chosen from sulfur-containing alpha or beta amino acids. The X in the MAP sequence can be any natural or non-natural amino acid.

[0045] In some embodiments, the invention provides peptide motifs that strongly bind with a select metal. In some embodiments, the metal is a select metal, or a specific metal. For example, a peptide can have selectivity for binding one metal over another, or one oxidation state of a metal over another oxidation state of the same metal or a different metal. Such peptides are referred to as metal abstraction peptides (MAP(s)). MAPs can be used, for example, to bind a metal in a composition. MAPs can be included in longer polypeptides and proteins at the N-terminus, C-terminus, or any position in between. In some embodiments, a MAP can be present in a polypeptide or protein configuration that presents the MAP for binding with a metal, such as being present in an external loop. The MAP can be covalently attached to a polypeptide or protein through a linker, such as at the N-terminus, C-terminus, or through a side-chain from the polypeptide or protein. For example, such linkers can include amide bonds, esters, polyamides, polyethers, and polyesters. The MAP can be attached to a non-peptide entity. Non-peptide entities include without limitation carbohydrates, glycoproteins, and/or covalent linkers, including polyethylene glycol. Additionally, more than one MAP can be present on a particular molecule. In some embodiments, one or more MAPs can be covalently linked to an antibody. In some embodiments, the MAP is a tripeptide capable of complexation with metal ions, as described in U.S. Patent Publication 2010/0221839.

Chemical Structure/Peptide Sequence.

[0046] As used herein, the abbreviations for the L-enantiomeric and D-enantiomeric amino acids are as follows: alanine (A, Ala); arginine (R, Arg); asparagine (N, Asn); aspartic acid (D, Asp); cysteine (C, Cys); glutamic acid (E, Glu); glutamine (Q, Gln); glycine (G, Gly); histidine (H, His); isoleucine (I, Ile); leucine (L, Leu); lysine (K, Lys); methionine (M, Met); phenylalanine (F, Phe); proline (P, Pro); serine (S, Ser); threonine (T, Thr); tryptophan (W, Trp); tyrosine (Y, Tyr); valine (V, Val). Typically, X or Xaa can indicate any amino acid. However, in some embodiments, X or Xaa is selected from a subset of amino acids. In some embodiments, the amino acid is a L-enantiomer. In some embodiments, the amino acid is a D-enantiomer.

[0047] In some embodiments, XC_1C_2 are L-amino acids or are achiral, such as glycine. Non-limiting examples of X include hydrophilic amino acids, hydrophobic amino acids, charged amino acids, uncharged amino acids, acidic amino acids, basic amino acids, neutral amino acids, aromatic amino acids, aliphatic amino acids, natural amino acids, and non-natural amino acids. In some embodiments, X is selected from N, Q, H, K, and R. In some embodiments, XC_1C_2 are L-amino acids, and X is selected from N, Q, H, K, and R. In some embodiments, X is achiral, for example, X is glycine. In some embodiments, X is not N, Q, H, K, R, or G. In some embodiments, X is not cysteine. In some embodiments, X is selected from alanine (A); aspartic acid (D); glutamic acid (E); isoleucine (I); leucine (L); methionine (M); phenylalanine (F); serine (S); threonine (T); tryptophan (W); tyrosine (Y); and valine (V).

[0048] A MAP tag can have a net charge of -5, -4, -3, -2, -1, 0, +1, +2, +3, +4, or +5. When the net charge is zero, the MAP can be uncharged or zwitterionic. Addition of a metal to a MAP can change the net charge of the complex, for example, by decreasing the net charge by 1, 2, 3, 4, or 5, or by increasing the net charge by 1, 2, 3, 4, or 5. In some embodiments, addition of a metal to a MAP does not change the net charge of the complex.

[0049] In some embodiments, the MAP tag can be attached to another molecule. For example, the MAP sequence also can be attached to a non-peptide entity (e.g., polymer, fluorophore, solid support, chemical linker, or sugar). In some embodiments, the MAP can be attached to a solid surface or substrate through a linker. In some embodiments, the solid surface or substrate can be polymeric, such as a resin bead or membrane. In some embodiments, the MAP is incorporated in a longer polypeptide which is further attached to a non-peptide entity, such as a polymeric solid surface. The attachment can be covalent, and can be affected through a linker. Additionally, more than one MAP sequence can be present on a particular molecule or macromolecule. In some embodiments, a lysine-derived linker is attached directly or indirectly to the MAP sequence.

[0050] In some embodiments, the MAP sequence is flanked by additional amino acids. For example, in addition to the MAP tag being an isolated tripeptide XC_1C_2 , the MAP tag can comprise a sequence selected from the group consisting of: $Z_1XC_1C_2$; $XC_1C_2Z_2$; and $Z_1XC_1C_2Z_2$, wherein X is any amino acid or amino acid analogue; C_1 and C_2 are the same or different and are a cysteine, or a cysteine-like non-natural amino acid, or a cysteine-like amino acid analogue; Z_1 is any amino acid or any sequence of amino acids, and Z_2 is any amino acid or sequence of amino acids that is equivalent or not equivalent to Z_1 . Non-natural and amino acids analogues can be included as Z_1 and Z_2 . In some embodiments, Z_1 and Z_2 are both natural amino acids or sequences of natural amino acids. In some embodiments, the MAP sequence is flanked by a lysine. For example, the MAP tag can comprise a sequence selected from the group consisting of

K-XC₁C₂ and XC₁C₂-K. In some embodiments, the MAP tag can comprise a sequence selected from the group consisting of KNCC and NCKK. In some embodiments, the MAP tag is included within a polypeptide that further comprises a lysine moiety, where the lysine moiety is further linked to a resin through the lysine side chain.

[0051] In some embodiments, a MAP tag of the present disclosure can be encoded in line with a gene or nucleotide sequence that provides for targeted delivery of the MAP tag, either before MAP tag complexation with a metal or after complexation with a metal. Targeted delivery can be accomplished using genes, peptides, or other motifs known to be useful for targeting.

[0052] In some embodiments, MAP tags can be incorporated onto solid surfaces or substrates via natural or synthetic linkers. Additionally, MAPs can be incorporated into a peptide or protein using any synthetic or biosynthetic method for peptide or protein production, wherein the polypeptide or protein is further linked to a polymer or a solid surface or substrate. In some embodiments, one or more MAPs are covalently linked to a synthetic polymer via a polyether linker, such as polyethylene glycol or polypropylene glycol.

[0053] In some embodiments, the MAP tag spontaneously reacts with a metal to form a peptide-metal complex, such as zinc during protein production to increase yield of the desired product. Metal-MAP complexes can form in solution or via transmetallation or any other process.

[0054] In some embodiments, compositions comprise an affinity tag in addition to MAP sequence. The affinity tag can be any affinity tag capable of binding to a substrate. The affinity tag can bind the substrate reversibly or substantially irreversibly. Non-limiting examples of suitable affinity tags include maltose binding protein (MBP), glutathione-S-transferase (GST), poly(His), biotin ligase tags, Strep, HaloTag, cellulose binding domain (CBD), glutathione transferases (GST-tag), and a glycan.

[0055] In some embodiments, the MAP is incorporated in a polypeptide, which further comprises an affinity tag. The present disclosure also provides compositions and methods that provide a substrate for the affinity tag. In such embodiments, the affinity tag and any contaminants that can be bound or retained by the substrate for the affinity tag are separated from the metal product of interest.

[0056] A MAP can be incorporated into a molecule, such as a biomolecule. Non-limiting examples of biomolecules include peptides, proteins, enzymes, growth factors, and antibodies. The MAP can be incorporated before or after binding a metal. The MAP can be incorporated one amino acid at a time, or the entire MAP can be incorporated in a single operation. The MAP can be attached to a wild type biomolecule, inserted into the wild type biomolecule such as by the insertion of amino acids into an amino acid sequence, or the MAP can substitute for a series of

amino acids in the wild type biomolecule. A MAP can be attached to a therapeutic molecule, such as any of the foregoing biomolecules, a drug, or drug candidate.

[0057] A MAP can be incorporated into a biomolecule by various techniques. A MAP can be incorporated by a chemical transformation, such as the formation of a covalent bond, such as an amide bond or a peptide bond. A MAP can be incorporated, for example, by solid phase or solution phase peptide synthesis. A MAP can be incorporated by preparing a nucleic acid sequence encoding the biomolecule, wherein the nucleic acid sequence includes a subsequence that encodes the MAP. The subsequence can be in addition to the sequence that encodes the biomolecule, or can substitute for a subsequence of the sequence that encodes the biomolecule.

[0058] A MAP can also be incorporated into a molecule having therapeutic or diagnostic use or potential, by the methods described above. In some embodiments, a therapeutic or diagnostic molecule is a biomolecule. A therapeutic or diagnostic molecule can be natural, synthetic, or semi-synthetic.

Metals.

[0059] A metal can be in elemental form, a metal atom, or a metal ion. Non-limiting examples of metals include transition metals, main group metals, and metals of Group 3, Group 4, Group 5, Group 6, Group 7, Group 8, Group 9, Group 10, Group 11, Group 12, Group 13, Group 14, and Group 15 of the Periodic Table. Non-limiting examples of metal include scandium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, yttrium, zirconium, niobium, molybdenum, technetium, ruthenium, rhodium, palladium, silver, cadmium, lanthanum, hafnium, tantalum, tungsten, rhenium, osmium, iridium, platinum, gold, mercury, tin, lead, and bismuth.

Linkers.

[0060] A tag of the invention, such as either a MAP tag or a small organic group, can be attached to a larger molecule, such as a peptide, a protein, or an antibody, through a linker, or directly, in the absence of a linker.

[0061] Direct attachment is possible by covalent attachment of a tag to a region of the larger molecule. For example, the tag could be attached to a terminus of the amino acid sequence of the larger molecule, or could be attached to a side chain, such as the side chain of a lysine, serine, threonine, cysteine, tyrosine, aspartic acid, or glutamic acid residue. The attachment can be via an amide bond, an ester bond, an ether bond, or a thioether bond.

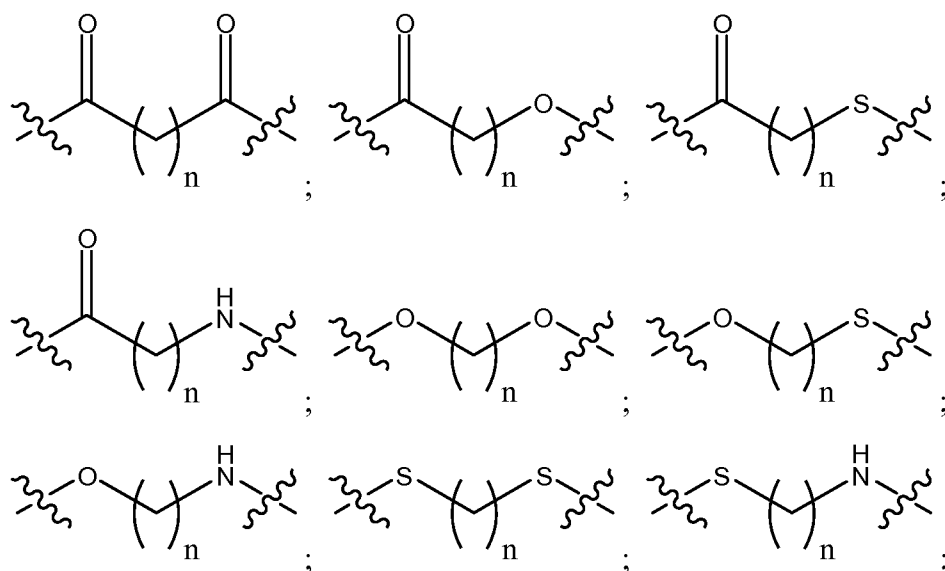
[0062] Attachment via a linker involves incorporation of a linker moiety between the larger molecule and the tag. The tag and the larger molecule can both be covalently attached to the

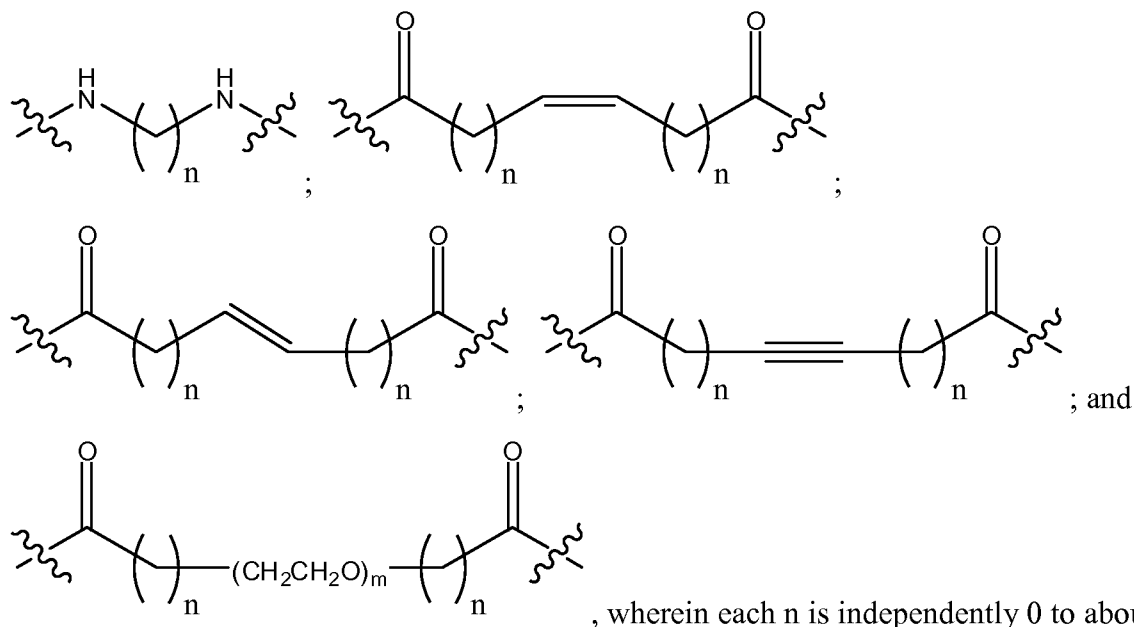
linker. The linker can be cleavable, non-cleavable, self-immolating, hydrophilic, or hydrophobic. The linker has at least two functional groups, one bonded to the larger molecule, and one bonded to the tag, and a linking portion between the two functional groups.

[0063] Non-limiting examples of the functional groups for attachment include functional groups capable of forming, for example, an amide bond, an ester bond, an ether bond, a carbonate bond, a carbamate bond, or a thioether bond. Non-limiting examples of functional groups capable of forming such bonds include amino groups; carboxyl groups; acid halides such as acid fluorides, chlorides, bromides, and iodides; acid anhydrides, including symmetrical, mixed, and cyclic anhydrides; carbonates; carbonyl functionalities bonded to leaving groups such as cyano, succinimidyl, and *N*-hydroxysuccinimidyl; hydroxyl groups; sulfhydryl groups; and molecules possessing, for example, alkyl, alkenyl, alkynyl, allylic, or benzylic leaving groups, such as halides, mesylates, tosylates, triflates, epoxides, phosphate esters, sulfate esters, and besylates.

[0064] Non-limiting examples of the linking portion include alkylene, alkenylene, alkynylene, polyether, such as polyethylene glycol (PEG), polyester, polyamide, and polyamine, any of which being unsubstituted or substituted with any number of substituents, such as halogens, hydroxyl groups, sulfhydryl groups, amino groups, nitro groups, nitroso groups, cyano groups, azido groups, sulfoxide groups, sulfone groups, sulfonamide groups, carboxyl groups, carboxaldehyde groups, imine groups, alkyl groups, halo-alkyl groups, alkenyl groups, halo-alkenyl groups, alkynyl groups, halo-alkynyl groups, alkoxy groups, aryl groups, aryloxy groups, aralkyl groups, arylalkoxy groups, heterocyclyl groups, acyl groups, acyloxy groups, carbamate groups, amide groups, urethane groups, epoxides, and ester groups.

[0065] Non-limiting examples of linkers include:





1 to about 1,000; 0 to about 500; 1 to about 500; 0 to about 250; 1 to about 250; 0 to about 200; 1 to about 200; 0 to about 150; 1 to about 150; 0 to about 100; 1 to about 100; 0 to about 50; 1 to about 50; 0 to about 40; 1 to about 40; 0 to about 30; 1 to about 30; 0 to about 25; 1 to about 25; 0 to about 20; 1 to about 20; 0 to about 15; 1 to about 15; 0 to about 10; 1 to about 10; 0 to about 5; or 1 to about 5. In some embodiments, each n is independently 0, about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, or about 50. In some embodiments, m is 1 to about 1,000; 1 to about 500; 1 to about 250; 1 to about 200; 1 to about 150; 1 to about 100; 1 to about 50; 1 to about 40; 1 to about 30; 1 to about 25; 1 to about 20; 1 to about 15; 1 to about 10; or 1 to about 5. In some embodiments, m is 0, about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, or about 50.

Effects of Tags on Formulation Properties.

[0066] In some embodiments, the addition of a tag of the invention can change the viscosity of a fluid. In some embodiments the addition of a tag can increase or decrease the viscosity of a fluid

by at least 0.001 Pascal-second (Pa.s), at least 0.001 Pa.s, at least 0.0009 Pa.s, at least 0.0008 Pa.s, at least 0.0007 Pa.s, at least 0.0006 Pa.s, at least 0.0005 Pa.s, at least 0.0004 Pa.s, at least 0.0003 Pa.s, at least 0.0002 Pa.s, at least 0.0001 Pa.s, at least 0.00005 Pa.s, or at least 0.00001 Pa.s.

[0067] In some embodiments, the addition of a tag of the invention to a molecule can increase or decrease the viscosity of a formulation of the molecule by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99%. In some embodiments, the addition of a tag can increase or decrease the viscosity by a percentage that is no greater than 5%, no greater than 10%, no greater than 15%, no greater than 20%, no greater than 25%, no greater than 30%, no greater than 35%, no greater than 40%, no greater than 45%, no greater than 50%, no greater than 55%, no greater than 60%, no greater than 65%, no greater than 70%, no greater than 75%, no greater than 80%, no greater than 85%, no greater than 90%, no greater than 95%, or no greater than 99%.

[0068] In some embodiments, the addition of a tag of the invention can change the density of a compound by increasing or decreasing the density of the compound. In some embodiments, the change in density can change the buoyancy of a compound by increasing or decreasing the buoyancy of the compound. In some embodiments, the addition of a tag can increase or decrease the density of a compound by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99%. In some embodiments, the addition of a tag can increase or decrease the density of a compound by a percentage that is no greater than 5%, no greater than 10%, no greater than 15%, no greater than 20%, no greater than 25%, no greater than 30%, no greater than 35%, no greater than 40%, no greater than 45%, no greater than 50%, no greater than 55%, no greater than 60%, no greater than 65%, no greater than 70%, no greater than 75%, no greater than 80%, no greater than 85%, no greater than 90%, no greater than 95%, or no greater than 99%.

[0069] In some embodiments, the addition of a tag of the invention can increase or decrease the solubility of a compound by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99%. In some embodiments, the addition of a tag can increase or decrease the solubility of a compound by a percentage that is no greater than 5%, no greater than 10%, no greater than 15%, no greater than 20%, no greater than 25%, no greater than 30%, no greater than 35%, no

greater than 40%, no greater than 45%, no greater than 50%, no greater than 55%, no greater than 60%, no greater than 65%, no greater than 70%, no greater than 75%, no greater than 80%, no greater than 85%, no greater than 90%, no greater than 95%, or no greater than 99%.

Affinity-based Constructs.

[0070] Affinity based methods generally comprise matrix-immobilization of a ligand that binds to a target, which is generally insoluble. The matrix can either be precoated or provided in an activated form for the attachment of specific affinity ligands. Affinity reagents/materials include, by way of example, antibody specific ligands, such as Protein A, Protein G and Protein A/G; antibodies; biotin and biotin derivative specific ligands, such as avidin and streptavidin; Nickel-NTA and cobalt immobilized metal affinity chromatography (IMAC) resins; glutathione and glutathione transferase (Glutathione S-Transferase; GST); maltose binding protein; poly(His); biotin ligase tags; Strep; HaloTag; cellulose binding domain; glycan and the like. In some embodiments, the examples of affinity reagents listed herein are used as affinity tags and a suitable binding partner is used as an affinity reagent on a matrix. Magnetic affinity separation particles, including paramagnetic particles and activated affinity purification beads can carry one or more ligands to be used as an affinity reagent/material during protein purification. In some embodiments, the affinity tags bind to affinity reagents/materials irreversibly. In some embodiments, the affinity tags can be reversibly removed from the affinity reagents/materials. In some embodiments, the affinity reagents/materials are discarded after a single use, two uses, three uses, four uses, or five uses.

[0071] In some embodiments, a polypeptide is a chimeric protein. By the association between the binding domain of the chimeric protein and the substrate of the binding domain which is immobilized on a solid matrix such as beads, resins, plates, etc, the desired products can be conveniently purified.

[0072] In some embodiments, an affinity tag is linked to a target polypeptide using a MAP sequence. Constructs with affinity tags can be immobilized on a substrate that is capable of binding to the affinity tag. In some embodiments, the target is released by changing the binding affinity of the target (*i.e.*, metal) with the MAP. For example, in some embodiments, a cycle of high/low pH provides for alternating affinity with the target (*i.e.*, metal). Some embodiments of the invention relate to compositions comprising the constructs discussed above. In some embodiments, MAP tag can be used to improve purification using ion exchange chromatography. Modulation of the metal affects the charge and improved separation can be accomplished by varying occupancy or the choice of metal. Further embodiments of the invention relate to

composition comprising nucleic acid sequences encoding the constructs discussed above.

[0073] Methods and compositions comprising functionalized substrates with affinity reagents are known in the art. U.S. Patent Publication 2004/0002081, incorporated herein by reference in its entirety, discloses the use of monolithic substrate for the isolation and purification of polynucleotides. The use of the same for the isolation and purification of polypeptides is envisioned in some embodiments of the invention. U.S. Patent Publication 2010/0213411, incorporated herein by reference in its entirety, further discloses manufacture and use of monoliths for chromatography. U.S. Patent 6,319,401, incorporated herein by reference in its entirety, discloses conversion of substrates both for analytical and preparative purposes in a flow-through and a cross flow reactor, a separation and/or conversion device, an analytical device as well as filtration device for carrying out such processes. U.S. Patent 6,664,305, incorporated herein by reference in its entirety, discloses manufacture and use of chromatography materials, including monoliths, with functional groups, e.g. chromatography ligands such as affinity ligands. Affinity ligands comprising specific ionic interactions, such as ion exchange interactions, and affinity ligands comprising biospecificity, immunoaffinity, enzyme-substrate affinity, receptor-ligand affinity or nucleotide affinity, such as hybridization are further described. U.S. Patent 6,736,973, incorporated herein by reference in its entirety, discloses porous self-supporting structures, wherein the surfaces of the pores are modified with functional groups, hydrophobic moieties, reactive groups for covalently binding of ligands, enzymes, immunoglobulins, antigens, lectins, sugars, nucleic acids cell organelles, or dyes. Affinity ligands, e.g. proteinaceous ligands, and functional groups comprising ion-exchange groups are also disclosed.

[0074] Nucleic acids encoding polypeptides or polypeptide fusion proteins/chimeric proteins described herein can be used to construct recombinant expression vectors capable of expressing the polypeptides or polypeptide fusion proteins/chimeric proteins of the present invention. In some embodiments, nucleic acid constructs capable of expressing the protein constructs described herein comprise nucleotide sequences containing transcriptional and translational regulatory information, and such sequences are operably linked to nucleotide coding sequences.

[0075] In some embodiments, host cells are capable of expressing one or more polypeptides or polypeptide fusion proteins/chimeric proteins described herein. The host cells of the present invention encompass cells in procaryotic, eucaryotic, and insect cells. In some embodiments, host cells are capable of modulating the expression of the inserted sequences, or modifying and processing the gene or protein product in the specific fashion desired. For example, expression from certain promoters can be elevated in the presence of certain inducers (e.g., zinc and

cadmium ions for metallothioneine promoters). In some embodiments, modifications (e.g., phosphorylation) and processing (e.g., cleavage) of protein products are important for the function of the protein. Host cells of the present invention can have characteristic and specific mechanisms for the post-translational processing and modification of a protein. Suitable cell lines or host systems to ensure the correct modification and processing of the expressed protein are well known in the art. In some embodiments, host cells secrete minimal amounts of proteolytic enzymes. In some embodiments, host systems of viral origin are utilized to perform the processes described for host cells herein.

[0076] Polypeptides can also be synthesized in a cell-free system.

Colloids.

[0077] As disclosed herein, a tag of the invention can provide a system in which finely divided particles are dispersed within a continuous medium in a manner that slows filtration or prevents rapid settling. In some embodiments, the covalent addition of a tag can provide colloid characteristics to, for example, a peptide, a protein, or a molecule. In some embodiments, the presence of a tag in a solution can provide colloid characteristics to a plurality of particles in the solution.

[0078] A tag can be covalently added to a peptide, protein, antibody, or molecule, such as a biomolecule. A tag that is covalently added to a peptide can interact with at least one additional protein, for example, by forming a hydrogen bond, forming a salt bridge, engaging in a hydrophobic interaction, or contributing to the overall van der Waals forces. In some embodiments, the colloid properties of a solution are at least partially mediated by the ability of a tag to contribute to intermolecular interactions. In some embodiments, the colloid properties of a solution are at least partially mediated by the ability of a tag to contribute to intramolecular interactions.

[0079] A particulate comprising a tag can be a homogeneous or a heterogeneous particulate. In some embodiments, the size of the particulate is at least 1 Å, at least 5 Å, at least 10 Å, at least 20 Å, at least 30 Å, at least 40 Å, at least 50 Å, at least 60 Å, at least 70 Å, at least 80 Å, at least 90 Å, at least 100 Å, at least 150 Å, at least 200 Å, at least 300 Å, at least 400 Å, at least 500 Å, at least 600 Å, at least 700 Å, at least 800 Å, at least 900 Å, at least 1,000 Å, at least 2,500 Å, at least 5,000 Å, or at least 10,000 Å.

[0080] In some embodiments, the size of the particulate is no greater than 1 Å, no greater than 5 Å, no greater than 10 Å, no greater than 20 Å, no greater than 30 Å, no greater than 40 Å, no greater than 50 Å, no greater than 60 Å, no greater than 70 Å, no greater than 80 Å, no greater

than 90 Å, no greater than 100 Å, no greater than 150 Å, no greater than 200 Å, no greater than 300 Å, no greater than 400 Å, no greater than 500 Å, no greater than 600 Å, no greater than 700 Å, no greater than 800 Å, no greater than 900 Å, no greater than 1,000 Å, no greater than 2,500 Å, no greater than 5,000 Å, or no greater than 10,000 Å.

Cellulose Binding Domain.

[0081] Some embodiments of the invention utilize a cellulose binding domain fused to a target MAP as an affinity tag. Cellulose can be used to bind to cellulose binding domains that are linked to target polypeptides for various applications including metal purification.

[0082] Cellulose, a major component of the cellular walls of plants, is a continuous linear glucose β -1,4 linkage polysaccharide which is readily available in the nature. Cellulase is a hydrolase of cellulose, wherein the cellulase can digest the cellulose by cleaving the β -1,4 glycosidic bonds of cellulose. The sequence encoding the cellulase comprises a cellulose-binding domain (CBD) allowing cellulose to bind to cellulose and subsequently cleave the β -1,4 glycosidic bonds of cellulose. U.S. Pat. No. 5,496,934, incorporated herein by reference in its entirety, discloses that cellulose binding domains have a high affinity for crystalline cellulose having a K_d ranging from 1.5 to about 0.8 μ M.

[0083] Chimeric proteins with a cellulose binding protein have been disclosed in several U.S. patents and literature. For example, U.S. Pat. No. 5,202,247, incorporated herein by reference in its entirety, discloses a cellulose binding fusion protein having a substrate binding region of cellulase of *Cellulomonas filmi* origin. Cellulose substrates comprising carboxymethyl cellulose, microcrystalline cellulose, paper or cotton are also disclosed. Cellulose binding domains devoid of any linked cellulase activity are also described. U.S. Pat. No. 5,137,819, incorporated herein by reference in its entirety, discloses cellulose binding fusion proteins for immobilization and purification of polypeptides. U.S. Pat. No. 5,340,731, incorporated herein by reference in its entirety, describes a method of preparing a β -1,4-glycan matrix containing a bound fusion protein. U.S. Pat. No. 5,837,814, incorporated herein by reference in its entirety, discloses CBDs having a high affinity for crystalline cellulose and chitin, along with methods for the molecular cloning and recombinant production thereof. Fusion products comprising the CBD and a second protein are likewise described.

[0084] Cellulose binding domains from other resources can be used as cellulose binding targets in various embodiments. US 6,407,208, incorporated herein by reference in its entirety, discloses a cellulose binding domain from eumycetes. The eumycetes cellulose binding domain is shorter than many of the known cellulose binding domains from bacteria with a denser structure.

[0085] Thus, some embodiments of the invention relate to compositions comprising a polypeptide sequence sufficiently similar to or derived from such CBDs. Further, compositions comprising nucleic acid sequences encoding polypeptide sequences sufficiently similar to or derived from CBD sequences are also used in some embodiments of the invention. Methods for binding to chimeric proteins comprising a polypeptide sequence sufficiently similar to or derived from CBD sequences are provided herein. Further, some embodiments of the invention relate to compositions comprising a nucleic acid sequence encoding a CBD, wherein a nucleic acid sequence encoding a desired polypeptide can be or is linked/incorporated, such as an empty vector or a vector with an insert encoding a target polypeptide. In some embodiments, the linked nucleic acid product encodes for a CBD as part of a polypeptide linked to a MAP sequence.

Homology.

[0086] A peptide of the invention can be prepared, for example, by peptide synthesis or expression of an appropriate nucleic acid molecule. Non limiting examples of peptide sequencing methods include: a) liquid-phase peptide synthesis; b) solid-phase peptide synthesis, with a polystyrene resin, a polyamide resin, a PEG hybrid polystyrene resin, a PEG base resin, and/or a combination of any solid phase support; and c) synthetic biology. Non-limiting examples of methods for the expression of an appropriate nucleic acid molecule include molecular cloning and recombinant DNA technologies.

[0087] A peptide of the invention prepared by peptide synthesis can have at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, at least 50%, at least 45%, at least 40%, at least 35%, or at least 30% homology with a naturally occurring peptide or a parent peptide. A peptide of the invention prepared by the expression of an appropriate nucleic acid molecule can have at least 99.99%, at least 99.9%, at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, at least 50%, at least 45%, at least 40%, at least 35%, or at least 30% homology with a naturally occurring peptide or a parent peptide.

[0088] A nucleic acid sequence encoding a peptide of the invention can have at least 99.99%, at least 99.9%, at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, at least 50%, at least 45%, at least 40%, at least 35%, or at least 30% homology with a naturally occurring nucleic acid sequence or a parent nucleic acid sequence. An appropriate nucleic acid sequence for the preparation of a peptide of the invention can be a degenerate sequence. The percent homology between sequences can be calculated using a plurality of algorithms.

[0089] A peptide of the invention can be covalently incorporated into a known molecule without disrupting certain biochemical properties of the molecule. For example, a peptide of the invention can be covalently incorporated into a molecule such that the structure of the original beta-sheet and alpha-helices of the molecule are not disrupted. A peptide of the invention can be covalently incorporated into a molecule such that the original three-dimensional structure of the molecule is preserved. A peptide of the invention can be covalently incorporated into a molecule such that the original arrangement of multi-subunit complexes, or quaternary structure, is preserved. In some embodiments, a peptide of the invention can change the solubility of an existing molecule by providing a method to change the net charge of a molecule without disturbing the original secondary, tertiary or quaternary structure.

Therapeutic Uses.

[0090] The incorporation of a metal abstracting peptide with a high affinity for binding metal can significantly improve the efficacy of a potential cancer therapeutic. A plurality of subjects in need or want of treatment for a cancer can benefit from the use of a greatly improved therapy. Subjects can be, for example, elderly adults, adults, adolescents, pre-adolescents, children, toddlers, infants.

[0091] In some embodiments, a peptide or a peptide/metal complex of the invention can be used in the treatment of a cancer. In some embodiments, the cancer is susceptible to treatment with a metal-based therapy, for example, a platinum-based therapy such as *cis*-platin. Non-limiting examples of cancers include: acute lymphoblastic leukemia, acute myeloid leukemia, adrenocortical carcinoma, AIDS-related cancers, AIDS-related lymphoma, anal cancer, appendix cancer, astrocytomas, basal cell carcinoma, bile duct cancer, bladder cancer, bone cancers, brain tumors, such as cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic glioma, breast cancer, bronchial adenomas, Burkitt lymphoma, carcinoma of unknown primary origin, central nervous system lymphoma, cerebellar astrocytoma, cervical cancer, childhood cancers, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, cutaneous T-cell lymphoma, desmoplastic small round cell tumor, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, germ cell tumors, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor, gliomas, hairy cell leukemia, head and neck cancer, heart cancer, hepatocellular (liver) cancer, Hodgkin lymphoma, Hypopharyngeal cancer, intraocular melanoma, islet cell carcinoma, Kaposi sarcoma, kidney cancer, laryngeal cancer, lip and oral

cavity cancer, liposarcoma, liver cancer, lung cancers, such as non-small cell and small cell lung cancer, lymphomas, leukemias, macroglobulinemia, malignant fibrous histiocyoma of bone/osteosarcoma, medulloblastoma, melanomas, mesothelioma, metastatic squamous neck cancer with occult primary, mouth cancer, multiple endocrine neoplasia syndrome, myelodysplastic syndromes, myeloid leukemia, nasal cavity and paranasal sinus cancer, nasopharyngeal carcinoma, neuroblastoma, non-Hodgkin lymphoma, non-small cell lung cancer, oral cancer, oropharyngeal cancer, osteosarcoma/malignant fibrous histiocyoma of bone, ovarian cancer, ovarian epithelial cancer, ovarian germ cell tumor, pancreatic cancer, pancreatic cancer islet cell, paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal astrocytoma, pineal germinoma, pituitary adenoma, pleuropulmonary blastoma, plasma cell neoplasia, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell carcinoma, renal pelvis and ureter transitional cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, sarcomas, skin cancers, skin carcinoma merkel cell, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, stomach cancer, T-cell lymphoma, throat cancer, thymoma, thymic carcinoma, thyroid cancer, trophoblastic tumor (gestational), cancers of unknown primary site, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenström macroglobulinemia, and Wilms tumor.

[0092] In some embodiments, a composition of the invention is used to treat a metal-susceptible cancer, for example a platinum-susceptible cancer. Non-limiting examples of cancers that can be amenable to treatment with a metal, such as platinum, include bladder cancers, cervical cancers, non-small cell lung cancer, ovarian cancer, head and neck squamous cell carcinoma, testicular cancer, and malignant mesothelioma. In some embodiments, a composition of the invention is used to prevent a metastasis of a metal-susceptible cancer, such as a platinum-susceptible cancer.

[0093] In some embodiments the therapy can comprise an antibody. **TABLE 1** lists exemplary antibody therapies.

TABLE 1

Antibody	Brand name	Target	Indication (Targeted disease)
Abciximab	ReoPro TM	inhibition of glycoprotein IIb/IIIa	Cardiovascular disease
Adalimumab	Humira TM	inhibition of TNF- α signaling	Several auto-immune disorders
Alemtuzumab	Campath TM	CD52	Chronic lymphocytic leukemia
Basiliximab	Simulect TM	IL-2R α receptor (CD25)	Transplant rejection
Belimumab	Benlysta TM	inhibition of B- cell activating factor	Systemic lupus erythematosus
Bevacizumab	Avastin TM	Vascular endothelial growth factor (VEGF)	Colorectal cancer, Age related macular degeneration (off-label)
Brentuximab vedotin	Adcetris TM	CD30	Anaplastic large cell lymphoma (ALCL) and Hodgkin lymphoma
Canakinumab	Ilaris TM	IL-1 β	Cryopyrin-associated periodic syndrome (CAPS)
Certolizumab pegol[19]	Cimzia TM	inhibition of TNF- α signaling	Crohn's disease
Cetuximab	Erbitux TM	epidermal growth factor receptor (EGFR)	Colorectal cancer, Head and neck cancer
Daclizumab	Zenapax TM	IL-2R α receptor (CD25)	Transplant rejection
Denosumab	Prolia , Xgeva TM	RANK Ligand inhibitor	Postmenopausal osteoporosis , Solid tumor's bony metastases
Eculizumab	Soliris TM	Complement system protein C5	Paroxysmal nocturnal hemoglobinuria
Efalizumab	Raptiva TM	CD11a	Psoriasis
Gemtuzumab	Mylotarg TM	CD33	Acute myelogenous leukemia (with calicheamicin)
Golimumab	Simponi TM	TNF-alpha inhibitor	Rheumatoid arthritis, Psoriatic arthritis, and Ankylosing spondylitis
Ibritumomab tiuxetan	Zevalin TM	CD20	Non-Hodgkin lymphoma (with yttrium-90 or indium-111)
Infliximab	Remicade TM	inhibition of TNF- α signaling	Several autoimmune disorders
Ipilimumab (MDX-101)	Yervoy TM	blocks CTLA-4	Melanoma
Muromonab-CD3	Orthoclone TM OKT3 TM	T cell CD3 Receptor	Transplant rejection
Natalizumab	Tysabri TM	alpha-4 (α 4) integrin,	Multiple sclerosis and Crohn's

			disease
Ofatumumab	Arzerra TM	CD20	Chronic lymphocytic leukemia
Omalizumab	Xolair TM	immunoglobulin E (IgE)	mainly allergy-related asthma
Palivizumab	Synagis TM	epitope of the RSV F protein	Respiratory Syncytial Virus
Panitumumab	Vectibix TM	epidermal growth factor receptor (EGFR)	Colorectal cancer
Ranibizumab	Lucentis TM	Vascular endothelial growth factor A (VEGF-A)	Macular degeneration
Rituximab	Rituxan TM Mabthera TM	CD20	Non-Hodgkin lymphoma
Tocilizumab (or Atlizumab)	Actemra TM RoActemra TM	Anti-IL-6R	Rheumatoid arthritis
Tositumomab	Bexxar TM	CD20	Non-Hodgkin lymphoma
Trastuzumab	Herceptin TM	ErbB2 (HER2)	Breast cancer

[0094] Therapeutic monoclonal antibodies (mAbs) can be used for treating cancer, immunological disorders, and a plurality of diseases. The major targets of current mAb products include, for example, Epidermal Growth Factor Receptor (EGFR), Human Epidermal Growth Factor receptor 2 (HER2), Vascular Endothelial Growth Factor (VEGF), and CD20. Numerous cancers are EGFR positive (EGFR+) and can be differentially treated according to an EGFR+ diagnosis. Cetuximab and panitumumab products are approved for the treatment of head and neck squamous cell carcinomas and can be used to treat metastatic colon cancer. Trastuzumab is approved by the FDA and Trastuzumab can be used as a primary therapy to treat HER2 positive (HER2+) breast cancers. In some embodiments, the MAP tag of the invention increases the efficacy of the therapies of Table 1 by modulating the colloidal properties of the antibody. In some embodiments a MAP tag of the invention can be added to an antibody to increase the efficacy of the antibody as a therapy.

[0095] Cell surface marker molecules, such as EGFR, HER2, and/or CD20 can be used to identify a population of cells. In some embodiments, a combination of cell surface markers, or a cell surface marker signature, can be used to identify a population of cells. In some embodiments, a cell surface marker, and/or a cell surface marker signature can be used in medical diagnosis.

[0096] In some embodiments, the invention provides a method of treating a cancer, the method comprising administering to a subject in need or want of relief thereof a composition comprising a therapeutically-effective amount of a peptide, or a pharmaceutically-acceptable salt thereof, at a

concentration, wherein the peptide comprises a sequence XC_1C_2 , wherein X is any natural or non-natural amino acid or amino acid analogue, and C_1 and C_2 are each individually chosen from a cysteine and a sulfur-containing alpha or beta amino acid, wherein a therapeutic molecule is bound to the peptide, wherein a metal is bound to the peptide, and wherein the composition has a viscosity less than that of a second composition, wherein the second composition comprises the therapeutic molecule at the concentration.

[0097] In some embodiments, the invention provides a method of formulating a medicament for treatment of a cancer, wherein the medicament comprises a therapeutically-effective amount of a peptide, or a pharmaceutically-acceptable salt thereof, at a concentration, wherein the peptide comprises a sequence XC_1C_2 , wherein X is any natural or non-natural amino acid or amino acid analogue, and C_1 and C_2 are each individually chosen from a cysteine and a sulfur-containing alpha or beta amino acid, wherein a therapeutic molecule is bound to the peptide, wherein a metal is bound to the peptide, and wherein the composition has a viscosity less than that of a second composition, wherein the second composition comprises the therapeutic molecule at the concentration.

[0098] In some embodiments, the invention provides a use of a unit dosage form for treatment of a cancer, the unit dosage form comprising a therapeutically-effective amount of a peptide, or a pharmaceutically-acceptable salt thereof, at a concentration, wherein the peptide comprises a sequence XC_1C_2 , wherein X is any natural or non-natural amino acid or amino acid analogue, and C_1 and C_2 are each individually chosen from a cysteine and a sulfur-containing alpha or beta amino acid, wherein a therapeutic molecule is bound to the peptide, wherein a metal is bound to the peptide, and wherein the composition has a viscosity less than that of a second composition, wherein the second composition comprises the therapeutic molecule at the concentration.

[0099] In some embodiments, the addition of a peptide comprising a sequence XC_1C_2 comprises a significant efficacy improvement over existing platinum-based antineoplastic drugs. An existing platinum based antineoplastic drug can be limited by, for example, chemical properties such as solubility in plasma. The incorporation of a peptide comprising a sequence XC_1C_2 of the invention can modulate the plasma solubility of an existing antineoplastic drug by modulating the solubility of a peptide or protein. The addition of a peptide comprising a sequence XC_1C_2 can, for example, incorporate charge into a protein or peptide, by permitting metal incorporation into the protein or peptide sequence. A change in charge can modulate the properties of a peptide system by modulating the hydrophobicity or hydrophilicity of a compound.

[00100] In some embodiments, a subject undergoing treatment with a platinum containing compound comprising a sequence XC_1C_2 of the invention can be spared of unwanted side effects

catalyzed by non-specific by controlling the delivery and release of the metal.

[00101] In some embodiments, the incorporation of a peptide sequence XC_1C_2 of the invention to an existing treatment can be modulate the viscosity of the existing treatment. The ability to modulate the viscosity, for example, by altering the net charge of a treatment, can decrease the viscosity of the existing treatment, increase the solubility of a molecule, and provide therapeutically effective blood plasma levels at significantly lower doses than the required doses for the unaltered existing therapy.

Pharmaceutical Formulations.

[00102] A pharmaceutical composition of the disclosure can be a combination of any pharmaceutical compounds described herein with other chemical components, such as carriers, stabilizers, diluents, dispersing agents, suspending agents, thickening agents, and/or excipients. The pharmaceutical composition facilitates administration of the compound to an organism. Pharmaceutical compositions can be administered in therapeutically-effective amounts as pharmaceutical compositions by any form and route known in the art including, for example, intravenous, subcutaneous, intramuscular, oral, rectal, parenteral, ophthalmic, pulmonary, transdermal, vaginal, otic, nasal, and topical administration.

[00103] A pharmaceutical composition can be administered in a local or systemic manner, for example, via injection of the compound directly into an organ, optionally in a depot or sustained release formulation. Pharmaceutical compositions can be provided in the form of a rapid release formulation, in the form of an extended release formulation, or in the form of an intermediate release formulation. A rapid release form can provide an immediate release. An extended release formulation can provide a controlled release or a sustained delayed release.

[00104] For oral administration, pharmaceutical compositions can be formulated by combining the active compounds with pharmaceutically acceptable carriers or excipients. Such carriers can be used to formulate liquids, gels, syrups, elixirs, slurries, suspensions and the like, for oral ingestion by a subject. Non-limiting examples of solvents used in an oral dissolvable formulation can include water, ethanol, isopropanol, saline, physiological saline, DMSO, dimethylformamide, potassium phosphate buffer, phosphate buffer saline (PBS), sodium phosphate buffer, 4-2-hydroxyethyl-1-piperazineethanesulfonic acid buffer (HEPES), 3-(N-morpholino)propanesulfonic acid buffer (MOPS), piperazine-N,N'-bis(2-ethanesulfonic acid) buffer (PIPES), and saline sodium citrate buffer (SSC). Non-limiting examples of co-solvents used in an oral dissolvable formulation can include sucrose, urea, cremaphor, DMSO, and potassium phosphate buffer.

[00105] Pharmaceutical preparations can be formulated for intravenous administration. The pharmaceutical compositions can be in a form suitable for parenteral injection as a sterile suspension, solution or emulsion in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Suspensions of the active compounds can be prepared as oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions can contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. The suspension can also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Alternatively, the active ingredient can be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

[00106] The active compounds can be administered topically and can be formulated into a variety of topically administrable compositions, such as solutions, suspensions, lotions, gels, pastes, medicated sticks, balms, creams, and ointments. Such pharmaceutical compositions can contain solubilizers, stabilizers, tonicity enhancing agents, buffers and preservatives.

[00107] The compounds can also be formulated in rectal compositions such as enemas, rectal gels, rectal foams, rectal aerosols, suppositories, jelly suppositories, or retention enemas, containing conventional suppository bases such as cocoa butter or other glycerides, as well as synthetic polymers such as polyvinylpyrrolidone, PEG, and the like. In suppository forms of the compositions, a low-melting wax such as a mixture of fatty acid glycerides, optionally in combination with cocoa butter, is first melted.

[00108] In practicing the methods of treatment or use provided herein, therapeutically-effective amounts of the compounds described herein are administered in pharmaceutical compositions to a subject having a disease or condition to be treated. In some embodiments, the subject is a mammal such as a human. A therapeutically-effective amount can vary widely depending on the severity of the disease, the age and relative health of the subject, the potency of the compounds used, and other factors.

[00109] Pharmaceutical compositions can be formulated using one or more physiologically-acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active compounds into preparations that can be used pharmaceutically. Formulation can be modified depending upon the route of administration chosen. Pharmaceutical compositions comprising compounds described herein can be manufactured in a

conventional manner, for example, by means of conventional mixing, dissolving, granulating, or emulsifying.

[00110] The pharmaceutical compositions can include at least one pharmaceutically acceptable carrier, diluent, or excipient and compounds described herein or pharmaceutically-acceptable salt form.

[00111] Methods for the preparation of compositions comprising the compounds described herein can include formulating the compounds with one or more inert, pharmaceutically-acceptable excipients. Liquid compositions include, for example, solutions in which a compound is dissolved, emulsions comprising a compound, or a solution containing liposomes, micelles, or nanoparticles comprising a compound as disclosed herein. Semi-solid compositions include, for example, gels, suspensions and creams. The compositions can be in liquid solutions or suspensions, solid forms suitable for solution or suspension in a liquid prior to use, or as emulsions. These compositions can also contain minor amounts of nontoxic, auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, and other pharmaceutically-acceptable additives.

[00112] Compounds can be delivered via liposomal technology. The use of liposomes as drug carriers can increase the therapeutic index of the compounds. Liposomes are composed of natural phospholipids, and can contain mixed lipid chains with surfactant properties (e.g., egg phosphatidylethanolamine). A liposome design can employ surface ligands for attaching to unhealthy tissue. Non-limiting examples of liposomes include the multilamellar vesicle (MLV), the small unilamellar vesicle (SUV), and the large unilamellar vesicle (LUV). Liposomal physicochemical properties can be modulated to optimize penetration through biological barriers and retention at the site of administration, and to prevent premature degradation and toxicity to non-target tissues. Optimal liposomal properties depend on the administration route: large-sized liposomes show good retention upon local injection, small-sized liposomes are better suited to achieve passive targeting. PEGylation reduces the uptake of the liposomes by liver and spleen, and increases the circulation time, resulting in increased localization at the inflamed site due to the enhanced permeability and retention (EPR) effect. Additionally, liposomal surfaces can be modified to achieve selective delivery of the encapsulated drug to specific target cells. Non-limiting examples of targeting ligands include monoclonal antibodies, vitamins, peptides, and polysaccharides specific for receptors concentrated on the surface of cells associated with the disease.

[00113] Non-limiting examples of dosage forms suitable for use in the disclosure include liquid, elixir, nanosuspension, aqueous or oily suspensions, drops, syrups, and any combination

thereof. Non-limiting examples of pharmaceutically-acceptable excipients suitable for use in the disclosure include granulating agents, binding agents, lubricating agents, disintegrating agents, sweetening agents, glidants, anti-adherents, anti-static agents, surfactants, anti-oxidants, gums, coating agents, coloring agents, flavouring agents, coating agents, plasticizers, preservatives, suspending agents, emulsifying agents, plant cellulosic material and spheronization agents, and any combination thereof.

[00114] Non-limiting examples of pharmaceutically-acceptable excipients can be found, for example, in *Remington: The Science and Practice of Pharmacy*, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., *Pharmaceutical Dosage Forms*, Marcel Decker, New York, N.Y., 1980; and *Pharmaceutical Dosage Forms and Drug Delivery Systems*, Seventh Ed. (Lippincott Williams & Wilkins 1999), each of which is incorporated by reference in its entirety.

[00115] Compositions of the invention can be packaged as a kit. In some embodiments, a kit includes written instructions on the administration/use of the composition. The written material can be, for example, a label. The written material can suggest conditions methods of administration. The instructions provide the subject and the supervising physician with the best guidance for achieving the optimal clinical outcome from the administration of the therapy. The written material can be a label. In some embodiments, the label can be approved by a regulatory agency, for example the U.S. Food and Drug Administration (FDA), the European Medicines Agency (EMA), or other regulatory agencies.

[00116] In some embodiments, a kit of the invention comprises: a) a peptide comprising a sequence XC_1C_2 , wherein X is any natural or non-natural amino acid or amino acid analogue, and C_1 and C_2 are each individually chosen from a cysteine and a sulfur-containing alpha or beta amino acid, wherein a molecule is bound to the peptide; b) a metal; and c) written instructions describing a use of the kit in treatment of a condition.

Dosing.

[00117] Pharmaceutical compositions described herein can be in unit dosage forms suitable for single administration of precise dosages. In unit dosage form, the formulation is divided into unit doses containing appropriate quantities of one or more compounds. The unit dosage can be in the form of a package containing discrete quantities of the formulation. Non-limiting examples are liquids in vials or ampoules. Aqueous suspension compositions can be packaged in single-dose non-reclosable containers. Multiple-dose reclosable containers can be

used, for example, in combination with a preservative. Formulations for parenteral injection can be presented in unit dosage form, for example, in ampoules, or in multi-dose containers with a preservative.

[00118] A compound described herein can be present in a composition in a range of from about 1 mg to about 2000 mg; from about 100 mg to about 2000 mg; from about 10 mg to about 2000 mg; from about 5 mg to about 1000 mg, from about 10 mg to about 500 mg, from about 50 mg to about 250 mg, from about 100 mg to about 200 mg, from about 1 mg to about 50 mg, from about 50 mg to about 100 mg, from about 100 mg to about 150 mg, from about 150 mg to about 200 mg, from about 200 mg to about 250 mg, from about 250 mg to about 300 mg, from about 300 mg to about 350 mg, from about 350 mg to about 400 mg, from about 400 mg to about 450 mg, from about 450 mg to about 500 mg, from about 500 mg to about 550 mg, from about 550 mg to about 600 mg, from about 600 mg to about 650 mg, from about 650 mg to about 700 mg, from about 700 mg to about 750 mg, from about 750 mg to about 800 mg, from about 800 mg to about 850 mg, from about 850 mg to about 900 mg, from about 900 mg to about 950 mg, or from about 950 mg to about 1000 mg.

[00119] A compound described herein can be present in a composition in an amount of about 1 mg, about 2 mg, about 3 mg, about 4 mg, about 5 mg, about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 35 mg, about 40 mg, about 45 mg, about 50 mg, about 55 mg, about 60 mg, about 65 mg, about 70 mg, about 75 mg, about 80 mg, about 85 mg, about 90 mg, about 95 mg, about 100 mg, about 125 mg, about 150 mg, about 175 mg, about 200 mg, about 250 mg, about 300 mg, about 350 mg, about 400 mg, about 450 mg, about 500 mg, about 550 mg, about 600 mg, about 650 mg, about 700 mg, about 750 mg, about 800 mg, about 850 mg, about 900 mg, about 950 mg, about 1000 mg, about 1050 mg, about 1100 mg, about 1150 mg, about 1200 mg, about 1250 mg, about 1300 mg, about 1350 mg, about 1400 mg, about 1450 mg, about 1500 mg, about 1550 mg, about 1600 mg, about 1650 mg, about 1700 mg, about 1750 mg, about 1800 mg, about 1850 mg, about 1900 mg, about 1950 mg, or about 2000 mg.

[00120] In some embodiments, a dose can be expressed in terms of an amount of the drug divided by the mass of the subject, for example, milligrams of drug per kilograms of subject body mass. In some embodiments, a platinum containing compound attached to a metal abstracting peptide (MAP) is present in a composition in an amount ranging from about 250 mg/kg to about 2000 mg/kg, about 10 mg/kg to about 800 mg/kg, about 50 mg/kg to about 400 mg/kg, about 100 mg/kg to about 300 mg/kg, or about 150 mg/kg to about 200 mg/kg.

[00121] The foregoing ranges are merely suggestive. Dosages can be altered depending

on a number of variables, including, for example, the activity of the compound used, the disease or condition to be treated, the mode of administration, the requirements of the individual subject, the severity of the disease or condition being treated, and the judgment of the practitioner.

Pharmacokinetic and Pharmacodynamic Measurements.

[00122] Pharmacokinetic and pharmacodynamic data can be obtained by various experimental techniques. Appropriate pharmacokinetic and pharmacodynamic profile components describing a particular composition can vary due to variations in drug metabolism in human subjects. Pharmacokinetic and pharmacodynamic profiles can be based on the determination of the mean parameters of a group of subjects. The group of subjects includes any reasonable number of subjects suitable for determining a representative mean, for example, 5 subjects, 10 subjects, 15 subjects, 20 subjects, 25 subjects, 30 subjects, 35 subjects, or more. The mean is determined by calculating the average of all subject's measurements for each parameter measured. A dose can be modulated to achieve a desired pharmacokinetic or pharmacodynamics profile, such as a desired or effective blood profile, as described herein.

[00123] The pharmacodynamic parameters can be any parameters suitable for describing compositions of the invention. For example, the pharmacodynamic profile can be obtained at a time after dosing of, for example, about zero minutes, about 1 minute, about 2 minutes, about 3 minutes, about 4 minutes, about 5 minutes, about 6 minutes, about 7 minutes, about 8 minutes, about 9 minutes, about 10 minutes, about 11 minutes, about 12 minutes, about 13 minutes, about 14 minutes, about 15 minutes, about 16 minutes, about 17 minutes, about 18 minutes, about 19 minutes, about 20 minutes, about 21 minutes, about 22 minutes, about 23 minutes, about 24 minutes, about 25 minutes, about 26 minutes, about 27 minutes, about 28 minutes, about 29 minutes, about 30 minutes, about 31 minutes, about 32 minutes, about 33 minutes, about 34 minutes, about 35 minutes, about 36 minutes, about 37 minutes, about 38 minutes, about 39 minutes, about 40 minutes, about 41 minutes, about 42 minutes, about 43 minutes, about 44 minutes, about 45 minutes, about 46 minutes, about 47 minutes, about 48 minutes, about 49 minutes, about 50 minutes, about 51 minutes, about 52 minutes, about 53 minutes, about 54 minutes, about 55 minutes, about 56 minutes, about 57 minutes, about 58 minutes, about 59 minutes, about 60 minutes, about zero hours, about 0.5 hours, about 1 hour, about 1.5 hours, about 2 hours, about 2.5 hours, about 3 hours, about 3.5 hours, about 4 hours, about 4.5 hours, about 5 hours, about 5.5 hours, about 6 hours, about 6.5 hours, about 7 hours, about 7.5 hours, about 8 hours, about 8.5 hours, about 9 hours, about 9.5 hours, about 10 hours, about 10.5 hours, about 11 hours, about 11.5 hours, about 12 hours, about 12.5 hours, about 13 hours, about 13.5

hours, about 14 hours, about 14.5 hours, about 15 hours, about 15.5 hours, about 16 hours, about 16.5 hours, about 17 hours, about 17.5 hours, about 18 hours, about 18.5 hours, about 19 hours, about 19.5 hours, about 20 hours, about 20.5 hours, about 21 hours, about 21.5 hours, about 22 hours, about 22.5 hours, about 23 hours, about 23.5 hours, or about 24 hours.

[00124] The pharmacokinetic parameters can be any parameters suitable for describing a compound. The $C_{\max}$ can be, for example, not less than about 1 ng/mL; not less than about 5 ng/mL; not less than about 10 ng/mL; not less than about 15 ng/mL; not less than about 20 ng/mL; not less than about 25 ng/mL; not less than about 50 ng/mL; not less than about 75 ng/mL; not less than about 100 ng/mL; not less than about 200 ng/mL; not less than about 300 ng/mL; not less than about 400 ng/mL; not less than about 500 ng/mL; not less than about 600 ng/mL; not less than about 700 ng/mL; not less than about 800 ng/mL; not less than about 900 ng/mL; not less than about 1000 ng/mL; not less than about 1250 ng/mL; not less than about 1500 ng/mL; not less than about 1750 ng/mL; not less than about 2000 ng/mL; or any other $C_{\max}$ appropriate for describing a pharmacokinetic profile of a compound described herein. The $C_{\max}$ can be, for example, about 1 ng/mL to about 5,000 ng/mL; about 1 ng/mL to about 4,500 ng/mL; about 1 ng/mL to about 4,000 ng/mL; about 1 ng/mL to about 3,500 ng/mL; about 1 ng/mL to about 3,000 ng/mL; about 1 ng/mL to about 2,500 ng/mL; about 1 ng/mL to about 2,000 ng/mL; about 1 ng/mL to about 1,500 ng/mL; about 1 ng/mL to about 1,000 ng/mL; about 1 ng/mL to about 900 ng/mL; about 1 ng/mL to about 800 ng/mL; about 1 ng/mL to about 700 ng/mL; about 1 ng/mL to about 600 ng/mL; about 1 ng/mL to about 500 ng/mL; about 1 ng/mL to about 450 ng/mL; about 1 ng/mL to about 400 ng/mL; about 1 ng/mL to about 350 ng/mL; about 1 ng/mL to about 300 ng/mL; about 1 ng/mL to about 250 ng/mL; about 1 ng/mL to about 200 ng/mL; about 1 ng/mL to about 150 ng/mL; about 1 ng/mL to about 125 ng/mL; about 1 ng/mL to about 100 ng/mL; about 1 ng/mL to about 90 ng/mL; about 1 ng/mL to about 80 ng/mL; about 1 ng/mL to about 70 ng/mL; about 1 ng/mL to about 60 ng/mL; about 1 ng/mL to about 50 ng/mL; about 1 ng/mL to about 40 ng/mL; about 1 ng/mL to about 30 ng/mL; about 1 ng/mL to about 20 ng/mL; about 1 ng/mL to about 10 ng/mL; about 1 ng/mL to about 5 ng/mL; about 10 ng/mL to about 4,000 ng/mL; about 10 ng/mL to about 3,000 ng/mL; about 10 ng/mL to about 2,000 ng/mL; about 10 ng/mL to about 1,500 ng/mL; about 10 ng/mL to about 1,000 ng/mL; about 10 ng/mL to about 900 ng/mL; about 10 ng/mL to about 800 ng/mL; about 10 ng/mL to about 700 ng/mL; about 10 ng/mL to about 600 ng/mL; about 10 ng/mL to about 500 ng/mL; about 10 ng/mL to about 400 ng/mL; about 10 ng/mL to about 300 ng/mL; about 10 ng/mL to about 200 ng/mL; about 10 ng/mL to about 100 ng/mL; about 10 ng/mL to about 50 ng/mL; about 25 ng/mL to about 500 ng/mL; about 25 ng/mL to about 100 ng/mL; about 50 ng/mL to about 500 ng/mL;

about 50 ng/mL to about 100 ng/mL; about 100 ng/mL to about 500 ng/mL; about 100 ng/mL to about 400 ng/mL; about 100 ng/mL to about 300 ng/mL; or about 100 ng/mL to about 200 ng/mL.

[00125] The $T_{\max}$ of a compound described herein can be, for example, not greater than about 0.5 hours, not greater than about 1 hours, not greater than about 1.5 hours, not greater than about 2 hours, not greater than about 2.5 hours, not greater than about 3 hours, not greater than about 3.5 hours, not greater than about 4 hours, not greater than about 4.5 hours, not greater than about 5 hours, or any other $T_{\max}$ appropriate for describing a pharmacokinetic profile of a compound described herein. The $T_{\max}$ can be, for example, about 0.1 hours to about 24 hours; about 0.1 hours to about 0.5 hours; about 0.5 hours to about 1 hour; about 1 hour to about 1.5 hours; about 1.5 hours to about 2 hour; about 2 hours to about 2.5 hours; about 2.5 hours to about 3 hours; about 3 hours to about 3.5 hours; about 3.5 hours to about 4 hours; about 4 hours to about 4.5 hours; about 4.5 hours to about 5 hours; about 5 hours to about 5.5 hours; about 5.5 hours to about 6 hours; about 6 hours to about 6.5 hours; about 6.5 hours to about 7 hours; about 7 hours to about 7.5 hours; about 7.5 hours to about 8 hours; about 8 hours to about 8.5 hours; about 8.5 hours to about 9 hours; about 9 hours to about 9.5 hours; about 9.5 hours to about 10 hours; about 10 hours to about 10.5 hours; about 10.5 hours to about 11 hours; about 11 hours to about 11.5 hours; about 11.5 hours to about 12 hours; about 12 hours to about 12.5 hours; about 12.5 hours to about 13 hours; about 13 hours to about 13.5 hours; about 13.5 hours to about 14 hours; about 14 hours to about 14.5 hours; about 14.5 hours to about 15 hours; about 15 hours to about 15.5 hours; about 15.5 hours to about 16 hours; about 16 hours to about 16.5 hours; about 16.5 hours to about 17 hours; about 17 hours to about 17.5 hours; about 17.5 hours to about 18 hours; about 18 hours to about 18.5 hours; about 18.5 hours to about 19 hours; about 19 hours to about 19.5 hours; about 19.5 hours to about 20 hours; about 20 hours to about 20.5 hours; about 20.5 hours to about 21 hours; about 21 hours to about 21.5 hours; about 21.5 hours to about 22 hours; about 22 hours to about 22.5 hours; about 22.5 hours to about 23 hours; about 23 hours to about 23.5 hours; or about 23.5 hours to about 24 hours.

[00126] The $AUC_{(0-\infty)}$ of a compound described herein can be, for example, not less than about 1 ng•hr/mL, not less than about 5 ng•hr/mL, not less than about 10 ng•hr/mL, not less than about 20 ng•hr/mL, not less than about 30 ng•hr/mL, not less than about 40 ng•hr/mL, not less than about 50 ng•hr/mL, not less than about 100 ng•hr/mL, not less than about 150 ng•hr/mL, not less than about 200 ng•hr/mL, not less than about 250 ng•hr/mL, not less than about 300 ng•hr/mL, not less than about 350 ng•hr/mL, not less than about 400 ng•hr/mL, not less than about 450 ng•hr/mL, not less than about 500 ng•hr/mL, not less than about 600 ng•hr/mL, not

less than about 700 ng•hr/mL, not less than about 800 ng•hr/mL, not less than about 900 ng•hr/mL, not less than about 1000 ng•hr/mL, not less than about 1250 ng•hr/mL, not less than about 1500 ng•hr/mL, not less than about 1750 ng•hr/mL, not less than about 2000 ng•hr/mL, not less than about 2500 ng•hr/mL, not less than about 3000 ng•hr/mL, not less than about 3500 ng•hr/mL, not less than about 4000 ng•hr/mL, not less than about 5000 ng•hr/mL, not less than about 6000 ng•hr/mL, not less than about 7000 ng•hr/mL, not less than about 8000 ng•hr/mL, not less than about 9000 ng•hr/mL, not less than about 10,000 ng•hr/mL, or any other $AUC_{(0-\infty)}$ appropriate for describing a pharmacokinetic profile of a compound described herein. The $AUC_{(0-\infty)}$ of a compound can be, for example, about 1 ng•hr/mL to about 10,000 ng•hr/mL; about 1 ng•hr/mL to about 10 ng•hr/mL; about 10 ng•hr/mL to about 25 ng•hr/mL; about 25 ng•hr/mL to about 50 ng•hr/mL; about 50 ng•hr/mL to about 100 ng•hr/mL; about 100 ng•hr/mL to about 200 ng•hr/mL; about 200 ng•hr/mL to about 300 ng•hr/mL; about 300 ng•hr/mL to about 400 ng•hr/mL; about 400 ng•hr/mL to about 500 ng•hr/mL; about 500 ng•hr/mL to about 600 ng•hr/mL; about 600 ng•hr/mL to about 700 ng•hr/mL; about 700 ng•hr/mL to about 800 ng•hr/mL; about 800 ng•hr/mL to about 900 ng•hr/mL; about 900 ng•hr/mL to about 1,000 ng•hr/mL; about 1,000 ng•hr/mL to about 1,250 ng•hr/mL; about 1,250 ng•hr/mL to about 1,500 ng•hr/mL; about 1,500 ng•hr/mL to about 1,750 ng•hr/mL; about 1,750 ng•hr/mL to about 2,000 ng•hr/mL; about 2,000 ng•hr/mL to about 2,500 ng•hr/mL; about 2,500 ng•hr/mL to about 3,000 ng•hr/mL; about 3,000 ng•hr/mL to about 3,500 ng•hr/mL; about 3,500 ng•hr/mL to about 4,000 ng•hr/mL; about 4,000 ng•hr/mL to about 4,500 ng•hr/mL; about 4,500 ng•hr/mL to about 5,000 ng•hr/mL; about 5,000 ng•hr/mL to about 5,500 ng•hr/mL; about 5,500 ng•hr/mL to about 6,000 ng•hr/mL; about 6,000 ng•hr/mL to about 6,500 ng•hr/mL; about 6,500 ng•hr/mL to about 7,000 ng•hr/mL; about 7,000 ng•hr/mL to about 7,500 ng•hr/mL; about 7,500 ng•hr/mL to about 8,000 ng•hr/mL; about 8,000 ng•hr/mL to about 8,500 ng•hr/mL; about 8,500 ng•hr/mL to about 9,000 ng•hr/mL; about 9,000 ng•hr/mL to about 9,500 ng•hr/mL; or about 9,500 ng•hr/mL to about 10,000 ng•hr/mL.

[00127] The plasma concentration of a compound described herein can be, for example, not less than about 1 ng/mL, not less than about 5 ng/mL, not less than about 10 ng/mL, not less than about 15 ng/mL, not less than about 20 ng/mL, not less than about 25 ng/mL, not less than about 50 ng/mL, not less than about 75 ng/mL, not less than about 100 ng/mL, not less than about 150 ng/mL, not less than about 200 ng/mL, not less than about 300 ng/mL, not less than about 400 ng/mL, not less than about 500 ng/mL, not less than about 600 ng/mL, not less than about 700 ng/mL, not less than about 800 ng/mL, not less than about 900 ng/mL, not less than about 1000 ng/mL, not less than about 1200 ng/mL, or any other plasma concentration of a

compound described herein. The plasma concentration can be, for example, about 1 ng/mL to about 2,000 ng/mL; about 1 ng/mL to about 5 ng/mL; about 5 ng/mL to about 10 ng/mL; about 10 ng/mL to about 25 ng/mL; about 25 ng/mL to about 50 ng/mL; about 50 ng/mL to about 75 ng/mL; about 75 ng/mL to about 100 ng/mL; about 100 ng/mL to about 150 ng/mL; about 150 ng/mL to about 200 ng/mL; about 200 ng/mL to about 250 ng/mL; about 250 ng/mL to about 300 ng/mL; about 300 ng/mL to about 350 ng/mL; about 350 ng/mL to about 400 ng/mL; about 400 ng/mL to about 450 ng/mL; about 450 ng/mL to about 500 ng/mL; about 500 ng/mL to about 600 ng/mL; about 600 ng/mL to about 700 ng/mL; about 700 ng/mL to about 800 ng/mL; about 800 ng/mL to about 900 ng/mL; about 900 ng/mL to about 1,000 ng/mL; about 1,000 ng/mL to about 1,100 ng/mL; about 1,100 ng/mL to about 1,200 ng/mL; about 1,200 ng/mL to about 1,300 ng/mL; about 1,300 ng/mL to about 1,400 ng/mL; about 1,400 ng/mL to about 1,500 ng/mL; about 1,500 ng/mL to about 1,600 ng/mL; about 1,600 ng/mL to about 1,700 ng/mL; about 1,700 ng/mL to about 1,800 ng/mL; about 1,800 ng/mL to about 1,900 ng/mL; or about 1,900 ng/mL to about 2,000 ng/mL.

Pharmaceutically Acceptable Salts.

[00128] The invention provides the use of pharmaceutically-acceptable salts of any therapeutic compound described herein. Pharmaceutically-acceptable salts include, for example, acid-addition salts and base-addition salts. The acid that is added to the compound to form an acid-addition salt can be an organic acid or an inorganic acid. A base that is added to the compound to form a base-addition salt can be an organic base or an inorganic base. In some embodiments, a pharmaceutically-acceptable salt is a metal salt. In some embodiments, a pharmaceutically-acceptable salt is an ammonium salt.

[00129] Metal salts can arise from the addition of an inorganic base to a compound of the invention. The inorganic base consists of a metal cation paired with a basic counterion, such as, for example, hydroxide, carbonate, bicarbonate, or phosphate. The metal can be an alkali metal, alkaline earth metal, transition metal, or main group metal. In some embodiments, the metal is lithium, sodium, potassium, cesium, cerium, magnesium, manganese, iron, calcium, strontium, cobalt, titanium, aluminum, copper, cadmium, or zinc.

[00130] In some embodiments, a metal salt is a lithium salt, a sodium salt, a potassium salt, a cesium salt, a cerium salt, a magnesium salt, a manganese salt, a iron salt, a calcium salt, a strontium salt, a cobalt salt, a titanium salt, an aluminum salt, a copper salt, a cadmium salt, or a zinc salt.

[00131] Ammonium salts can arise from the addition of ammonia or an organic amine to a

compound of the invention. In some embodiments, the organic amine is triethyl amine, diisopropyl amine, ethanol amine, diethanol amine, triethanol amine, morpholine, N-methylmorpholine, piperidine, N-methylpiperidine, N-ethylpiperidine, dibenzylamine, piperazine, pyridine, pyrrazole, pipyrrazole, imidazole, pyrazine, or pipyrazine.

[00132] In some embodiments, an ammonium salt is a triethyl amine salt, a diisopropyl amine salt, an ethanol amine salt, a diethanol amine salt, a triethanol amine salt, a morpholine salt, an N-methylmorpholine salt, a piperidine salt, an N-methylpiperidine salt, an N-ethylpiperidine salt, a dibenzylamine salt, a piperazine salt, a pyridine salt, a pyrrazole salt, a pipyrrazole salt, an imidazole salt, a pyrazine salt, or a pipyrazine salt.

[00133] Acid addition salts can arise from the addition of an acid to a compound of the invention. In some embodiments, the acid is organic. In some embodiments, the acid is inorganic. In some embodiments, the acid is hydrochloric acid, hydrobromic acid, hydroiodic acid, nitric acid, nitrous acid, sulfuric acid, sulfurous acid, a phosphoric acid, isonicotinic acid, lactic acid, salicylic acid, tartaric acid, ascorbic acid, gentisinic acid, gluconic acid, glucaronic acid, saccharic acid, formic acid, benzoic acid, glutamic acid, pantothenic acid, acetic acid, propionic acid, butyric acid, fumaric acid, succinic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, p-toluenesulfonic acid, citric acid, oxalic acid, or maleic acid.

[00134] In some embodiments, the salt is a hydrochloride salt, a hydrobromide salt, a hydroiodide salt, a nitrate salt, a nitrite salt, a sulfate salt, a sulfite salt, a phosphate salt, isonicotinate salt, a lactate salt, a salicylate salt, a tartrate salt, an ascorbate salt, a gentisinate salt, a gluconate salt, a glucaronate salt, a saccharate salt, a formate salt, a benzoate salt, a glutamate salt, a pantothenate salt, an acetate salt, a propionate salt, a butyrate salt, a fumarate salt, a succinate salt, a methanesulfonate (mesylate) salt, an ethanesulfonate salt, a benzenesulfonate salt, a p-toluenesulfonate salt, a citrate salt, an oxalate salt, or a maleate salt.

EXAMPLES

Example 1: Incorporation of Pd(II) into a MAP and general experimental conditions.

[00135] To determine solubility of a series of MAP-containing peptides with and without metal, palladium was chosen as a representative metal for comprehensive screening and was incorporated using a series of solution conditions. As illustrated in **FIGURE 1**, Pd(II) nitrilotriacetic acid (PD-NTA) was used to incorporate Pd(II) into the peptides of **FIGURE 2**.

[00136] Peptides were dissolved in 50 mM potassium phosphate (KPi) buffer at pH 7.4. Potassium tetrachloropalladate was pre-complexed with nitrilotriacetic acid (NTA) in 50 mM KPi at pH 7.4 (Pd-NTA). One equivalent of Pd-NTA was added to half of each peptide solution;

both apo- and Pd-peptides were allowed to incubate for 1 hour, 24 hours, and 2 weeks before collecting absorbance data. To avoid light scattering of samples that were not fully soluble, all samples were centrifuged to pellet any remaining solid and the supernatant was analyzed.

[00137] Percent solubility for the apo peptide was determined by monitoring the absorbance at 220 nm (peptide backbone) and was normalized against the highest A_{220} measured. Percent palladium incorporation was determined by monitoring the absorbance at 380 nm. Pd-MAP complex formation was confirmed by visible absorption profile and as such the intensity of the complex was measured and quantified using the A_{380} peak. Data were normalized against the highest A_{380} measured.

[00138] Palladium incorporation into marginally soluble peptides that contain hydrophobic residues, such as NCCGW (SEQ ID No. 5), indicated that *in situ* creation of a charged species improved peptide solubility. The peptides ac-WKCCW-am (SEQ ID No. 3) and ac-KCCLW-am (SEQ ID No. 4) showed decreased solubility, however, which was likely due to crossing of the neutral charge point during metal incorporation, indicating crossing the neutral point is dominant over hydrophobicity in determining overall solubility of the product.

[00139] Incorporation of Pd(II) into the MAP resulted in an overall change in net charge of the complex by -2. **FIGURE 2** lists the peptides used (ac: acyl; am: amino), the number of charges in the MAP, certain chemical properties (basicity; number of tryptophan (W) residues; number of leucine (L) residues), the net charge of the MAP in the absence of Pd, and the net charge of the MAP/Pd complex.

Example 2: Aqueous solubility of MAPs with differing charge profiles.

[00140] Four MAPs (KCC-am, **FIGURE 3**); (ac-KCC-am, **FIGURE 4**); (KGNCC, SEQ ID No. 1, **FIGURE 5**); and (ac-KGNCC, SEQ ID No. 2, **FIGURE 6**) were tested for aqueous solubility. The solubility of the peptides alone (50 mM, pH 7.4 potassium phosphate buffer) over a two-week period (measured by UV absorption at 220 nm) is tabulated in **FIGURE 7**. KCC-am, having a net charge of +2, showed the lowest solubility. ac-KCC-am, having a only a positive charge, was significantly more soluble. KGNCC (SEQ ID No. 1), having three charges with a net charge of +1, demonstrated longer lasting solubility. ac-KGNCC (SEQ ID No. 2), a zwitterionic peptide, possessed the best combination of short and long term solubility.

Example 3: Incorporation of Pd(II) into MAP complexes.

[00141] The incorporation of Pd(II) into the MAPs of **Example 2** upon exposure to Pd(II)-NTA (50 mM, pH 7.4 potassium phosphate buffer) over a two-week period (measured by UV

absorption at 380 nm) is tabulated in **FIGURE 8**. KCC-am, having a net charge of +2, exhibited almost no solubility upon incorporation. ac-KCC-am, having a only a positive charge, showed initial incorporation, but showed loss due to insolubility over time. KGNCC (SEQ ID No. 1), having three charges with a net charge of +1, demonstrated poor incorporation and/or solubility. ac-KGNCC (SEQ ID No. 2), a zwitterionic peptide, possessed the best initial and long term incorporation and solubility, which increased over time. The zwitterionic MAP demonstrated the most desirable combination of solubility and metal incorporation.

Example 4: Effect of hydrophobic residues on MAP solubility.

[00142] Four MAPs (ac-WKCCW-am, SEQ ID No. 3, **FIGURE 9**); (ac-KCCLW-am, SEQ ID No. 4, **FIGURE 10**); (NCCGW, SEQ ID No. 5, **FIGURE 11**); and (NCCGG, SEQ ID No. 6 **FIGURE 12**) were tested for aqueous solubility. The solubility of the peptides alone (50 mM, pH 7.4 potassium phosphate buffer) over a two-week period (measured by UV absorption at 280 nm) is tabulated in **FIGURE 13**. ac-WKCCW-am (SEQ ID No. 3), having two hydrophobic side chains and only a single positive charge, showed the lowest solubility. ac-KCCLW-am (SEQ ID No. 4), having one hydrophobic side chain and only a single positive charge, was slightly more soluble. NCCGW (SEQ ID No. 5), a zwitterionic peptide having one hydrophobic side chain, demonstrated significantly greater solubility. NCCGG (SEQ ID No. 6), a zwitterionic peptide having no hydrophobic side chains, possessed the best combination of short and long term solubility.

Example 5: Effect of hydrophobic residues on incorporation of Pd(II) into MAP complexes.

[00143] The incorporation of Pd(II) into the MAPS of **Example 4** upon exposure to Pd(II)-NTA (50 mM, pH 7.4 potassium phosphate buffer) over a two-week period (measured by UV absorption at 380 nm) is tabulated in **FIGURE 14**. ac-WKCCW-am (SEQ ID No. 3), having two hydrophobic side chains and only a single positive charge, exhibited almost no incorporation. ac-KCCLW-am (SEQ ID No. 4), having one hydrophobic side chain and only a single positive charge, exhibited almost no incorporation. NCCGW (SEQ ID No. 5), a zwitterionic peptide having one hydrophobic side chain, demonstrated good short term and excellent long term incorporation. NCCGG (SEQ ID No. 6), a zwitterionic peptide having no hydrophobic side chains, exhibited very good incorporation with little fluctuation over time. The zwitterionic MAPs with zero or one hydrophobic side chain demonstrated the most desirable combination of solubility and metal incorporation.

Example 6: Effect of cosolvents on the aqueous solubility of MAPs with hydrophobic residues.

[00144] For the cosolvent study, excipients were prepared in 50 mM KPi at pH 7.4. Cosolvent conditions screened include a buffer control, 10% dimethylsulfoxide (DMSO), 0.02% cremophor, 3 M urea, and 20% sucrose. The peptides GGNCC (SEQ ID No. 7, **FIGURE 15**) and WGNCC (SEQ ID No. 8, **FIGURE 16**) were suspended in each solution, and palladium was incorporated into half of each sample as described above. The cosolvents urea and DMSO prevented reading the absorbance at 220 nm, so the solubility percentage for these samples was not quantitative.

[00145] The aqueous solubility of GGNCC (SEQ ID No. 7) cosolvated with: i) 50 mM, pH 7.4 potassium phosphate buffer; ii) 20% sucrose; iii) 3M urea; iv) 0.02% cremaphor; or v) 10% DMSO, is tabulated in **FIGURE 17**. Urea and DMSO prevented detection in their respective experiments. In the other three cases, solubility was high over two weeks. The aqueous solubility of WGNCC (SEQ ID No. 8) cosolvated with: i) 50 mM, pH 7.4 potassium phosphate buffer; ii) 20% sucrose; iii) 3M urea; iv) 0.02% cremaphor; or v) 10% DMSO, is tabulated in **FIGURE 18**. Solubility was high over two weeks in all cases.

Example 7: Effect of cosolvents on incorporation of Pd(II) into MAP complexes with hydrophobic residues.

[00146] The incorporation of Pd(II) into GGNCC (SEQ ID No. 7) upon exposure to Pd(II)- NTA in the presence of a cosolvent over a two-week period (measured by UV absorption at 380 nm) is tabulated in **FIGURE 19**. Incorporation in the presence of 50 mM, pH 7.4 potassium phosphate buffer and: i) buffer alone; ii) 20% sucrose; iii) 3M urea; iv) 0.02% cremaphor; or v) 10% DMSO, is tabulated. Potassium phosphate buffer provided increasing incorporation over time. Urea provided decreasing incorporation over time. Sucrose, cremaphor, and DMSO provided less consistent incorporation.

[00147] The incorporation of Pd(II) into WGNCC (SEQ ID No. 8) upon exposure to Pd(II)- NTA in the presence of a cosolvent over a two-week period (measured by UV absorption at 380 nm) is tabulated in **FIGURE 20**. Incorporation in the presence of 50 mM, pH 7.4 potassium phosphate buffer and: i) buffer alone; ii) 20% sucrose; iii) 3M urea; iv) 0.02% cremaphor; or v) 10% DMSO, is tabulated. Potassium phosphate buffer and sucrose provided decreasing incorporation over time. Urea provided excellent incorporation. Cremaphor and DMSO provided good initial incorporation, which then decayed.

[00148] Table 2 summarizes exemplary peptides of the invention.

Table 2	
Peptide	SEQ ID No.
KGNC *	1
ac-KGNC *	2
ac-WKCCW-am *	3
ac-KCCLW-am *	4
NCCGW	5
NCCGG	6
GGNC	7
WGNCC	8

* ac: acyl; am: amino

Example 8: Incorporation of a small organic moiety into an antibody.

[00149] To a solution of succinic anhydride (0.05 mmol) in methylene chloride at 0 °C under argon is added Bevacizumab (0.05 mmol), and the solution is slowly warmed to room temperature and stirred for 10 hours. The solution is concentrated to dryness, filtered through celite, and the resultant antibody complex is purified by high performance liquid chromatography. The presence of Bevacizumab labeled with at least one succinyl group is confirmed by high resolution mass spectrometry or MALDI. One of the products to be isolated from the reaction mixture is the complex illustrated in **FIGURE 21**.

Example 9: Incorporation of a small organic moiety into an antibody via linker.

[00150] Step 1. To a solution of suberoyl chloride (0.01 mmol) in methylene chloride at 0 °C under argon is added *N*-hydroxy succinamide (NOS; 0.02 mmol), triethyl amine (0.05 mmol), and 4-dimethylaminopyridine (DMAP; 0.0005 mmol), and the resultant mixture is warmed to room temperature and stirred for 5 hours. The mixture is concentrated to dryness and filtered through dry silica gel under nitrogen. The filtrate is concentrated to a residue and taken into dimethylsulfoxide to provide a solution of disuccinimidyl suberate.

[00151] Step 2. To the solution of disuccinimidyl suberate in dimethylsulfoxide is added a mixture of 2-aminobutyric acid (0.01 mmol) in buffer (20 mM sodium phosphate, 0.15 M NaCl, PBS, pH 7.5) at 0 °C under argon, and the resultant mixture is warmed to room temperature and stirred for 16 hours. To the resultant mixture is added Bevacizumab (0.01 mmol) in buffer (20 mM sodium phosphate, 0.15 M NaCl, PBS, pH 7.5) at 0 °C under argon, and the resultant mixture is warmed to room temperature and stirred for another 16 hours. The resultant mixture is

washed with ether, concentrated to dryness, and the resultant antibody complex is purified by high performance liquid chromatography. The presence of Bevacizumab labeled with at least one 2-butyric acid group is confirmed by high resolution mass spectrometry or MALDI. One of the products to be isolated from the reaction mixture is the complex illustrated in **FIGURE 22**.

EMBODIMENTS

[00152] The following non-limiting embodiments provide illustrative examples of the invention, but do not limit the scope of the invention.

[00153] Embodiment 1. A composition comprising a concentration of a synthetic complex, or a pharmaceutically-acceptable salt thereof, wherein the synthetic complex comprises a therapeutic molecule covalently bound to an additional group, wherein the composition has a viscosity less than that of a second composition, wherein the second composition comprises the therapeutic molecule at the concentration in absence of the covalently-bound additional group.

[00154] Embodiment 2. The composition of Embodiment 1, wherein the therapeutic molecule is a biomolecule.

[00155] Embodiment 3. The composition of any one of Embodiments 1 and 2, wherein the therapeutic molecule is a peptide.

[00156] Embodiment 4. The composition of any one of Embodiments 1-3, wherein the therapeutic molecule is a protein.

[00157] Embodiment 5. The composition of any one of Embodiments 1-4, wherein the therapeutic molecule is an antibody.

[00158] Embodiment 6. The composition of any one of Embodiments 1-5, wherein the synthetic complex is more soluble than is the therapeutic molecule.

[00159] Embodiment 7. The composition of any one of Embodiments 1-6, wherein the additional group is a non-peptide organic group.

[00160] Embodiment 8. The composition of any one of Embodiments 1-7, wherein the additional group is a peptide.

[00161] Embodiment 9. The composition of Embodiment 8, wherein the peptide comprises two neighboring sulfur-containing amino acid residues.

[00162] Embodiment 10. The composition of any one of Embodiments 1-9, wherein one or both of the neighboring sulfur-containing amino acid residues is cysteine.

[00163] Embodiment 11. The composition of any one of Embodiments 1-9, wherein the peptide binds a metal.

- [00164] Embodiment 12. The composition of Embodiment 11, wherein the peptide binds the metal in a square planar geometry.
- [00165] Embodiment 13. The composition of Embodiment 11, wherein the peptide binds the metal in a square pyramidal geometry.
- [00166] Embodiment 14. The composition of Embodiment 11, wherein the peptide binds the metal through two sulfur-containing amino acid residues.
- [00167] Embodiment 15. The composition of any one of Embodiments 1-14, wherein the additional group has a molecular weight no greater than 2,000 Daltons.
- [00168] Embodiment 16. The composition of any one of Embodiments 1-15, wherein the additional group has a negative charge.
- [00169] Embodiment 17. The composition of any one of Embodiments 1-16, wherein the additional group is covalently bound to the therapeutic molecule through a linker.
- [00170] Embodiment 18. The composition of any one of Embodiments 1-17, wherein the composition is an emulsion.
- [00171] Embodiment 19. The composition of any one of Embodiments 1-18, wherein the composition is a colloid.
- [00172] Embodiment 20. The composition of any one of Embodiments 1-19, wherein the composition is a suspension.
- [00173] Embodiment 21. The composition of any one of Embodiments 1-20, wherein the composition is a liposome.
- [00174] Embodiment 22. A synthetic complex, or a pharmaceutically-acceptable salt thereof, comprising a therapeutic molecule covalently bound to an additional group, wherein a composition comprising the complex at a concentration has a viscosity less than that of a second composition, wherein the second composition comprises the therapeutic molecule at the concentration in absence of the covalently-bound additional group.
- [00175] Embodiment 23. The complex of Embodiment 22, wherein the therapeutic molecule is a biomolecule.
- [00176] Embodiment 24. The complex of any one of Embodiments 22 and 23, wherein the therapeutic molecule is a peptide.
- [00177] Embodiment 25. The complex of any one of Embodiments 22-24, wherein the therapeutic molecule is a protein.
- [00178] Embodiment 26. The complex of any one of Embodiments 22-25, wherein the therapeutic molecule is an antibody.

- [00179] Embodiment 27. The complex of any one of Embodiments 22-26, wherein the synthetic complex is more soluble than is the therapeutic molecule.
- [00180] Embodiment 28. The complex of any one of Embodiments 22-27, wherein the additional group is a non-peptide organic group.
- [00181] Embodiment 29. The complex of any one of Embodiments 22-28, wherein the additional group is a peptide.
- [00182] Embodiment 30. The complex of Embodiment 29, wherein the peptide comprises two neighboring sulfur-containing amino acid residues.
- [00183] Embodiment 31. The complex of any one of Embodiments 22-29, wherein one or both of the neighboring sulfur-containing amino acid residues is cysteine.
- [00184] Embodiment 32. The complex of any one of Embodiments 29-31, wherein the peptide binds a metal.
- [00185] Embodiment 33. The complex of any one of Embodiments 29-32, wherein the peptide binds the metal in a square planar geometry.
- [00186] Embodiment 34. The complex of any one of Embodiments 29-33, wherein the peptide binds the metal in a square pyramidal geometry.
- [00187] Embodiment 35. The complex of any one of Embodiments 29-34, wherein the peptide binds the metal through two sulfur-containing amino acid residues.
- [00188] Embodiment 36. The complex of any one of Embodiments 22-35, wherein the additional group has a molecular weight no greater than 2,000 Daltons.
- [00189] Embodiment 37. The complex of any one of Embodiments 22-36, wherein the additional group has a negative charge.
- [00190] Embodiment 38. The complex of any one of Embodiments 22-37, wherein the additional group is covalently bound to the therapeutic molecule through a linker.
- [00191] Embodiment 39. The complex of any one of Embodiments 22-38, wherein the composition is an emulsion.
- [00192] Embodiment 40. The complex of any one of Embodiments 22-39, wherein the composition is a colloid.
- [00193] Embodiment 41. The complex of any one of Embodiments 22-40, wherein the composition is a suspension.
- [00194] Embodiment 42. The complex of any one of Embodiments 22-41, wherein the composition is a liposome.
- [00195] Embodiment 43. A method of treating a condition, the method comprising administering to a subject in need or want of relief thereof a composition comprising a

therapeutically-effective amount of a synthetic complex, wherein the synthetic complex comprises a therapeutic molecule and an additional group, and wherein the composition has a viscosity less than that of a second composition, wherein the second composition comprises the therapeutic molecule at the concentration in absence of the covalently-bound additional group.

[00196] Embodiment 44. The method of Embodiment 43, wherein the therapeutic molecule is a biomolecule.

[00197] Embodiment 45. The method of any one of Embodiments 43 and 44, wherein the therapeutic molecule is a peptide.

[00198] Embodiment 46. The method of any one of Embodiments 43-45, wherein the therapeutic molecule is a protein.

[00199] Embodiment 47. The method of any one of Embodiments 43-46, wherein the therapeutic molecule is an antibody.

[00200] Embodiment 48. The method of any one of Embodiments 43-47, wherein the synthetic complex is more soluble than is the therapeutic molecule.

[00201] Embodiment 49. The method of any one of Embodiments 43-48, wherein the additional group is a non-peptide organic group.

[00202] Embodiment 50. The method of any one of Embodiments 43-49, wherein the additional group is a peptide.

[00203] Embodiment 51. The method of Embodiment 50, wherein the peptide comprises two neighboring sulfur-containing amino acid residues.

[00204] Embodiment 52. The method of Embodiments 51, wherein one or both of the neighboring sulfur-containing amino acids is cysteine.

[00205] Embodiment 53. The method of any one of Embodiments 50-52, wherein the peptide binds a metal.

[00206] Embodiment 54. The method of any one of Embodiments 50-53, wherein the peptide binds the metal in a square planar geometry.

[00207] Embodiment 55. The method of any one of Embodiments 50-54, wherein the peptide binds the metal in a square pyramidal geometry.

[00208] Embodiment 56. The method of any one of Embodiments 50-55, wherein both sulfur-containing amino acid residues bind the metal.

[00209] Embodiment 57. The method of any one of Embodiments 43-56, wherein the additional group has a molecular weight no greater than 2,000 Daltons.

[00210] Embodiment 58. The method of any one of Embodiments 43-57, wherein the additional group has a negative charge.

- [00211] Embodiment 59. The method of any one of Embodiments 43-58, wherein the additional group is covalently bound to the therapeutic molecule through a linker.
- [00212] Embodiment 60. The method of any one of Embodiments 43-59, wherein the composition is an emulsion.
- [00213] Embodiment 61. The method of any one of Embodiments 43-60, wherein the composition is a colloid.
- [00214] Embodiment 62. The method of any one of Embodiments 43-61, wherein the composition is a suspension.
- [00215] Embodiment 63. The method of any one of Embodiments 43-62, wherein the composition is a liposome.
- [00216] Embodiment 64. The method of any one of Embodiments 43-63, wherein the composition is a unit dosage form comprising a pharmaceutically-acceptable carrier.
- [00217] Embodiment 65. The method of any one of Embodiments 43-64, wherein the administration is oral.
- [00218] Embodiment 66. The method of any one of Embodiments 43-65, wherein the administration is intravenous.
- [00219] Embodiment 67. The method of any one of Embodiments 43-66, wherein the condition is a cancer.
- [00220] Embodiment 68. The method of Embodiment 67, wherein the cancer is susceptible to treatment by a metal-based therapy.
- [00221] Embodiment 69. The method of Embodiment 67, wherein the cancer is susceptible to treatment by a platinum-based therapy.
- [00222] Embodiment 70. The method of any one of Embodiments 67-69, wherein the cancer is EGRF positive.
- [00223] Embodiment 71. The method of any one of Embodiments 67-70, wherein the cancer is HER2 positive.

CLAIMS

WHAT IS CLAIMED IS:

1. A composition comprising a concentration of a synthetic complex, or a pharmaceutically-acceptable salt thereof, wherein the synthetic complex comprises a therapeutic molecule covalently bound to an additional group, wherein the composition has a viscosity less than that of a second composition, wherein the second composition comprises the therapeutic molecule at the concentration in absence of the covalently-bound additional group.
2. The composition of claim 1, wherein the therapeutic molecule is a biomolecule.
3. The composition of claim 1, wherein the therapeutic molecule is a peptide.
4. The composition of claim 1, wherein the therapeutic molecule is a protein.
5. The composition of claim 1, wherein the therapeutic molecule is an antibody.
6. The composition of claim 1, wherein the synthetic complex is more soluble than is the therapeutic molecule.
7. The composition of claim 1, wherein the additional group is a non-peptide organic group.
8. The composition of claim 1, wherein the additional group is a peptide.
9. The composition of claim 8, wherein the peptide comprises two neighboring sulfur-containing amino acid residues.
10. The composition of claim 9, wherein one or both of the neighboring sulfur-containing amino acid residues is cysteine.
11. The composition of claim 8, wherein the peptide binds a metal.
12. The composition of claim 11, wherein the peptide binds the metal in a square planar

geometry.

13. The composition of claim 11, wherein the peptide binds the metal in a square pyramidal geometry.
14. The composition of claim 11, wherein the peptide binds the metal through two sulfur-containing amino acid residues.
15. The composition of claim 1, wherein the additional group has a molecular weight no greater than 2,000 Daltons.
16. The composition of claim 1, wherein the additional group has a negative charge.
17. The composition of claim 1, wherein the additional group is covalently bound to the therapeutic molecule through a linker.
18. The composition of claim 1, wherein the composition is an emulsion.
19. The composition of claim 1, wherein the composition is a colloid.
20. The composition of claim 1, wherein the composition is a suspension.
21. The composition of claim 1, wherein the composition is a liposome.
22. A synthetic complex, or a pharmaceutically-acceptable salt thereof, comprising a therapeutic molecule covalently bound to an additional group, wherein a composition comprising the complex at a concentration has a viscosity less than that of a second composition, wherein the second composition comprises the therapeutic molecule at the concentration in absence of the covalently-bound additional group.
23. The complex of claim 22, wherein the therapeutic molecule is a biomolecule.
24. The complex of claim 22, wherein the therapeutic molecule is a peptide.

25. The complex of claim 22, wherein the therapeutic molecule is a protein.
26. The complex of claim 22, wherein the therapeutic molecule is an antibody.
27. The complex of claim 22, wherein the synthetic complex is more soluble than is the therapeutic molecule.
28. The complex of claim 22, wherein the additional group is a non-peptide organic group.
29. The complex of claim 22, wherein the additional group is a peptide.
30. The complex of claim 29, wherein the peptide comprises two neighboring sulfur-containing amino acid residues.
31. The complex of claim 30, wherein one or both of the neighboring sulfur-containing amino acid residues is cysteine.
32. The complex of claim 29, wherein the peptide binds a metal.
33. The complex of claim 32, wherein the peptide binds the metal in a square planar geometry.
34. The complex of claim 32, wherein the peptide binds the metal in a square pyramidal geometry.
35. The complex of claim 32, wherein the peptide binds the metal through two sulfur-containing amino acid residues.
36. The complex of claim 22, wherein the additional group has a molecular weight no greater than 2,000 Daltons.
37. The complex of claim 22, wherein the additional group has a negative charge.
38. The complex of claim 22, wherein the additional group is covalently bound to the

therapeutic molecule through a linker.

39. The complex of claim 22, wherein the composition is an emulsion.
40. The complex of claim 22, wherein the composition is a colloid.
41. The complex of claim 22, wherein the composition is a suspension.
42. The complex of claim 22, wherein the composition is a liposome.
43. A method of treating a condition, the method comprising administering to a subject in need or want of relief thereof a composition comprising a therapeutically-effective amount of a synthetic complex, wherein the synthetic complex comprises a therapeutic molecule and an additional group, and wherein the composition has a viscosity less than that of a second composition, wherein the second composition comprises the therapeutic molecule at the concentration in absence of the covalently-bound additional group.
44. The method of claim 43, wherein the therapeutic molecule is a biomolecule.
45. The method of claim 43, wherein the therapeutic molecule is a peptide.
46. The method of claim 43, wherein the therapeutic molecule is a protein.
47. The method of claim 43, wherein the therapeutic molecule is an antibody.
48. The method of claim 43, wherein the synthetic complex is more soluble than is the therapeutic molecule.
49. The method of claim 43, wherein the additional group is a non-peptide organic group.
50. The method of claim 43, wherein the additional group is a peptide.
51. The method of claim 50, wherein the peptide comprises two neighboring sulfur-containing amino acid residues.

52. The method of claim 51, wherein one or both of the neighboring sulfur-containing amino acids is cysteine.
53. The method of claim 50, wherein the peptide binds a metal.
54. The method of claim 53, wherein the peptide binds the metal in a square planar geometry.
55. The method of claim 53, wherein the peptide binds the metal in a square pyramidal geometry.
56. The method of claim 53, wherein the peptide binds the metal through two sulfur-containing amino acid residues.
57. The method of claim 43, wherein the additional group has a molecular weight no greater than 2,000 Daltons.
58. The method of claim 43, wherein the additional group has a negative charge.
59. The method of claim 43, wherein the additional group is covalently bound to the therapeutic molecule through a linker.
60. The method of claim 43, wherein the composition is an emulsion.
61. The method of claim 43, wherein the composition is a colloid.
62. The method of claim 43, wherein the composition is a suspension.
63. The method of claim 43, wherein the composition is a liposome.
64. The method of claim 43, wherein the composition is a unit dosage form comprising a pharmaceutically-acceptable carrier.
65. The method of claim 43, wherein the administration is oral.

- 66. The method of claim 43, wherein the administration is intravenous.
- 67. The method of claim 43, wherein the condition is a cancer.
- 68. The method of claim 67, wherein the cancer is susceptible to treatment by a metal-based therapy.
- 69. The method of claim 67, wherein the cancer is susceptible to treatment by a platinum-based therapy.
- 70. The method of claim 67, wherein the cancer is EGRF positive.
- 71. The method of claim 67, wherein the cancer is HER2 positive.

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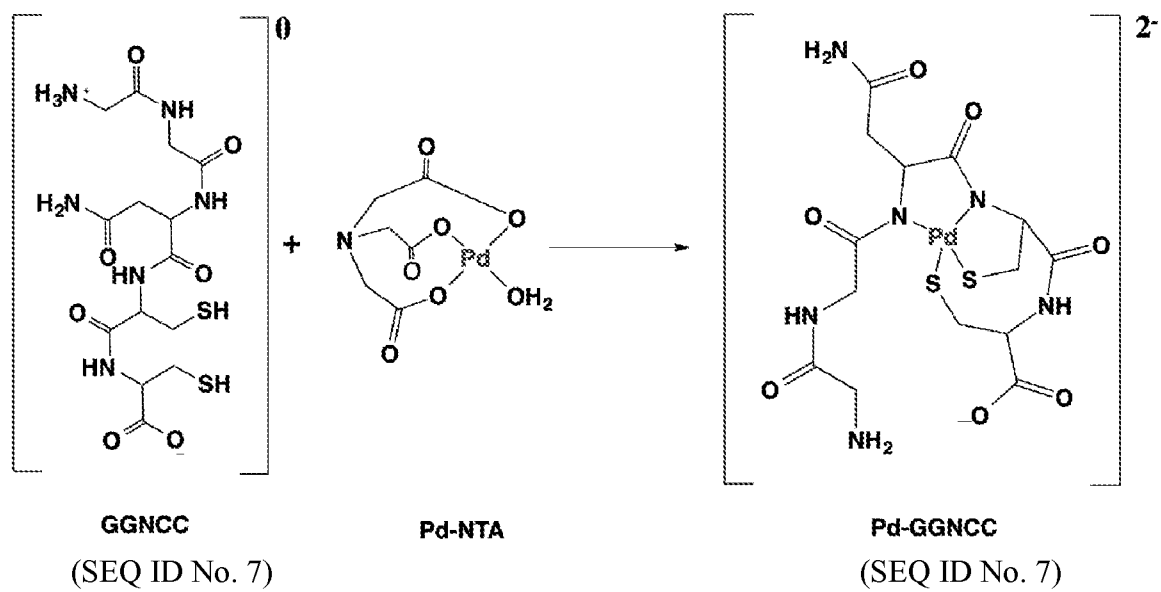


FIGURE 1

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Peptide	# Of Charged Moieties	Chemical Properties	Net Charge (- Metal)	Net Charge (+ Metal)
KCC-am	2	Basic	+ 2	0
ac-KCC-am	1	Basic	+ 1	-1
KGNC (SEQ ID No. 1)	3	Basic	+ 1	-1
ac-KGNC (SEQ ID No. 2)	2	Basic	0	-2
ac-WKCCW-am (SEQ ID No. 3)	1	2W	+1	-1
ac-KCCLW-am (SEQ ID No. 4)	1	Basic, 1W, 1L	+1	-1
NCCGW (SEQ ID No. 5)	2	1 W	0	-2
WGNCC (SEQ ID No. 8)	2	1 W	0	-2
NCCGG (SEQ ID No. 6)	2	-	0	-2
GGNC (SEQ ID No. 7)	2	-	0	-2

FIGURE 2

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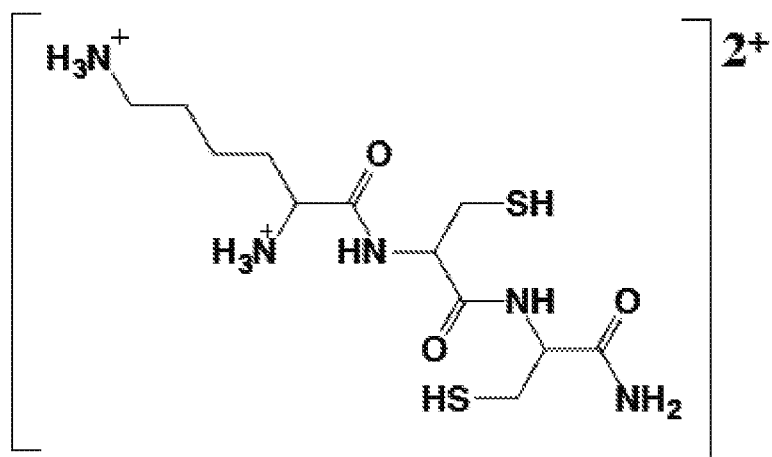
**KCC-am**

FIGURE 3

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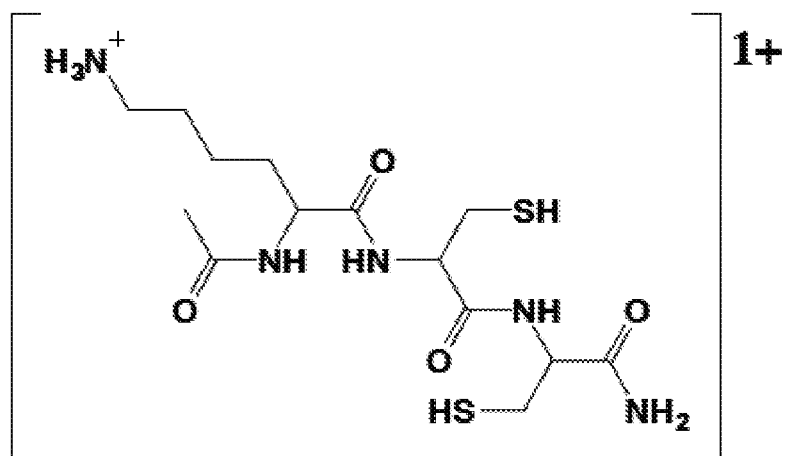
**ac-KCC-am**

FIGURE 4

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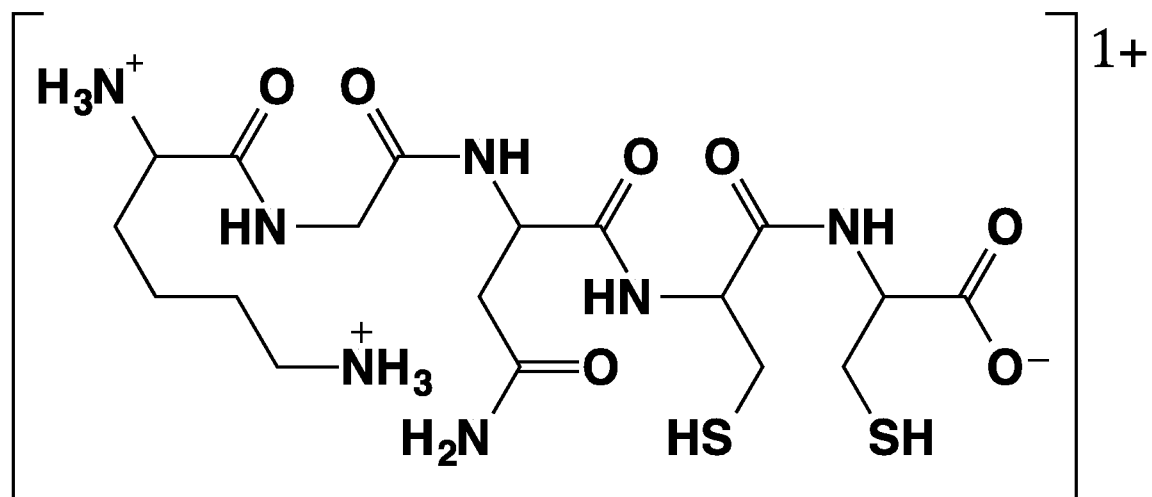
**KGNCC** (SEQ ID No. 1)

FIGURE 5

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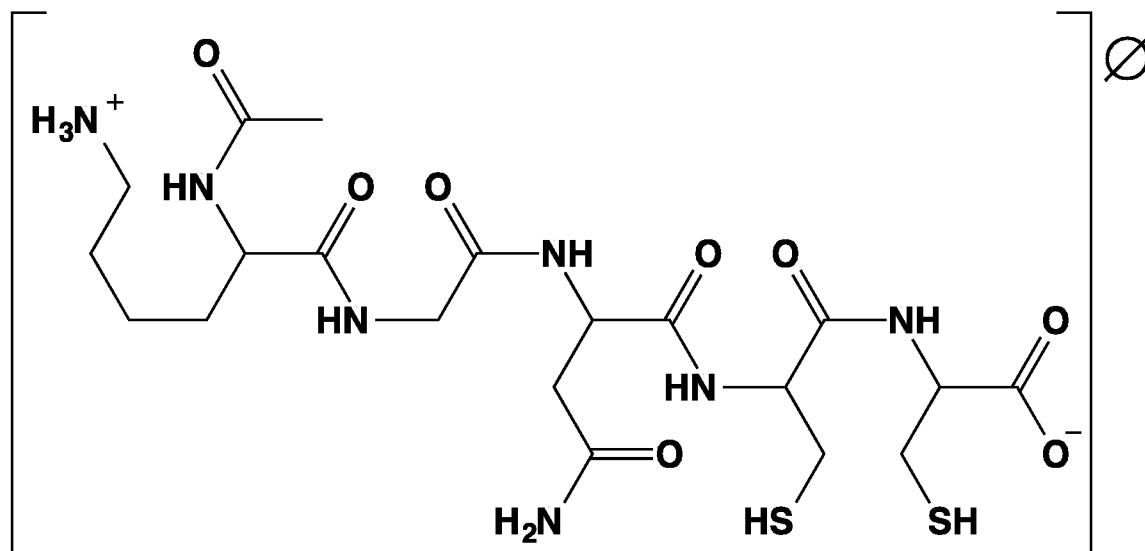
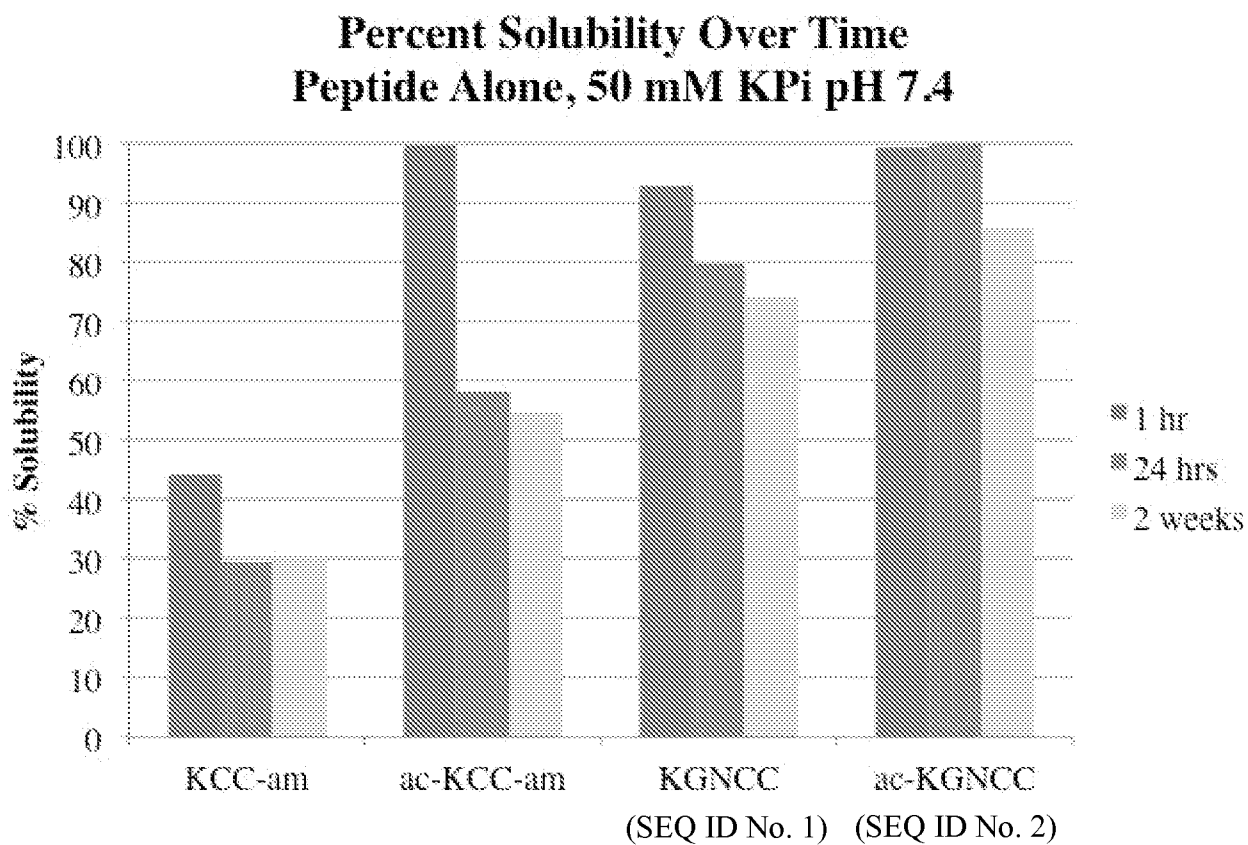
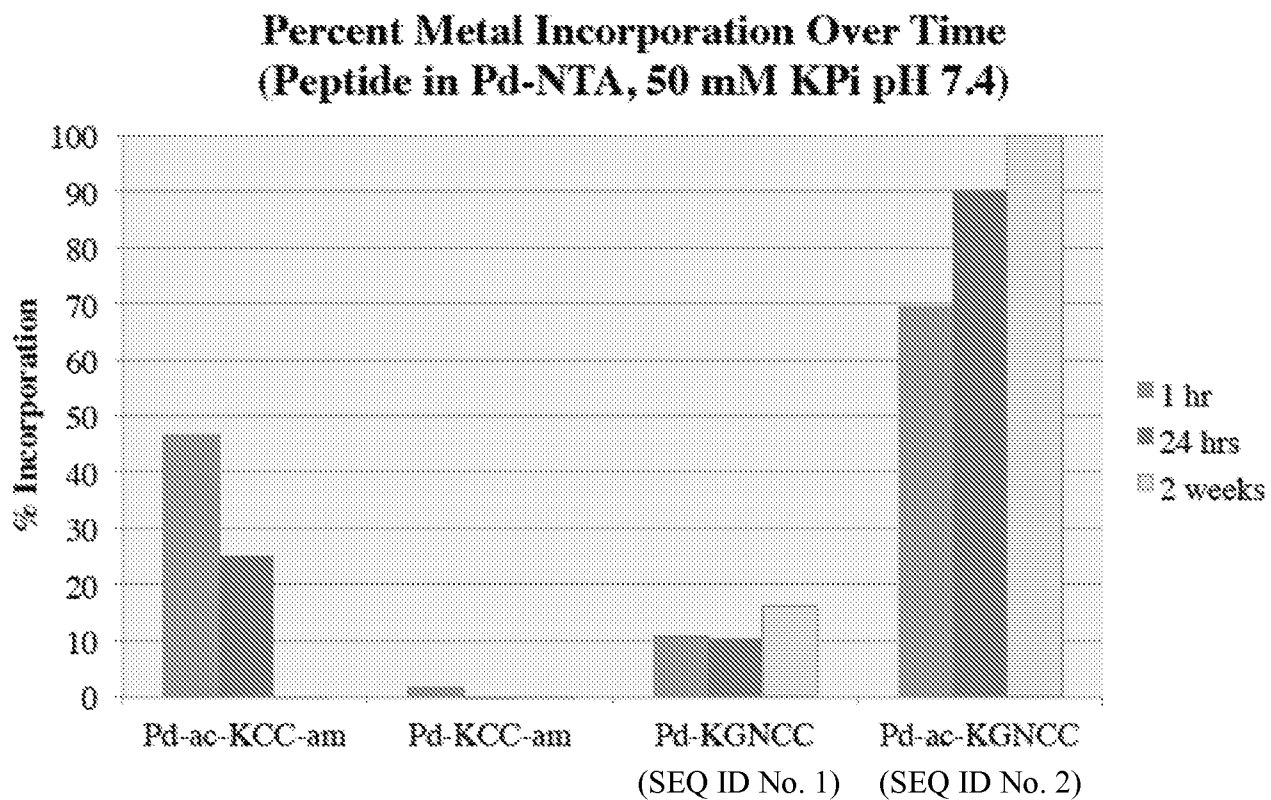
**ac-KGNCC** (SEQ ID No. 2)

FIGURE 6

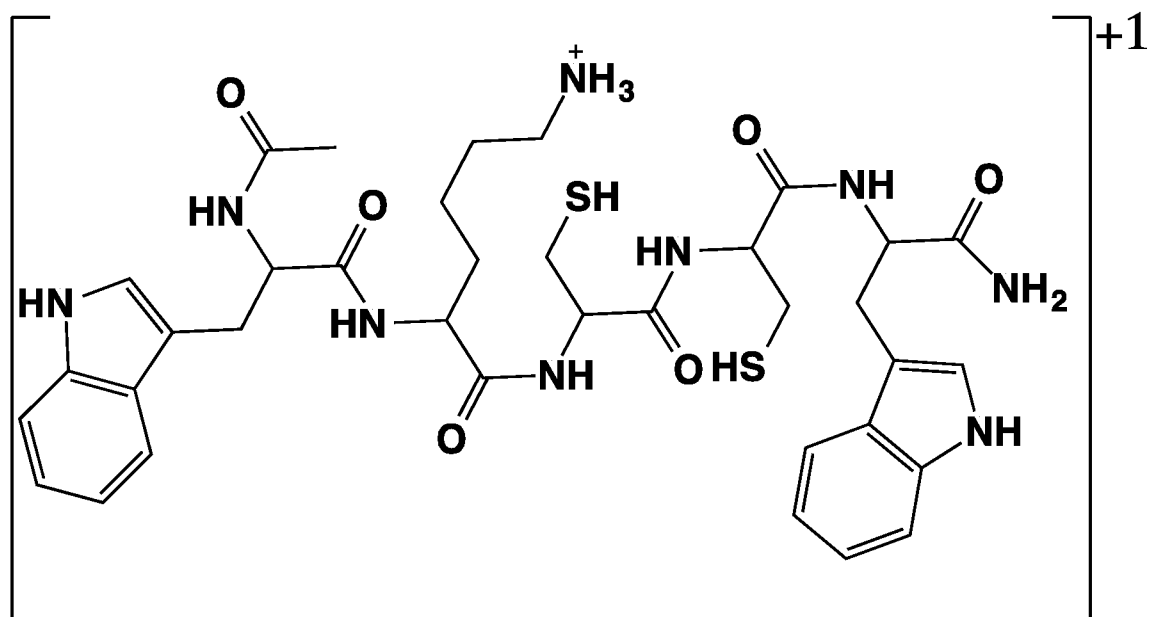
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**FIGURE 7**

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**FIGURE 8**

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**ac-WKCCW-am**

(SEQ ID No. 3)

FIGURE 9

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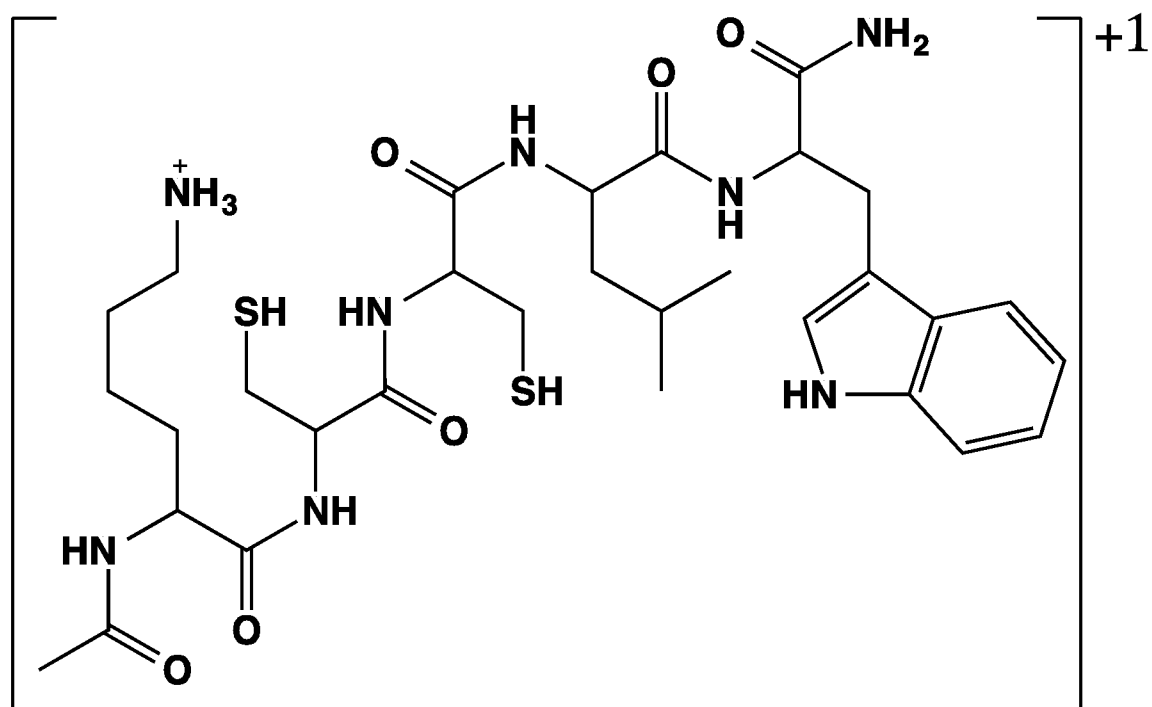
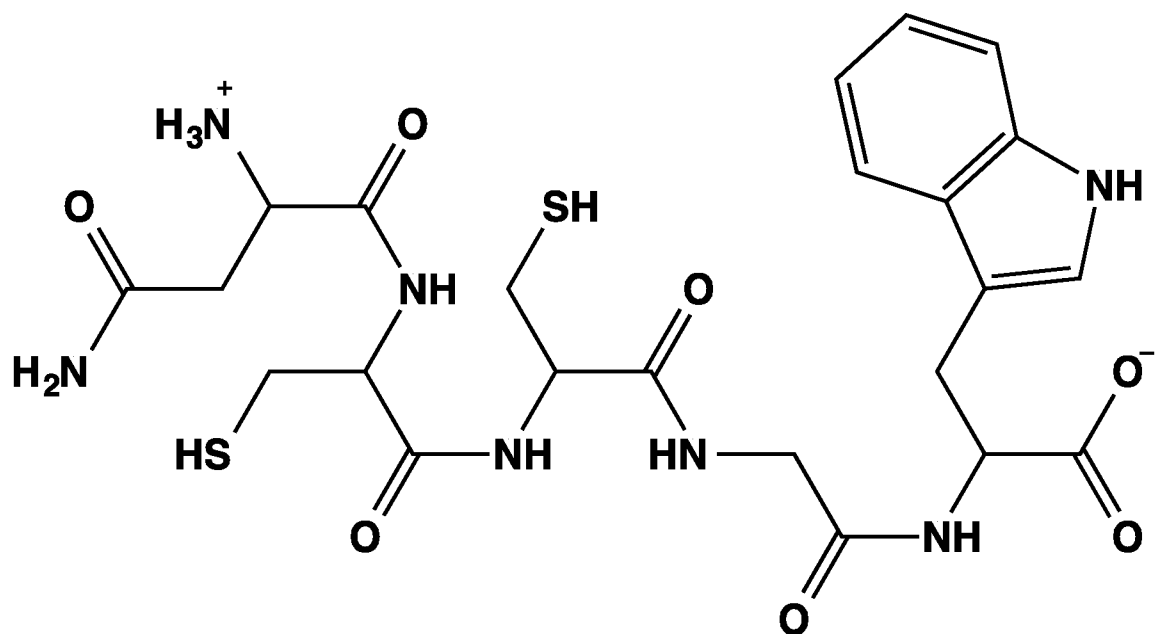
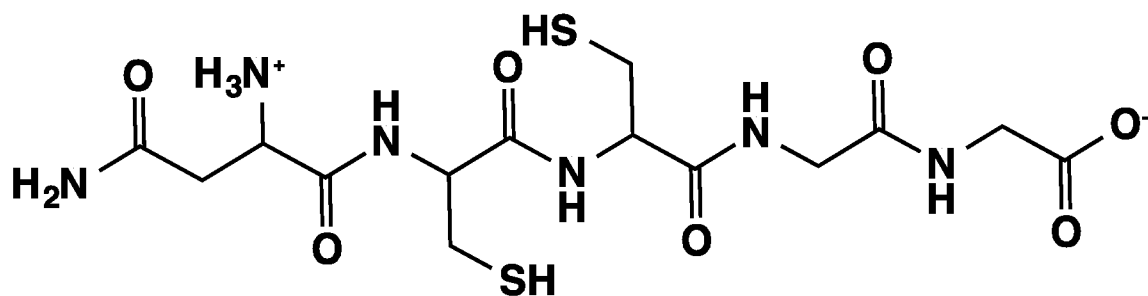
**ac-KCCLW-am****(SEQ ID No. 4)**

FIGURE 10

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**NCCGW****(SEQ ID No. 5)****FIGURE 11**

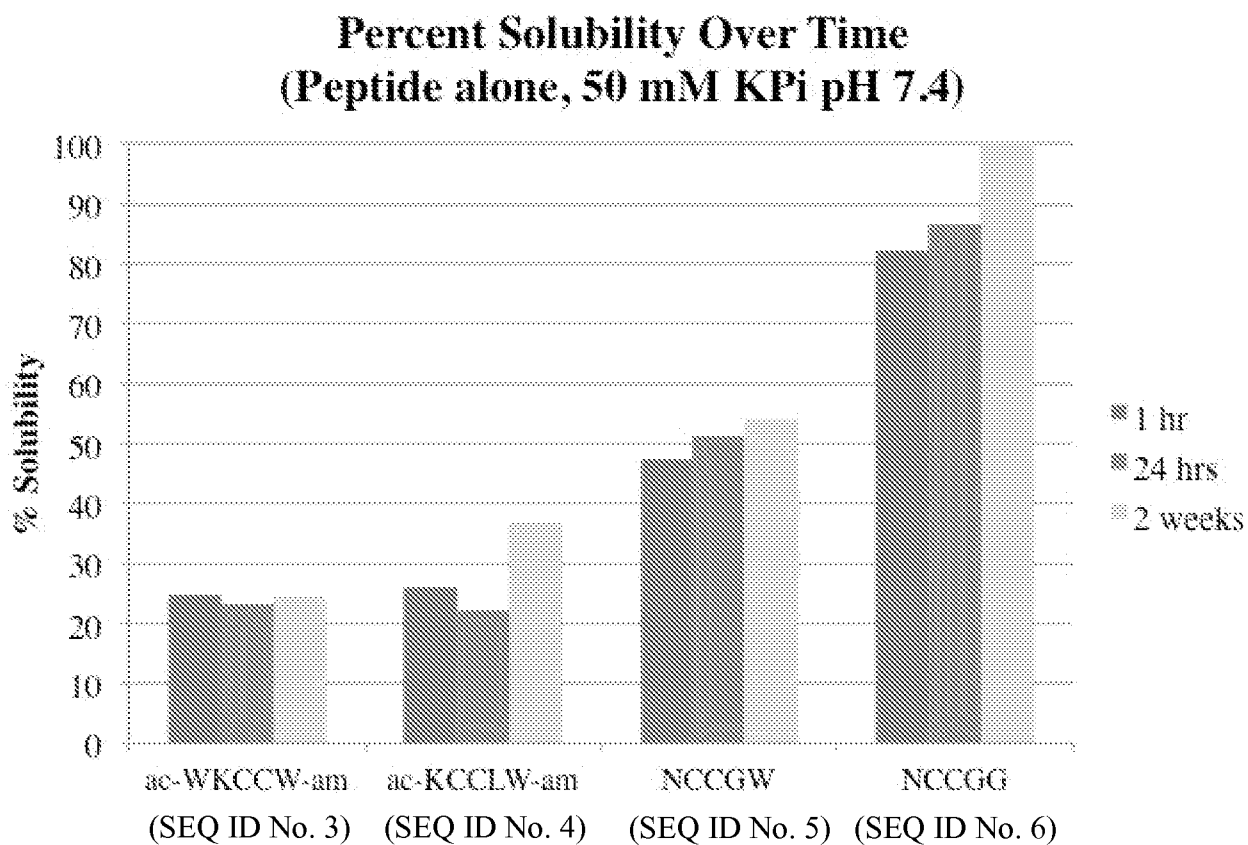
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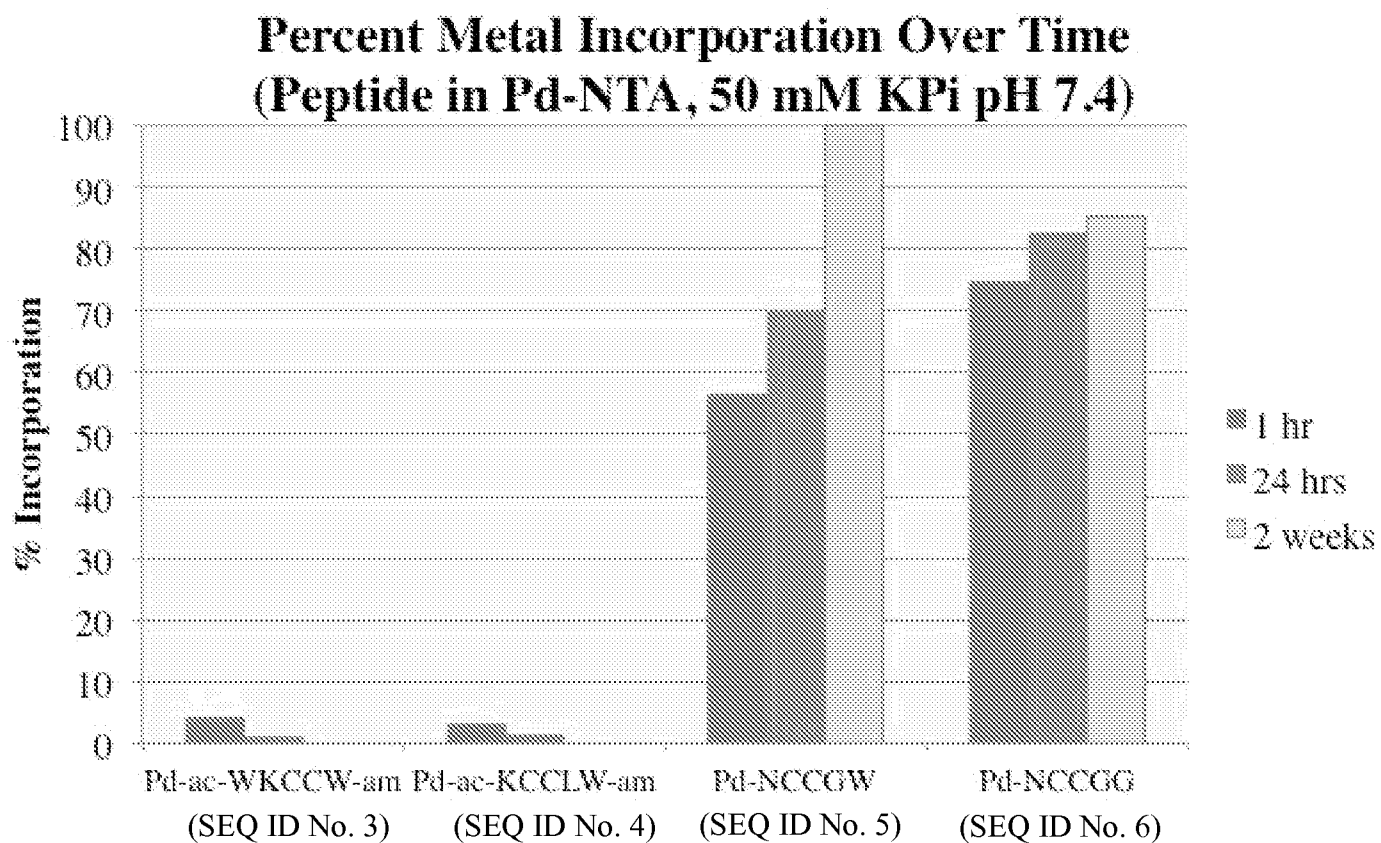
NCCGG
(SEQ ID No. 6)

FIGURE 12

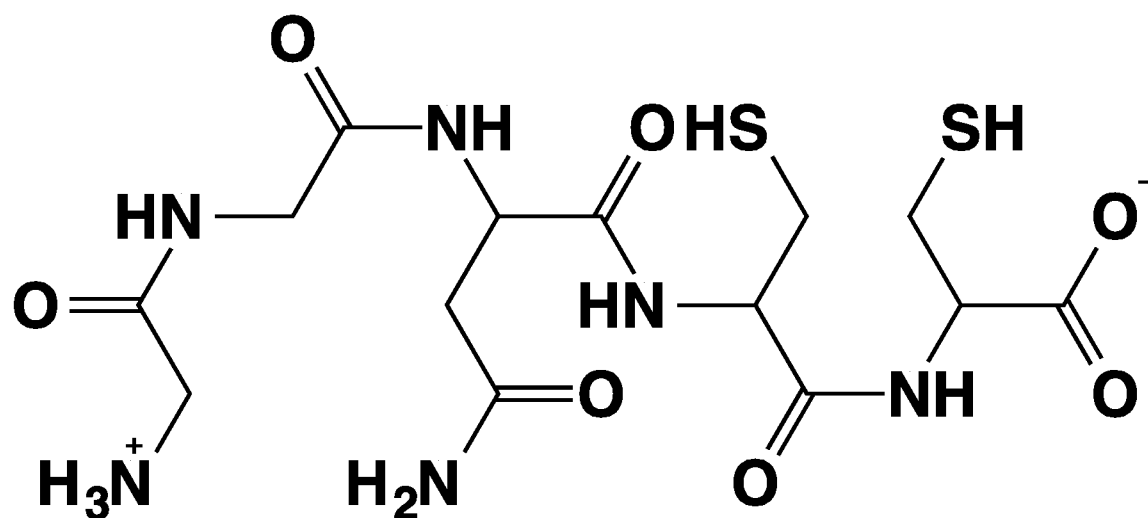
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**FIGURE 13**

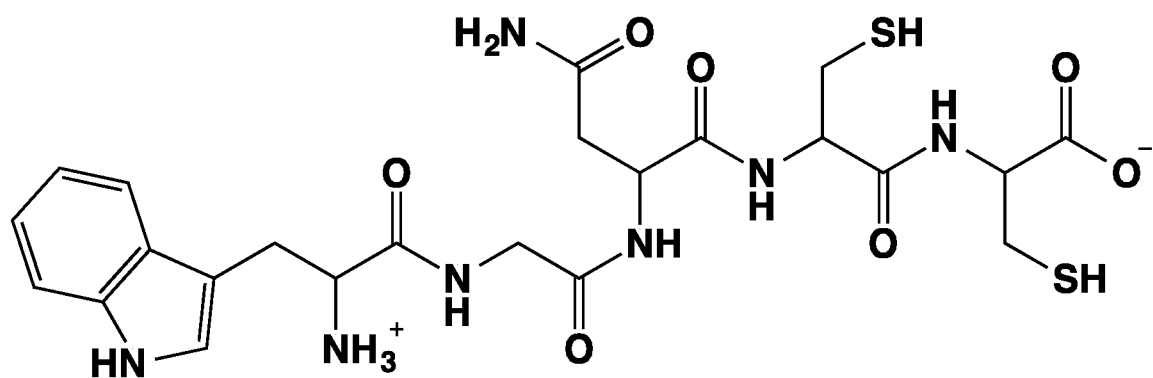
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**FIGURE 14**

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**GGNCC****(SEQ ID No. 7)****FIGURE 15**

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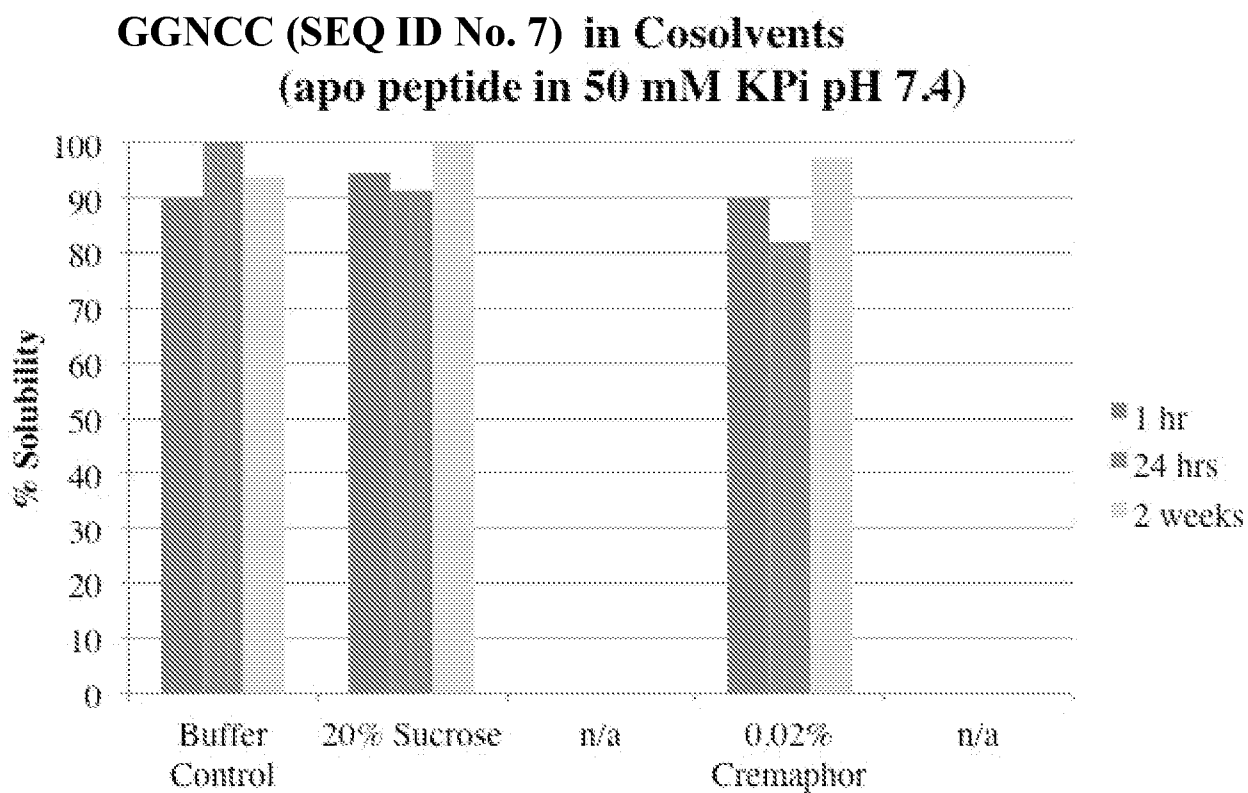


WGNCC

(SEQ ID No. 8)

FIGURE 16

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**FIGURE 17**

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WGNCC (SEQ ID No. 8) in Cosolvents
(apo peptide in 50 mM KPi pH 7.4)

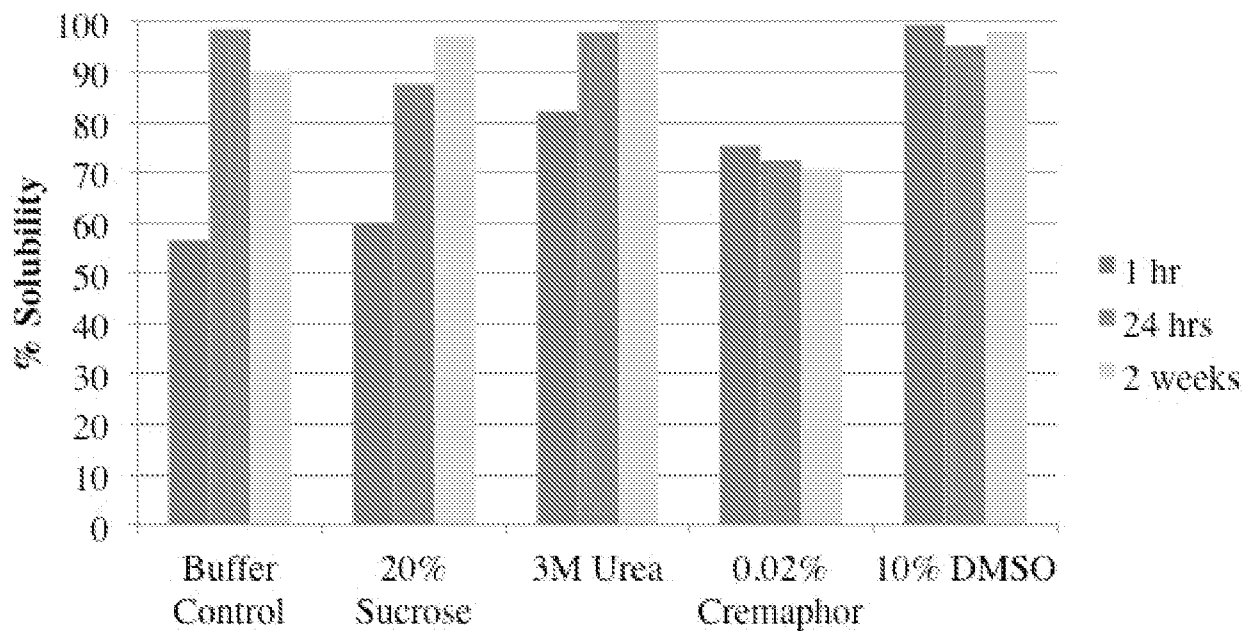


FIGURE 18

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Pd-GGNCC (SEQ ID No. 7) in Cosolvents
(Peptide in Pd-NTA, 50 mM KPi pH 7.4)

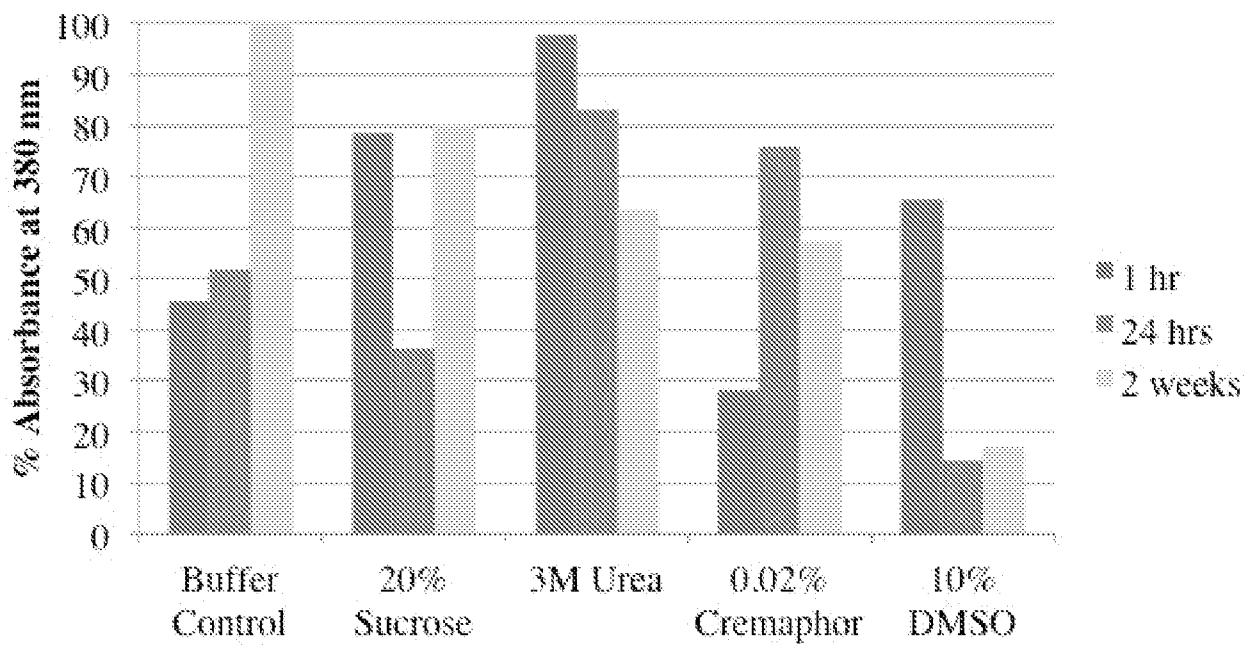


FIGURE 19

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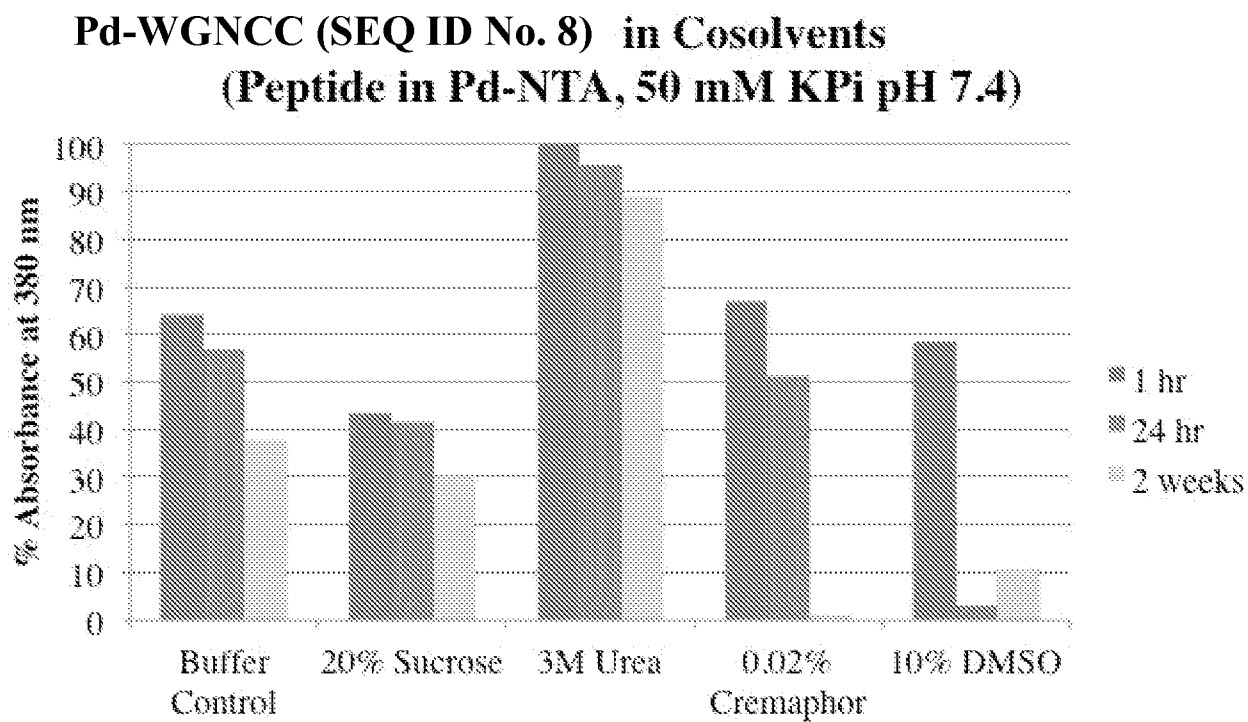


FIGURE 20

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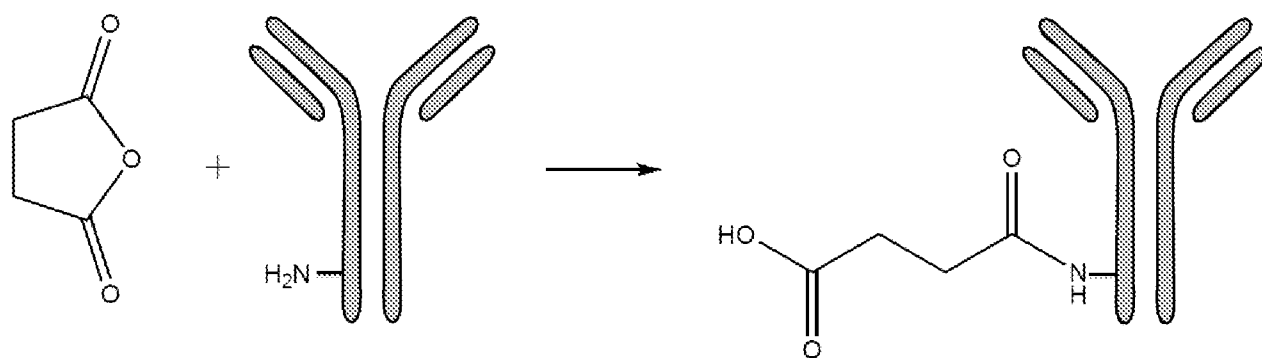


FIGURE 21

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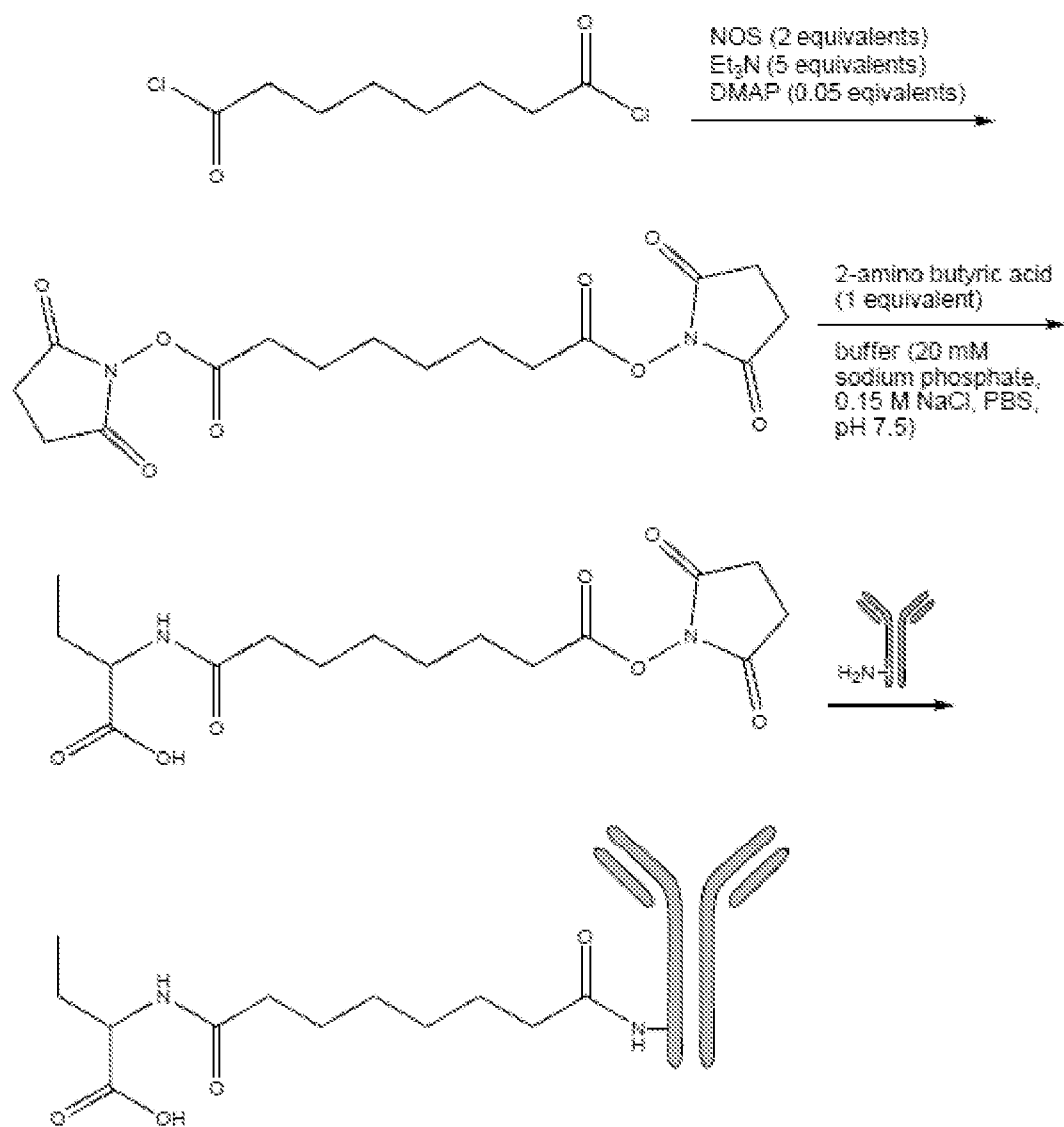


FIGURE 22

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2014/033700

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 5/00 (2014.01)

USPC - 436/73

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)

IPC(8) - A61K 31/28, 31/282, 38/00, 38/06, 38/07, 38/08; C07K 5/00, 5/062, 5/078, 5/083, 5/097; G01N 33/20, 33/68 (2014.01)

USPC - 75/392; 424/649; 436/73, 74; 530/331

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

CPC - C07K 5/0819, 7/06, 2319/20 (2014.06)

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

Orbit, Google Patents, Google Scholar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/0012028 A1 (CHAN et al) 08 January 2009 (08.01.2009) entire document	1-8, 5-29, 36-50, 57-67, 70, 71

Y		9-14, 30-35, 51-56, 68, 69
Y	US 2010/0221839 A1 (LAURENCE et al) 02 September 2010 (02.08.2010) entire document	9-14, 30-35, 51-56, 68, 69
A	WO 2012/138958 A1 (MONCK et al) 11 October 2012 (11.10.2012) entire document	1-71
A	US 2011/0112029 A1 (NIELSEN et al) 12 May 2011 (12.05.2011) entire document	1-71
A	US 2010/0093896 A1 (SPRAUL et al) 15 April 2010 (15.04.2010) entire document	1-71

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"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

11 August 2014

Date of mailing of the international search report

02 SEP 2014

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