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(54) Title: ALBUMIN BINDING CONJUGATE COMPOSITIONS AND METHODS OF MAKING AND USING SAME

(57) Abstract: The present invention concerns compositions of albumin binding conjugates comprising extended recombinant polypeptides (XTEN) with cysteine residues linked to albumin binding subunits with binding affinity to human serum albumin. The invention also provides compositions of albumin binding conjugates comprising therapeutic proteins, therapeutic drugs, or the combination of therapeutic proteins and therapeutic drugs. The invention also provides methods of making and using the albumin binding conjugates compositions.

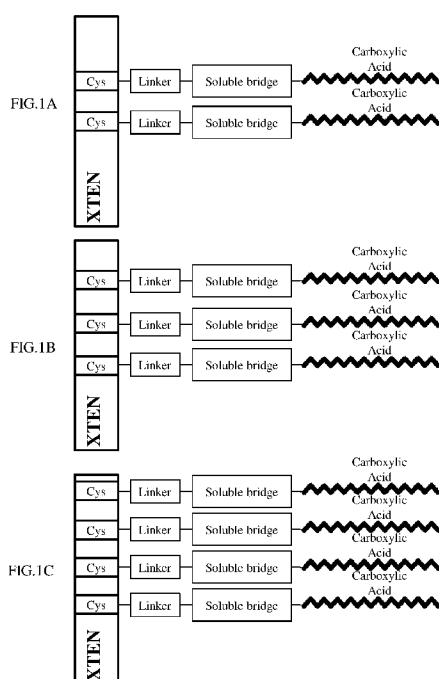


FIG. 1

Published:

— *with international search report (Art. 21(3))*

ALBUMIN BINDING CONJUGATE COMPOSITIONS AND METHODS OF MAKING AND USING SAME

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/334,815, filed May 11, 2016, which application is incorporated herein by reference.

BACKGROUND

[0002] Extending the half-life of a therapeutic agent, whether being a therapeutic protein, peptide or small molecule drug, often requires specialized formulations or modifications to the therapeutic agent itself. Conventional modification methods such as PEGylation, adding to the therapeutic agent an antibody fragment or an albumin molecule, suffer from a number of drawbacks. While these modified forms can be prepared on a large scale, conventional methods are generally plagued by high cost of goods, complex manufacturing processes, and low purity of the final product. Oftentimes, it is difficult to purify the target entity to an acceptable homogeneity. This is particularly true for pegylation, where precise control of the reaction to generate a homogenous population of pegylated agents that carry the same number or mass of polyethylene-glycol is not possible. Further, the metabolites of these PEGylated agents can have severe side effects. For example, PEGylated proteins have been observed to cause renal tubular vacuolation in animal models (Bendele, A., Seely, J., Richey, C., Sennello, G. & Shopp, G. Short communication: renal tubular vacuolation in animals treated with polyethylene-glycol-conjugated proteins. *Toxicol. Sci.* 1998. 42, 152–157). Renally cleared PEGylated proteins or their metabolites can accumulate in the kidney, causing formation of PEG hydrates that interfere with normal glomerular filtration. In addition, animals and humans can be induced to make antibodies to PEG (Sroda, K. et al. Repeated injections of PEG-PE liposomes generate anti-PEG antibodies. *Cell. Mol. Biol. Lett.* 2005.10, 37–47).

[0003] Albumin is a multifunctional transport protein that binds reversibly a wide variety of endogenous substances, therapeutic proteins and drugs. Because of the restricted passage of albumin-protein complexes across membranes, the pharmacokinetic properties of therapeutic proteins or drugs can be enhanced by attaching albumin to the therapeutic proteins or drug, either directly or indirectly by moieties that have binding affinity for albumin. However, the majority of such compositions evaluated to date have certain limitations, including the potential for allergic reactions, transmission of infections, and hyper oncotic albumin may cause kidney damage (Bairagi U, et al. Albumin: A Versatile Drug Carrier. *Austin Therapeutics.* 2015; 2(2): 1021).

[0004] Thus, there remains a considerable need for agents that can be attached to therapeutic proteins to enhance pharmacokinetic properties yet have pharmaceutical properties that permit their preparation as soluble, high concentration formulations having sufficient half-life and enhanced properties such that the overall therapeutic index is improved.

SUMMARY

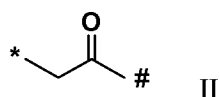
[0005] The present disclosure provides albumin binding conjugates (ABC) and compositions thereof, albumin binding conjugates comprising payloads such as therapeutic proteins or therapeutic drugs (e.g., small molecule drugs) or both, methods of making such compositions, and methods of using such compositions in the treatment of diseases.

[0006] The conjugates, compositions and methods disclosed herein not only are useful as therapeutics but are also particularly useful as research tools for preclinical and clinical development of a candidate therapeutic agent. In some aspects, the present disclosure addresses this need by, in part, generating albumin binding conjugates with linked proteins, drugs, as well as antibody fragments that target tissues bearing certain ligands. The albumin binding conjugates with the therapeutic payloads are superior in one or more aspects including, but not limited to, enhanced terminal half-life and enhanced pharmaceutical properties compared to unconjugated therapeutic agents.

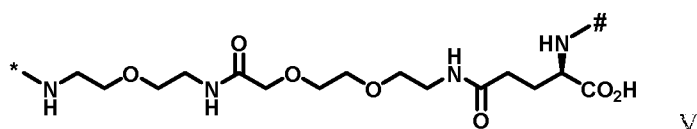
[0007] It is specifically contemplated that the disclosed albumin binding conjugate embodiments can exhibit one or more or any combination of the properties disclosed herein. It is further contemplated that the methods of treatment provided herein can exhibit one or more or any combination of the properties disclosed herein.

[0008] The subject albumin binding conjugates typically comprises an extended recombinant polypeptide having 2, or 3, or 4 cysteine residues, to which individual albumin binding subunits are conjugated to the thiol group of each cysteine residue. The individual albumin binding subunits disclosed comprise a linker moiety conjugated to a soluble bridge moiety conjugated to a carboxylic acid moiety, each of which is described more fully below. A therapeutic protein, or a therapeutic drug, or, optionally, both, can be linked or fused to the XTEN portion of the albumin binding conjugate, resulting in a composition that is useful in the treatment or prevention of disease and that exhibits enhanced properties relative to the unmodified therapeutic protein, or a therapeutic drug not linked to an albumin binding conjugate.

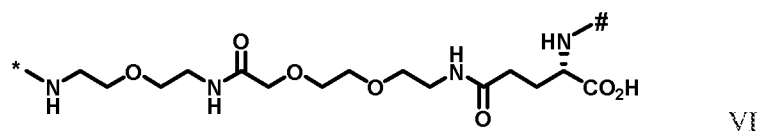
[0009] In a first aspect, the disclosure relates to compositions of albumin binding conjugates. In one embodiment, the disclosure provides an albumin binding conjugate comprising: an extended polypeptide (XTEN) having three cysteine residues, wherein the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity, or is identical to a sequence set forth in Table 1, when optimally aligned; three linker moieties, each of which has the structure of formula II



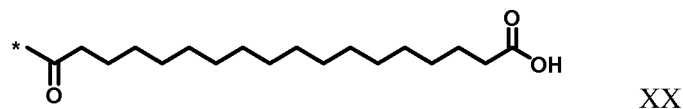
three soluble bridge moieties, each of which has the structure of formula V



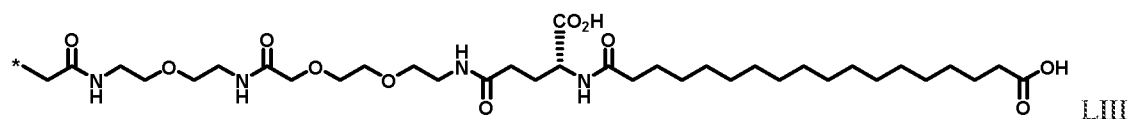
or formula VI



and three carboxylic acid moieties, each of which has the structure of formula XX

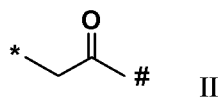


wherein the XTEN, three linker moieties, three soluble bridge moieties, and three carboxylic acid moieties are configured according the configuration set forth in FIG. 1B (the * and # symbols for all structures disclosed herein indicate the sites of conjugation for that molecule; e.g., the * location of the carboxylic acid is conjugated to the # location of the bridge moiety). In another embodiment, the albumin binding conjugate comprises three albumin binding subunits comprising the linker moiety, the soluble bridge moiety, and the carboxylic moiety, with each albumin binding subunit having the structure of formula LIII

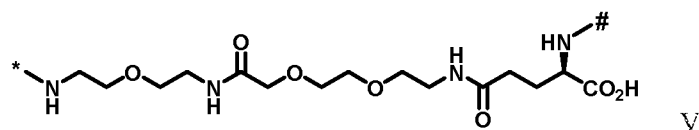


wherein each of the albumin binding subunits is linked to a thiol group of a cysteine residue of the XTEN.

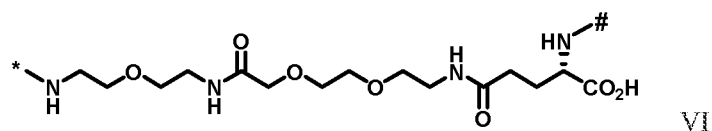
[0010] In another embodiment, the albumin binding conjugate comprises an extended polypeptide (XTEN) having two cysteine residues, wherein the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity, or is identical to a sequence set forth in Table 1, when optimally aligned; at least two linker moieties, each of which has the structure of formula II



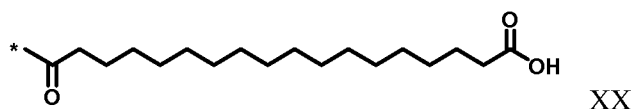
at least two soluble bridge moieties, each of which has the structure of formula V



or formula VI

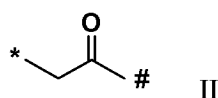


and at least two carboxylic acid moieties, each of which has the structure of formula XX

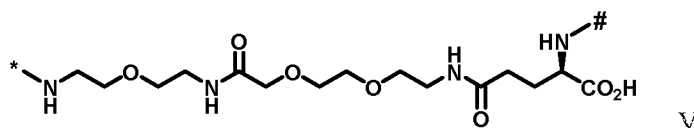


wherein the XTEN, two linker moieties, two soluble bridge moieties, and two carboxylic acid moieties are configured according to the configuration set forth in FIG. 1A.

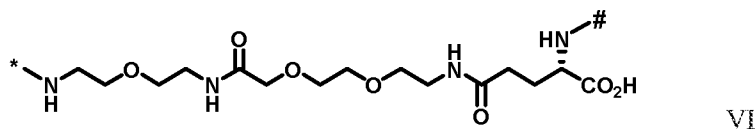
[0011] In another embodiment, an albumin binding conjugate comprises: an extended polypeptide (XTEN) having four cysteine residues, wherein the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity or is identical to a sequence set forth in Table 1, when optimally aligned; at least four linker moieties, each of which has the structure of formula II



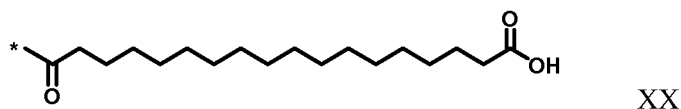
at least four soluble bridge moieties, each of which has the structure of formula V



or formula VI



and at least four carboxylic acid moieties, each of which has the structure of formula XX



wherein the XTEN, four linker moieties, four soluble bridge moieties, and four carboxylic acid moieties are configured according to the configuration set forth in FIG. 1C.

[0012] In a preferred embodiment, the XTEN of the albumin binding conjugates exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity, or is identical to sequence LX-31 or LX-40 set forth in Table 1. In one embodiment, the XTEN of a subject albumin binding conjugate is LX-40 and the composition is configured according to the structure set forth in FIG. 4. In another embodiment, the XTEN of a subject albumin binding conjugate is LX-31 and the composition is configured according to the structure set forth in FIG. 14.

[0013] In another aspect, the disclosure relates to compositions of albumin binding conjugates linked to payloads of therapeutic proteins, therapeutic drugs or, optionally, both, as well as antibody fragments that target tissues bearing certain ligands. The albumin binding conjugates with the therapeutic payloads

are superior in one or more aspects including enhanced terminal half-life and enhanced pharmaceutical properties compared to unconjugated product. In some embodiments, the disclosure provides an albumin binding conjugate of any one of the preceding embodiments that further comprises a single atom residue of a first therapeutic protein attached to the N-terminus of the XTEN, wherein the single atom residue is carbon, nitrogen, sulfur or oxygen. In another embodiment, the disclosure provides an albumin binding conjugate of any one of the preceding embodiments that further comprises the first therapeutic protein. The therapeutic proteins employed in the subject compositions are selected from the group consisting of cytokines, interleukins, growth factors, growth hormones, endocrine hormones, exocrine hormones, coagulation factors, glucose-regulating peptides, enzymes, receptor agonists, receptor antagonists, kinases, antibodies, antibody fragments and toxins. In one embodiment, the therapeutic protein is selected from the group consisting of the therapeutic proteins of Table 4. In other embodiments, the disclosure provides an albumin binding conjugate that further comprises a single atom residue of a first therapeutic drug attached to the N-terminus of the XTEN via a cross-linker, wherein the single atom residue is carbon, nitrogen, sulfur or oxygen. In another embodiment, the disclosure provides an albumin binding conjugate of any one of the preceding embodiments that further comprises the first therapeutic drug. The therapeutic drugs employed in the subject compositions are selected from the group consisting of hypnotics, sedatives, psychic energizers, tranquilizers, respiratory drugs, anticonvulsants, muscle relaxants, antiparkinson agents, analgesics, anti-inflammatories, antianxiety drugs, anxiolytics, appetite suppressants, antimigraine agents, muscle contractants, anti-infectives, antiarthritics, antimalarials, antiemetics, antiepileptics, bronchodilators, anti-cancer agents, antithrombotic agents, antihypertensives, cardiovascular drugs, antiarrhythmics, antioxidants, anti-asthma agents, hormonal agents, sympathomimetics, diuretics, lipid regulating agents, antiandrogenic agents, antiparasitics, anticoagulants, neoplastics, antineoplastics, hypoglycemics, antienteritis agents, diagnostic agents, contrasting agents, and radioactive imaging agents. In one embodiment, the therapeutic drug is selected from the group consisting of the drugs of Table 5.

[0014] In some embodiments, the subject compositions of albumin binding conjugates comprising therapeutic proteins, therapeutic drugs or antibody fragments exhibit enhanced half-life when administered to a subject compared to the unmodified therapeutic protein, therapeutic drug or antibody fragments. In one embodiment, an albumin binding conjugate linked to a therapeutic protein, therapeutic drug, or an antibody fragment has a terminal half-life when administered to a subject that is at least 2-fold, or at least 3-fold, or at least 4-fold, or at least 5-fold, or at least 6-fold, or at least 7-fold, or at least 8-fold, or at least 9-fold, or at least 10-fold longer compared to an unmodified therapeutic protein, or an unmodified therapeutic drug, or an unmodified antibody fragment when administered to a subject at a comparable molar dose. In another embodiment, an albumin binding conjugate linked to a therapeutic protein, therapeutic drug, or an antibody fragment has a terminal half-life when administered to a subject that is at least 12 h, or at least 24 h, or at least 36 h, or at least 48 h, or at least 72 h, or at least 96 h, or at least 120 h, or at least 144 h, or at least 7 days, or at least 10 days, or at least 14 days, or at least 21 days.

[0015] In some embodiments, the subject compositions of albumin binding conjugates comprising therapeutic proteins, therapeutic drugs or antibody fragments exhibit enhanced binding to human serum albumin. The subject compositions can be designed to offer advantages compared to other albumin-binding compositions used for half-life extension of payload peptides, proteins and drugs due to the incorporation of the multivalent albumin binding subunits; i.e., the inclusion of 2, 3 or 4 albumin binding subunits increases the binding affinity to albumin and thus reduces the dissociation of the composition from albumin once bound. The enhanced albumin-binding capacity of the subject compositions can be demonstrated in *in vitro* assays or as a result of administration to a subject. In one embodiment, an albumin binding conjugate of the embodiments described herein binds to human serum albumin in an *in vitro* assay with a K_d of less than 1×10^{-4} M, or a K_d of less than 3.3×10^{-4} M, or a K_d of less than 1×10^{-5} M, or a K_d of less than 3.3×10^{-5} M, or a K_d of less than 1×10^{-6} M, or a K_d of less than 3.3×10^{-6} M, or a K_d of less than 1×10^{-7} M, or a K_d of less than 3.3×10^{-7} M, or a K_d of less than 1×10^{-8} M, or a K_d of less than 3.3×10^{-8} M, or a K_d of less than 1×10^{-9} M, or a K_d of less than 1×10^{-10} M. In another embodiment, an albumin binding conjugate with a linked first therapeutic protein or a first therapeutic drug of the embodiments described herein binds to human serum albumin in an *in vitro* assay with at least 2-fold, or at least 3-fold, or at least 4-fold, or at least 5-fold, or at least 6-fold, or at least 7-fold, or at least 8-fold, or at least 9-fold, or at least 10-fold, or at least 20-fold, or at least 50-fold, or at least 100-fold greater affinity compared to a binding affinity of a first therapeutic protein or a first therapeutic drug conjugated to a single carboxylic acid moiety that is comparable to a carboxylic acid moiety incorporated into the albumin binding conjugate. In another embodiment, an albumin binding conjugate with a linked first therapeutic protein or a first therapeutic drug of the embodiments described herein binds to human serum albumin with a K_d of 10^{-1} M or less, or a K_d of 10^{-2} M or less, or a K_d of 10^{-3} M or less, in an *in vitro* assay compared to a K_d of a first therapeutic protein or a first therapeutic drug conjugated to a single carboxylic acid moiety comparable to the carboxylic acid moieties incorporated into the albumin binding conjugate.

[0016] In some embodiments, the subject compositions of albumin binding conjugates comprising linked therapeutic proteins, therapeutic drugs or antibody fragments are able to be formulated in a solution to a higher molar concentration than compositions of the therapeutic proteins, therapeutic drugs or antibody fragments linked only to carboxylic acids. In one embodiment, the disclosure provides an albumin binding conjugate with a linked first therapeutic protein or a first therapeutic drug or a first antibody fragment, wherein the conjugate is capable of being formulated in a saline or buffer solution at a molar concentration that is at least 10%, or at least 20%, or at least 30%, or at least 40%, or at least 50% higher than that which can be achieved for a first therapeutic protein or a first therapeutic drug or a first antibody fragment conjugated to a single carboxylic acid moiety comparable to the carboxylic acid moieties incorporated into the albumin binding conjugate.

[0017] In another aspect, the disclosure relates to pharmaceutical compositions of albumin binding conjugates. In one embodiment, the disclosure provides a pharmaceutical composition comprising an albumin binding conjugate with a linked first therapeutic protein or a first therapeutic drug or a first

antibody fragment and optionally, suitable formulations of carrier, stabilizers and/or excipients. In another embodiment, the pharmaceutical composition is suitable for subcutaneous, intravenous, or intramuscular administration. In another embodiment, the pharmaceutical composition is in a liquid form. In another embodiment, the liquid pharmaceutical composition is in a pre-filled syringe for a single injection to a subject. In another embodiment, the disclosure provides liquid pharmaceutical compositions wherein the composition is formulated in a saline buffer solution at a concentration of at least 1 mM, or at least 2 mM, or at least 3 mM, or at least 4 mM, or at least 5 mM, or at least 6 mM, or at least 7 mM, or at least 8 mM, or at least 9 mM, or at least 10 mM and wherein the saline buffer solution comprising the composition can be passed through a 25, 26, 27, 28, 29, 30, 31, or 32 gauge needle for intravenous, intramuscular, intraarticular, or subcutaneous administration. In another embodiment, the disclosure provides liquid pharmaceutical compositions wherein the composition is formulated in a saline or buffer solution at a concentration of at least 1 mM, or at least 2 mM, or at least 3 mM, or at least 4 mM, or at least 5 mM, or at least 6 mM, or at least 7 mM, or at least 8 mM, or at least 9 mM, or at least 10 mM and has a viscosity of less than 10 cP, or less than 15 cP, or less than 20 cP, or less than 25 cP, or less than 30 cP.

[0018] In another embodiment, the disclosure provides use of the albumin binding conjugate linked to a first therapeutic protein or a first therapeutic drug or a first antibody fragment in the preparation of a medicament for use in a subject in need thereof.

[0019] In yet another aspect, the disclosure relates to methods of treating a disease in a subject using the pharmaceutical composition embodiments disclosed herein wherein the method comprises administering to a subject with a disease a therapeutically effective dose of the pharmaceutical composition. Where desired, the method comprises administering to the subject in need thereof a therapeutically effective dose of a pharmaceutical composition comprising the pharmaceutical composition of conjugates provided herein. In another embodiment of the method of treatment, the pharmaceutical composition is administered to the subject as one or more therapeutically effective doses. In one embodiment, the therapeutically effective dose is administered every week, every two weeks, every three weeks, or monthly. In another embodiment of the method of treatment, the pharmaceutical composition is administered to the subject subcutaneously, intravenously, intraperitoneally, or intramuscularly.

[0020] The disclosure provides a pharmaceutical composition for use in a treatment regimen for the treatment of a disease, comprising administration to a subject with the disease the pharmaceutical composition in two or more consecutive doses using a therapeutically effective dose.

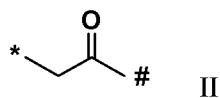
[0021] In another aspect, the disclosure provides kits comprising the pharmaceutical composition. In one embodiment, the disclosure provides a kit comprising the pharmaceutical composition of any one of embodiments disclosed herein, a container and a label or package insert on or associated with the container. In another embodiment, the disclosure provides a kit comprising a pre-filled syringe

containing the pharmaceutical composition of any one of the embodiments disclosed herein, and a label or package insert on or associated with the syringe.

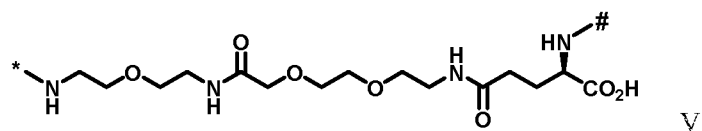
[0022] In another aspect, the disclosure relates to extended polypeptides (XTEN) comprising cysteine residues utilized in the subject albumin binding conjugates. In one embodiment, the disclosure provides an extended polypeptide (XTEN) comprising at least 2, at least 3, or at least 4 cysteine residues, wherein the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity or is identical to a sequence set forth in Table 1, when optimally aligned. In one embodiment, the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity or is identical to sequence LX-40 set forth in Table 1. In another embodiment, the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity or is identical to sequence LX-31 set forth in Table 1.

[0023] In another aspect, the disclosure relates to nucleic acids encoding extended polypeptides (XTEN) comprising cysteine residues utilized in the subject albumin binding conjugates. In one embodiment, the nucleic acid encodes an XTEN comprising at least 2, at least 3, or at least 4 cysteine residues, wherein the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity or is identical to a sequence set forth in Table 1, or a complement thereof. In another embodiment, the nucleic acid encodes the amino acid sequence LX-40 set forth in Table 1, or a complement thereof. In another embodiment, the nucleic acid encodes the amino acid sequence LX-31 set forth in Table 1, or a complement thereof. The disclosure also relates to expression vectors. In one embodiment, the disclosure provides an expression vector comprising a nucleic acid encoding an XTEN comprising at least 2, at least 3, or at least 4 cysteine residues, wherein the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity or is identical to a sequence set forth in Table 1, wherein the vector further comprises a recombinant regulatory sequence operably linked to the nucleic acid. In another embodiment, the disclosure provides an isolated host cell comprising the expression vector of the foregoing embodiment. In another embodiment, the disclosure provides use of a nucleic acid in the making of the XTEN embodiments provided herein or the complement thereof.

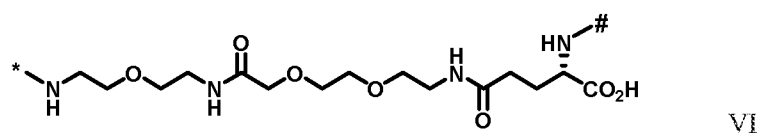
[0024] In another aspect, the disclosure provides compositions of albumin binding conjugates in which the carboxylic acid moiety integrated into the conjugate is of varying length. In one embodiment, the disclosure provides an albumin binding conjugate comprising: an extended polypeptide (XTEN) having 2, or 3, or 4 cysteine residues, wherein the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity, or is identical to a sequence set forth in Table 1, when optimally aligned; 2, or 3, or 4 linker moieties, each of which has the structure of formula II



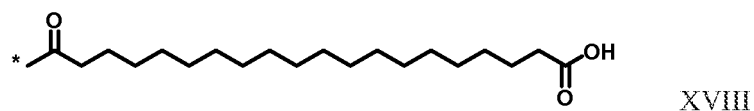
2, or 3, or 4 soluble bridge moieties, each of which has the structure of formula V



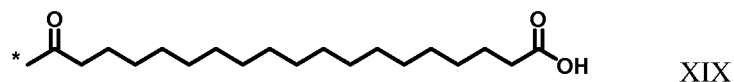
or formula VI



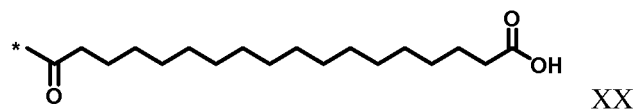
and 2, 3, or 4 carboxylic acid moieties, each of which has the structure of formula XVIII



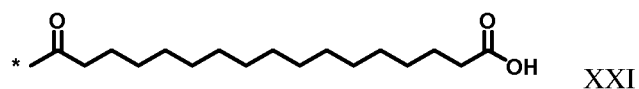
or formula XIX



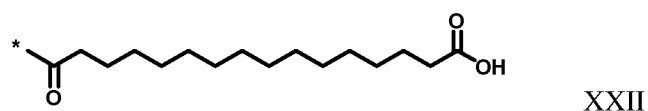
or formula XX



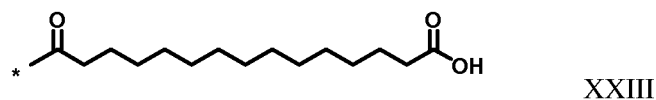
or formula XXI



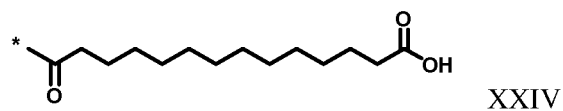
or formula XXII



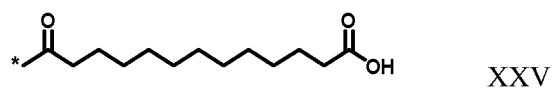
or formula XXIII



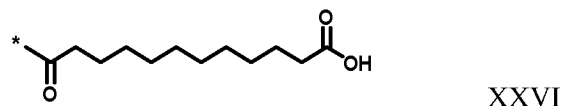
or formula XXIV



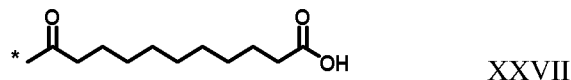
or formula XXV



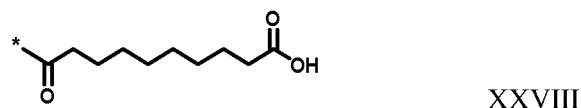
or formula XXVI



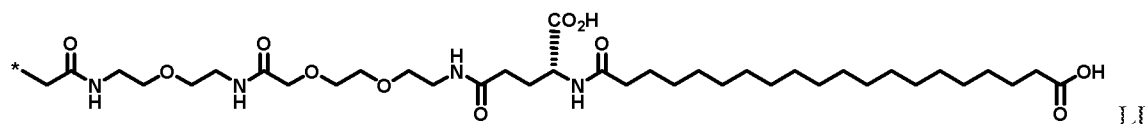
or formula XXVII



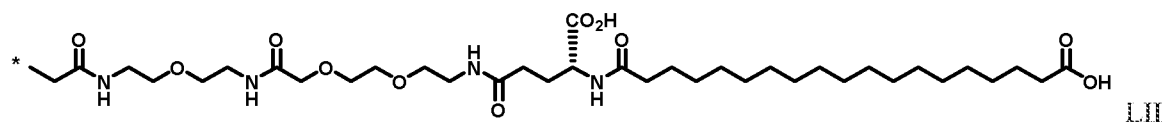
or formula XXVIII



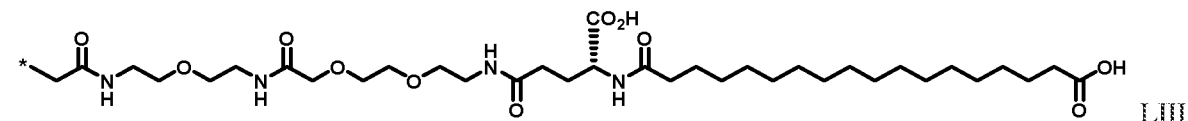
wherein the XTEN, linker moieties, soluble bridge moieties, and carboxylic acid moieties are configured according the configurations set forth in FIG. 1A for the albumin binding conjugate having 2 cysteine residues, 2 linker moieties, 2 soluble bridge moieties, and 2 carboxylic acids, or the configurations set forth in FIG. 1B for the albumin binding conjugate having 3 cysteine residues, 3 linker moieties, 3 soluble bridge moieties, and 3 carboxylic acids, or the configurations set forth in FIG. 1C for the albumin binding conjugate having 4 cysteine residues, 4 linker moieties, 4 soluble bridge moieties, and 4 carboxylic acids. In another embodiment, the disclosure provides an albumin binding conjugate comprising: an extended polypeptide (XTEN) having 2, or 3, or 4 cysteine residues, wherein the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity, or is identical to a sequence set forth in Table 1, when optimally aligned and a corresponding number of albumin binding subunits (2, 3, or 4), each of which has the structure of formula LI



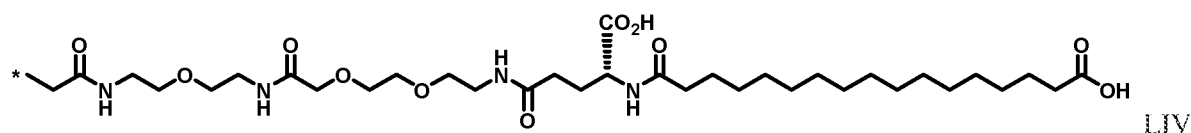
or formula LII



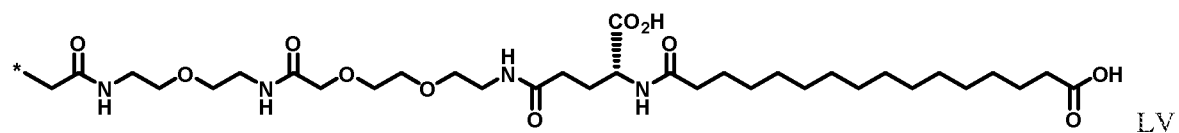
or formula LIII



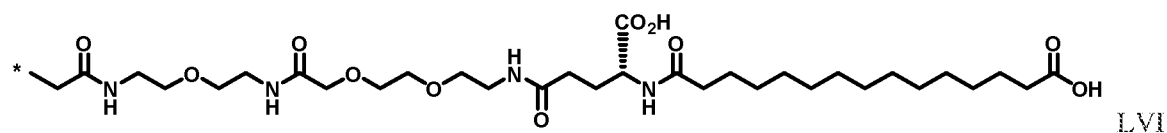
or formula LIV



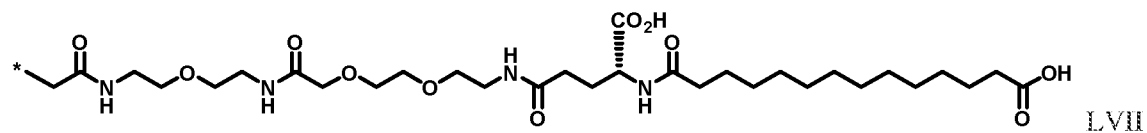
or formula LV



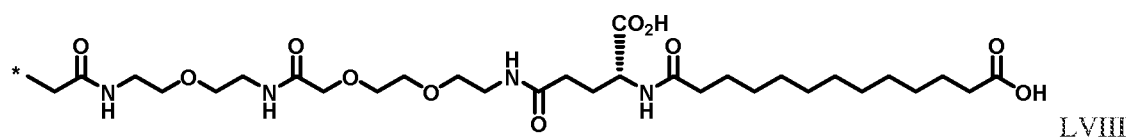
or formula LVI



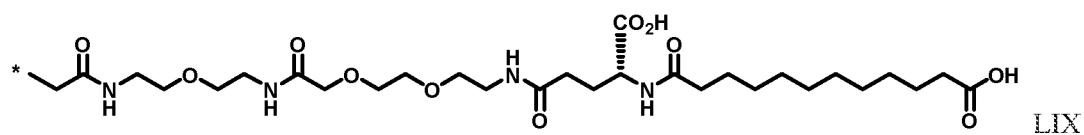
or formula LVII



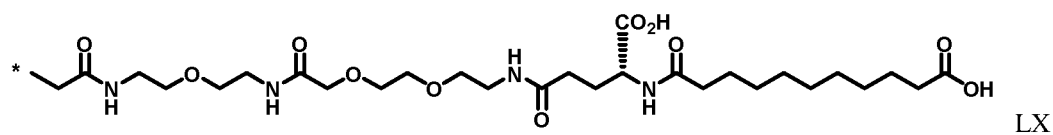
or formula LVIII



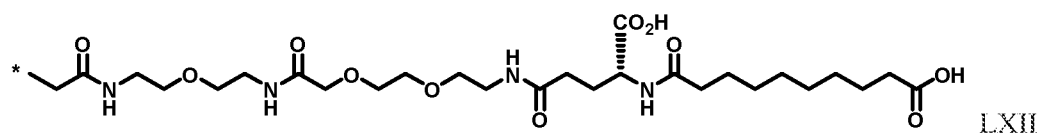
or formula LIX



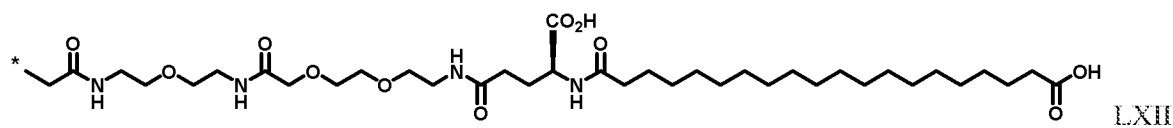
or formula LX



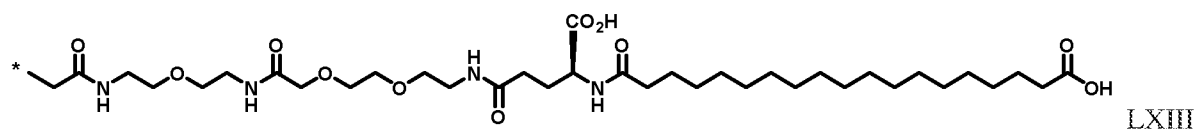
or formula LXI



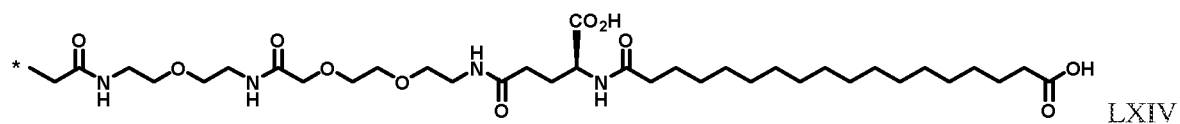
or formula LXII



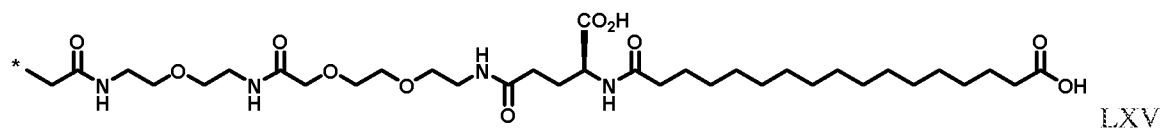
or formula LXIII



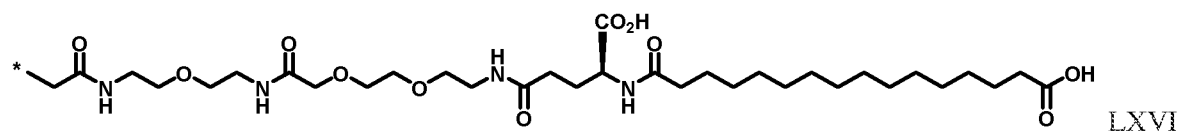
or formula LXIV



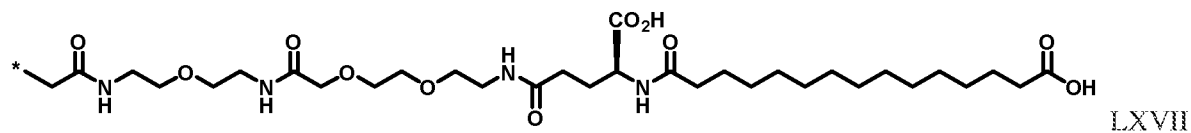
or formula LXV



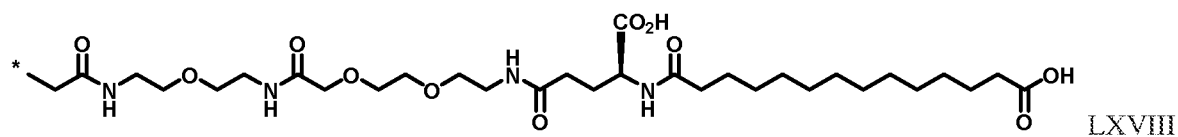
or formula LXVI



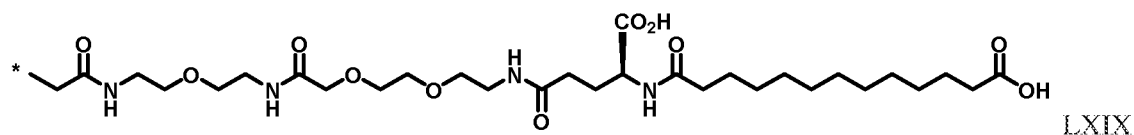
or formula LXVII



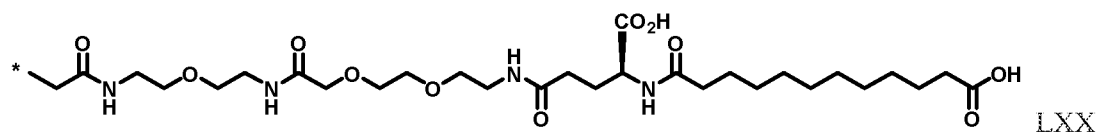
or formula LXVIII



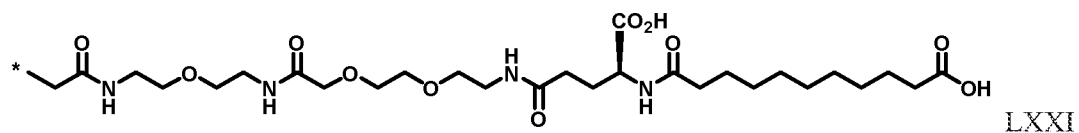
or formula LXIX



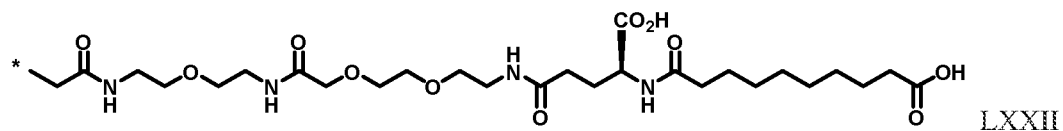
or formula LXX



or formula LXXI



or formula LXXII



wherein each individual albumin binding subunit is linked to a thiol group of a cysteine residue of the XTEN (i.e., an albumin binding conjugate having 2 cysteine residues would have 2 linked albumin binding subunits; an albumin binding conjugate having 3 cysteine residues would have 3 linked albumin binding subunits; an albumin binding conjugate having 4 cysteine residues would have 4 linked albumin binding subunits). In some embodiments of the conjugates provided herein, the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity, or is identical to sequence LX-31 or LX-40 set forth in Table 1. In one embodiment, the XTEN is the LX-40 sequence set forth in Table 1. In another embodiment, the XTEN is the LX-31 sequence set forth in Table 1. In some embodiments, the albumin binding conjugate further comprises a single atom residue of a first therapeutic protein attached to the N-terminus of the XTEN, wherein the single atom residue is carbon, nitrogen, sulfur or oxygen. In one embodiment, the albumin binding conjugate comprises the first therapeutic protein. In one embodiment, the first therapeutic protein is selected from the group consisting of cytokines, interleukins, growth factors, growth hormones, endocrine hormones, exocrine hormones, coagulation factors, glucose-regulating peptides, enzymes, receptor agonists, receptor antagonists, kinases, antibodies, antibody fragments and toxins. In another embodiment, the therapeutic protein is selected from the group consisting of the therapeutic proteins of Table 4. In other embodiments, the albumin binding conjugate further comprises a single atom residue of a first therapeutic drug attached to the N-terminus of the XTEN via a cross-linker, wherein the single atom residue is carbon, nitrogen, sulfur or oxygen. In one embodiment, the albumin binding conjugate comprises the first therapeutic drug. In one embodiment, the first therapeutic drug is selected from the group consisting of hypnotics, sedatives, psychic energizers, tranquilizers, respiratory drugs, anticonvulsants, muscle relaxants, antiparkinson agents, analgesics, anti-inflammatories, antianxiety drugs, anxiolytics, appetite suppressants, antimigraine agents, muscle contractants, anti-infectives, antiarthritics, antimalarials, antiemetics, antiepileptics, bronchodilators, anti-cancer agents, antithrombotic agents, antihypertensives, cardiovascular drugs, antiarrhythmics, antioxidants, anti-asthma agents, hormonal agents, sympathomimetics, diuretics, lipid regulating agents, antiandrogenic agents, antiparasitics, anticoagulants, neoplastics, antineoplastics, hypoglycemics, antienteritis agents, diagnostic agents, contrasting agents, and radioactive imaging agents. In another embodiment, the first therapeutic drug is selected from the group consisting of the drugs of Table 5. In some embodiments of the albumin binding conjugates linked to a therapeutic protein or therapeutic drug, the albumin binding conjugate conjugates linked to a therapeutic protein or therapeutic drug has a terminal half-life when administered to a subject that is at least 2-fold, or at least 3-fold, or at least 4-fold, or at least 5-fold, or at least 6-fold, or at least 7-fold, or at least 8-fold, or at least 9-fold, or at least 10-fold longer compared to an unmodified first therapeutic protein or an unmodified first therapeutic drug. In other embodiments of the albumin

binding conjugates linked to a therapeutic protein or therapeutic drug, the conjugate linked to a therapeutic protein or therapeutic drug has a terminal half-life when administered to a subject of at least 12 h, or at least 24 h, or at least 36 h, or at least 48 h, or at least 72 h, or at least 96 h, or at least 120 h, or at least 144 h, or at least 7 days, or at least 10 days, or at least 14 days, or at least 21 days. In other embodiments of the foregoing albumin binding conjugates, the albumin binding conjugate binds to human serum albumin in an *in vitro* assay with a K_d of less than 1×10^{-4} M, or a K_d less than 3.3×10^{-4} M, or a K_d less than 1×10^{-5} M, or a K_d less than 3.3×10^{-5} M, or a K_d less than 1×10^{-6} M, or a K_d less than 3.3×10^{-6} M, or a K_d less than 1×10^{-7} M, or a K_d less than 3.3×10^{-7} M, or a K_d less than 1×10^{-8} M, or a K_d less than 3.3×10^{-8} M, or a K_d less than 1×10^{-9} M, or a K_d less than 1×10^{-10} M. In other embodiments of the foregoing albumin binding conjugates, the albumin binding conjugate binds to human serum albumin in an *in vitro* assay with at least 2-fold, or at least 3-fold, or at least 4-fold, or at least 5-fold, or at least 6-fold, or at least 7-fold, or at least 8-fold, or at least 9-fold, or at least 10-fold, or at least 20-fold, or at least 50-fold, or at least 100-fold greater affinity compared to a first therapeutic protein or a first therapeutic drug conjugated to a single carboxylic acid moiety comparable to the carboxylic acid moieties incorporated into the albumin binding conjugate. In other embodiments of the foregoing albumin binding conjugates, the albumin binding conjugate linked to a therapeutic protein or therapeutic drug binds to human serum albumin with a K_d of 10^{-1} M or less, or a K_d of at least 10^{-2} M or less, or a K_d of 10^{-3} M or less in an *in vitro* assay compared to a K_d of a first therapeutic protein or a first therapeutic drug conjugated to a single carboxylic acid moiety comparable to the carboxylic acid moieties incorporated into the albumin binding conjugate. In other embodiments of the foregoing albumin binding conjugates, the conjugate is capable of being formulated in a saline or buffer solution at a molar concentration that is at least 10%, or at least 20%, or at least 30%, or at least 40%, or at least 50% higher than that which can be achieved for a first therapeutic protein or a first therapeutic drug conjugated to a single carboxylic acid moiety comparable to the carboxylic acid moieties incorporated into the albumin binding conjugate. The disclosure provides pharmaceutical compositions of the foregoing albumin binding conjugates comprising a first therapeutic protein or a first therapeutic drug of this paragraph and optionally, suitable formulations of carrier, stabilizers and/or excipients. The pharmaceutical composition is suitable for subcutaneous, intravenous, or intramuscular administration to a subject. In one embodiment, the composition is in a liquid form. In another embodiment, the pharmaceutical composition is in a pre-filled syringe for a single injection. In another embodiment, the pharmaceutical composition is formulated in a saline or buffer solution at a concentration of at least 1 mM, or at least 2 mM, or at least 3 mM, or at least 4 mM, or at least 5 mM, or at least 6 mM, or at least 7 mM, or at least 8 mM, or at least 9 mM, or at least 10 mM and wherein the saline buffer solution comprising the composition can be passed through a 25, 26, 27, 28, 29, 30, 31, or 32 gauge needle for intravenous, intramuscular, intraarticular, or subcutaneous administration. In another embodiment, the pharmaceutical composition is formulated in a saline or buffer solution at a concentration of at least 1 mM, or at least 2 mM, or at least 3 mM, or at least 4 mM, or at least 5 mM, or at least 6 mM, or at least 7 mM, or at least 8 mM, or at least 9 mM, or at least 10 mM and has a viscosity

of less than 10 cP, or less than 15 cP, or less than 20 cP, or less than 25 cP, or less than 30 cP. In another embodiment, the disclosure provides use of the albumin binding conjugates comprising a first therapeutic protein or a first therapeutic drug in the preparation of a medicament for use in a subject in need thereof. The disclosure provides a method of treating a disease in a subject, the method comprising administering to a subject with a disease one or more therapeutically effective doses of a pharmaceutical composition comprising any of the foregoing albumin binding conjugates and compositions. In one embodiment of the method of treating a disease in a subject, the therapeutically effective dose is administered every week, every two weeks, every three weeks, or monthly. In another embodiment of the method of treating a disease in a subject, the therapeutically effective dose is administered subcutaneously, intravenously, intraperitoneally, or intramuscularly. The disclosure provides a pharmaceutical composition for use in a treatment regimen for the treatment of a disease, comprising any pharmaceutical composition provided herein for administration to a subject with the disease in two or more consecutive doses using a therapeutically effective dose.

[0025] In another aspect, the disclosure provides bifunctional albumin binding conjugates comprising a therapeutic protein and a therapeutic drug linked to the XTEN of the albumin binding conjugates by cross-linkers. In one embodiment, the albumin binding conjugates comprise a first single atom residue of a therapeutic protein attached to the N-terminus of the XTEN component by a cross-linker and a second single atom of a therapeutic drug attached to the ϵ -amino group of a C-terminal lysine of the XTEN component by a cross-linker, wherein the single atom residue of each is carbon, nitrogen, sulfur or oxygen. In another embodiment, the albumin binding conjugates comprise a therapeutic protein attached to the N-terminus of the XTEN component by a cross-linker and a therapeutic drug attached to the ϵ -amino group of a C-terminal lysine of the XTEN component by a cross-linker wherein the therapeutic protein is selected from the group consisting of cytokines, interleukins, growth factors, growth hormones, growth factors, endocrine hormones, exocrine hormones, coagulation factors, glucose-regulating peptides, enzymes, receptor agonists, receptor antagonists, kinases, antibodies, antibody fragments and toxins, and wherein the therapeutic drug is selected from the group consisting of hypnotics, sedatives, psychic energizers, tranquilizers, respiratory drugs, anticonvulsants, muscle relaxants, antiparkinson agents, analgesics, anti-inflammatories, antianxiety drugs, anxiolytics, appetite suppressants, antimigraine agents, muscle contractants, anti-infectives, antiarthritics, antimalarials, antiemetics, anepileptics, bronchodilators, anti-cancer agents, antithrombotic agents, antihypertensives, cardiovascular drugs, antiarrhythmics, antioxidants, anti-asthma agents, hormonal agents, sympathomimetics, diuretics, lipid regulating agents, antiandrogenic agents, antiparasitics, anticoagulants, neoplastics, antineoplastics, hypoglycemics, antienteritis agents, diagnostic agents, contrasting agents, and radioactive imaging agents. In another embodiment, the albumin binding conjugates comprise a therapeutic protein attached to the N-terminus of the XTEN component by a cross-linker and a therapeutic drug attached to the ϵ -amino group of a C-terminal lysine of the XTEN component by a cross-

linker wherein the therapeutic protein is selected from the group consisting of the therapeutic proteins of Table 4 and the therapeutic drug is selected from the group consisting of the therapeutic drugs of Table 5.

[0026] The disclosure provides albumin binding conjugates with different configurations and with different XTEN sequences. In one embodiment, the albumin binding conjugate has the configuration according to the configuration set forth FIG. 4. In another embodiment, the albumin binding conjugate has the configuration according to the configuration set forth FIG. 5. In another embodiment, the albumin binding conjugate has the configuration according to the configuration set forth FIG. 6. In another embodiment, the albumin binding conjugate has the configuration according to the configuration set forth FIG. 7. In another embodiment, the albumin binding conjugate has the configuration according to the configuration set forth FIG. 8. In another embodiment, the albumin binding conjugate has the configuration according to the configuration set forth FIG. 9. In another embodiment, the albumin binding conjugate has the configuration according to the configuration set forth FIG. 10. In another embodiment, the albumin binding conjugate has the configuration according to the configuration set forth FIG. 11. In another embodiment, the albumin binding conjugate has the configuration according to the configuration set forth FIG. 12. In another embodiment, the albumin binding conjugate has the configuration according to the configuration set forth FIG. 13. In another embodiment, the albumin binding conjugate has the configuration according to the configuration set forth FIG. 14. In one embodiment, the disclosure also provides the albumin binding conjugates having the configurations according to the configuration set forth in FIGS. 4-14, further comprising a single atom residue of a first therapeutic protein attached to the N-terminus of the XTEN by a cross-linker, wherein the single atom residue is carbon, nitrogen, sulfur or oxygen. In another embodiment, the disclosure provides the albumin binding conjugates having the configurations according to the configuration set forth in FIGS. 4-14, further comprising a single atom residue of a first therapeutic protein attached to an ϵ -amino group of a C-terminal lysine of the XTEN by a cross-linker, wherein the single atom residue is carbon, nitrogen, sulfur or oxygen. In another embodiment, the disclosure provides the albumin binding conjugates having the configurations according to the configuration set forth in FIGS. 4-14, further comprising the first therapeutic protein attached to the N-terminus of the XTEN by a cross-linker. In another embodiment, the disclosure provides the albumin binding conjugates having the configurations according to the configuration set forth in FIGS. 4-14, further comprising the first therapeutic protein attached to the ϵ -amino group of a C-terminal lysine of the XTEN by a cross-linker. In still other embodiments, the disclosure provides the albumin binding conjugates of FIGS. 4-14 further comprising a first single atom residue of a first therapeutic protein attached to the N-terminus of the XTEN by a cross-linker and a second single atom residue of a second therapeutic protein attached to an ϵ -amino group of a C-terminal lysine of the XTEN by a cross-linker, wherein the first and second single atom residues are selected from carbon, nitrogen, sulfur and oxygen. In still other embodiments, the disclosure provides the albumin binding conjugates having the configurations according to the configuration set forth in FIGS. 4-14 further comprising a first therapeutic protein attached to the N-terminus of the XTEN by a cross-linker and a second therapeutic protein

attached to an ϵ -amino group of a C-terminal lysine of the XTEN by a cross-linker, wherein the first and second proteins are identical or different (and thus are a bifunctional) and are selected from the group consisting of cytokines, interleukins, growth factors, growth hormones, endocrine hormones, exocrine hormones, coagulation factors, glucose-regulating peptides, enzymes, receptor agonists, receptor antagonists, kinases, antibodies, antibody fragments and toxins. In still other embodiments, the disclosure provides the albumin binding conjugates having the configurations according to the configuration set forth in FIGS. 4-14 further comprising a first therapeutic protein attached to the N-terminus of the XTEN by a cross-linker and a second therapeutic protein attached to an ϵ -amino group of a C-terminal lysine of the XTEN by a cross-linker, wherein the first and second proteins are identical or different (and thus are a bifunctional) and are selected from the group consisting of the therapeutic proteins of Table 4. In another embodiment, the disclosure also provides the albumin binding conjugates having the configurations according to the configuration set forth in FIGS. 4-14, further comprising a single atom residue of a first therapeutic drug attached to the N-terminus of the XTEN by a cross-linker, wherein the single atom residue is carbon, nitrogen, sulfur or oxygen. In another embodiment, the disclosure provides the albumin binding conjugates having the configurations according to the configuration set forth in FIGS. 4-14, further comprising a single atom residue of a first therapeutic drug attached to an ϵ -amino group of a C-terminal lysine of the XTEN by a cross-linker, wherein the single atom residue is carbon, nitrogen, sulfur or oxygen. In another embodiment, the disclosure provides the albumin binding conjugates having the configurations according to the configuration set forth in FIGS. 4-14, further comprising the first therapeutic drug attached to the N-terminus of the XTEN by a cross-linker. In another embodiment, the disclosure provides the albumin binding conjugates having the configurations according to the configuration set forth in FIGS. 4-14, further comprising the first therapeutic drug attached to the ϵ -amino group of a C-terminal lysine of the XTEN by a cross-linker. In still other embodiments, the disclosure provides the albumin binding conjugates having the configurations according to the configuration set forth in FIGS. 4-14 further comprising a first single atom residue of a first therapeutic drug attached to the N-terminus of the XTEN by a cross-linker and a second single atom residue of a second therapeutic drug attached to an ϵ -amino group of a C-terminal lysine of the XTEN by a cross-linker, wherein the first and second single atom residues are selected from carbon, nitrogen, sulfur and oxygen. In still other embodiments, the disclosure provides the albumin binding conjugates having the configurations according to the configuration set forth in FIGS. 4-14 further comprising a first therapeutic drug attached to the N-terminus of the XTEN by a cross-linker and a second therapeutic drug attached to an ϵ -amino group of a C-terminal lysine of the XTEN by a cross-linker, wherein the first and second drugs are identical or different (and thus are a bifunctional) and are selected from the group consisting of hypnotics, sedatives, psychic energizers, tranquilizers, respiratory drugs, anticonvulsants, muscle relaxants, antiparkinson agents, analgesics, anti-inflammatories, antianxiety drugs, anxiolytics, appetite suppressants, antimigraine agents, muscle contractants, anti-infectives, antiarthritics, antimalarials, antiemetics, anepileptics, bronchodilators, anti-cancer agents, antithrombotic agents,

antihypertensives, cardiovascular drugs, antiarrhythmics, antioxidants, anti-asthma agents, hormonal agents, sympathomimetics, diuretics, lipid regulating agents, antiandrogenic agents, antiparasitics, anticoagulants, neoplastics, antineoplastics, hypoglycemics, antienteritis agents, diagnostic agents, contrasting agents, and radioactive imaging agents. In another embodiment, the disclosure provides the albumin binding conjugates having the configurations according to the configuration set forth in FIGS. 4-14, further comprising the first therapeutic drug attached to the ϵ -amino group of a C-terminal lysine of the XTEN by a cross-linker. In still other embodiments, the disclosure provides the albumin binding conjugates having the configurations according to the configuration set forth in FIGS. 4-14 further comprising a first single atom residue of a therapeutic protein attached to the N-terminus of the XTEN by a cross-linker and a second single atom residue of a therapeutic drug attached to an ϵ -amino group of a C-terminal lysine of the XTEN by a cross-linker, wherein the first and second single atom residues are selected from carbon, nitrogen, sulfur and oxygen. In still other embodiments, the disclosure provides the albumin binding conjugates having the configurations according to the configuration set forth in FIGS. 4-14 further comprising a therapeutic protein attached to the N-terminus of the XTEN by a cross-linker and a therapeutic drug attached to an ϵ -amino group of a C-terminal lysine of the XTEN by a cross-linker (and thus are a bifunctional) and wherein the therapeutic protein is selected from the group consisting of cytokines, interleukins, growth factors, growth hormones, growth factors, endocrine hormones, exocrine hormones, coagulation factors, glucose-regulating peptides, enzymes, receptor agonists, receptor antagonists, kinases, antibodies, antibody fragments and toxins, and wherein the therapeutic drug is selected from the group consisting of hypnotics, sedatives, psychic energizers, tranquilizers, respiratory drugs, anticonvulsants, muscle relaxants, antiparkinson agents, analgesics, anti-inflammatories, antianxiety drugs, anxiolytics, appetite suppressants, antimigraine agents, muscle contractants, anti-infectives, antiarthritics, antimalarials, antiemetics, anepileptics, bronchodilators, anti-cancer agents, antithrombotic agents, antihypertensives, cardiovascular drugs, antiarrhythmics, antioxidants, anti-asthma agents, hormonal agents, sympathomimetics, diuretics, lipid regulating agents, antiandrogenic agents, antiparasitics, anticoagulants, neoplastics, antineoplastics, hypoglycemics, antienteritis agents, diagnostic agents, contrasting agents, and radioactive imaging agents. In still other embodiments, the disclosure provides the albumin binding conjugates having the configurations according to the configuration set forth in FIGS. 4-14 further comprising a therapeutic protein attached to the N-terminus of the XTEN by a cross-linker and a therapeutic drug attached to an ϵ -amino group of a C-terminal lysine of the XTEN by a cross-linker (and thus are a bifunctional) and wherein the therapeutic protein is selected from the group consisting of the therapeutic proteins of Table 4 and the therapeutic drug is selected from the group consisting of the therapeutic drugs of Table 5.

INCORPORATION BY REFERENCE

[0027] 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

[0028] The features and advantages of the invention may be further explained by reference to the following detailed description and accompanying drawings that sets forth illustrative embodiments.

[0029] FIG. 1 are schematics of the various configurations of albumin binding conjugate (ABC) compositions. FIG. 1A is a schematic diagram of an ABC composition with two albumin binding subunits that are each made by the linkage of a linker moiety, a soluble bridge, and a carboxylic acid, wherein the albumin binding subunits are linked to the thiol groups of cysteine residues in the XTEN polypeptide, as depicted. FIG. 1B is a schematic diagram of an ABC composition with three albumin binding subunits that are each made by the linkage of a linker moiety, a soluble bridge, and a carboxylic acid, wherein the albumin binding subunits are linked to the thiol groups of cysteine residues in the XTEN polypeptide, as depicted. FIG. 1C is a schematic diagram of an ABC composition with four albumin binding subunits that are each made by the linkage of a linker moiety, a soluble bridge, and a carboxylic acid, wherein the albumin binding subunits are linked to the thiol groups of cysteine residues in the XTEN polypeptide, as depicted.

[0030] FIG. 2 are schematics of ABC composition molecules with an incorporated therapeutic payload. FIG. 2A has the ABC configuration of FIG. 1B with 3 albumin binding subunits in which the therapeutic payload is conjugated using the N-terminal amino group of the XTEN moiety via a suitable cross-linker for the payload of interest. FIG. 2B is a schematic of an ABC composition molecule bearing two copies of a therapeutic payload. The therapeutic payload is conjugated to both the N-terminal amino group of the XTEN moiety and the ϵ amino group of the C-terminal lysine both via suitable cross-linkers for the payload of interest.

[0031] FIG. 3 is a schematic depiction of the assembly of an ABC composition from its component parts, with the albumin binding subunit first assembled from linker moiety, soluble bridge, and carboxylic acid linked together as depicted and then conjugated to the XTEN at the thiol groups of the incorporated cysteine residues.

[0032] FIG. 4 depicts the structure of an exemplary ABC utilizing the LX-40 XTEN. The structure is produced from conjugation of the *R* enantiomer of the albumin binding subunit to the LX-40 XTEN sequence described herein; the completed composition is referred to elsewhere herein as UHLX-40. The amino acid sequence is shown with the cysteine groups expanded to display their chemical structure. The moiety labeled "Z" represents the albumin binding subunit, the chemical structure of which is shown, and which is linked to each of the thiol group of the cysteine residues of the XTEN. The two Z compositions are enantiomers, representing the two stereochemistries of the incorporated glutamate moiety such that either can be selected and utilized in the linkage to the XTEN.

[0033] FIG. 5 depicts the structure of an exemplary ABC originating from the LX-41 XTEN sequence described herein. The amino acid sequence is shown with the cysteine groups expanded to display their whole chemical structure. The moiety labeled "Z" represents the albumin binding subunit, the chemical structure of which is shown, which is linked to the thiol group of the cysteine residues of the

XTEN. The two structures are enantiomers, representing the two stereochemistries of the incorporated glutamate moiety such that either can be selected and utilized in the linkage to the XTEN.

[0034] FIG. 6 depicts the structure of an exemplary ABC composition originating from LX-42 XTEN sequence described herein. The amino acid sequence is shown with the cysteine groups expanded to display their whole chemical structure. The moiety labeled “Z” represents the albumin binding subunit, the chemical structure of which is shown, which is linked to the thiol group of the cysteine residues of the XTEN. The two albumin binding subunit structures are enantiomers, representing the two stereochemistries of the incorporated glutamate moiety such that either can be selected and utilized in the linkage to the XTEN.

[0035] FIG. 7 depicts the structure of an exemplary ABC composition originating from LX-45 XTEN sequence described herein. The amino acid sequence is shown with the cysteine groups expanded to display their whole chemical structure. The moiety labeled “Z” represents the albumin binding subunit, the chemical structure of which is shown, which is linked to the thiol group of the cysteine residues of the XTEN. The two albumin binding subunit structures are enantiomers, representing the two stereochemistries of the incorporated glutamate moiety such that either can be selected and utilized in the linkage to the XTEN.

[0036] FIG. 8 depicts the structure of an exemplary ABC composition originating from LX-50 XTEN sequence described herein. The amino acid sequence is shown with the cysteine groups expanded to display their whole chemical structure. The moiety labeled “Z” represents the albumin binding subunit, the chemical structure of which is shown, which is linked to the thiol group of the cysteine residues of the XTEN. The two albumin binding subunit structures are enantiomers, representing the two stereochemistries of the incorporated glutamate moiety such that either can be selected and utilized in the linkage to the XTEN.

[0037] FIG. 9 depicts the structure of an exemplary ABC composition originating from LX-51 XTEN sequence. The amino acid sequence is shown with the cysteine groups expanded to display their whole chemical structure. The moiety labeled “Z” represents the albumin binding subunit, the chemical structure of which is shown, which is linked to the thiol group of the cysteine residues of the XTEN. The two albumin binding subunit structures are enantiomers, representing the two stereochemistries of the incorporated glutamate moiety such that either can be selected and utilized in the linkage to the XTEN.

[0038] FIG. 10 depicts the structure of an exemplary ABC composition originating from LX-54 XTEN sequence. The amino acid sequence is shown with the cysteine groups expanded to display their whole chemical structure. The moiety labeled “Z” represents the albumin binding subunit, the chemical structure of which is shown, which is linked to the thiol group of the cysteine residues of the XTEN. The two albumin binding subunit structures are enantiomers, representing the two stereochemistries of the incorporated glutamate moiety such that either can be selected and utilized in the linkage to the XTEN.

[0039] FIG. 11 depicts the structure of an exemplary ABC composition originating from LX-57 XTEN sequence described herein. The amino acid sequence is shown with the cysteine groups expanded to

display their whole chemical structure. The moiety labeled “Z” represents the albumin binding subunit, the chemical structure of which is shown, which is linked to the thiol group of the cysteine residues of the XTEN. The two albumin binding subunit structures are enantiomers, representing the two stereochemistries of the incorporated glutamate moiety such that either can be selected and utilized in the linkage to the XTEN.

[0040] FIG. 12 depicts the structure of an exemplary ABC composition originating from LX-58 XTEN sequence. The amino acid sequence is shown with the cysteine groups expanded to display their whole chemical structure. The moiety labeled “Z” represents the albumin binding subunit, the chemical structure of which is shown, which is linked to the thiol group of the cysteine residues of the XTEN. The two albumin binding subunit structures are enantiomers, representing the two stereochemistries of the incorporated glutamate moiety such that either can be selected and utilized in the linkage to the XTEN.

[0041] FIG. 13 depicts the structure of an exemplary ABC composition originating from LX-62 XTEN sequence described herein. The amino acid sequence is shown with the cysteine groups expanded to display their whole chemical structure. The moiety labeled “Z” represents the albumin binding subunit, the chemical structure of which is shown, which is linked to the thiol group of the cysteine residues of the XTEN. The two albumin binding subunit structures are enantiomers, representing the two stereochemistries of the incorporated glutamate moiety such that either can be selected and utilized in the linkage to the XTEN.

[0042] FIG. 14 depicts the structure of an exemplary ABC composition originating from LX-31 XTEN sequence, prepared as described in Example 2. The amino acid sequence is shown with the cysteine groups expanded to display their whole chemical structure. The moiety labeled “Z” represents the albumin binding subunit, the chemical structure of which is shown, which is linked to the thiol group of the cysteine residues of the XTEN. The two albumin binding subunit structures are enantiomers, representing the two stereochemistries of the incorporated glutamate moiety such that either can be selected and utilized in the linkage to the XTEN.

[0043] FIG. 15 shows HPLC chromatograms of various conditions for conjugation of the albumin binding subunit to the LX-31 XTEN in order to produce UHLX-31, as described in Example 3.

[0044] FIG. 16 shows data from a pharmacokinetic evaluation of ABC compositions in rats, as described in Example 4. Concentration is plotted as a function of time after injection.

[0045] FIG. 17 shows analysis by RP-HPLC of reaction mixture between an LX-40 XTEN and FTA_01-IA, as described in Example 5.

[0046] FIG. 18 shows analyses of the UHLX-40 product, as described in Example 5. FIG. 18A shows a chromatogram of the gradient elution of the material from an anion exchange column. FIG. 18B depicts results from RP-HPLC analysis of the material before purification. FIG. 18C depicts results from RP-HPLC analysis of the material after elution from the column and pooling desired fractions containing the UHLX-40 product.

[0047] FIG. 19 depicts the comparison of viscosity at various concentrations using UHLX-40, 40 kDa branched PEG, and HSA, as described in Example 6.

[0048] FIG. 20 shows the volume distribution profile from DLS analysis of a 5 mM solution of the UHLX-40 albumin binding conjugate, as described in Example 7.

[0049] FIG. 21 depicts results from an albumin binding evaluation performed by size exclusion chromatography (SEC), as described in Example 8. FIG. 20A in each panel shows, from top to bottom, the ABC composition run alone, HSA run alone, the stoichiometric mixture of the two materials analyzed with the absorbance detector set to 215 nm, the stoichiometric mixture of the two materials analyzed with the absorbance detector set to 280 nm. FIG. 21B is a graph showing the results of integrating the peaks in the 280 nm absorbance versus retention time traces shown in FIG. 21A. The value shown corresponds to the percent of the area under the curve present in the peak corresponding to the complex divided by the total area under the curve.

[0050] FIG. 22 shows results from analysis of the albumin binding experiment, as described in Example 8. FIG. 22A depicts results from the SEC-MALS analysis of the stoichiometric mixture of UHLX-40 and HSA. FIG. 22B depicts results from the SEC-MALS analysis of a mixture with a 10-fold excess of HSA over UHLX-40.

DETAILED DESCRIPTION

[0051] Before the embodiments of the disclosure are described, it is to be understood that such embodiments are provided by way of example only, and that various alternatives to the embodiments of the disclosure described herein may be employed. Numerous variations, changes, and substitutions will now occur to those skilled in the art.

[0052] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Although methods and materials similar or equivalent to those described herein can be used to make and evaluate the conjugates and compositions disclosed herein, suitable methods and materials are described below. The materials, methods, and examples are illustrative only and not intended to be limiting. Numerous variations, changes, and substitutions will now occur to those skilled in the art.

DEFINITIONS

[0053] In the context of the present application, the following terms have the meanings ascribed to them unless specified otherwise:

[0054] As used throughout the specification and claims, the terms “a”, “an” and “the” are used in the sense that they mean “at least one”, “at least a first”, “one or more” or “a plurality” of the referenced components or steps, except in instances wherein an upper limit is thereafter specifically stated. Therefore, a “moiety”, as used herein, means “at least a first moiety” but includes a plurality of moieties. The operable limits and parameters of combinations, as with the amounts of any single agent, will be known to those of ordinary skill in the art in light of the present disclosure.

[0055] As used herein, the term “fatty acid chain” and “carboxylic acid” or “carboxylic acid moiety” are used interchangeably and refer to the hydrocarbon backbone of fatty acids containing 2 to 40 carbon atoms. Preferably, the carboxylic acid chain for use in the compositions of the instant disclosure contains between 6 and 40 carbon atoms, more preferably between 10 and 30 carbon atoms, even more preferably between 15 and 25 carbon atoms. It will be appreciated that carboxylic acid chain length may be selected on the basis of the intended properties of the resulting conjugate or composition and preferred half-life and/or pharmaceutical properties. Preferably the carboxylic acid chain for use in the present disclosure is a straight chain of between 14 and 24 carbon atoms. The carboxylic acids for use in the conjugates and compositions provided herein have either one carboxylic acid group at one terminus of the hydrocarbon chain (“monocarboxylic acids”) or may have a second carboxylic acid at or near the other terminus (“dicarboxylic acids”). Carboxylic acids for use in the conjugates and compositions provided herein may be saturated or may contain one or more units of unsaturation. Non-limiting examples of carboxylic acids suitable for use in the conjugates and compositions herein or for use in making the conjugates and compositions include, for example, n-dodecanoate (C12, laurate), n-tetradecanoate (C14, myristate), n-hexadecanoate (C16, palmitate), n-octadecanoate (C18, stearate), n-eicosanoate (C20, arachidate), n-docosanoate (C22, behenate), n-tetracosanoate (C24), n-triacontanoate (C30), n-tetracontanoate (C40).

[0056] The terms “polypeptide”, “peptide”, and “protein” are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids (such as D-amino acids), and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified, for example, by disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation, such as conjugation with another component or molecule.

[0057] As used herein, the term “amino acid” refers to either natural and/or unnatural or synthetic amino acids, including but not limited to both the D or L optical isomers, and amino acid analogs and peptidomimetics. Standard single or three letter codes are used to designate amino acids.

[0058] The term “natural L-amino acid” refers to the L optical isomer forms of glycine (G), proline (P), alanine (A), valine (V), leucine (L), isoleucine (I), methionine (M), cysteine (C), phenylalanine (F), tyrosine (Y), tryptophan (W), histidine (H), lysine (K), arginine (R), glutamine (Q), asparagine (N), glutamic acid (E), aspartic acid (D), serine (S), and threonine (T).

[0059] The term “non-naturally occurring,” as applied to sequences and as used herein, refers to polypeptide or polynucleotide sequences that do not have a counterpart to, are not complementary to, or do not have a high degree of homology with a wild-type or naturally-occurring sequence found in a mammal. For example, a non-naturally occurring polypeptide or fragment may share no more than 99%, 98%, 95%, 90%, 80%, 70%, 60%, 50% or even less amino acid sequence identity to a natural sequence when suitably aligned.

[0060] A “therapeutic protein” includes any protein or peptide composition of matter desired to be delivered to a subject that provides or is expected to provide some pharmacologic, often beneficial, effect that can be demonstrated *in vivo* or *in vitro*.

[0061] A “therapeutic drug” includes any non-protein small molecule composition of matter desired to be delivered to a subject that provides or is expected to provide some pharmacologic, often beneficial, effect that can be demonstrated *in vivo* or *in vitro*.

[0062] A “fragment” when applied to a therapeutic protein, generally refers to a truncated form of the biologically active protein that retains at least a portion of the therapeutic and/or biological activity. A “variant,” when applied to a therapeutic protein, generally refers to a protein with sequence homology to the native biologically active protein that retains at least a portion of the therapeutic and/or biological activity of the therapeutic protein. For example, a variant protein may share at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% amino acid sequence identity to the reference, biologically active protein. As used herein, the term “therapeutic protein variant” includes proteins modified deliberately, as for example, by site directed mutagenesis, synthesis of the encoding gene, insertions, or accidentally through mutations and that retain activity.

[0063] The term “sequence variant” refers to polypeptides that have been modified compared to their native or original sequence by one or more amino acid insertions, deletions, or substitutions. Insertions may be located at either or both termini of the protein, and/or may be positioned within internal regions of the amino acid sequence. A non-limiting example is substitution of an amino acid in an therapeutic protein with a different amino acid. In deletion variants, one or more amino acid residues in a polypeptide as described herein can be removed. Deletion variants, therefore, can include all fragments of a described polypeptide sequence. In substitution variants, one or more amino acid residues of a polypeptide can be removed and replaced with alternative residues. In one aspect, the substitutions are conservative in nature and conservative substitutions of this type are known in the art.

[0064] The terms “hydrophilic” and “hydrophobic” generally refer to the degree of affinity that a substance has with water. A hydrophilic substance, for example, has a strong affinity for water, tending to dissolve in, mix with, or be wetted by water, while a hydrophobic substance, for example, substantially lacks affinity for water, tending to repel and not absorb water and tending not to dissolve in, mix with, or be wetted by water. Amino acids can be characterized based on their hydrophobicity. A number of scales have been developed. An example is a scale developed by Levitt, M, et al., J Mol Biol (1976) 104:59, which is listed in Hopp, TP, et al., Proc Natl Acad Sci U S A (1981) 78:3824. Examples of “hydrophilic amino acids” are arginine, lysine, threonine, alanine, asparagine, and glutamine. Of particular interest are the hydrophilic amino acids aspartate, glutamate, and serine, and glycine. Examples of “hydrophobic amino acids” are tryptophan, tyrosine, phenylalanine, methionine, leucine, isoleucine, and valine.

[0065] The term “moiety” refers to a component of a larger composition or that is intended to be incorporated into a larger composition, such as a carboxylic acid joined to a soluble bridge.

[0066] “Activity” as applied to form(s) of a conjugate or composition provided herein, generally refers to an action or effect, including but not limited to receptor binding, antagonist activity, agonist activity, a cellular or physiologic response, cell lysis, cell death, or an effect generally known in the art for the effector component of the composition, whether measured by an *in vitro*, *ex vivo* or *in vivo* assay or a clinical effect.

[0067] As used herein, the term “ELISA” refers to an enzyme-linked immunosorbent assay as described herein or as otherwise known in the art.

[0068] A “host cell” includes an individual cell or cell culture which can be or has been a recipient for vectors of the present disclosure, for example, those into which exogenous nucleic acid has been introduced, such as nucleic acids described herein. Host cells include progeny of a single host cell. The progeny may not necessarily be completely identical (in morphology or genetically) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected *in vivo* with a vector of this disclosure.

[0069] “Isolated,” when used to describe the various polypeptides disclosed herein, generally refers to a polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that can interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. As is apparent to those of skill in the art, a non-naturally occurring polynucleotide, peptide, polypeptide, protein, antibody, or fragments thereof, does not require “isolation” to distinguish it from its naturally occurring counterpart. In addition, a “concentrated”, “separated” or “diluted” polynucleotide, peptide, polypeptide, protein, antibody, or fragments thereof, can be distinguishable from its naturally occurring counterpart in that the concentration or number of molecules per volume is generally greater than that of its naturally occurring counterpart. In general, a polypeptide made by recombinant means and expressed in a host cell is considered to be “isolated.”

[0070] An “isolated” nucleic acid refers to a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the polypeptide-encoding nucleic acid. For example, an isolated polypeptide-encoding nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated polypeptide-encoding nucleic acid molecules therefore are distinguished from the specific polypeptide-encoding nucleic acid molecule as it exists in natural cells. However, an isolated polypeptide-encoding nucleic acid molecule includes polypeptide-encoding nucleic acid molecules contained in cells that ordinarily express the polypeptide where, for example, the nucleic acid molecule is in a chromosomal or extra-chromosomal location different from that of natural cells.

[0071] A “chimeric” protein contains at least one fusion polypeptide comprising at least one region in a different position in the sequence than that which occurs in nature. The regions may normally exist in separate proteins and are brought together in the fusion polypeptide; or they may normally exist in the same protein but are placed in a new arrangement in the fusion polypeptide. A chimeric protein may be

created, for example, by chemical synthesis, or by creating and translating a polynucleotide in which the peptide regions are encoded in the desired relationship.

[0072] “Fused” and “fusion” are used interchangeably herein and refer to the joining together of two or more peptide or polypeptide sequences by recombinant means. A “fusion protein” or “chimeric protein” comprises a first amino acid sequence linked to a second amino acid sequence with which it is not naturally linked in nature.

[0073] “Crosslinking,” “conjugating,” “link,” “linking” and “joined to” are used interchangeably herein, and refer to the covalent joining of two different molecules by a chemical reaction. The linking can occur in one or more chemical reactions.

[0074] The term “conjugation partner” as used herein, refers to the individual components that can be linked or are linked in a conjugation reaction.

[0075] The term “conjugate” as used herein, refers to the heterogeneous molecule formed as a result of covalent linking of conjugation partners one to another, e.g., a carboxylic acid covalently linked to a soluble bridge.

[0076] “Cross-linker” and “cross-linking agent” are used interchangeably and, in their broadest context, refer to a chemical entity used to covalently join two or more entities. For example, a cross-linker joins a therapeutic protein, peptide, or a drug to an XTEN. It will be understood by one of skill in the art that a cross-linker can refer to the covalently-bound reaction product remaining after the crosslinking of the reactants. The cross-linker can also comprise one or more reactants which have not yet reacted but which are capable to react with another entity.

[0077] In the context of polypeptides, a “linear sequence” or a “sequence” refers to an order of amino acids in a polypeptide in an amino to carboxyl terminus direction in which residues that neighbor each other in the sequence are contiguous in the primary structure of the polypeptide. A “partial sequence” refers to a linear sequence of part of a polypeptide that is known to comprise additional residues in one or both directions.

[0078] “Heterologous” refers to that which is derived from a genotypically distinct entity from the rest of the entity to which it is being compared. For example, a glycine rich sequence removed from its native coding sequence and operatively linked to a coding sequence other than the native sequence is a heterologous glycine rich sequence. The term “heterologous” as applied to a polynucleotide or a polypeptide, refers to a polynucleotide or polypeptide that is derived from a genotypically distinct entity from that of the rest of the entity to which it is being compared.

[0079] The terms “polynucleotides”, “nucleic acids”, “nucleotides” and “oligonucleotides” are used interchangeably. They refer to nucleotides of any length, encompassing a singular nucleic acid as well as plural nucleic acids, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: coding or non-coding regions of a gene or gene fragment, loci (locus) defined from linkage analysis, exons, introns, messenger RNA (mRNA), transfer

RNA, ribosomal RNA, ribozymes, aptamers, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component.

[0080] The term “complement of a polynucleotide” refers to a polynucleotide molecule having a complementary base sequence and reverse orientation as compared to a reference sequence, such that it could hybridize with a reference sequence with complete fidelity.

[0081] “Recombinant,” as applied to a polynucleotide, refers to a polynucleotide which is the product of various combinations of recombination steps which may include cloning, restriction and/or ligation steps, and other procedures that result in expression of a recombinant protein in a host cell.

[0082] The terms “gene” and “gene fragment” are used interchangeably herein. They refer to a polynucleotide containing at least one open reading frame that is capable of encoding a particular protein after being transcribed and translated. A gene or gene fragment may be genomic or cDNA, as long as the polynucleotide contains at least one open reading frame, which may cover the entire coding region or a segment thereof. A “fusion gene” is a gene composed of at least two heterologous polynucleotides that are linked together.

[0083] As used herein, a “coding region” or “coding sequence” refers to a portion of polynucleotide which consists of codons translatable into amino acids. Although a “stop codon” (TAG, TGA, or TAA) is typically not translated into an amino acid, it may be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, and the like, generally are not part of a coding region. The boundaries of a coding region are typically determined by a start codon at the 5' terminus, encoding the amino terminus of the resultant polypeptide, and a translation stop codon at the 3' terminus, encoding the carboxyl terminus of the resulting polypeptide. Two or more coding regions of the present invention can be present in a single polynucleotide construct, e.g., on a single vector, or in separate polynucleotide constructs, e.g., on separate (different) vectors. It follows, then, that a single vector can contain just a single coding region, or comprise two or more coding regions, e.g., a single vector can separately encode a first moiety and a second moiety of a fusion protein. In addition, a vector, polynucleotide, or nucleic acid of the disclosure can encode heterologous coding regions, either fused or unfused to a nucleic acid encoding a binding domain of the disclosure. Heterologous coding regions include, without limitation, specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain.

[0084] The term “downstream” refers to a nucleotide sequence that is located 3' to a reference nucleotide sequence. In certain embodiments, downstream nucleotide sequences relate to sequences that

follow the starting point of transcription. For example, the translation initiation codon of a gene is located downstream of the start site of transcription.

[0085] The term “upstream” refers to a nucleotide sequence that is located 5’ to a reference nucleotide sequence. In certain embodiments, upstream nucleotide sequences relate to sequences that are located on the 5’ side of a coding region or starting point of transcription. For example, most promoters are located upstream of the start site of transcription.

[0086] “Homology” or “homologous” refers to sequence similarity or interchangeability between two or more polynucleotide sequences or between two or more polypeptide sequences. When using a program such as BestFit to determine sequence identity, similarity or homology between two different amino acid sequences, the default settings may be used, or an appropriate scoring matrix, such as blosum45 or blosum80, may be selected to optimize identity, similarity or homology scores. Preferably, polynucleotides that are homologous are those which hybridize under stringent conditions as defined herein and have at least 70%, preferably at least 80%, more preferably at least 90%, more preferably 95%, more preferably 97%, more preferably 98%, and even more preferably 99% sequence identity compared to those sequences. Polypeptides that are homologous preferably have sequence identities that are at least 70%, preferably at least 80%, even more preferably at least 90%, even more preferably at least 95-99% identical.

[0087] “Ligation” as applied to polynucleic acids refers to the process of forming phosphodiester bonds between two nucleic acid fragments or genes, linking them together. To ligate the DNA fragments or genes together, the ends of the DNA must be compatible with each other. In some cases, the ends will be directly compatible after endonuclease digestion. However, it may be necessary to first convert the staggered ends commonly produced after endonuclease digestion to blunt ends to make them compatible for ligation.

[0088] The terms “stringent conditions” or “stringent hybridization conditions” includes reference to conditions under which a polynucleotide will hybridize to its target sequence, to a detectably greater degree than other sequences (e.g., at least 2-fold over background). Generally, stringency of hybridization is expressed, in part, with reference to the temperature and salt concentration under which the wash step is carried out. Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short polynucleotides (e.g., 10 to 50 nucleotides) and at least about 60°C for long polynucleotides (e.g., greater than 50 nucleotides)—for example, “stringent conditions” can include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and three washes for 15 min each in 0.1×SSC/1% SDS at 60°C to 65°C. Alternatively, temperatures of about 65°C, 60°C, 55°C, or 42°C may be used. SSC concentration may be varied from about 0.1 to 2×SSC, with SDS being present at about 0.1%. Such wash temperatures are typically selected to be about 5°C to 20°C lower than the thermal melting point for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence

hybridizes to a perfectly matched probe. An equation for calculating T_m and conditions for nucleic acid hybridization are well known and can be found in Sambrook, J. *et al.*, "Molecular Cloning: A Laboratory Manual," 3rd edition, Cold Spring Harbor Laboratory Press, 2001. Typically, blocking reagents are used to block non-specific hybridization. Such blocking reagents include, for instance, sheared and denatured salmon sperm DNA at about 100-200 $\mu\text{g/ml}$. Organic solvent, such as formamide at a concentration of about 35-50% v/v, may also be used under particular circumstances, such as for RNA:DNA hybridizations. Useful variations on these wash conditions will be readily apparent to those of ordinary skill in the art.

[0089] The terms "percent identity," "percentage of sequence identity," and "% identity," as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences. Percent identity may be measured over the length of an entire defined polynucleotide sequence, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polynucleotide sequence, for instance, a fragment of at least 45, at least 60, at least 90, at least 120, at least 150, at least 210 or at least 450 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured. The percentage of sequence identity is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of matched positions (at which identical residues occur in both polypeptide sequences), dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. When sequences of different length are to be compared, the shortest sequence defines the length of the window of comparison. Conservative substitutions are not considered when calculating sequence identity.

[0090] "Percent sequence identity," or "sequence identity" with respect to the polypeptide sequences identified herein, refers to the percentage of amino acid residues in a query sequence that are identical with the amino acid residues of a second, reference polypeptide sequence or a portion thereof, after optimally aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity over comparable lengths of the polypeptides, and not considering any conservative substitutions as part of the sequence identity, thereby resulting in optimal alignment. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve optimal alignment over the full length of the sequences being compared. Percent identity may be measured over

the length of an entire defined polypeptide sequence, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

[0091] “Repetitiveness” used in the context of polynucleotide sequences refers to the degree of internal homology in the sequence such as, for example, the frequency of identical nucleotide sequences of a given length. Repetitiveness can, for example, be measured by analyzing the frequency of identical sequences.

[0092] The term “expression” as used herein refers to a process by which a polynucleotide produces a gene product, for example, an RNA or a polypeptide. It includes without limitation transcription of the polynucleotide into messenger RNA (mRNA), transfer RNA (tRNA), small hairpin RNA (shRNA), small interfering RNA (siRNA) or any other RNA product, and the translation of an mRNA into a polypeptide. Expression produces a “gene product.” As used herein, a gene product can be either a nucleic acid, e.g., a messenger RNA produced by transcription of a gene, or a polypeptide which is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, e.g., polyadenylation or splicing, or polypeptides with post translational modifications, e.g., methylation, glycosylation, the addition of lipids, association with other protein subunits, or proteolytic cleavage.

[0093] The terms “vector” or “expression vector” are used interchangeably and refer to a nucleic acid molecule, preferably self-replicating in an appropriate host, which, in some cases, can transfer an inserted nucleic acid molecule into and/or between host cells. The term includes vectors that function primarily for insertion of DNA or RNA into a cell, replication of vectors that function primarily for the replication of DNA or RNA, and expression vectors that function for transcription and/or translation of the DNA or RNA. Also included are vectors that provide more than one of the above functions. An “expression vector,” in some cases, refers to a polynucleotide which, when introduced into an appropriate host cell, can be transcribed and translated into a polypeptide(s). An “expression system” usually connotes a suitable host cell comprised of an expression vector that can function to yield a desired expression product.

[0094] “Serum degradation resistance,” as applied to a polypeptide, refers to the ability of the polypeptides to withstand degradation in blood or components thereof, which typically involves proteases in the serum or plasma. The serum degradation resistance can be measured by combining the protein with human (or mouse, rat, monkey, as appropriate) serum or plasma, typically for a range of days (e.g. 0.25, 0.5, 1, 2, 4, 8, 16 days), typically at about 37°C. The samples for these time points can be run on a Western blot assay and the protein is detected with an antibody. The antibody can be targeted to a tag in the protein. If the protein shows a single band on the western, where the protein’s size is identical to that

of the injected protein, then it may be concluded that no degradation has occurred. In this exemplary method, the time point where 50% of the protein is degraded, as judged by Western blots or equivalent techniques, can be referred to as the serum degradation half-life or “serum half-life” of the protein.

[0095] The terms “ $t_{1/2}$ ”, “half-life”, “terminal half-life”, “elimination half-life” and “circulating half-life” are used interchangeably herein and, as used herein refer to the terminal half-life calculated as $\ln(2)/K_{el}$. K_{el} is the terminal elimination rate constant calculated by linear regression of the terminal linear portion of the log concentration vs. time curve. Half-life typically refers to the time required for half the quantity of an administered substance deposited in a living organism to be metabolized or eliminated by normal biological processes. When a clearance curve of a given polypeptide is constructed as a function of time, the curve is usually biphasic with a rapid α -phase and longer β -phase. The typical β phase half-life of a human antibody in humans is 21 days.

[0096] “Active clearance” refers to the mechanisms by which a protein is removed from the circulation other than by filtration, and which includes removal from the circulation mediated by cells, receptors, metabolism, or degradation of the protein.

[0097] “Apparent molecular weight factor” and “apparent molecular weight” are related terms referring to a measure of the relative increase or decrease in apparent molecular weight exhibited by a particular amino acid or polypeptide sequence. The apparent molecular weight can be determined using size exclusion chromatography (SEC) or similar methods by comparing to globular protein standards, and can be measured in “apparent kDa” units. The apparent molecular weight factor is the ratio between the apparent molecular weight and the actual molecular weight; the latter predicted by adding, based on amino acid composition, the calculated molecular weight of each type of amino acid in the composition or by estimation from comparison to molecular weight standards, for example, in an SDS electrophoresis gel.

[0098] The terms “hydrodynamic radius” or “Stokes radius” refer to the effective radius (R_h in nm) of a molecule in a solution measured by assuming that it is a body moving through the solution and resisted by the solution’s viscosity. In embodiments of the disclosure, the hydrodynamic radius measurements of the XTEN polypeptides correlate with the “apparent molecular weight factor” which is a more intuitive measure. The “hydrodynamic radius” of a protein affects its rate of diffusion in aqueous solution as well as its ability to migrate in gels of macromolecules. The hydrodynamic radius of a protein is determined by its molecular weight as well as by its structure, including shape and compactness. Methods for determining the hydrodynamic radius are well known in the art, such as by the use of size exclusion chromatography (SEC), as described in U.S. Patent Nos. 6,406,632 and 7,294,513. Most proteins have globular structure, which is the most compact three-dimensional structure a protein can have with the smallest hydrodynamic radius. Some proteins adopt a random and open, unstructured, or ‘linear’ conformation and as a result have a much larger hydrodynamic radius compared to typical globular proteins of similar molecular weight.

[0099] “Diffusion coefficient” refers to the magnitude of the molar flux through a surface per unit concentration gradient out-of-plane. In dilute species transport, the flux due to diffusion is given by Fick's first law, which only depends on a single property of the solute's interaction with the solvent: the diffusion coefficient.

[00100] “Physiological conditions” refers to a set of conditions in a living host as well as *in vitro* conditions, including temperature, salt concentration, pH, that mimic those conditions of a living subject. A host of physiologically relevant conditions for use in *in vitro* assays have been established. Generally, a physiological buffer contains a physiological concentration of salt and is adjusted to a neutral pH ranging from about 6.5 to about 7.8, and preferably from about 7.0 to about 7.5. A variety of physiological buffers are listed in Sambrook et al. (2001). Physiologically relevant temperature ranges from about 25°C to about 38°C, and preferably from about 35°C to about 37°C.

[00101] The term “binding domain,” as used herein, includes the categories of antibodies or antibody fragments that have specific binding affinity for a target antigen or ligand such as cell-surface receptors or antigens or glycoproteins, oligonucleotides, enzymatic substrates, antigenic determinants, or binding sites that may be present in or on the surface of a target tissue or cell.

[00102] The term “antibody” is used herein in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

[00103] The term “monoclonal antibody,” as used herein, refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is generally directed against a single determinant on an antigen. Thus, the modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being known in the art or described herein.

[00104] An “antibody fragment” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody and that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂, diabodies, linear

antibodies, single-chain antibody molecules (e.g., scFv), and multispecific antibodies formed from antibody fragments.

[00105] The terms “antigen”, “target antigen” and “immunogen” are used interchangeably herein to refer to the structure or binding determinant that an antibody, antibody fragment or an antibody fragment-based molecule binds to or has specificity against.

[00106] The term “epitope” refers to the particular site on an antigen molecule to which an antibody or binding domain binds.

[00107] The terms “specific binding” or “specifically bind” are used interchangeably herein to refer to the high degree of binding affinity of a binding domain to its corresponding target. Typically, specific binding as measured by one or more of the assays disclosed herein would have a dissociation constant or K_d of less than about 10^{-4} M.

[00108] “Affinity” refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an albumin binding conjugate) and its binding partner (e.g., human serum albumin (HSA)). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., albumin binding conjugate and HSA). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_d).

[00109] “Inhibition constant” or “ K_i ” are used interchangeably and refer to the dissociation constant of the enzyme-inhibitor complex, or the reciprocal of the binding affinity of the inhibitor to the enzyme.

[00110] “Dissociation constant” or “ K_d ” are used interchangeably and refer to the affinity between a ligand “L” and a protein “P”; i.e. how tightly a ligand binds to a particular protein. It can be calculated using the formula $K_d = [L][P]/[LP]$, where [P], [L] and [LP] represent molar concentrations of the protein, ligand and complex, respectively. The term “ k_{on} ”, as used herein, is intended to refer to the on rate constant for association of an albumin binding conjugate to the HSA to form the complex. The term “ k_{off} ”, as used herein, is intended to refer to the off rate constant for dissociation of an albumin binding conjugate from the complex.

[00111] The term “antagonist,” as used herein, includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native polypeptide disclosed herein. Methods for identifying antagonists of a polypeptide may comprise contacting a native polypeptide with a candidate antagonist molecule and measuring a detectable change in one or more biological activities normally associated with the native polypeptide. In the context of the present invention, antagonists may include proteins, nucleic acids, carbohydrates, antibodies or any other molecules that decrease the effect of a biologically active protein.

[00112] A “target,” as used herein, refers to the ligand of a binding domain or antibody, such as cell-surface receptors, antigens, glycoproteins, oligonucleotides, enzymatic substrates, antigenic determinants, or binding sites that may be present on the surface of a target tissue or cell.

[00113] A “target tissue,” as used herein, refers to a tissue that is the cause of or is part of a disease condition such as, but not limited to cancer or inflammatory conditions. Sources of diseased target tissue include a body organ, a tumor, a cancerous cell, bone, skin, cells that produce cytokines or factors contributing to a disease condition.

[00114] A “defined medium,” as used herein, refers to a medium comprising nutritional and hormonal requirements necessary for the survival and/or growth of the cells in culture such that the components of the medium are known. Traditionally, the defined medium has been formulated by the addition of nutritional and growth factors necessary for growth and/or survival. Typically, the defined medium provides at least one component from one or more of the following categories: a) all essential amino acids, and usually the basic set of twenty amino acids, b) an energy source, usually in the form of a carbohydrate such as glucose; c) vitamins and/or other organic compounds required at low concentrations; d) free carboxylic acids; and e) trace elements, where trace elements are defined as inorganic compounds or naturally occurring elements that are typically required at very low concentrations, usually in the micromolar range. The defined medium may also optionally be supplemented with one or more components from any of the following categories: a) one or more mitogenic agents; b) salts and buffers as, for example, calcium, magnesium, and phosphate; c) nucleosides and bases such as, for example, adenosine and thymidine, hypoxanthine; and d) protein and tissue hydrolysates.

[00115] The term “agonist” is used in the broadest sense and includes any molecule that mimics a biological activity of a native polypeptide disclosed herein. Suitable agonist molecules specifically include agonist antibodies or antibody fragments, fragments or amino acid sequence variants of native polypeptides, peptides, small organic molecules, etc. Methods for identifying agonists of a native polypeptide may comprise contacting a native polypeptide with a candidate agonist molecule and measuring a detectable change in one or more biological activities normally associated with the native polypeptide.

[00116] As used herein, “treatment” or “treating,” or “palliating” or “ameliorating” is used interchangeably herein. These terms refer to an approach for obtaining beneficial or desired results including, but not limited to, a therapeutic benefit and/or a prophylactic benefit. By therapeutic benefit is meant eradication or amelioration of the underlying disorder being treated. Also, a therapeutic benefit is achieved with the eradication or amelioration of one or more of the physiological symptoms associated with the underlying disorder such that an improvement is observed in the subject, notwithstanding that the subject may still be afflicted with the underlying disorder. For prophylactic benefit, the compositions may be administered to a subject at risk of developing a particular disease, or to a subject reporting one or more of the physiological symptoms of a disease, even though a diagnosis of this disease may not have been made.

[00117] A “therapeutic effect” or “therapeutic benefit,” as used herein, refers to a physiologic effect, including but not limited to the mitigation, amelioration, or prevention of disease in humans or other

animals, or to otherwise enhance physical or mental wellbeing of humans or animals, resulting from administration of a conjugate or composition of the disclosure other than the ability to induce the production of an antibody against an antigenic epitope possessed by the biologically active protein. For prophylactic benefit, the conjugate or composition may be administered to a subject at risk of developing a particular disease, a recurrence of a former disease, condition or symptom of the disease, or to a subject reporting one or more of the physiological symptoms of a disease, even though a diagnosis of this disease may not have been made.

[00118] The terms “therapeutically effective amount” and “therapeutically effective dose”, as used herein, refer to an amount of a therapeutic agent, such as a drug or a biologically active protein, either alone or as a part of a polypeptide composition, that is capable of having any detectable, beneficial effect on any symptom, aspect, measured parameter or characteristics of a disease state or condition when administered in one or repeated doses to a subject. Such effect need not be absolute to be beneficial. Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[00119] The term “therapeutically effective dose regimen”, as used herein, refers to a schedule for consecutively administered multiple doses (i.e., at least two or more) of a therapeutic agent such as a biologically active protein, either alone or as a part of a polypeptide composition, wherein the doses are given in therapeutically effective amounts to result in sustained beneficial effect on any symptom, aspect, measured parameter or characteristics of a disease state or condition.

I). GENERAL TECHNIQUES

[00120] The practice of the present invention employs, unless otherwise indicated, conventional techniques of immunology, biochemistry, chemistry, molecular biology, microbiology, cell biology, genomics and recombinant DNA, which are within the skill of the art. *See* Sambrook, J. *et al.*, “Molecular Cloning: A Laboratory Manual,” 3rd edition, Cold Spring Harbor Laboratory Press, 2001; “Current protocols in molecular biology”, F. M. Ausubel, *et al.* eds., 1987; the series “Methods in Enzymology,” Academic Press, San Diego, CA.; “PCR 2: a practical approach”, M.J. MacPherson, B.D. Hames and G.R. Taylor eds., Oxford University Press, 1995; “Antibodies, a laboratory manual” Harlow, E. and Lane, D. eds., Cold Spring Harbor Laboratory, 1988; “Goodman & Gilman’s The Pharmacological Basis of Therapeutics,” 11th Edition, McGraw-Hill, 2005; and Freshney, R.I., “Culture of Animal Cells: A Manual of Basic Technique,” 4th edition, John Wiley & Sons, Somerset, NJ, 2000, the contents of which are incorporated in their entirety herein by reference.

II). ALBUMIN BINDING CONJUGATES

[00121] The present disclosure relates to compounds capable of binding albumin for use in extending the *in vivo* serum half-life of therapeutic proteins to which they are attached, as well as enhancing certain pharmaceutical properties of the resulting conjugates such that the conjugates and compositions thereof can be formulated at high concentrations for administration to a subject in need thereof. More

specifically the disclosure relates to albumin binding conjugates to which one or more therapeutic proteins, or therapeutic drugs, or both are attached.

[00122] The use of fatty acids or carboxylic acids, that bind albumin to increase *in vivo* half-life of insulin has been described by Kurtzhals et al., Biochem. J., 312, 725-731 (1995), in which insulin derivatives were produced by acylation of insulin with fatty acids. The resulting compositions were demonstrated to have affinity for albumin and, in particular, it was demonstrated that increasing the number of carbon atoms in the acyl chain increased the affinity of the binding to albumin.

[00123] The present disclosure provides new conjugates and compositions thereof that are capable of binding albumin with greater affinity than single carboxylic acid compositions for use in extending the half-life of therapeutic proteins to which they are attached, as well as enhancing certain pharmaceutical properties, resulting in improved formulations. Methods for the production of such albumin binding conjugates, methods for the production of albumin binding conjugates comprising therapeutic proteins, and pharmaceutical compositions containing them are also provided, as well as methods of use of the compositions in the treatment or prevention of diseases.

[00124] It is an object of the disclosure to provide albumin binding conjugates useful in the preparation of compositions comprising therapeutic proteins that have enhanced pharmacokinetic and pharmaceutical properties. In a first aspect, the invention provides albumin binding conjugates having four components; 1) an extended polypeptide (XTEN) comprising two, three or four cysteine residues interspersed along the polypeptide chain and, optionally, a single lysine residue at or near the C-terminus; 2) a linker; 3) a soluble bridge moiety; and 4) a long-chain carboxylic acid moiety; the latter three components are joined together (hereinafter referred to as “albumin binding subunit”) and linked to each cysteine residue of the XTEN. Each component is described more fully, below. Alternatively, polypeptides such as PAS or elastin-like proteins can be substituted for XTEN in the conjugates.

1. XTEN

[00125] In one aspect, it is an object of the disclosure to provide XTEN polypeptides comprising cysteine residues to which albumin binding subunits are linked by chemical conjugation, resulting in albumin binding conjugates. In one embodiment, the resulting albumin binding conjugates can be linked to a therapeutic protein or a single amino acid residue of a therapeutic protein at the N-terminus of the XTEN by a cross-linker. In another embodiment, the XTEN of the resulting albumin binding conjugates further comprise a lysine residue at or near the C-terminus to which a therapeutic protein or a single amino acid residue of a therapeutic protein is linked by a cross-linker.

[00126] The XTEN of the subject conjugates and compositions thereof are extended length polypeptides with non-naturally occurring, substantially non-repetitive sequences that are composed mainly of small hydrophilic or neutral amino acids, with the sequence having a low degree or no secondary or tertiary structure under physiologic conditions, having two, three or four cysteine residues for the conjugation of the albumin binding subunits (further described below). Exemplary methods for making the XTEN utilized in the subject conjugates and compositions disclosed herein are presented in

the Examples and are similar to methods describe in U.S. Patent Application Publication No. US20150037359A1. In the subject conjugates and compositions disclosed herein, XTEN confers certain desirable pharmacokinetic, physicochemical and pharmaceutical properties when linked to a therapeutic protein and/or an albumin binding subunit. Such desirable properties include, but are not limited to, enhanced pharmacokinetic parameters, conformational flexibility, enhanced aqueous solubility, high degree of protease resistance, low immunogenicity, low binding to mammalian receptors, the ability to formulate product at high concentration but with low viscosity, and increased hydrodynamic (or Stokes) radii. As used herein, “XTEN” specifically excludes antibodies or antibody fragments such as single-chain antibodies or Fc fragments of a light chain or a heavy chain.

[00127] Typically, the XTEN component of the subject conjugates and compositions are designed to behave like denatured peptide sequences under physiological conditions, despite the length of the polymer. Denatured describes the state of a peptide in solution that is characterized by a large conformational freedom of the peptide backbone. Most peptides and proteins adopt a denatured conformation in the presence of high concentrations of denaturants or at elevated temperature. Peptides in denatured conformation have, for example, characteristic circular dichroism (CD) spectra and are characterized by a lack of long-range interactions as determined by NMR. “Denatured conformation” and “unstructured conformation” are used synonymously herein. In some cases, the disclosure provides XTEN sequences that, under physiologic conditions, can resemble denatured sequences substantially devoid of secondary structure under physiologic conditions. “Substantially devoid,” as used in this context, generally means that at least about 80%, or about 90%, or about 95%, or at least about 99% of the XTEN amino acid residues of the XTEN sequence do not contribute to alpha helices or beta-sheets, as measured or determined by the methods described herein.

[00128] A variety of methods have been established in the art to discern the presence or absence of secondary and tertiary structures in a given polypeptide. In particular, secondary structure can be measured spectrophotometrically, e.g., by circular dichroism spectroscopy in the “far-UV” spectral region (190-250 nm). Secondary structure elements, such as alpha-helix and beta-sheet, each give rise to a characteristic shape and magnitude of CD spectra. Secondary structure can also be predicted for a polypeptide sequence via certain computer programs or algorithms, such as the well-known Chou-Fasman algorithm (Chou, P. Y., et al. (1974) *Biochemistry*, 13: 222-45) and the Garnier-Osguthorpe-Robson (“GOR”) algorithm (Garnier J, Gibrat JF, Robson B. (1996), GOR method for predicting protein secondary structure from amino acid sequence. *Methods Enzymol* 266:540-553), as described in US Patent Application Publication No. US20030228309. For a given sequence, the algorithms can predict whether there exists some or no secondary structure at all, expressed as the total and/or percentage of residues of the sequence that form, for example, alpha-helices, beta-sheets or beta-turns, or are predicted to result in random coil formation (which lacks secondary structure).

[00129] In some cases, the XTEN sequences of the conjugates and compositions herein can have an alpha-helix percentage ranging from 0% to less than about 5% and a beta-sheet percentage ranging from

0% to less than about 5% as determined by a Chou-Fasman algorithm, such as that found in the World Wide Web at fasta.bioch.virginia.edu/fasta_www2/fasta_www.cgi?rm=misc1 as it existed on April 14, 2016. In other cases, the XTEN sequences of the conjugates and compositions herein can have a high degree of random coil percentage, as determined by a GOR algorithm, provided by Pole Informatique Lyonnais at the Network Protein Sequence Analysis internet site, URL located on the World Wide Web at [.npsa-pbil.ibcp.fr/cgi-bin/secpred_gor4.pl](http://npsa-pbil.ibcp.fr/cgi-bin/secpred_gor4.pl) as it existed on April 14, 2016. In some embodiments, an XTEN sequence can have at least about 80%, more preferably at least about 90%, more preferably at least about 91%, more preferably at least about 92%, more preferably at least about 93%, more preferably at least about 94%, more preferably at least about 95%, more preferably at least about 96%, more preferably at least about 97%, more preferably at least about 98%, and most preferably at least about 99% random coil, as determined by a GOR algorithm. In a preferred embodiment, the XTEN of the subject conjugates and compositions has less than 2% alpha-helices and less than 2% beta-sheets as determined by the Chou-Fasman algorithm, and greater than 95% random coil formation as determined by the GOR algorithm.

[00130] XTEN sequences of the subject conjugates and compositions can be substantially non-repetitive; e.g. no three contiguous amino acids in the sequence are identical amino acid types unless the amino acid is serine, in which case no more than three contiguous amino acids are serine residues. In general, repetitive amino acid sequences have a tendency to aggregate or form higher order structures, as exemplified by natural repetitive sequences such as collagens and leucine zippers, or form contacts resulting in crystalline or pseudocrystalline structures. In contrast, the low tendency of non-repetitive sequences to aggregate enables the design of long-sequence XTENs with a relatively low frequency of charged amino acids that would be likely to aggregate if the sequences were otherwise repetitive.

[00131] It has been established that the non-repetitive characteristic of XTEN of the present disclosure together with the particular types of amino acids that predominate in the XTEN, rather than the absolute primary sequence, confers the enhanced physicochemical and biological properties of the XTEN and the resulting conjugates and compositions comprising XTEN. Accordingly, while the sequences of Table 1 are exemplary, they are not intended to be limiting, as sequences having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the sequences of Table 1 exhibit the enhanced properties of XTEN. These enhanced properties include, but are not limited to, a high degree of expression of the XTEN protein in a host cell, greater genetic stability of the genes encoding XTEN, the XTEN confers a greater degree of solubility, less tendency to aggregate, and enhanced pharmacokinetics of the conjugates and compositions comprising therapeutic proteins compared to therapeutic proteins not linked to XTEN. In addition, the unstructured characteristic and large hydrodynamic radius also contributed to the enhanced pharmacokinetic properties and reduced extravasation out of the circulatory system in normal tissue. These enhanced properties permit more efficient manufacturing, greater uniformity of the final product, lower cost of goods, and/or facilitate the formulation of pharmaceutical preparations containing extremely high protein concentrations; in some cases exceeding 100 mg/ml, as well as an improved safety profile and reduced dosing interval, described more fully below.

[00132] In one embodiment, the disclosure provides XTEN polypeptides comprising two cysteine residues. In another embodiment, the disclosure provides XTEN polypeptides comprising three cysteine residues. In another embodiment, the disclosure provides XTEN polypeptides comprising four cysteine residues. In another embodiment, the disclosure provides XTEN polypeptides comprising two, three, or four cysteine residue and a single lysine residue at or near the C-terminus of the XTEN. In some embodiments, the cysteine residues are interspersed along the length of the XTEN polypeptide, each being separated from an adjacent cysteine residue by between at least about 14 to about 96 residues, or between at least about 24 to about 72 residues, or between at least about 36 to about 48 residues, or between at least about 36 to about 51 residues, depending on the length of the XTEN. In some embodiments, the cysteine residues are concentrated on the C-terminal end of the XTEN, separated from each other by 9 to about 24 residues.

[00133] In preferred embodiments, the XTEN lengths for incorporation into the albumin binding conjugates range from about 72 amino acids to about 288 amino acids in length. In some embodiments, the XTEN component of the albumin binding conjugates has about 72 amino acids, or about 108 amino acids, or about 144 amino acids, or about 180 amino acids, or about 216 amino acids, or about 244 amino acids. In a preferred embodiment, the XTEN component of the albumin binding conjugates has 144 amino acids of which two, three, or four residues are cysteines. It is specifically contemplated that the design of the albumin binding conjugates with such lengths of XTEN result in enhanced pharmaceutical properties compared to conjugates having long lengths of XTEN (e.g, 432 or 864 amino acids), including reduced molecular weight and reduced viscosity, yet still retain high solubility, reduced aggregation, and enhanced terminal half-life, permitting formulations (when combined with a therapeutic protein, as described below) with high concentration of final drug product.

[00134] In one embodiment, the XTEN of the subject albumin binding conjugates has two cysteine residues and has at least 90%, or 91%, or 92%, or 93%, or 94%, or 95%, or 96%, or 97%, or 98%, or 99% identity to or is identical to a sequence set forth in Table 1, when optimally aligned. In another embodiment, the XTEN of the subject albumin binding conjugates has three cysteine residues and has at least 90%, or 91%, or 92%, or 93%, or 94%, or 95%, or 96%, or 97%, or 98%, or 99% identity to or is identical to a sequence set forth in Table 1, when optimally aligned. In still another embodiment, the XTEN of the subject albumin binding conjugates has four cysteine residues and has at least 90%, or 91%, or 92%, or 93%, or 94%, or 95%, or 96%, or 97%, or 98%, or 99% identity to or is identical to a sequence set forth in Table 1, when optimally aligned. In still other embodiments, the XTEN of the subject albumin binding conjugates has two, or three, or four cysteine residues interspersed in the XTEN sequence, has a single lysine residue at the C-terminus, and has at least 90%, or 91%, or 92%, or 93%, or 94%, or 95%, or 96%, or 97%, or 98%, or 99% identity to or is identical to a sequence set forth in Table 1, when optimally aligned. In one embodiment, the XTEN of the subject albumin binding conjugates has two cysteine residues and has at least 90%, or 91%, or 92%, or 93%, or 94%, or 95%, or 96%, or 97%, or 98%, or 99% identity to or is identical to the LX-40 sequence set forth in Table 1, when optimally

			EGSASK
LX-41	72	C18, C36, C54	GSPGAGSPAGSPTSTEEGCGEESATPESGPGTSTEGCGESAPGSPAG SPTSTEEGCGETEPSEGSAPGTESASR
LX-42	288	C72, C144, C216	GSPGAGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAG SPTSTEEGTSTEPSEGSAPGTSTEGCGESAPGTSESATPESGPGSE PATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSESATPESGPG TSTEGCGESAPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGS PGTSTEPSEGSAPGTSESATPESGPGTSTEGCGESAPGTSESATPE SGPGSEPATSGSETPGTSTEPSEGSAPGTSTEPSEGSAPGTSESAT PESGPGTESASR
LX-43	108	C36, C72	GSPGAGSPAGSPTSTEEGTSESATPESGPGTSTEGCGESAPGSPAG SPTSTEEGTSTEPSEGSAPGTSTEGCGESAPGTSESATPESGPGSE PATSGSETPGSESASR
LX-44	144	C48, C96	GSPGAGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAS GCGSTEEGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGSE PAGCGSETPGSEPATSGSETPGSPAGSPTSTEEGTSESATPESGPG TESASR
LX-45	108	C27, C54, C81	GSPGAGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAG SPTSTEEGCGETEPSEGSAPGTSTEPSEGSAPGEGCGATPESGPGSE PATSGSETPGSESASR
LX-46	108	C21, C42, C63, C84	GSPGAGSPAGSPTSTEEGEGCGATPESGPGTSTEPSEGSAGCGPAG SPTSTEEGTSTEPSEGCPTSTEPSEGSAPGTSESGCGESGPGSE PATSGSETPGSESASR
LX-47	144	C28, C56, C84, C112	GSPGAGSPAGSPTSTEEGTSESATPEGCGPTSTEPSEGSAPGSPAG SPTSTEEGTGCGEPSEGSAPGTSTEPSEGSAPGTSESGCGESGPGSE PATSGSETPGSEPATSGEGCGPSPAGSPTSTEEGTSESATPESGPG TESASR
LX-48	108	C88, C98	GSPGAGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAG SPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPEGCGPSE PATSGCGEPGSESASR
LX-49	144	C125, C135	GSPGAGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAG SPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGSE PATSGSETPGSEPATSGSETPGSPAGSPTTEEGCGTSESATEGCGPG TESASR
LX-50	108	C78, C88, C98	GSPGAGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAG SPTSTEEGTSTEPSEGSAPGTSTEPSEGSAGCGSESATPEGCGPSE PATSGCGEPGSESASR
LX-51	144	C115, C125, C135	GSPGAGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAG SPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGSE PATSGSETPGSEPATSGSETPGCGAGSPTTEEGCGTSESATEGCGPG TESASR
LX-52	108	C68, C78, C88, C98	GSPGAGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAG SPTSTEEGTSTEPSEGSAPTGCCEPSEGSAGCGSESATPEGCGPSE PATSGCGEPGSESASR
LX-53	144	C105, C115, C125, C135	GSPGAGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAG SPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGSE PATSGSETPGEGCGTSGSETPGCGAGSPTTEEGCGTSESATEGCGPG TESASR
LX-54	144	C31, C81, C135	GSPGAGSPAGSPTSTEEGTSESATPESGPGCGTEPSEGSAPGSPAG SPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGEGCGATPESGPGSE PATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSESATEGCGPG TESASR
LX-55	108	C36, C72	GSPGAGSPAGSPTSTEEGTSESATPESGPGTSTEGCGESAPGSPAG SPTSTEEGTSTEPSEGSAPGTSTEGCGESAPGTSESATPESGPGSE PATSGSETPGSESASK
LX-56	144	C48, C96	GSPGAGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAS GCGSTEEGTSTEPSEGSAPGTSTEPSEGSAPGTSESATPESGPGSE PAGCGSETPGSEPATSGSETPGSPAGSPTSTEEGTSESATPESGPG TESASK
LX-57	108	C27, C54, C81	GSPGAGSPAGSPTSTEEGTSESATEGCGPGTSTEPSEGSAPGSPAG SPTSTEEGCGETEPSEGSAPGTSTEPSEGSAPGEGCGATPESGPGSE PATSGSETPGSESASK

LX-58	144	C36, C72, C108	GSPGAGSPAGSPTSTEEGTSESATPESGPGTSTEGCGESAPGSPAG SPTSTEEGTSTEPSEGSAPGTSTEGCGESAPGTSESATPESGPGSE PATSGSETPGSEPAGCGSETPGSPAGSPTSTEEGTSESATPESGPG TESASK
LX-59	108	C21, C42, C63, C84	GSPGAGSPAGSPTSTEEGEGCGATPESGPGTSTEPSEGSAGCGPAG SPTSTEEGTSTEPSEGCGETPGTSTEPSEGSAPGTSESGCGESGPGSE PATSGSETPGSESASK
LX-60	144	C28, C56, C84, C112	GSPGAGSPAGSPTSTEEGTSESATPEGCGPTSTEPSEGSAPGSPAG SPTSTEEGTGCGEPSEGSAPGTSTEPSEGSAPGTSESGCGESGPGSE PATSGSETPGSEPATSGEGCGPSPAGSPTSTEEGTSESATPESGPG TESASK
LX-61	144	C31, C81, C135	GSPGAGSPAGSPTSTEEGTSESATPESGPGCTEPSEGSAPGSPAG SPTSTEEGTSTEPSEGSAPGTSTEPSEGSAPGEGCGATPESGPGSE PATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSESATGCGPG TESASK
LX-62	288	C72, C144, C216	GSPGAGSPAGSPTSTEEGTSESATPESGPGTSTEPSEGSAPGSPAG SPTSTEEGTSTEPSEGSAPGTSTEGCGESAPGTSESATPESGPGSE PATSGSETPGSEPATSGSETPGSPAGSPTSTEEGTSESATPESGPG TSTEGCGESAPGTSTEPSEGSAPGSPAGSPTSTEEGTSTEPSEGS PGTSTEPSEGSAPGTSESATPESGPGTSTEGCGESAPGTSESATPE SGPGSEPATSGSETPGTSTEPSEGSAPGTSTEPSEGSAPGTSESAT PESGPGTESASK

[00135] In some embodiments, wherein the XTEN of the subject conjugates and compositions have less than 100% of its amino acids consisting of amino acid selected from glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P) and the 2, 3, or 4 cysteine residues incorporated for the linking to albumin binding subunits, the other amino acid residues of the XTEN are selected from any of the other 14 natural L-amino acids, but are preferentially selected from hydrophilic amino acids such that the XTEN sequence contains at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% hydrophilic or neutral amino acids. An individual amino acid or a short sequence of amino acids other than glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P) may be incorporated into the XTEN to achieve a needed property, such as to permit incorporation of a restriction enzyme site by the encoding nucleotides or the ability to link a therapeutic protein. As one exemplary embodiment, the disclosure provides XTEN that incorporate a single lysine residue at the C-terminus wherein the reactive ϵ -amino group of the lysine is utilized for linking to a second therapeutic payload, as described herein. As hydrophobic amino acids impart structure and/or result in MHC-II epitopes, the disclosure provides that the content of hydrophobic amino acids in the XTEN of the subject conjugates and compositions will typically be less than 5%, or less than 2%, or less than 1% hydrophobic amino acid content. In some embodiments, the XTEN of the subject conjugates and compositions will contain no hydrophobic amino acid residues. Hydrophobic residues that are less favored in construction of XTEN include tryptophan, phenylalanine, tyrosine, leucine, isoleucine, valine, and methionine. In some embodiments, the XTEN of the subject conjugates and compositions will contain no F, I, L, M, V, W, or Y amino acid residues, which can minimize the formation of MHC-II epitopes in the conjugates and composition. Additionally, one can design the XTEN sequences to contain less than 5% or less than 4% or less than 3% or less than 2% or less than 1% or none of the following amino acids: methionine (to avoid oxidation), asparagine and glutamine (to avoid deamidation). In some embodiments, the amino

acid content of methionine and tryptophan in the XTEN component used in the subject conjugates and compositions is typically less than 5%, or less than 2%, and most preferably less than 1%. In other embodiments, the XTEN of the subject XTEN conjugates and compositions will have a sequence that has less than 5% amino acid residues with a positive charge, or less than about 2%, or less than about 1% amino acid residues with a positive charge, the sum of methionine and tryptophan residues will be less than 2%, and the sum of asparagine and glutamine residues will be less than 5% of the total XTEN sequence.

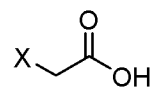
[00136] By controlling the number and position of cysteine residues, one can control the number and position of albumin binding subunits that are conjugated to XTEN. This enables one to adjust the degree of albumin binding of the resulting conjugates, depending on the properties desired with a given therapeutic protein that is to be linked to a given conjugate.

[00137] Alternates to XTEN can include PAS (polymers adopting random coil conformation under physiological conditions comprising a plurality of amino acid repeats consist of alanine, serine, and proline residues, such as described in U.S. patent no. 8,563,521) or elastin-like proteins, such as described in U.S. Patent No. 8,367,626, into which 2, 3, or 4 cysteine residues are incorporated.

2. Linker

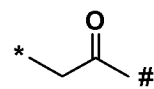
[00138] In an aspect of the disclosure, linker moieties are provided to join the soluble bridge component to the thiol of the cysteine residues of the XTEN.

[00139] In one embodiment, the linker used in the conjugation of the soluble bridge to the thiol of the cysteine residue has the configuration of formula I:



wherein X is Br or I.

[00140] In one embodiment, the linker component of the synthesized albumin binding conjugate has the configuration of formula II:

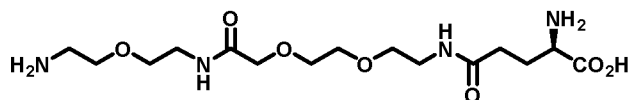


wherein * denotes the attachment point to the thiol of a cysteine residue of the XTEN and wherein # denotes the attachment point to the * nitrogen of the soluble bridge (with the * nitrogen indicated in the soluble bridge formulae below).

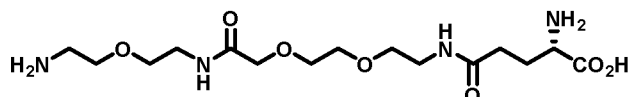
3. Soluble Bridge Moieties

[00141] In an aspect of the disclosure, soluble bridge moieties are provided to join the carboxylic acid component to the linker that is linked to the thiol of the XTEN cysteine residues.

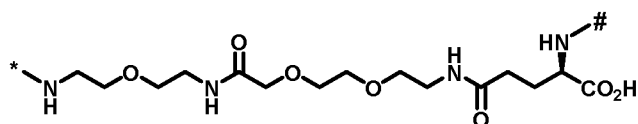
[00142] In one embodiment, the soluble bridge moiety used in the conjugation of the carboxylic acid to the linker has the configuration of formula III, with the *R* stereochemistry at the chiral center:



[00143] In one embodiment, the soluble bridge moiety used in the conjugation of the carboxylic acid to the linker has the configuration of formula IV, with the *S* stereochemistry at the chiral center:

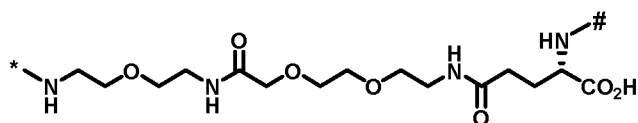


[00144] In one embodiment, the soluble bridge component of the synthesized albumin binding conjugate has the configuration of formula V, with the *R* stereochemistry at the chiral center:



wherein * denotes the attachment point to the carbon of the linker and wherein # denotes the attachment point to the * carbon of the carboxylic acid moiety (with the * indicated in the carboxylic formulae below).

[00145] In one embodiment, the soluble bridge component of the synthesized albumin binding conjugate has the configuration of formula VI, with the *S* stereochemistry at the chiral center:



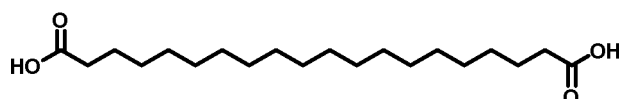
wherein * denotes the attachment point to the carbon of the linker and wherein # denotes the attachment point to the * carbon of the carboxylic acid moiety (with the * indicated in the carboxylic formulae below).

4. Carboxylic acid moieties

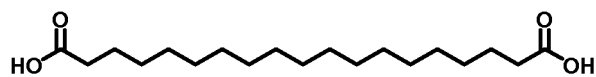
[00146] In some embodiments, the carboxylic acid components used in the making of the subject conjugates and compositions comprise a carboxylic acid chain of 2 to 40 carbon atoms and at least one carboxylic acid group at one terminus of the hydrocarbon chain. In other embodiments, the carboxylic acid used in the making of the subject conjugates and compositions comprise a diacid containing a second carboxylic acid group at the opposite end of the carboxylic acid chain from the first. The second carboxylic acid can be derivatized to an amide for the incorporation of a water soluble linker using standard synthetic techniques. Preferably, the carboxylic acid for use in the disclosure contains between 9 and 27 carbon atoms, and more preferably between 14 and 20 carbon atoms. The carboxylic acid length may be selected with regard to the desired pharmacokinetic characteristics. Carboxylic acids for use in the disclosure may be saturated or may contain one or more units of unsaturation. Suitable carboxylic acids for use in the disclosure include, for example, decanoate (C10, caproate), undecanoate (C11, undecylate), dodecanoate (C12, laurate), tridecanoate (C13, tridecylate), tetradecanoate (C14, myristate), pentadecanoate (C15, pentadecylate), hexadecanoate (C16, palmitate), heptadecanoate (C17, margarate),

octadecanoate (C18, stearate), nonadecanoate (C19, nonadecylate), eicosanate (C20, arachidate), decanedioate (C10, ω -carboxycaproate), undecanedioate (C11, ω -carboxyundecylate), dodecanedioate (C12 diacid, ω -carboxylaurate), tridecanedioate (C13 diacid, ω -carboxytridecylate), tetradecanedioate (C14 diacid, ω -carboxymyristate), pentadecanedioate (C15 diacid, ω -carboxypentadecylate), hexadecanedioate (C16 diacid, ω -carboxypalmitate) heptadecandioate (C17 diacid, ω -carboxyheptadecanoate), octadecanedioate (C18 diacid, ω -carboxystearate), nonadecanedioate (C19, ω -carboxynonadecylate), and eicosadioate (C20, ω -carboxyarachidate). Preferably the carboxylic acid for use in the disclosure consists of straight chain of between 11 and 26 carbon atoms originating from a diacid, or at least retaining a free carboxylic acid moiety after derivatization with a soluble bridge moiety. In one preferred embodiment, the carboxylic acid used in the making of the albumin binding conjugate is octadecanedioic acid, an 18 carbon diacid. In this embodiment, one of the two carboxylic acid groups on the parent carboxylic acid is linked via amidation to a soluble bridge moiety, and the latter is linked to a reactive linker moiety that allows conjugation to the thiol groups of the cysteine residues of the XTEN, resulting in an albumin binding conjugate, described more fully, below.

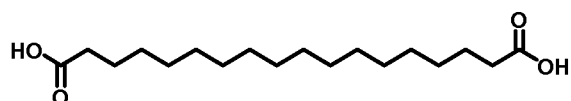
[00147] In one embodiment, the dicarboxylic acid used in the conjugation to the soluble bridge has the configuration of formula VII:



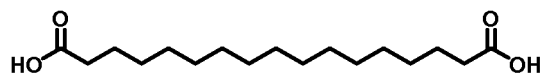
[00148] In one embodiment, the dicarboxylic acid used in the conjugation to the soluble bridge has the configuration of formula VIII:



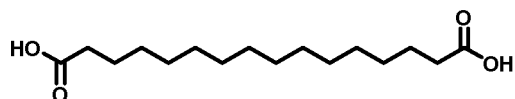
[00149] In one embodiment, the dicarboxylic acid used in the conjugation to the soluble bridge has the configuration of formula IX:



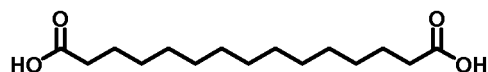
[00150] In one embodiment, the dicarboxylic acid used in the conjugation to the soluble bridge has the configuration of formula X:



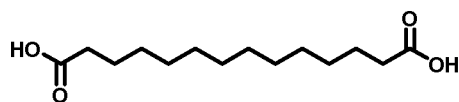
[00151] In one embodiment, the dicarboxylic acid used in the conjugation to the soluble bridge has the configuration of formula XI:



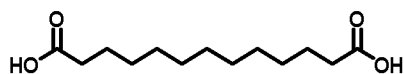
[00152] In one embodiment, the dicarboxylic acid used in the conjugation to the soluble bridge has the configuration of formula XII:



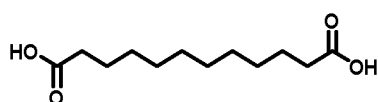
[00153] In one embodiment, the dicarboxylic acid used in the conjugation to the soluble bridge has the configuration of formula XIII:



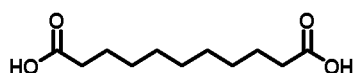
[00154] In one embodiment, the dicarboxylic acid used in the conjugation to the soluble bridge has the configuration of formula XIV:



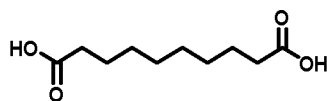
[00155] In one embodiment, the dicarboxylic acid used in the conjugation to the soluble bridge has the configuration of formula XV:



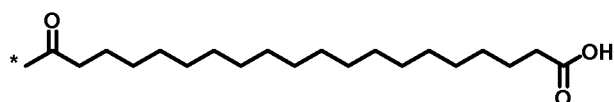
[00156] In one embodiment, the dicarboxylic acid used in the conjugation to the soluble bridge has the configuration of formula XVI:



[00157] In one embodiment, the dicarboxylic acid used in the conjugation to the soluble bridge has the configuration of formula XVII:

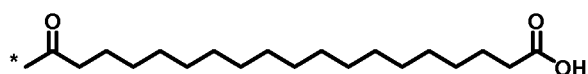


[00158] In one embodiment, the carboxylic acid component of the synthesized albumin binding conjugate composition has the configuration of formula XVIII:



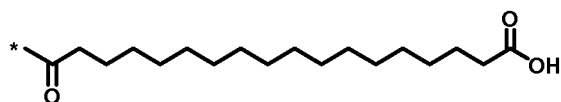
wherein * denotes the attachment point to the nitrogen of the soluble bridge.

[00159] In one embodiment, the carboxylic acid component of the synthesized albumin binding conjugate composition has the configuration of formula XIX:



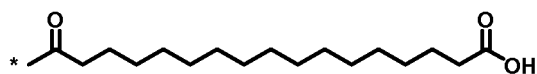
wherein * denotes the attachment point to the * nitrogen of the soluble bridge moiety (with the * indicated in the soluble bridge formulae above).

[00160] In one embodiment, the carboxylic acid component of the synthesized albumin binding conjugate composition has the configuration of formula XX:



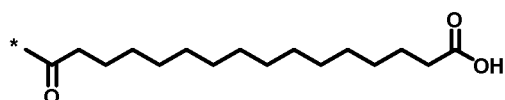
wherein * denotes the attachment point to the * nitrogen of the soluble bridge moiety (with the * indicated in the soluble bridge formulae above).

[00161] In one embodiment, the carboxylic acid component of the synthesized albumin binding conjugate composition has the configuration of formula XXI:



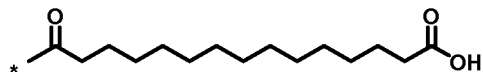
wherein * denotes the attachment point to the * nitrogen of the soluble bridge moiety (with the * indicated in the soluble bridge formulae above).

[00162] In one embodiment, the carboxylic acid component of the synthesized albumin binding conjugate composition has the configuration of formula XXII:



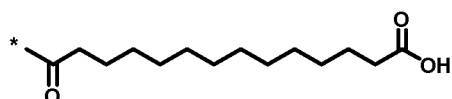
wherein * denotes the attachment point to the * nitrogen of the soluble bridge moiety (with the * indicated in the soluble bridge formulae above).

[00163] In one embodiment, the carboxylic acid component of the synthesized albumin binding conjugate composition has the configuration of formula XXIII:



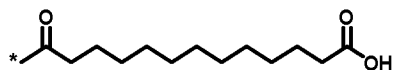
wherein * denotes the attachment point to the * nitrogen of the soluble bridge moiety (with the * indicated in the soluble bridge formulae above).

[00164] In one embodiment, the carboxylic acid component of the synthesized albumin binding conjugate composition has the configuration of formula XXIV:



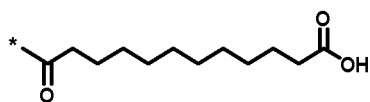
wherein * denotes the attachment point to the * nitrogen of the soluble bridge moiety (with the * indicated in the soluble bridge formulae above).

[00165] In one embodiment, the carboxylic acid component of the synthesized albumin binding conjugate composition has the configuration of formula XXV:



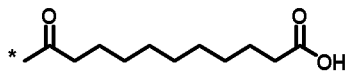
wherein * denotes the attachment point to the * nitrogen of the soluble bridge moiety (with the * indicated in the soluble bridge formulae above).

[00166] In one embodiment, the carboxylic acid component of the synthesized albumin binding conjugate composition has the configuration of formula XXVI:



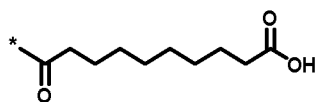
wherein * denotes the attachment point to the * nitrogen of the soluble bridge moiety (with the * indicated in the soluble bridge formulae above).

[00167] In one embodiment, the carboxylic acid component of the synthesized albumin binding conjugate composition has the configuration of formula XXVII:



wherein * denotes the attachment point to the * nitrogen of the soluble bridge moiety (with the * indicated in the soluble bridge formulae above).

[00168] In one embodiment, the carboxylic acid component of the synthesized albumin binding conjugate composition has the configuration of formula XXVIII:



wherein * denotes the attachment point to the * nitrogen of the soluble bridge moiety (with the * indicated in the soluble bridge formulae above).

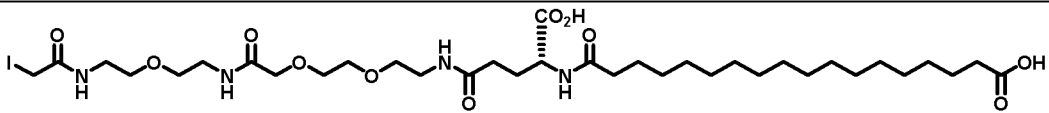
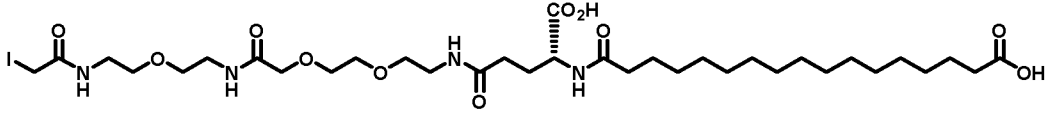
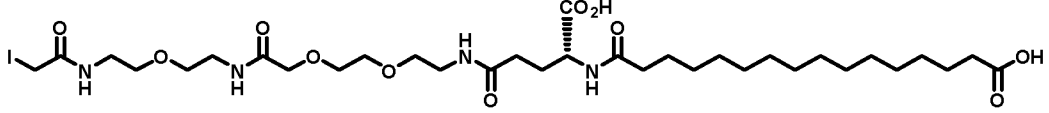
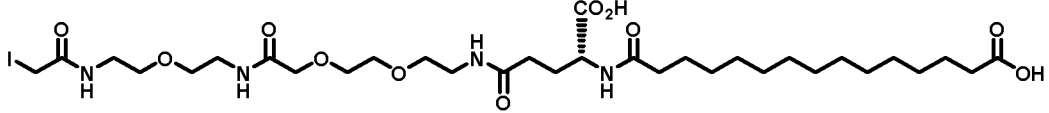
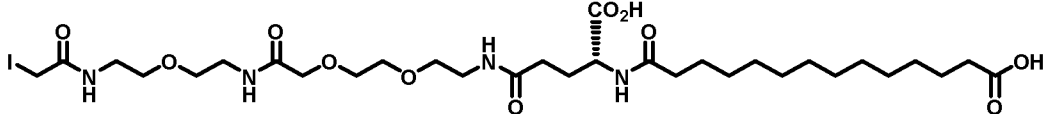
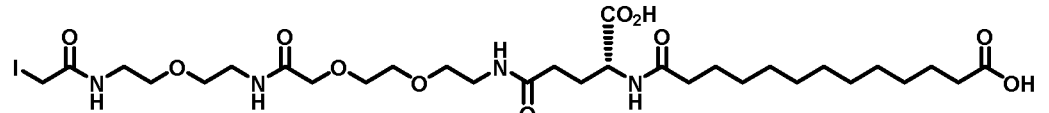
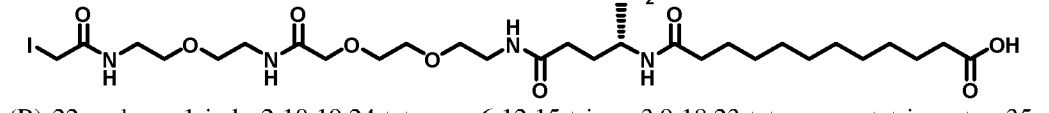
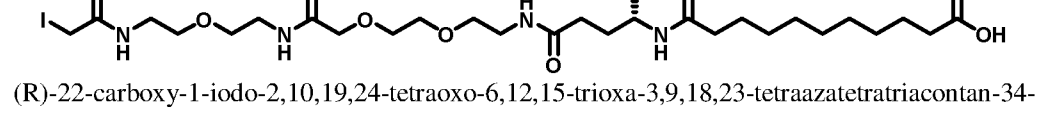
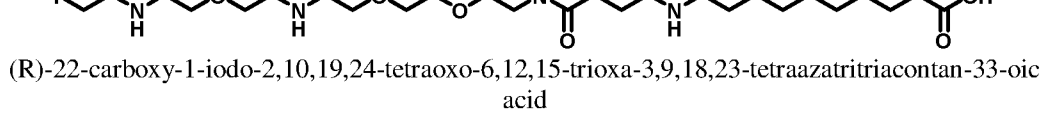
5. Albumin binding subunits for conjugation to XTEN

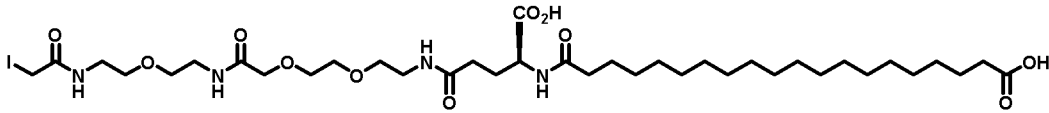
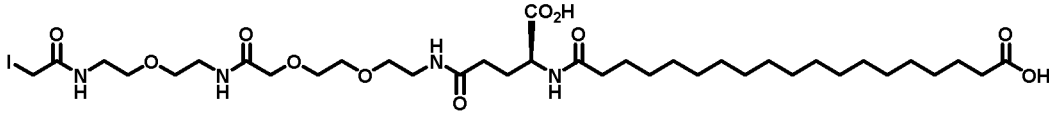
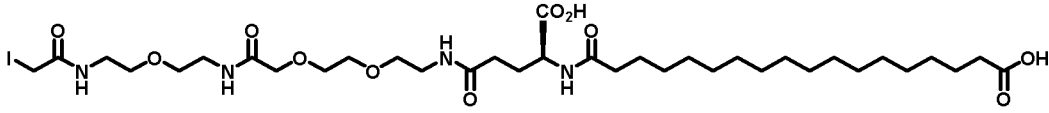
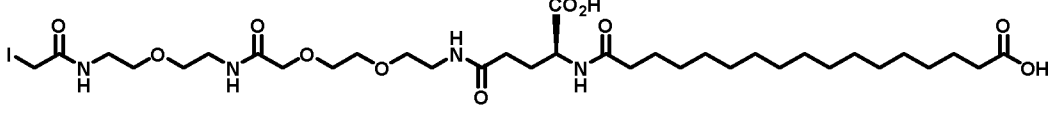
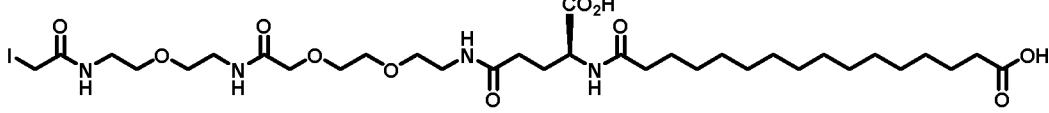
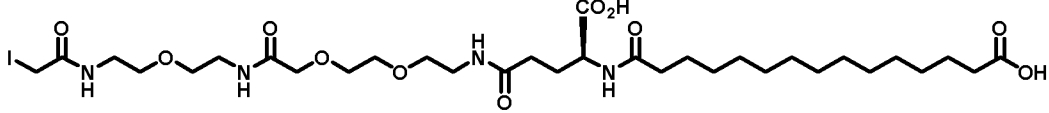
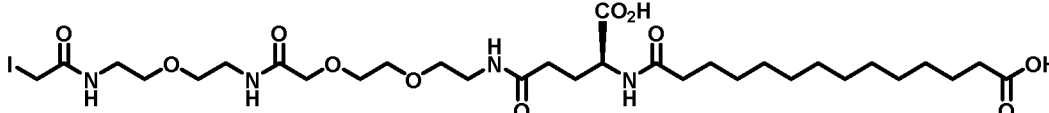
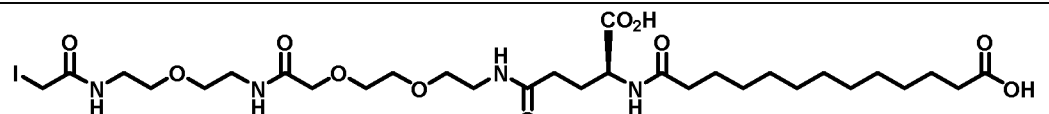
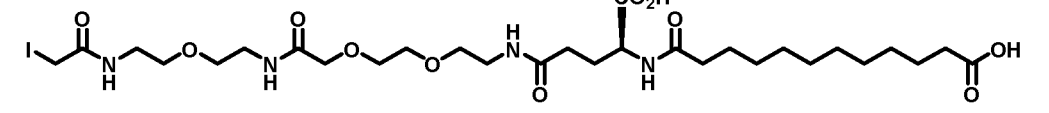
[00169] In an aspect, the disclosure provides reactive compositions of a linker, a soluble bridge moiety, and a carboxylic acid linked together (hereinafter “albumin binding subunit” or “albumin binding subunit precursor”), which, in turn, are conjugated to an XTEN to result in an albumin binding conjugate. In a preferred embodiment, the albumin binding subunit precursor is arranged in an A-B-C linked configuration, with A being the linker, B being the soluble bridge moiety, and C being a long-chain carboxylic acid. The joining of these components in this configuration is depicted schematically in the first step of FIG. 3. It will be understood that the albumin binding subunit precursor is created by conjugation of the reactive components of the linker of formula I, the soluble bridge of either formula III or IV, and one dicarboxylic acid of formulae VII-XVII.

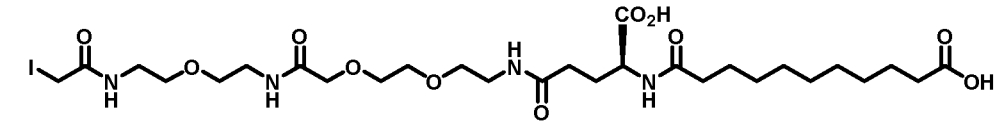
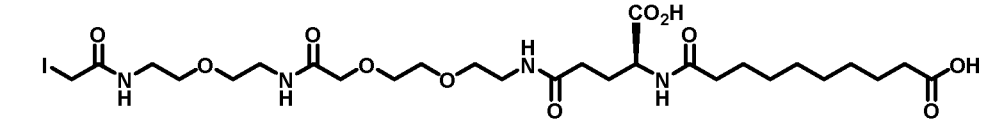
[00170] Non-limiting embodiments of albumin binding subunit precursors useful for conjugation to an XTEN include the structures shown in Table 2.

Table 2: Albumin Binding Subunit Precursors for Conjugation to XTEN

Formula #	Structure and Compound Name
XXIX	(R)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazatritetracontan-43-oic acid
XXX	(R)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazadotetracontan-42-

	oic acid
XXXI	 (R)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazahentetracontan-41-oic acid
XXXII	 (R)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazatetracontan-40-oic acid
XXXIII	 (R)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazanonatriacontan-39-oic acid
XXXIV	 (R)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetrazaoctatriacontan-38-oic acid
XXXV	 (R)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazaheptatriacontan-37-oic acid
XXXVI	 (R)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazahexatriacontan-36-oic acid
XXXVII	 (R)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazapentatriacontan-35-oic acid
XXXVIII	 (R)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazatetratriacontan-34-oic acid
XXXIX	 (R)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazatritriacontan-33-oic acid

XL	 (S)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazatritetracontan-43-oic acid
XLI	 (S)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazadotetracontan-42-oic acid
XLII	 (S)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazahentetracontan-41-oic acid
XLIII	 (S)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazatetracontan-40-oic acid
XLIV	 (S)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazanonatriacontan-39-oic acid
XLV	 (S)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazaoctatriacontan-38-oic acid
XLVI	 (S)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazaheptatriacontan-37-oic acid
XLVII	 (S)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazahexatriacontan-36-oic acid
XLVIII	 (S)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazapentatriacontan-35-oic acid

XLIX	 (S)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazatetratriacontan-34-oic acid
L	 (S)-22-carboxy-1-iodo-2,10,19,24-tetraoxo-6,12,15-trioxa-3,9,18,23-tetraazatritriacontan-33-oic acid

[00171] In a preferred embodiment, the disclosure provides albumin binding conjugates comprising i) an XTEN polypeptide comprising three cysteine residues wherein the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity or is identical to the sequence of LX-31 or LX-40 set forth in Table 1; ii) three linker moieties wherein the linker moiety has the structure of formula II; iii) three soluble bridge moieties wherein each bridge moiety has the structure of formula VI; and iv) three carboxylic acid moieties wherein each carboxylic acid moiety has the structure of formula XX, wherein the composition is configured according to the structure of FIG. 14 for LX-31 or FIG. 4 for LX-40.

6. Configurations and properties of albumin binding conjugates

[00172] In an aspect, the disclosure provides compositions of albumin binding subunits of Table 3 linked to XTEN (herein after an “albumin binding conjugates”). The joining of these components to make the subject compositions described herein is depicted schematically in FIG. 3.

[00173] As schematically illustrated in FIG. 1, the albumin binding conjugates of the disclosure are conjugates having different configurations, with either two, three, or four albumin binding subunits, with the albumin binding subunits conjugated to a cysteine residue of the XTEN. It is specifically contemplated that the subject conjugates and compositions are designed such that they have enhanced properties or combinations of properties compared to compositions that bind albumin with a single carboxylic acid or compared to compositions that are conjugated to albumin.

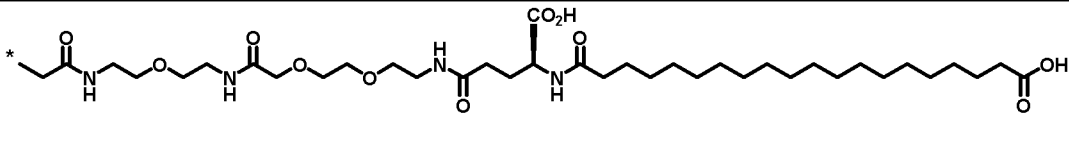
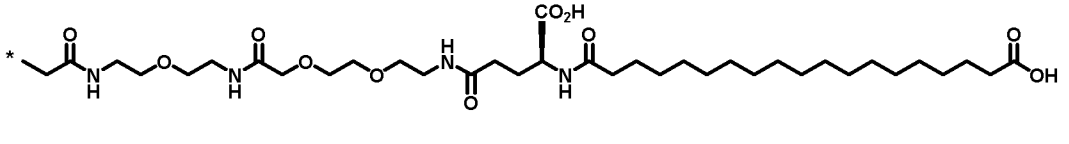
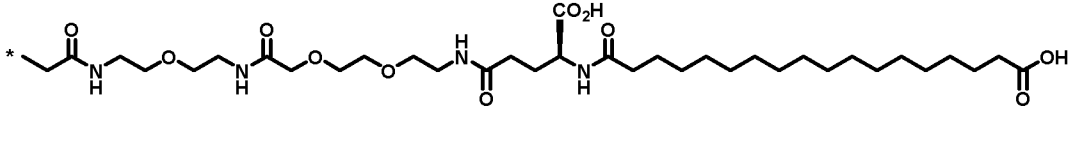
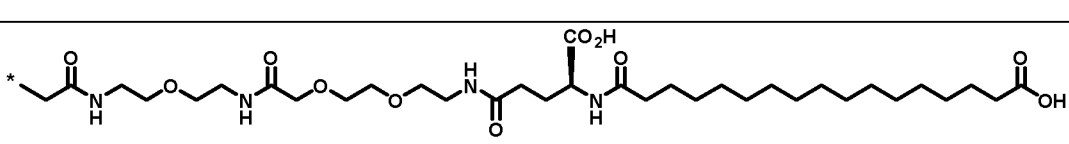
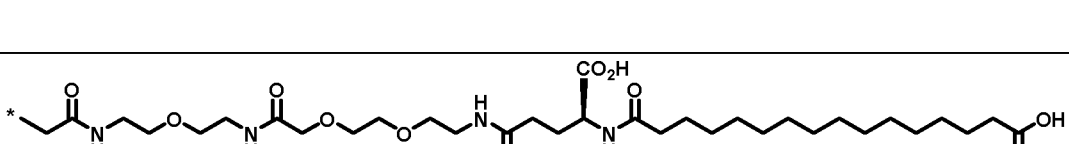
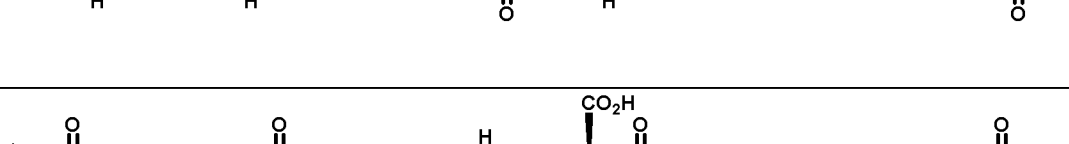
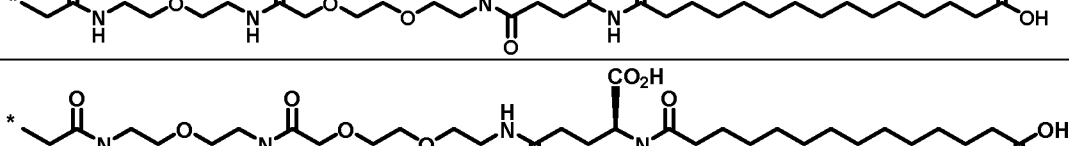
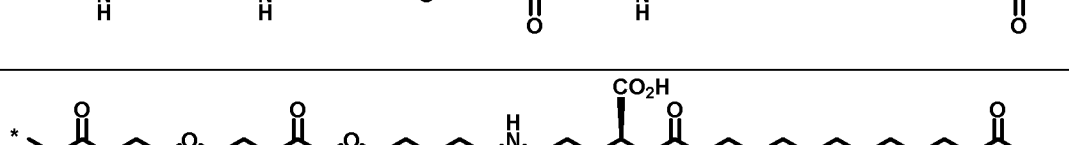
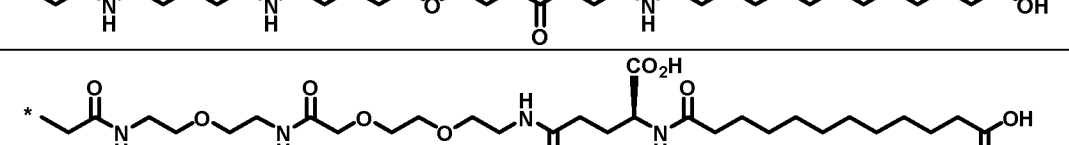
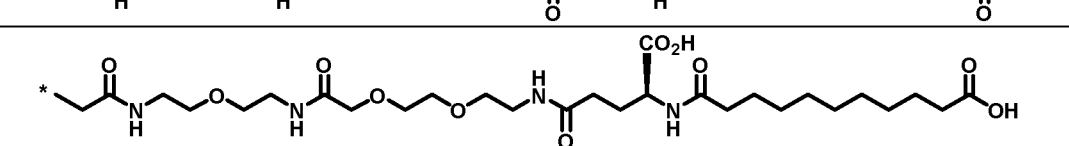
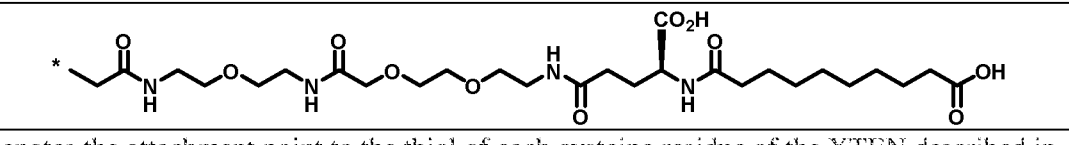
[00174] In one embodiment, the albumin binding conjugate comprises an XTEN having at least 90%, or 91%, or 92%, or 93%, or 94%, or 95%, or 96%, or 97%, or 98%, or 99% identity to or is identical to a sequence set forth in Table 1, when optimally aligned, and further comprises two, three, or four identical albumin binding subunits selected from the group consisting of the albumin binding subunit structures of Table 3, wherein the albumin binding subunits are linked to the thiol group of each cysteine residue, resulting in an albumin binding conjugate having two, three, or four albumin binding subunits, depending on the number of cysteine residues of the XTEN component (in other words, an albumin binding conjugate with an XTEN having three cysteines can have three albumin binding subunits linked to the thiol groups of the XTEN cysteine residues).

[00175] In a preferred embodiment, the disclosure provides albumin binding conjugates comprising i) an XTEN polypeptide comprising three cysteine residues wherein the XTEN exhibits at least 90%, at

least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity or is identical to the sequence of LX-31 or LX-40 set forth in Table 1; and
 ii) an albumin binding subunit linked to each of the three cysteine residues wherein each albumin binding subunit has the structure of formula LIII.

Table 3: Albumin Binding Subunits

Formula #	Structure
LI	
LII	
LIII	
LIV	
LV	
LVI	
LVII	
LVIII	
LIX	
LX	
LXI	

LXII	
LXIII	
LXIV	
LXV	
LXVI	
LXVII	
LXVIII	
LXIX	
LXX	
LXXI	
LXXII	

wherein * denotes the attachment point to the thiol of each cysteine residue of the XTEN described in Table 1.

[00176] In an aspect, the disclosure provides albumin binding conjugates that further comprise single atom residues of carbon, nitrogen, sulfur, or oxygen linked to the N-terminus of the XTEN of the albumin

binding conjugates. In one embodiment, a single atom residue of carbon, nitrogen, sulfur, or oxygen is that of a therapeutic protein disclosed herein that is linked to the XTEN recombinantly. In another embodiment, a single atom residue of carbon, nitrogen, sulfur, or oxygen is that of a therapeutic drug disclosed herein that is linked to the XTEN by a cross-linker. It will be understood that a “single atom residue” is that residue of a therapeutic protein or drug connected to a suitable cross-linker that is the attached to the XTEN after conjugation. Examples of suitable cross-linkers useful for such conjugation of the therapeutic proteins or drugs to XTEN include, but are not limited to, azidoacetic acid NHS ester, succinamidyl iodoacetic acid (SIA), and SPDP (succinimidyl 3-(2-pyridyldithio)propionate).

[00177] In an embodiment, the albumin binding conjugate comprises a single atom residue selected from the group consisting of carbon, nitrogen, sulfur, and oxygen linked to a cross-linker conjugated to the N-terminus of an XTEN having at least 90%, or 91%, or 92%, or 93%, or 94%, or 95%, or 96%, or 97%, or 98%, or 99% identity to or is identical to a sequence set forth in Table 1, when optimally aligned, and further comprises an albumin binding subunit selected from the group consisting of the structures of Table 3 linked to the thiol group of each cysteine residue of the XTEN, resulting in an albumin binding conjugate composition. In one embodiment, the single atom residue selected from the group consisting of carbon, nitrogen, sulfur, and oxygen is that of a therapeutic protein disclosed herein. In another embodiment, the single atom residue selected from the group consisting of carbon, nitrogen, sulfur, and oxygen is that of a therapeutic drug disclosed herein.

[00178] In an embodiment, the albumin binding conjugates comprising the first single atom residue of a therapeutic protein further comprises a second a single atom residue selected from the group consisting of carbon, nitrogen, sulfur, and oxygen of a therapeutic protein attached to a suitable cross-linker conjugated to an ϵ -amino group of a C-terminal lysine of an XTEN having at least 90%, or 91%, or 92%, or 93%, or 94%, or 95%, or 96%, or 97%, or 98%, or 99% identity to or is identical to a sequence set forth in Table 1, when optimally aligned, and further comprises an albumin binding subunit selected from the group consisting of the structures of Table 3.

[00179] Human serum albumin (HSA) has 585 amino acids and a molecular mass of 66,500 Da. The concentration of HSA in human plasma is about 0.6 mM. Albumin binds a wide variety of endogenous substances and drugs with K_d binding constants that are typically in the order of 10^{-4} M to 10^{-6} M for anions, and about 10^{-8} M for long-chain carboxylic acids (Peters, T., Adv. Protein Chem. 17:161-245 (1985); Kragh-Hansen, U. Dan. Med. Bul. 37:57-84 (1990); Carter, D.C. and Ho, J.X. Adv. Protein Chem. 45:153-203 (1994)).

[00180] Long-chain carboxylic acids have a strong tendency to bind to HSA, and the number of endogenous long-chain carboxylic acids bound to circulating HSA can depend on the certain conditions of the human body. At least seven binding sites for long-chain carboxylic acids have been identified on HSA. Carboxylic acid sites 2, 4 and 5 bind long-chain carboxylic acids with high affinity, while sites 1, 3, 6 and 7 exhibit low affinity (J. Mol. Biol. 361:336-351 (2006)). Ordinarily, long-chain carboxylic acid content in circulating HSA is 1–2 molecules per HSA molecule, however, upon fasting or after hard

exercise, up to 6–7 molecules of long-chain carboxylic acids can bind to HSA (Varshney A, et al. Chirality 22:77–87 (2010); Curry S. Drug Metab. Pharmacokinet. 24:342–357 (2009); Simard JR, et al. J. Mol. Biol. 361:336–351 (2006).

[00181] It has been surprisingly discovered in experimental *in vivo* models that albumin binding conjugates comprising multiple carboxylic acids linked to short XTEN have longer terminal half-life than a composition comprising a long XTEN linked to a single carboxylic acid. Subsequent *in vitro* experiments have resulted in the surprising finding that albumin binding conjugates comprising multiple carboxylic acids bind to single albumin molecules rather than two or more albumin molecules and bind with enhanced affinity. Without being bound to a particular theory, it is believed that the inclusion of two or more carboxylic acids into the albumin binding conjugates permits the conjugate and compositions thereof to bind simultaneously to multiple carboxylic acid sites on the same HSA molecule, resulting in binding with enhanced affinity.

[00182] In some embodiments, the albumin binding conjugates of the disclosure have binding affinity to human serum albumin (HSA), as measured by determination of the K_d binding constant in the biochemical assay, with a K_d of less than 1×10^{-4} M, or less than 3.3×10^{-4} M, or less than 1×10^{-5} M, or less than 3.3×10^{-5} M, or less than 1×10^{-6} M, or less than 3.3×10^{-6} M, or less than 1×10^{-7} M, or less than 3.3×10^{-7} M, or less than 1×10^{-8} M, or less than 3.3×10^{-8} M, or less than 1×10^{-9} M, or less than 3.3×10^{-9} M, or less than 1×10^{-10} M. In another embodiment, an albumin binding conjugate of the disclosure has binding affinity to human serum albumin, as measured by determination of the K_d binding constant in the *in vitro* assay between about 1×10^{-4} M to about 1×10^{-10} M.

[00183] In one embodiment, the albumin binding conjugates of the disclosure comprising two albumin binding subunits have the ability to bind HSA in an *in vitro* assay with a K_d of less than 1×10^{-4} M, or less than 3.3×10^{-4} M, or less than 1×10^{-5} M, or less than 3.3×10^{-5} M, or less than 1×10^{-6} M, or less than 3.3×10^{-6} M, or less than 1×10^{-7} M, or less than 3.3×10^{-7} M, or less than 1×10^{-8} M, or less than 3.3×10^{-8} M, or less than 1×10^{-9} M, or less than 3.3×10^{-9} M, or less than 1×10^{-10} M. In another embodiment, the albumin binding conjugates of the disclosure comprising three albumin binding subunits have the ability to bind HSA in an *in vitro* assay with a K_d of less than 1×10^{-4} M, or less than 3.3×10^{-4} M, or less than 1×10^{-5} M, or less than 3.3×10^{-5} M, or less than 1×10^{-6} M, or less than 3.3×10^{-6} M, or less than 1×10^{-7} M, or less than 3.3×10^{-7} M, or less than 1×10^{-8} M, or less than 3.3×10^{-8} M, or less than 1×10^{-9} M, or less than 3.3×10^{-9} M, or less than 1×10^{-10} M. In another embodiment, the albumin binding conjugates of the disclosure comprising three albumin binding subunits have the ability to bind two HSA molecules in an *in vitro* assay with a K_d of less than 1×10^{-4} M or less than 3.3×10^{-4} M, or less than 1×10^{-5} M, or less than 3.3×10^{-5} M, or less than 1×10^{-6} M, or less than 3.3×10^{-6} M, or less than 1×10^{-7} M, or less than 3.3×10^{-7} M, or less than 1×10^{-8} M, or less than 3.3×10^{-8} M, or less than 1×10^{-9} M, or less than 3.3×10^{-9} M, or less than 1×10^{-10} M. In another embodiment, the albumin binding conjugates of the disclosure comprising three albumin binding subunits have the ability to bind at least three HSA molecules in an *in vitro* assay with a K_d of less than 1×10^{-4} M. In another embodiment, the albumin binding conjugates of the disclosure

comprising four albumin binding subunits have the ability to bind HSA in an *in vitro* assay with a K_d of less than 1×10^{-4} M, or less than 3.3×10^{-4} M, or less than 1×10^{-5} M, or less than 3.3×10^{-5} M, or less than 1×10^{-6} M, or less than 3.3×10^{-6} M, or less than 1×10^{-7} M, or less than 3.3×10^{-7} M, or less than 1×10^{-8} M, or less than 3.3×10^{-8} M, or less than 1×10^{-9} M, or less than 3.3×10^{-9} M, or less than 1×10^{-10} M. In another embodiment, the albumin binding conjugates of the disclosure comprising four albumin binding subunits have the ability to bind at least two HSA molecules in an *in vitro* assay with a K_d of less than 1×10^{-4} M. In another embodiment, the albumin binding conjugates of the disclosure comprising four albumin binding subunits have the ability to bind at least three HSA molecules in an *in vitro* assay with a K_d of less than 1×10^{-4} M. In another embodiment, the albumin binding conjugates of the disclosure comprising four albumin binding subunits have the ability to bind at least four HSA molecules in an *in vitro* assay with a K_d of less than 1×10^{-4} M. In another embodiment, an albumin binding conjugates of the disclosure binds HSA with at least 2-fold, or at least 3-fold, or at least 5-fold, or at least 7-fold, or at least 9-fold, or at least 10-fold, or at least 50-fold, or at least 100-fold, or at least 500-fold, or at least 1000-fold greater affinity in an *in vitro* assay compared to a compound bearing a single carboxylic acid comparable to the carboxylic acids incorporated into the albumin binding conjugate. In another embodiment, an albumin binding conjugates of the disclosure binds HSA with a K_d of 10^{-1} M or less, or of 10^{-2} M or less, or of 10^{-3} M or less, in an *in vitro* assay, compared to a compound (or payload such as a therapeutic protein or drug) bearing a single carboxylic acid comparable to the carboxylic acids incorporated into the albumin binding conjugate.

7. Compositions and configurations of albumin binding conjugates with linked therapeutic proteins (TP-ABC)

[00184] In an aspect, the disclosure provides compositions of therapeutic proteins linked by a suitable cross-linker to albumin binding conjugates (hereinafter “TP-ABC”). It is contemplated that the subject conjugates and compositions are designed such that they have enhanced properties or combinations of properties compared both to the native therapeutic proteins or to therapeutic proteins linked to carboxylic acids or polyethyleneglycol (PEG), including, but not limited to enhanced binding to human serum albumin, increased terminal half-life, tunable terminal half-life, enhanced solubility, decreased viscosity, lower molecular weight, lack of aggregation during recovery and in final product, and high thermal stability. In addition, the subject compositions have enhanced properties compared to XTENylated therapeutic proteins with long XTEN sequences, including lower molecular weight, lower viscosity, and increased terminal half-life.

[00185] As illustrated in FIG. 1, the albumin-binding conjugates of the disclosure can have different configurations, with either two, three, or four albumin binding subunits conjugated to each cysteine residue of the XTEN. In three embodiments, a TP-ABC comprises a single therapeutic protein linked by a suitable cross-linker to the N-terminus of the XTEN component of the albumin binding conjugates, such that three different configurations are possible; a TP-ABC with 2 albumin binding subunits, a TP-ABC with 3 albumin binding subunits, as illustrated schematically in FIG. 2A, and a TP-ABC with 4 albumin

binding subunits. In three other embodiments, a TP-ABC comprises two therapeutic proteins, with one linked by a suitable cross-linker to the N-terminus of the XTEN component of the albumin binding conjugate and a second linked by a suitable cross-linker to the ϵ -amino group of a lysine residue at the C-terminus of the XTEN component, such that three different configurations are possible; a TP-ABC with 2 albumin binding subunits, a TP-ABC with 3 albumin binding subunits, as illustrated schematically in FIG. 2B, and a TP-ABC with 4 albumin binding subunits. In three other embodiments, a TP-ABC comprises a single therapeutic protein linked by a suitable cross-linker to the ϵ -amino group of a lysine residue at the C-terminus of the XTEN component of the albumin binding conjugate such that three different configurations are possible; a TP-ABC with 2 albumin binding subunits, a TP-ABC with 3 albumin binding subunits, and a TP-ABC with 4 albumin binding subunits. In another embodiment, a TP-ABC comprises two identical therapeutic proteins, with one linked by a suitable cross-linker to the N-terminus of the XTEN and a second linked by a suitable cross-linker to the ϵ -amino group of a lysine residue at the C-terminus of the XTEN component of the albumin binding conjugate. In still another embodiment, a TP-ABC comprises two different therapeutic proteins, with one linked by a suitable cross-linker to the N-terminus of the XTEN and a second different therapeutic protein linked by a suitable cross-linker to the ϵ -amino group of a lysine residue at the C-terminus of the XTEN component of the albumin binding conjugate, resulting in a bifunctional composition. In all of the foregoing embodiments, the therapeutic protein can be conjugated to the XTEN using a suitable cross-linker, described more fully, below.

[00186] The therapeutic proteins for inclusion in the TP-ABC compositions can include any protein or peptide of biologic, therapeutic, prophylactic, or diagnostic interest or function, or that is useful for mediating a biological activity or preventing or ameliorating a disease, disorder or conditions when administered to a subject. A therapeutic protein for inclusion in the TP-ABC can be a native, full-length protein or can be a fragment or a sequence variant of a protein that retains at least a portion of the biological activity of the native protein. Categories of therapeutic proteins intended for use in the TP-ABC include, but are not limited to cytokines, interleukins, growth factors, growth hormones, endocrine hormones, exocrine hormones, coagulation factors, glucose-regulating peptides, enzymes, receptor agonists, receptor antagonists, and toxins. A therapeutic protein for inclusion in the TP-ABC compositions can be an IgG antibody or an antibody fragment, such as a Fab fragment, a F(ab')₂ fragment, a scFv, a scFab, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody.

[00187] Of particular interest are therapeutic proteins for which an increase in a pharmacokinetic parameter, increased solubility, increased stability, or some other enhanced pharmaceutical property is sought, or those for which increasing the terminal half-life would improve efficacy, safety, or result in reduce dosing frequency and/or improve patient compliance. Thus, the TP-ABC compositions are prepared with various objectives in mind, including improving the therapeutic efficacy of the bioactive compound by, for example, increasing the in vivo exposure or the length that the TP-ABC remains within

the therapeutic window when administered to a subject, compared to a therapeutic protein not linked to an albumin binding conjugate provided herein.

[00188] In one embodiment, the conjugated therapeutic protein component of the TP-ABC retains at least 10%, or at least 20%, or at least 30%, or at least 40%, or at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 90%, or at least 100% of the functional activity of the corresponding unmodified therapeutic protein, and the TP-ABC has an extended terminal half-life when administered to a subject. The extended terminal half-life, for example, is at least 2-fold greater, or at least 3-fold greater, or at least 4-fold greater, or at least 5-fold greater, or at least 6-fold greater, or at least 8-fold greater, or at least 10-fold greater than the corresponding unmodified therapeutic protein products not linked to an ABC. In another embodiment, the TP-ABC has a terminal half-life when administered to a subject of at least 72 h, or at least 96 h, or at least 120 h, or at least 7 days, or at least 10 day, or at least 14 days, or at least 21 days, or at least 1 month. In the foregoing embodiment, the subject is selected from mouse, rat, dog, monkey, pig, bovine, or human. In another embodiment, the conjugated therapeutic protein component in the TP-ABC composition retains at least 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, or 150 percent (%) of the biological activity relative to the corresponding unmodified therapeutic protein.

[00189] In an embodiment, the TP-ABC or a composition thereof, comprises one molecule of a peptide or protein that includes, but is not limited to, a peptide or polypeptide selected from Table 4, or a sequence variant thereof that retains at least a portion of the activity of the biologically active protein ,wherein the albumin binding component has the configuration shown in FIG. 2A. In another embodiment, the TP-ABC or a composition thereof, comprises two identical molecules of a peptide or protein that includes, but is not limited to, a peptide or polypeptide selected from Table 4, or a sequence variant thereof that retains at least a portion of the activity of the biologically active protein, wherein the albumin binding component has the configuration shown in FIG. 2B. In another embodiment, the TP-ABC or a composition thereof, comprises a first and a second different peptide or protein that includes, but is not limited to, a peptide or polypeptide selected from Table 4, or a sequence variant thereof that retains at least a portion of the activity of the biologically active protein, wherein the albumin binding component has the configuration shown in FIG. 2B. By “sequence variant,” it is meant that the peptide or protein exhibits at least about 80%, or 90%, or 91%, or 92%, or 93%, or 94%, or 95%, or 96%, or 97%, or 98%, or 99% sequence identity, when optimally aligned, to that of the known peptide or polypeptide, such as those that are listed in Table 4 or the sequences described herein.

Table 4: Proteins for linking to XTEN

Protein/Peptide Name
EPO, IFN-α, IFN-β, IFN-γ, factor VII, factor VIIa, factor VIII, factor IX, IL-1, IL-2, remicade (infliximab), Rituxan (rituximab), Enbrel (etanercept), Synagis (palivizumab), Reopro (abciximab), Herceptin (trastuzimab), tPA, Cerizyme (imiglucerase), rDNase, alpha-1 proteinase inhibitor, C-peptide, fuzeon, G-CSF, GM-CSF, growth hormone, somatropin, glucagon, GLP-1, GLP-2, FSH, TNF-receptor, uricase, VEGF, PTH, asparaginase, amdoxovir (DAPD), antide, becaplermin, calcitonins, cyanovirin, denileukin diftitox, erythropoietin (EPO), ceredase, cerezyme, alpha-glucosidase, collagen, cyclosporin, alpha defensins, beta defensins, exendin-4, granulocyte colony stimulating factor (GCSF), thrombopoietin (TPO), alpha-1 proteinase inhibitor, elcatonin, granulocyte macrophage colony stimulating factor (GMCSF), fibrinogen, filgrastim, human growth hormone (hGH), GRO-beta, GRO-beta antibody, bone morphogenic proteins such as bone morphogenic protein-2, bone morphogenic protein-6, OP-1; acidic fibroblast growth factor, basic fibroblast growth factor, CD-40 ligand, heparin, human serum albumin, low molecular weight heparin (LMWH), interferons such as interferon alpha, interferon beta, interferon gamma, interferon omega, interferon tau, consensus interferon; interleukin-1 receptor, interleukin-2, interleukin-1 receptor antagonist, interleukin-3, interleukin-4, interleukin-4 receptor, interleukin-6, interleukin-8, interleukin-12, interleukin-13 receptor, interleukin-17 receptor; lactoferrin and lactoferrin fragments, luteinizing hormone releasing hormone (LHRH), amylin, somatostatin, somatostatin analogs including octreotide, vasopressin, follicle stimulating hormone (FSH), influenza vaccine, insulin-like growth factor (IGF), insulintropin, macrophage colony stimulating factor (M-CSF), plasminogen activators such as alteplase, urokinase, reteplase, streptokinase, pamiteplase, lanoteplase, and tenecteplase; nerve growth factor (NGF), osteoprotegerin, platelet-derived growth factor, tissue growth factors, transforming growth factor-1, vascular endothelial growth factor, leukemia inhibiting factor, keratinocyte growth factor (KGF), glial growth factor (GGF), T Cell receptors, CD molecules/antigens, tumor necrosis factor (TNF), monocyte chemoattractant protein-1, endothelial growth factors, parathyroid hormone (PTH), thymosin alpha 1, thymosin alpha 1 IIb/IIIa inhibitor, thymosin beta 10, thymosin beta 9, thymosin beta 4, alpha-1 antitrypsin, phosphodiesterase (PDE) compounds, VLA-4 (very late antigen-4), VLA-4 inhibitors, bisphosphonates, respiratory syncytial virus antibody, cystic fibrosis transmembrane regulator (CFTR) gene, deoxyribonuclease (Dnase), bactericidal/permeability increasing protein (BPI), anti-CMV antibody, etanercept, abciximab, afeliomomab, basiliximab, daclizumab, infliximab, ibritumomab tiuxetan, mitumomab, muromonab-CD3, iodine 131 tositumomab conjugate, olizumab, rituximab, trastuzumab (herceptin), boxtox, Dysport, alglucosidase alfa, daptomycin, YH-16, choriogonadotropin alfa, filgrastim, cetorelix, aldesleukin, teceleukin, denileukin diftitox, interferon alfa-n3 (injection), interferon alfa-n1, DL-8234, interferon, Suntory (gamma-1a), interferon gamma, thymosin alpha 1, tasonermin, DigiFab, ViperaTAb, EchiTAb, CroFab, nesiritide, abatacept, alefacept, Rebif, eptoterminal alfa, teriparatide (osteoporosis), calcitonin injectable, bone disease), calcitonin (nasal, osteoporosis), etanercept, hemoglobin glutamer 250 (bovine), drotrecogin alfa, collagenase, carperitide, recombinant human epidermal growth factor (topical gel, wound healing), DWP-401, darbepoetin alfa, epoetin omega, epoetin beta, epoetin alfa, desirudin, lepirudin, bivalirudin, nonacog alpha, Mononine, eptacog alfa (activated), recombinant Factor VIII + VWF, Recombinate, recombinant Factor VIII, Alphanate, octocog alfa, palifermin, Indikinase, tenecteplase, alteplase, pamiteplase, reteplase, nateplase, monteplase, follitropin alfa, rFSH, hpFSH, micafungin, pegfilgrastim, lenograstim, nartograstim, sermorelin, glucagon, exenatide, pramlintide, imiglucerase, galsulfase, Leucotropin, molgramostim, triptorelin acetate, histrelin, histrelin acetate, deslorelin, nafarelin, leuprolide sustained release depot (ATRIGEL), leuprolide implant (DUROS), somatropin, Eutropin, KP-102 program, somatropin, somatropin, mecasermin (growth failure), enfuvirtide, Org-33408, mecasermin rinfabate, anakinra, celmoleukin, 99mTc-apcitide injection, myelopid, Betaseron, glatiramer acetate, Gepon, oprelvekin, human leukocyte-derived alpha interferons, Bilive, mecasermin, Roferon-A, interferon-alpha 2, Alfaferone, interferon alfacon-1, interferon alpha, Avonex' recombinant human luteinizing hormone, dornase alfa, trafermin, ziconotide, taltirelin, diboterminal alfa, atosiban, becaplermin, eptifibatide, Zemaira, CTC-111, Shanvac-B, HPV vaccine (quadrivalent), NOV-002, octreotide, lanreotide, ancestim, agalsidase beta, agalsidase alfa, laronidase, prezatide copper acetate (topical gel), rasburicase, ranibizumab, Actimmune, PEG-Intron, Tricomin, recombinant house dust mite allergy desensitization injection, recombinant human parathyroid hormone (PTH) 1-84 (sc, osteoporosis), epoetin delta, transgenic antithrombin III, Granditropin, Vitrase, interferon-alpha (oral lozenge), GEM-21S, vapreotide, idursulfase, omapatrilat, recombinant serum albumin, certolizumab pegol, glucarpidase, human recombinant C1 esterase inhibitor (angioedema), lanoteplase, enfuvirtide, VGV-1, interferon-alpha, lucinactant, ariaptadil (inhaled, pulmonary disease), icatibant, ecallantide, omiganan, Aurograb, pexiganan acetate, ADI-PEG-20, LDI-200, degarelix, cintredekin besudotox, FavId, MDX-1379, ISAtx-247, liraglutide, teriparatide, tifacogin, AA-4500, T4N5 liposome lotion, catumaxomab, DWP-413, ART-123, Chrysalin, desmoteplase, amediopase, corifollitropin alfa, TH-9507, teduglutide, Diamyd, DWP-412, recombinant G-CSF, RGN-303, DiaPep277, interferon-beta, interferon alfa-n3 (oral), belatacept, AMG-531, MBP-8298, Xerecept, opebacan, AIDSVAX, GV-1001, LymphoScan, ranpirnase, Lipoxysan, lusupultide, MP52 (beta-tricalciumphosphate carrier, bone regeneration), melanoma vaccine,

Protein/Peptide Name

sipuleucel-T, CTP-37, Insegia, vitespen, human thrombin (frozen, surgical bleeding), thrombin, TransMID, alfineprase, Puricase, terlipressin, EUR-1008M, recombinant FGF-1, BDM-E, rotigaptide, ETC-216, P-113, MBI-594AN, duramycin, SCV-07, OPI-45, Endostatin, Angiostatin, ABT-510, Bowman Birk Inhibitor Concentrate, XMP-629, 99mTc-Hynic-Annexin V, kahalalide F, CTCE-9908, teverelix (extended release), ozarelix, romidepsin, BAY-50-4798, interleukin-4, PRX-321, Pepscan, iboctadekin, rh lactoferrin, TRU-015, IL-21, ATN-161, cilengtide, Albuferon, Biphasix, IRX-2, omega interferon, PCK-3145, CAP-232, pasireotide, huN901-DM1, ovarian cancer immunotherapeutic vaccine, SB-249553, Oncovax-CL, OncoVax-P, BLP-25, CerVax-16, nemifitide, rAAT (inhaled), rAAT (dermatological), CGRP (inhaled, asthma), pegsunercept, thymosin beta-4, plitidepsin, GTP-200, ramoplanin, GRASPA, OBI-1, AC-100, salmon calcitonin (oral, eligen), calcitonin (oral, osteoporosis), examorelin, capromorelin, Cardeva, velafermin, 131I-TM-601, KK-220, TP-10, ularitide, depelestat, hematide, Chrysalin (topical), rNAPc2, recombinant Factor VIII (PEGylated liposomal), bFGF, PEGylated recombinant staphylokinase variant, V-10153, SonoLysis Prolyse, NeuroVax, CZEN-002, islet cell neogenesis therapy, GLP-1, BIM-51077, LY-548806, exenatide, AVE-0010, GA-GCB, avorelin, AOD-9604, linacotide acetate, CETi-1, Hemospan, VAL, recombinant methionyl human leptin, pitrakinra subcutaneous injection, eczema, pitrakinra, Multikine, RG-1068, MM-093, NBI-6024, AT-001, PI-0824, Org-39141, Cpn10, rEV-131 (ophthalmic), rEV-131 (respiratory disease), RPI-78M, CYT-99007 CTLA4-Ig, DTY-001, valategrast, IRX-3, RDP-58, Tauferon, bile salt stimulated lipase, Merispace, alkaline phosphatase, EP-2104R, Melanotan-II, bremelanotide, ATL-104, recombinant human microplasmin, AX-200, SEMAX, ACV-1, Xen-2174, CJC-1008, dynorphin A, SI-6603, AER-002, CHS-13340, PTH(1-34) liposomal cream (Novasome), Ostabolin-C, PTH analog (topical, psoriasis), MBRI-93.02, IDD-5, CDX-110, Pentrys, Norelin, CytoFab, P-9808, VT-111, icrocaptide, telbermin, rupintrivir, reticulose, rGRF, P1A, alpha-galactosidase A, ACE-011, ALTU-140, CGX-1160, D-4F, ETC-642, APP-018, rhMBL, SCV-07, DRF-7295, ABT-828, ErbB2-specific immunotoxin (anticancer), DT388IL-3, TST-10088, PRO-1762, Combotox, cholecystokinin-B/gastrin-receptor binding peptides, 111In-hEGF, AE-37, trastuzumab-DM1, Antagonist G, IL-12 (recombinant), PM-02734, IMP-321, rhIGF-BP3, BLX-883, CUV-1647 (topical), L-19 based radioimmunotherapeutics (cancer), Re-188-P-2045, AMG-386, DC/1540/KLH vaccine (cancer), VX-001, AVE-9633, AC-9301, NY-ESO-1 vaccine (peptides), NA17.A2 peptides, melanoma vaccine (pulsed antigen therapeutic), prostate cancer vaccine, CBP-501, recombinant human lactoferrin (dry eye), FX-06, AP-214, WAP-8294A2 (injectable), ACP-HIP, SUN-11031, peptide YY [3-36] (obesity, intranasal), FGLL, atacicept, BR3-Fc, BN-003, BA-058, human parathyroid hormone 1-34 (nasal, osteoporosis), F-18-CCR1, AT-1001 (celiac disease/diabetes), JPD-003, PTH(7-34) liposomal cream (Novasome), duramycin, CAB-2, CTCE-0214, erythropoietin, EPO-Fc, CNTO-528, AMG-114, JR-013, Factor XIII, aminocandin, PN-951, 716155, SUN-E7001, TH-0318, BAY-73-7977, teverelix (immediate release), EP-51216, hGH, OGP-I, sifuvirtide, TV-4710, ALG-889, Org-41259, rhCC10, F-991, thymopentin (pulmonary diseases), r(m)CRP, subalin, L19-IL-2 fusion protein, elafin, NMK-150, ALTU-139, EN-122004, rhTPO, thrombopoietin receptor agonist, AL-108, AL-208, nerve growth factor antagonists (pain), SLV-317, CGX-1007, INNO-105, oral teriparatide (eligen), GEM-OS1, AC-162352, PRX-302, LFn-p24 fusion vaccine (Therapore), EP-1043, hPTH(1-34), 768974, SYN-101, PGN-0052, aviscumine, BIM-23190, enkastim, APC-8024, GI-5005, ACC-001, TTS-CD3, TNF, desmopressin, onercept, TP-9201, AC165198, Activin receptor type IIA, Adenosine deaminase, Adipotide, Afostase alfa (alkaline phosphatase), Alpha melanocyte stimulating hormone, Alpha-1 antitrypsin, Alpha-galactosidase, Angiotensin, Anti-angiopoietin-1 inhibitor, Anti-angiopoietin-2 inhibitor, Apolipoprotein A1, Arcalyst, Arginine deiminase, Asparaginase, Atilmotin, AZD2820, Bradykinin receptor antagonist, Calcitonin, Calcitonin gene-related peptide, Cenderitide, Cholecystokinin, Ciliary neurotrophic factor (CNTF), Ciligenitide, Coagulation factor IX, Coagulation factor VII, Coagulation factor VIII, Coagulation factor X, Coagulation factor XIII, Collagenase, Complement C1 esterase inhibitor (conestat alfa), Complement factor C3 inhibitor, Complement factor C5 inhibitor, Corticotropin-releasing factor, C-peptide, C-type natriuretic peptide, Defensins, DiaPep277, Diphenhydramine, Ecaltantide, Endostatin, Eptifibatide, Fibrinogen, Fibroblast growth factor receptor agonist, Follicle-stimulating hormone (FSH), Follistatin, FP-1039 (FGF trap), Fuzeon, Gastrin, Ghrelin, Ghrelin antagonist, GIP-1, GIP-1/GLP-1 dual agonist, Glucagon, Glucagon-like peptide (GLP) 1, Glucagon-like peptide (GLP) 2, Glucocerebrosidase (Cerezyme), Glutamate carboxypeptidase (carboxypeptidase G2), Glutaminase, Granulocyte colony stimulating factor (GCSF), Hematide, Heparinase, Hirudin, Human chorionic gonadotropin (hCG), Human deoxyribonuclease I, Humanin, Hyaluronidase, Icatibant, Iduronate-2-sulfatase (Elaprase), INGAP (Exsulim), Irisin, KAI-4169, Lepirudin (Refludan), Leukemia inhibiting factor, L-iduronidase, LRP5 inhibitor, LRP6 inhibitor, Luteinizing hormone (LH), Macrophage inflammatory protein 2 (GroBeta-T CXC chemokine), Mannose binding lectin, Melanocortin stimulating hormone, Methioninase (METase), Mirostipen, MUCI inhibitor, Myostatin inhibitor, N-acetylgalactosamine 4-sulfatase (Naglazyme), Nerve growth factor (Cerebrolysin), Neuropeptide Y2, Neurophilin, NU206, Onconase (Ranpirnase), Ontak (IL-2-toxin), Opioid growth factor, Oxyntomodulin, Oxytocin, Paliperidone, Pancreatic polypeptide, PanCyte, Parathyroid hormone (PTH), Parathyroid hormone related protein (PTHrP), Peptide YY (3-36), Phenylalanine ammonia lyase (PAL), Phenylalanine hydroxylase (PAH), Pituitary adenylate

Protein/Peptide Name

cyclase-activating polypeptide (PACAP), Platelet-activating factor acetylhydrolase, POT-4 (APL-1), Pramlintide, P-Selectin, Relaxin, rhDNase (Pulmozyme), RNase, Sanvar, Secretin, SN38, Somatostatin (Octreotide, Pasireotide, Sandostatin etc.), Somavert (human growth hormone receptor antagonist), Stem cell growth factor, Superoxide dismutase, TACI, Thrombin inhibitor (direct), Thrombomodulin, Thrombopoietin (TPO), Thymosin alpha 1 (Thymalfasin), Thyroid stimulating hormone (TSH), Thyrotropin releasing hormone, Tigapotide, Tissue plasminogen activator (tPA), TLN-232, Tripeptidyl peptidase 1, Tumour necrosis factor receptor, Tyrosine kinase receptor (TrkA), UGP281, Urate oxidase, Uricase, Urocortin 2, Urokinase plasminogen activator, Vascular endothelial growth factor (VEGF) inhibitor, Vasoactive intestinal peptide, Vasopressin, von Willebrand Factor (vWF), Ziconotide (Prialt), Zinc protoporphyrin, Adrenal corticotrophin hormone (ACTH), CD25, Interleukin-1 receptor, Interleukin-21, ABCF1, ACVR1, ACVR1B, ACVR2, ACVR2B, ACVRL1, ADORA2A, Aggrecan, AGR2, AICDA, AIF1, AIG1, AKAP1, AKAP2, AMH, AMHR2, ANGPT1, ANGPT2, ANGPTL3, ANGPTL4, ANPEP, APC, APOC1, APRIL, AR, AZGP1 (zinc-a-glycoprotein), A4 integrin, B7, B7.1, B7.2, BAD, BAFF, BAG1, BAI1, BCL2, BCL6, BDNF, BLNK, BLR1 (MDR15), BlyS, BMP1, BMP2, BMP3B (GDF10), BMP4, BMP6, BMP8, BMPR1A, BMPR1B, BMPR2, BPAG1 (plectin), BRCA1, C19orf10 (IL27w), C3, C4A, C5, C5R1, CANT1, CASP1, CASP4, CAV1, CCBP2 (D6/JAB61), CCL1 (1-309), CCL11 (eotaxin), CCL13 (MCP-4), CCL15 (MIP-1d), CCL16 (HCC-4), CCL17 (TARC), CCL18 (PARC), CCL19 (MIP-3b), CCL2 (MCP-1), MCAF, CCL20 (MIP-3a), CCL21 (MIP-2), SLC, exodus-2, CCL22 (MDC/STC-1), CCL23 (MPIF-1), CCL24 (MPIF-2/eotaxin-2), CCL25 (TECK), CCL26 (eotaxin-3), CCL27 (CTACK/ILC), CCL28, CCL3 (MIP-1a), CCL4 (MIP-1b), CCL5 (RANTES), CCL7 (MCP-3), CCL8 (mcp-2), CCNA1, CCNA2, CCND1, CCNE1, CCNE2, CCR1 (CKR1/HM145), CCR2 (mcp-1RB/RA), CCR3 (CKR3/CMKBR3), CCR4, CCR5 (CMKBR5/ChemR13), CCR6 (CMKBR6/CKR-L3/STRL22/DRY6), CCR7 (CKR7/EBI1), CCR8 (CMKBR8/TER1/CKR-L1), CCR9 (GPR-9-6), CCRL1 (VSHK1), CCRL2 (L-CCR), CD164, CD19, CD1C, CD20, CD200, CD-22, CD24, CD28, CD3, CD37, CD38, CD3E, CD3G, CD3Z, CD4, CD11a (LFA-1 integrin alphaL), CD40, CD40L, CD44, CD45RB, CD52, CD69, CD72, CD74, CD79A, CD79B, CD8, CD80, CD81, CD83, CD86, CD340, CDH1 (E-cadherin), CDH10, CDH12, CDH13, CDH18, CDH19, CDH20, CDH5, CDH7, CDH8, CDH9, CDK2, CDK3, CDK4, CDK5, CDK6, CDK7, CDK9, CDKN1A (p21Wap1/Cip1), CDKN1B (p27Kip1), CDKN1C, CDKN2A (p16INK4a), CDKN2B, CDKN2C, CDKN3, CEBPB, CER1, CHGA, CHGB, Chitinase, CHST10, CKLFSF2, CKLFSF3, CKLFSF4, CKLFSF5, CKLFSF6, CKLFSF7, CKLFSF8; CLDN3; CLDN7 (claudin-7); CLN3; CLU (clusterin); cMET; CMKLR1; CMKOR1 (RDC1); CNR1; COL18A1; COL1A1; COL4A3; COL6A1; CR2; CRP; CSF1 (M-CSF); CSF2 (GM-CSF); CSF3 (GCSF); CTLA4; CTNNB1 (b-catenin); CTSB (cathepsin B); CX3CL1 (SCYD1); CX3CR1 (V28); CXCL1 (GRO1); CXCL10 (IP-10); CXCL11 (I-TAC/IP-9); CXCL12 (SDF1); CXCL13; CXCL14; CXCL16; CXCL2 (GRO2); CXCL3 (GRO3); CXCL5 (ENA-78/LIX); CXCL6 (GCP-2); CXCL9 (MIG); CXCR3 (GPR9/CKR-L2); CXCR4; CXCR6 (TYMSTR/STRL33/Bonzo); CYB5; CYC1; CYSLTR1; DAB2IP; DES; DKFZp451J0118; DNCL1; DPP4; E2F1; ECGF1; EDG1; EFNA1; EFNA3; EFN2; EGF; EGFR; ELAC2; elastase; ENG; ENO1; ENO2; ENO3; EPHB4; EPO; ERBB-2 (Her2); EREG; ERK8; ESR1; ESR2; F3 (TF); FADD; FasL; FASN; FCER1A; FCER2; FCGR3A; FGF; FGF1 (aFGF); FGF10; FGF11; FGF12; FGF12B; FGF13; FGF14; FGF16; FGF17; FGF18; FGF19; FGF2 (bFGF); FGF20; FGF21; FGF22; FGF23; FGF3 (int-2); FGF4 (HST); FGF5; FGF6 (HST-2); FGF7 (KGF); FGF8; FGF9; FGF9R3; FGF9 (VEGFD); FIL1 (EPSILON); FIL1 (ZETA); FLJ12584; FLJ25530; FLRT1 (fibronectin); FLT1; FOS; FOSL1 (FRA-1); FY (DARC); GABRP (GABAa); GAGEB1; GAGEC1; GALNAC4S-6ST; GATA3; GDF5; GFI1; GGT1; GM-CSF; GNAS1; GNRH1; GPR2 (CCR10); GPR31; GPR44; GPR81 (FKSG80); GRCC10 (C10); GRP; GSN (Gelsolin); GSTP1; HAVCR2; HDAC4; HDAC5; HDAC7A; HDAC9; HER2; HGF; HIF1A; HIP1; histamine and histamine receptors; HLA-A; HLA-DRA; HM74; HMOX1; HUMCYT2A; ICEBERG; ICOSL; ID2; IFN-a; IFNA1; IFNA2; IFNA4; IFNA5; IFNA6; IFNA7; IFNB1; IFNgamma; IFNW1; IGBP1; IGF1; IGF1R; IGF2; IGFBP2; IGFBP3; IGFBP6; IL-1; IL10; IL10RA; IL10RB; IL11; IL11RA; IL-12; IL12A; IL12B; IL12RB1; IL12RB2; IL13; IL13RA1; IL13RA2; IL14; IL15; IL15RA; IL16; IL17; IL17B; IL17C; IL17R; IL18; IL18BP; IL18R1; IL18RAP; IL19; IL1A; IL1B; IL1F10; IL1F5; IL1F6; IL1F7; IL1F8; IL1F9; IL1HY1; IL1R1; IL1R2; IL1RAP; IL1RAPL1; IL1RAPL2; IL1RL1; IL1RL2; IL1RN; IL2; IL20; IL20RA; IL21R; IL22; IL22R; IL22RA2; IL23; IL24; IL25; IL26; IL27; IL28A; IL28B; IL29; IL31; IL32 alpha; IL33; IL2RA; IL2RB; IL2RG; IL3; IL30; IL3RA; IL4; IL4R; IL5; IL5RA; IL6; IL6R; IL6ST (glycoprotein 130); IL7; IL7R; IL8; IL8RA; IL8RB; IL8RB; IL9; IL9R; ILK; INHA; INHBA; INSL3; INSL4; IRAK1; IRAK2; ITGA1; ITGA2; ITGA3; ITGA6 (a6 integrin); ITGAV; ITGB3; ITGB4 (b 4 integrin); JAG1; JAK1; JAK3; JUN; K6HF; KAI1; KDR; KITLG; KLF5 (GC Box BP); KLF6; KLK10; KLK12; KLK13; KLK14; KLK15; KLK3; KLK4; KLK5; KLK6; KLK9; KRT1; KRT19 (Keratin 19); KRT2A; KRTHB6 (hair-specific type II keratin); LAMA5; LEP (leptin); LFA3; LIGHT; Lingo-p75; Lingo-Troy; LPS; LTA (TNF-b); LTB; LTBR (GPR16); LTBR2; LTBR; MACMARCKS; MAG or Omgp; MAP2K7 (c-Jun); MDK; MIB1; midkine; MIF; MIP-2; MKI67 (Ki-67); MMP2; MMP9; MS4A1; MSMB; MT3 (metallothionein-III); MTSS1; MUC1 (mucin); MYC; MYD88; NCK2; neurocan; NFKB1; NFKB2; NGFB (NGF); NGFR; NgR-Lingo; NgR-Nogo66 (Nogo); NgR-p75; NgR-Troy; NME1 (NM23A); NOX5; NPPB; NR0B1; NR0B2; NR1D1; NR1D2; NR1H2; NR1H3; NR1H4; NRII2; NRII3; NR2C1; NR2C2; NR2E1; NR2E3; NR2F1; NR2F2; NR2F6; NR3C1; NR3C2; NR4A1; NR4A2; NR4A3;

Protein/Peptide Name
NR5A1; NR5A2; NR6A1; NRP1; NRP2; NT5E; NTN4; ODZ1; OPRD1; P2RX7; PAP; PART1; PATE; PAWR; PCA3; PCNA; PDGFA; PDGFB; PECAM1; PF4 (CXCL4); PGF; PGR; phosphacan; PIAS2; PIK3CG; PLAU (uPA); PLG; PLXDC1; PPBP (CXCL7); PPID; PR1; PRKCQ; PRKD1; PRL; PROC; PROK2; PSAP; PSCA; PTAFR; PTEN; PTGS2 (COX-2); PTN; RAC2 (p21Rac2); RANKL; RARB; RGS1; RGS13; RGS3; RNF110 (ZNF144); ROBO2; RSV; SI00A2; SCGB1D2 (lipophilin B); SCGB2A1 (mammaglobin 2); SCGB2A2 (mammaglobin 1); SCYE1 (endothelial Monocyte-activating cytokine); SDF2; SERPINA1; SERPINA3; SERPINB5 (maspin); SERPINE1 (PAI-1); SERPINF1; SHBG; SLA2; SLC2A2; SLC33A1; SLC43A1; SLIT2; SPP1; SPRR1B (Spr1); ST6GAL1; STAB1; STAT6; STEAP; STEAP2; TB4R2; TBX21; TCP10; TDGF1; TEK; TGFA; TGFBI; TGFBI1; TGFBI2; TGFBI3; TGFBI; TGFBR1; TGFBR2; TGFBR3; TH1L; THBS1 (thrombospondin-1); THBS2; THBS4; THPO; TIE (Tie-1); TIMP3; tissue factor; TLR10; TLR2; TLR3; TLR4; TLR5; TLR6; TLR7; TLR8; TLR9; TNF; TNF-a; TNFAIP2 (B94); TNFAIP3; TNFRSF11A; TNFRSF1A; TNFRSF1B; TNFRSF21; TNFRSF5; TNFRSF6 (Fas); TNFRSF7; TNFRSF8; TNFRSF9; TNFSF10 (TRAIL); TNFSF11 (TRANCE); TNFSF12 (APO3L); TNFSF13 (April); TNFSF13B; TNFSF14 (HVEM-L); TNFSF15 (VEGI); TNFSF18; TNFSF4 (OX40 ligand); TNFSF5 (CD40 ligand); TNFSF6 (FasL); TNFSF7 (CD27 ligand); TNFSF8 (CD30 ligand); TNFSF9 (4-1BB ligand); TOLLIP; Toll-like receptors (TLR1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 to TLR-13); TOP2A (topoisomerase lia); TP53; TPM1; TPM2; TRADD; TRAF1; TRAF2; TRAF3; TRAF4; TRAF5; TRAF6; TREM1; TREM2; TRPC6; TSLP; TWEAK; VAP1; VEGF; VEGFB; VEGFC; versican; VHL C5; VLA-1; VLA-4; XCL1 (lymphotactin); XCL2 (SCM-1b); XCR1 (GPR5/CCXCR1); YY1; ZFPM2.

(i) Exemplary Therapeutic Proteins

[00190] Proteinacious compounds that are specifically contemplated for inclusion in the subject conjugates and compositions are the following peptides and proteins:

[00191] “C-type Natriuretic peptide” or “CNP,” which refers to the human protein (UniProt No. P23582) encoded by the NPPC gene that is cleaved to the 22 amino acid peptide C-type natriuretic peptide (CNP), having the sequence GLSKGCFGLKLDRIGSMSGLGC, as well as species and synthetic variations thereof, having at least a portion of the biological activity of the native peptide. CNP is a selective agonist for the natriuretic peptide receptor B (NPRB) and is reported to be a potent stimulator of endochondral bone growth. CNP binds to its receptor, initiates intracellular signals & ultimately inhibits the overactive FGFR3 pathway. Use of CNP is indicated for achondroplasia, a common form of skeletal dysplasia or short-limbed dwarfism, and human disorders caused by FGFR3 mutations, including syndromes affecting skeletal development; e.g., hypochondroplasia [HCH], ACH, thanatophoric dysplasia [TD]), skin (epidermal nevi, seborrhaeic keratosis, acanthosis nigricans), and cancer (multiple myeloma [MM], prostate and bladder carcinoma, seminoma) (Foldynova-Trantirkova S. Hum Mutat. (2012) 33:29). The half-life of CNP-22 is reported to be 2.6 min, being rapidly metabolized by neutral endopeptidase and cleared by a clearance receptor (Prickett T., 2004, Clinical Science, 106:535), thereby limiting its utility. In one embodiment, TP-ABC compositions comprising CNP have utility in the treatment of metabolic disorders, skeletal dysplasias and cancer.

[00192] “Luteinizing hormone-releasing hormone” or “LHRH,” which refers to the human protein (UniProt No. P01148) encoded by the *GNRHI* gene that is processed in the preoptic anterior hypothalamus from a 92-amino acid preprohormone into the linear decapeptide end-product having the sequence pyroGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂, as well as species and synthetic variations thereof, having at least a portion of the biological activity of the native peptide. LHRH plays a pivotal role in the regulation of the pituitary/gonadal axis, and thus reproduction. LHRH exerts its effects through binding to high-affinity receptors on the pituitary gonadotroph cells and subsequent release of

FSH and LH. LHRH is found in organs outside of the hypothalamus and pituitary, and because a high percentage of certain cancer tissues have LHRH binding sites and because sex steroids have been implicated in the development of breast and prostate cancers, hormonal therapy with LHRH agonists are approved or are considered for the treatment of sex-steroid-dependent conditions such as estrogen-dependent breast cancer, ovarian cancer, endometrial cancer, bladder cancer and androgen-dependent prostate carcinoma. Because the half-life is reported to be less than 4 minutes, (Redding TW, et al. The Half-life, Metabolism and Excretion of Tritiated Luteinizing Hormone-Releasing Hormone (LH-RH) in Man. *J Clin Endocrinol. Metab.* (1973) 37:626-631), its utility as a therapeutic may be limited. In one embodiment, TP-ABC compositions comprising LHRH have utility in the treatment of cancer.

[00193] “Cilengitide,” which refers to the synthetic cyclic RGD pentapeptide having the sequence Arg-Gly-Asp-Dphe-NmeVal or the chemical name 2-[(2S,5R,8S,11S)-5-benzyl-11-{3-[(diaminomethylidene)amino]propyl}-7-methyl-3,6,9,12,15-pentaoxo-8-(propan-2-yl)-1,4,7,10,13-pentaaazacyclopentadecan-2-yl]acetic acid (CAS No. 188968-51-6). Cilengitide is selective for α_v integrins, which are important in angiogenesis (forming new blood vessels). The binding of such ligands activates the integrins to regulate tumor cell invasion, migration, proliferation, survival & angiogenesis. Hence, the use of cilengitide is under investigation for the treatment of glioblastoma by inhibiting angiogenesis (Burke P, et al. Cilengitide targeting of $\alpha_v\beta_3$ integrin receptor synergizes with radioimmunotherapy to increase efficacy and apoptosis in breast cancer xenografts". *Cancer Res* (2002) 62(15): 4263–4272). Because cilengitide has a short half-life of 3-5 h, and poor solubility limiting the maximum drug concentration to 15mg/mL (O'Donnell PH. A phase I study of continuous infusion cilengitide in patients with solid tumors. *Invest New Drugs* (2012) 30:604), its utility as a therapeutic may be limited. In one embodiment, TP-ABC compositions comprising cilengitide have utility in the treatment of tumors and in preventing or limiting angiogenesis in certain tissues and cancers.

[00194] “Exendin-4,” which refers to a glucose regulating peptide found in the saliva of the Gila-monster *Heloderma suspectum*, as well as species and sequence variants thereof, and includes the native 39 amino acid sequence His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser and homologous sequences and peptide mimetics, and variants thereof; natural sequences, such as from primates and non-natural having at least a portion of the biological activity of native exendin-4. Exendin-4 is an incretin polypeptide hormone that decreases blood glucose, promotes insulin secretion, slows gastric emptying and improves satiety, providing a marked improvement in postprandial hyperglycemia. The exendins have some sequence similarity to members of the glucagon-like peptide family, with the highest identity being to GLP-1 (Goke, et al., *J. Biol. Chem.*, 268:19650-55 (1993)). A variety of homologous sequences can be functionally equivalent to native exendin-4 and GLP-1. Conservation of GLP-1 sequences from different species are presented in *Regulatory Peptides* 2001 98 p. 1–12. Table 2 shows the sequences from a wide variety of species, while Table 3 shows a list of synthetic GLP-1 analogs; all of which are contemplated for use as glucose regulating peptides in the GPXTEN described

herein. Exendin-4 binds at GLP-1 receptors on insulin-secreting β TC1 cells, and also stimulates somatostatin release and inhibits gastrin release in isolated stomachs (Goke, et al., *J. Biol. Chem.* 268:19650-55, 1993). As a mimetic of GLP-1, exendin-4 displays a similar broad range of biological activities, yet has a longer half-life than GLP-1, with a mean terminal half-life of 2.4 h. Exenatide is a synthetic version of exendin-4, marketed as Byetta. However, due to its short half-life, exenatide is currently dosed twice daily, and its therapeutic utility may be limited. Exendin-4-containing TP-ABC compositions have utility in the treatment of diabetes (Type I and Type II) and insulin resistance disorders.

[00195] “Peptide YY” and “PYY,” which refers to human peptide YY polypeptide (UniProt No. P10082), synthetic versions and species and non-natural sequence variants having at least a portion of the biological activity of mature PYY. As used herein, “PYY” includes both major forms of the human full length, 36 amino acid peptide, PYY₁₋₃₆ and the predominant circulating form PYY₃₋₃₆ (“PYY3-36”) which have the PP fold structural motif. PYY3-36 has the amino acid sequence IKPEAPGEDASPEELNRYRYASLRHYLNLVTRQRY-NH₂. PYY is produced by specialized endocrine cells (L-cells) in the gut after a person eats and inhibits gastric motility and increases water and electrolyte absorption in the colon. PYY may also suppress pancreatic secretion. The naturally occurring PYY3-36 is a nonselective Y₁, Y₂, & Y₅ agonist. PYY-containing TP-ABC may find particular use in the treatment of diabetes for glucose regulation, insulin-resistance disorders, and obesity. Analogs of PYY have been prepared, as described in U.S. Patent Nos. 5,604,203, U.S. Patent No. 5,574,010 and U.S. Patent No. 7,166,575. Because the half-life is reported to be less than 1 h, (Addison ML. A role for metalloendopeptidases in the breakdown of the gut hormone, PYY 3-36. *Endocrinology* (2011) 152(12):4630-4640) and is typically administered by the intranasal route three times daily, its therapeutic utility may be limited. In one embodiment, TP-ABC compositions comprising PYY have utility in the treatment of diabetes and insulin resistance.

[00196] “Leptin,” which refers to the naturally occurring leptin (UnitProt No. P41159) encoded by the *Ob(Lep)* gene, synthetic versions and species and non-natural sequence variants having at least a portion of the biological activity of the mature leptin. Leptin has the amino acid sequence VPIQKVQDDTKTLIKTIVTRINDISHTQSVSSKQKVTGLDFIPGLHPILTLKMDQTLAVYQQILTS MPSRNVIQISNDLENLRDLLHVLAFSKCHLPWASGLETLDLGGVLEASGYSTEVVALSRLQGS LQDMLWQLDLSPGC, and has a disulfide bridge between residues 97 and 147. Leptin plays a key role in regulating energy intake and energy expenditure, including appetite, metabolism, and body weight. Leptin-containing TP-ABC compositions may find particular use in the treatment of conditions such as diabetes for glucose regulation, insulin-resistance disorders, obesity, congenital/acquired lipodystrophy, HAART-induced lipodystrophy, and hypothalamic amenorrhea. Leptin has been cloned, as described in U.S. Patent No. 7,112,659, and leptin analogs and fragments in U.S. Patent No. 5,521,283, U.S. Patent No. 5,532,336, PCT/US96/22308 and PCT/US96/01471. Because the commercially available form metreleptin has a half-life reported to be 8-30 min (Klein S., et al. Adipose tissue leptin production and

plasma leptin kinetics in humans. Diabetes (1996) 45:984–987) and the majority of current leptin therapies require 1x-2x/day dosing, its therapeutic utility may be limited. In one embodiment, TP-ABC compositions comprising leptin have utility in the treatment of disorders related to regulating energy intake and energy expenditure, including appetite, metabolism, and body weight.

[00197] “Pramlintide,” which refers to the synthetic amylin mimetic having the amino acid sequence KCNTATCATNRLANFLVHSSNNFGPILPPTNVGSNTY-NH₂, and sequence variants having at least a portion of the biological activity of pramlintide or native amylin. The pramlintide has a sequence wherein amino acids from the rat amylin sequence are substituted for amino acids in the human amylin sequence. Amylin is a 37aa peptide secreted by pancreatic beta-cells that is co-released with insulin in pulsatile fashion, typically in a molar ratio of 100 insulin to 1 amylin. Amylin functions to inhibit gastric emptying, glucagon secretion, promote satiety and meal termination (Kong MF, et al. Infusion of pramlintide, a human amylin analogue, delays gastric emptying in men with IDDM. Diabetologia. (1997) 40:82–88). Pramlintide is used as an adjunct to insulin therapy in Type I diabetes and Type II diabetes and shows improvement in glycemic control and reduction in insulin requirements, and also demonstrate modest reduction in body weight (Neary MT, Batterham RL. Gut hormones: Implications for the treatment of obesity. Pharmacology & Therapeutics (2009)124:44-56). Because pramlintide has a half-life reported to be 20 min (McQueen, J. Pramlintide acetate. Am. J. Health-System Pharmacy (2005) 22:2363-2372) and requires 2x-3x/day dosing, its therapeutic utility may be limited. In one embodiment, TP-ABC compositions comprising pramlintide have utility in the treatment of diabetes, including Type I and Type II.

[00198] “Oxytocin,” which refers to the mammalian hormone peptide (UniProt No. P01178) having the amino acid sequence CYIQNCPLG-NH₂ and a disulfide bridge between residues 1 and 6, and synthetic versions, such as pitocin. Oxytocin acts primarily as a neuromodulator in the brain, having a structure very similar to that of vasopressin, which are the only known hormones released by the human posterior pituitary gland to act at a distance. Oxytocin has uterine-contracting properties mediated by specific, high-affinity oxytocin receptors expressed in the mammary gland and the uterus; hence its role in parturition and lactation. In one embodiment, TP-ABC compositions comprising oxytocin may find particular use in the treatment of autism, fragile X syndrome, chronic daily headache, and male infertility.

[00199] “Relaxin,” which refers to the protein hormone that is a heterodimer of two peptide chains of 24 and 29 amino acids linked by disulfide bridges created from the 185 amino acid precursor protein (UniProt No. P04090); the B chain having the amino acid sequence DSWMEEVIKLCGRELVRAQIAICGMSTWS and the A chain having the amino acid sequence QLYSALANKCCHVGCTKRSLARFC, with the disulfide bridges between B10-A10 and B23-A24, and includes synthetic and recombinant versions. Relaxin is produced by the corpus luteum during the menstrual cycle and pregnancy in women and by the prostate in men. Relaxin orchestrates many of the maternal physiological responses to pregnancy, acts as a systemic and renal vasodilator, is a cardioprotective and antifibrotic agent. Relaxin binds to relaxin receptor (GPCR), increases cAMP and

activates PKC, PI3K and endothelin type B receptor resulting in increased nitric oxide production, and also activates MAPK, which may play a role in relaxin induced VEGF expression. Relaxin-containing polypeptides of the invention may find particular use in the treatment of acute decompensated heart failure (ADHF). Because the reported half-life of relaxin in humans is less than 10 min (Dschietzig T, et al. Intravenous recombinant human relaxin in compensated heart failure: a safety, tolerability, and pharmacodynamic trial. *J Card Fail.* 2009;15:182–190), the therapeutic utility of the unmodified protein may be limited. In one embodiment, TP-ABC compositions comprising relaxin have utility in the treatment of cardiovascular disease, including acute heart failure, congestive heart failure, compensated heart failure or decompensated heart failure and in the treatment of an autoimmune disorder such as, but not limited to, scleroderma, diffuse scleroderma or systemic scleroderma. The disease or condition may be an inflammatory disease. The inflammatory disease may be fibromyalgia. The disease or condition may be fibrosis. Alternatively, the disease or condition may be pregnancy.

[00200] “Cenderitide” and “CD-NP,” which refers to a human C-type natriuretic peptide-(32-53)-peptide (CNP-22) with eastern green mamba (*Dendroaspis angusticeps*) natriuretic peptide-(24-38)-peptide having the amino acid sequence GLSKGCFGLKLDRI GMSGLGCPSLRDPRPNAPSTSA, with disulfide bridges between residues 6 and 22. The chimeric peptide has vasoprotective and RAAS suppressing actions via activation of the receptors guanylyl cyclase (GC)-A and GC-B, and may potentiate renal enhancement and cardiac unloading while having minimal hypotensive effects. Accordingly, TP-ABC compositions comprising cenderitide may have use in treatment of cardiorenal disease such as acute decompensated heart failure (ADHF) and acute myocardial infarction (AMI), particularly during the “post-acute” treatment period.

[00201] “Peginesatide” or “hematide,” which refers to a peptide composed of two synthetic 21 amino-acid peptides having the amino acid sequence GlyGlyLeuTyrAlaCysHisMetGlyProIleThr1NalValCysGlnProLeuArgSarLys that are linked at lysine with TP-ABC compositions. Peginesatide is a novel analog of erythropoietin that has erythropoietic properties and is being developed for medical use as a treatment for anemia due to chronic kidney disease (CKD) in patients not on dialysis. In one embodiment, TP-ABC compositions comprising hematide have utility in the treatment of anemia.

[00202] “Oxyntomodulin” or “OXM,” which refers to human oxyntomodulin, synthetic versions and sequence variants thereof, having at least a portion of the biological activity of mature oxyntomodulin. Oxyntomodulin is a 37 amino acid peptide having the amino acid sequence HSQGTFTSDYSKYLD SRRRAQDFVQWLMNTKRN RNNIA, is produced postprandially from intestinal L-cells in the colon and contains the 29 amino acid sequence of glucagon followed by an 8 amino acid carboxyterminal extension. Oxyntomodulin is an agonist at both the glucagon receptor and the GLP-1R, with its anorectic effect likely mediated via the latter receptor. OXM has been found to suppress appetite. OXM-containing polypeptides of the TP-ABC compositions may find particular use in the treatment of diabetes for glucose regulation, insulin-resistance disorders, obesity, and can be used as a weight loss

treatment. As native oxyntomodulin has been reported to have a half-life of ~12 min in human plasma (measured with a cross-reacting glucagon assay; Schjoldager BT. Oxyntomodulin: a potential hormone from the distal gut. Pharmacokinetics and effects on gastric acid and insulin secretion in man. Eur J Clin Invest. (1988) 18(5):499-503.), the therapeutic utility of the unmodified protein may be limited. In one embodiment, TP-ABC compositions comprising oxyntomodulin have utility in the treatment of diabetes, obesity and insulin-resistance.

[00203] “POT4” or “APL-1,” which refers to the synthetic cyclic peptide having the sequence H-Ile-[Cys-Val-Val-Gln-Asp-Trp-Gly-His-His-Arg-Cys]-Thr-NH₂. POT4 is a more potent C3 complement inhibitor than compstatin, which inhibits the cleavage of native C3 to its active fragments C3a and C3b, and has extended circulating in vivo half-life of 8 hours. It is considered for use to prevent inflammation, damage and upregulation of angiogenic factors like VEGF in diseases like age-related macular degeneration (AMD), paroxysmal nocturnal hemoglobinuria (PNH), asthma and COPD. In one embodiment, TP-ABC compositions comprising POT4 have utility in the treatment of inflammation, damage and upregulation of angiogenic factors like VEGF in diseases like age-related macular degeneration (AMD), paroxysmal nocturnal hemoglobinuria (PNH), asthma and COPD.

[00204] “Interferon-lambda”, “IFN- λ ”, interleukin-29” and “IL-29,” which refers to the human interleukin (UniProt No. Q8IU54 (20-200)) encoded by the *IL29* gene having the amino acid sequence GPVPTSKPTTTGKGCHIGRFKSLSPQELASFKKARDALEESLKLKNWSCSSPVFPGNWDRLRLQV RERPVALEAELALTLKVLEAAAGPALEDVLDQPLHTLHHILSQLQACIQPQPTAGPRPRGRLHHW LHRLQEAPKKESAGCLEASVTFLNLFRLTRDLKYVADGNLCLRTSTHPEST, recombinant and synthetic versions and sequence variants thereof, having at least a portion of the biological activity of mature IL-29. A type III interferon, IL-29 signals through a heterodimer receptor complex (IL-10R2 & IL-28R α receptor chains) distinct from type I IFN (IFNAR1/IFNAR2 receptor complex), and plays an important role in anti-viral immunity. Notably, the IL-29 receptor is highly expressed on hepatocytes, the primary site of HCV infection, but is not significantly expressed on immune or bone marrow cells. Pegylated versions have an estimated half-life of 50-70 h. In one embodiment, TP-ABC compositions comprising interferon-lambda have utility in the treatment of viral diseases.

[00205] “Interferon-beta” or “IFN- β ,” which refers to the human protein encoded by the *IFNB1* gene having the amino acid sequence MSYNLLGFLQRSSNFQCQKLLWQLNGRLEYCLKDRMNFDIPEEIKQLQQFQKEDAALTIYEMLQ NIFAIQRQDSSSTGWNETIVENLLANVYHQINHLKTVLEEKLEKEDFTRGKLMSSLHLKRYYYGRIL HYLKAKEYSHCAWTIVRVEILRNIFYFINRLTGylRN, and recombinant and synthetic versions and sequence variants thereof, having at least a portion of the biological activity of mature IFN- β . IFN- β is produced by various cell types including fibroblasts and macrophages, and mediates antiviral, antiproliferative and immunomodulatory activities in response to viral infection and other biological inducers. The binding of IFN- β to specific receptors on the surface of human cells initiates a cascade of intracellular events that leads to the expression of numerous interferon-induced gene products such as 2',

5'-oligoadenylate synthetase, β 2-microglobulin, and neopterin. These gene products are routinely used as biomarkers in clinical setting. IFN- β can be used in treatment of various forms of multiple sclerosis (MS), including relapse remitting MS, secondary progressive MS, primary progressive MS, juvenile onset MS, and clinically isolated syndromes suggestive of MS. Commercially-available forms of IFN- β have reported half-lives of 4 to 67 h and require frequent dosing, which can limit their therapeutic utility. In one embodiment, TP-ABC compositions comprising interferon-beta have utility in the treatment of immunologic disorders, including MS.

[00206] “C-peptide,” which refers to the human pancreatic protein having the amino acid sequence EAEDLQVGQVELGGGPGAGSLQPLALEGSLQ, and recombinant and synthetic versions and sequence variants thereof, having at least a portion of the biological activity of native C-peptide. C-peptide is the middle segment of proinsulin that is between the N-terminal B-chain and the C-terminal A-chain, and is cleaved from preproinsulin as mature insulin is formed and secreted. Circulating C-peptide binds to a receptor that is likely G-protein-coupled, and the signal activates Ca^{2+} -dependent intracellular signaling pathways such as MAPK, PLC γ , and PKC, leading to upregulation of a range of transcription factors as well as eNOS and Na+K+ATPase activities. C-peptide is considered for use in diabetic complications and diabetic nephropathy. Since the reported half-life is about 30 minutes (Matthews DR. The half-life of endogenous insulin and C-peptide in man assessed by somatostatin suppression. Clin Endocrinol (Oxf). (1985) 23(1):71-79), the therapeutic utility of the unmodified protein may be limited. In one embodiment, TP-ABC compositions comprising C-peptide are useful in the treatment of diabetes.

[00207] “Ghrelin,” which refers to the human hormone having the amino acid sequence GSSFLSPEHQRVQQRKESKKPPAKLQPR, truncated versions, recombinant and synthetic versions and sequence variants thereof, having at least a portion of the biological activity of native ghrelin, including the native, processed 27 or 28 amino acid sequence and homologous sequences. Ghrelin induces satiation, or species and non-natural sequence variants having at least a portion of the biological activity of mature ghrelin, including the native, processed 27 or 28 amino acid sequence and homologous sequences. Ghrelin is produced mainly by P/D1 cells lining the fundus of the human stomach and epsilon cells of the pancreas that stimulates hunger, and is considered the counterpart hormone to leptin. Ghrelin levels increase before meals and decrease after meals, and can result in increased food intake and increase fat mass by an action exerted at the level of the hypothalamus. Ghrelin also stimulates the release of growth hormone. Ghrelin is acylated at a serine residue by n-octanoic acid; this acylation is important for binding to the GHS1 a receptor and for the agonist activity and the GH-releasing capacity of ghrelin. In one embodiment, ghrelin-containing TP-ABC compositions find particular use as agonists, e.g., to selectively stimulate motility of the GI tract in gastrointestinal motility disorder, to accelerate gastric emptying, or to stimulate the release of growth hormone. The disclosure encompasses unacylated forms and sequence variants of ghrelin, which can act as antagonists. Ghrelin analogs with sequence substitutions or truncated variants, such as described in U.S. Patent No. 7,385,026, may find particular use in TP-ABC compositions for use as antagonists for improved glucose homeostasis, treatment of

insulin resistance and treatment of obesity, cancer cachexia, post-operative ileus, bowel disorders, and gastrointestinal disorders. The isolation and characterization of ghrelin has been reported (Kojima M, *et al.*, Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*. 1999;402(6762):656-660) and synthetic analogs have been prepared by peptide synthesis, as described in U.S. Pat. No. 6,967,237. As ghrelin has a reported terminal half-life of 10-30 min (Akamizu T, *et al.* Pharmacokinetics, safety, and endocrine and appetite effects of ghrelin administration in young healthy subjects. *Eur J. Endocrinology* (2004)150(4):447-455), the therapeutic utility of the unmodified protein may be limited, and analogs with, at position 3, the native serine amino acid with an octyl side group instead of the native octanoyl side group, may confer added resistance to proteases.

[00208] “Follistatin,” also known as “activin-binding protein” or “FSH-suppressing protein (FSP),” refers to the protein that, in humans, is encoded by the *FST* gene. As used herein, “follistatin” includes homologs, species variants, sequence variants and fragments thereof. The mature protein form in humans has 315 amino acids, is referred to as FS-315 and has been cloned (US Pat Nos. 5,041,538 and 5,182,375). Follistatin contains two potential N-glycosylation sites, Asn95 and Asn259, however it has been demonstrated that mutation at these sites followed by testing of the recombinant product for their ability to inhibit FSH secretion and to bind activin resulted in each mutant having a similar property as the non-mutated recombinant hFS-315, suggesting that glycosylation of the follistatin molecule has no effect in these functions (Inouye, S., *et al.* Site-specific mutagenesis of human follistatin. *BBRC* (1991) 179(1):352-358). Porcine follistatin is disclosed in Ueno *et al.*, *PNAS:USA* 84:8282-8286 (1987) and bovine follistatin is disclosed in Robertson *et al.*, *Biochem. Biophys. Res. Commun.* 149:744-749 (1987). As bone morphogenetic proteins and growth/differentiation factors such as activin and myostatin have the ability to induce the growth, formation, differentiation and maintenance of various tissues, including bone, cartilage, tendon/ligament, muscle, neural, and various organs, their neutralization by follistatin and follistatin agonists can have therapeutic value (U.S. Patent No. 5,545,616, U.S. Patent No. 5,041,538, and AU9675056). As follistatin administered to a subject is rapidly eliminated from the circulation, with a terminal half-life of just over 2 hours in rats (Kogure K, *et al.* Intravenous administration of follistatin: delivery to the liver and effect on liver regeneration after partial hepatectomy. *Hepatology*. (1996) 24(2):361-366), the therapeutic utility of the unmodified protein can be limited. In one embodiment, TP-ABC compositions comprising follistatin are useful in the treatment of the growth, formation, differentiation and maintenance of various tissues.

[00209] “Vasoactive intestinal peptide” and “VIP,” which refers to the 28 amino acid peptide hormone (UniProt No. P01282 (125-152)) encoded by the *VIP* gene residues having the amino acid sequence HSDAVFTDNYTRLRKQMAVKKYLNSILN-NH₂ and recombinant and synthetic versions and sequence variants thereof, having at least a portion of the biological activity of native VIP. The VIP peptide is produced in many tissues, including the gut, pancreas and suprachiasmatic nuclei of the hypothalamus in the brain. VIP stimulates contractility in the heart, causes vasodilation, increases glycogenolysis, lowers arterial blood pressure and relaxes the smooth muscle of trachea, stomach and gall

bladder. Changes in concentration are associated with myocardial fibrosis, heart failure, cardiomyopathy and pulmonary hypertension, and its deficiency in the respiratory system is considered to be a pathogenetic factor in pulmonary disease (Said SI, 2007, *Circulation*, 115: 1260; Said SI, 2008, *Ann N Y Acad Sci*, 1144:148; Petkov V et.al., 2003, *J Clin Invest*, 111:1339). VIP is considered for use in treating resistant hypertension, primary pulmonary arterial hypertension (PAH), asthma, COPD, diabetes, erectile dysfunction, and female sexual dysfunction. As its half-life is reported to be approximately 1 minute (Domschke S, et al. Vasoactive intestinal peptide in man: pharmacokinetics, metabolic and circulatory effects. *Gut* (1978) 19:1049–1053), the therapeutic utility of the unmodified protein may be limited. In one embodiment, TP-ABC compositions comprising VIP are useful in the treatment of cardiac, pulmonary and GI diseases.

[00210] “Fuzeon,” which refers to the 36 amino acid peptide derived from the gp41 of HIV, a viral protein involved in fusion of HIV to CD4+ T cells, having the amino acid sequence YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF, and recombinant and synthetic versions and sequence variants thereof, having at least a portion of the binding activity of native gp41. Fuzeon and multimers thereof or conjugates with related peptides are used or are being considered for use in treating resistant forms of HIV infection. As fuzeon has a half-life of 3.8h in patients, requiring frequent injection administrations, its therapeutic utility may be limited. In one embodiment, TP-ABC compositions comprising fuzeon have utility in the treatment of HIV.

[00211] “KAI-4169,” which refers to the peptide agonist of the human cell surface calcium-sensing receptor (CaSR) under development by KAI Pharma for the treatment of secondary hyperparathyroidism (SHPT) in kidney disease patients and bone disorder (CKD-MBD) patients. In one embodiment, TP-ABC compositions comprising KAI-4169 have utility in the treatment of secondary hyperparathyroidism and related diseases.

[00212] “Pasireotide,” which refers to the a somatostatin analog having the chemical name [(3S,6S,9S,12R,15S,18S,20R)-9-(4-aminobutyl)-3-benzyl-12-(1H-indol-3-ylmethyl)-2,5,8,11,14,17-hexaoxo-15-phenyl-6-[(4-phenylmethoxyphenyl)methyl]-1,4,7,10,13,16-hexazabicyclo[16.3.0]henicosan-20-yl] N-(2-aminoethyl)carbamate used for the treatment of Cushing's disease. Pasireotide is a multi-receptor somatostatin analogue with high binding affinity for somatostatin-R-subtypes R1, 2, 3 & 5 that suppresses growth hormone, IGF-1 and adrenocorticotrophic hormone secretion. In addition to treatment of Cushing's Disease, it is also considered for use in acromegaly, neuroendocrine disease, liver disease, symptomatic polycystic liver disease, neuroendocrine tumor, lymphangioliomyomatosis, congenital hyperinsulinism, recurrent or progressive meningioma, and other endocrine disorders. As a commercially-available form has a reported half-life of 12 to 17 h (Petersenn, S. et al. Tolerability and Dose Proportional Pharmacokinetics of Pasireotide Administered as a Single Dose or Two Divided Doses in Healthy Male Volunteers: A Single-Center, Open-Label, Ascending-Dose Study. *Clinical Therapeutics* (2012) 34:677-688), its therapeutic utility may be limited. In one embodiment, TP-ABC compositions comprising pasireotide have utility in the treatment of endocrine disorders.

[00213] “Irisin,” which refers to the cleavage product of the protein encoded by the FNDC5 gene having the amino acid sequence DSPSAPVNVTVRHLKANSVVSWDVLDEDEVVIGFAISQQKKDVRMLRFIQEVNTTTRSCALWDL EEDTEYIVHVQAISIQGQSPASEPVLFKTPREAEKMASKNKDEVMTMKE, and recombinant and synthetic versions and sequence variants thereof, having at least a portion of the biological activity of native irisin. Irisin mediates beneficial effects of muscular exercise, and induces browning of white adipose tissue by up-regulating UCP1 expression through activation of the nuclear receptor PPARA. Mildly increased irisin levels have been shown to result in increased energy expenditure, reduced body weight and improved diet-induced insulin resistance (Bostrom P, 2012, Nature, 481:463). In one embodiment, TP-ABC compositions comprising irisin have utility in treating obesity, diabetes, and metabolic disorders.

[00214] “TXA127” and “PanCyte,” which refer to analogs of angiotensin (1-7), with TXA127 having the amino acid sequence NRVYIHP. PanCyte is an cyclic analog linking the 4th and 7th residues with dAla and Ala, respectively, with the result that it is more resistant to degradation and has a longer half-life. The analogs bind to MAS receptor and stimulate early hematopoietic precursor cells in bone marrow, and also have vasodilation, anti-trophic, antifibrotic, natriuresis, anti-inflammatory, and anti-thrombotic effects. The compounds are considered for use in acceleration of platelet recovery following stem cell transplant for patients with hematological cancers, such as acute myelogenous leukemia (AML), myelodysplastic syndrome (MDS), acute lymphocytic leukemia (ALL), chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), Hodgkin's lymphoma (HL), non-Hodgkin's lymphoma (NHL), and multiple myeloma, and use in treating pulmonary fibrosis, acute lung injury, pulmonary arterial hypertension, and fibrosis of the kidney and liver. In one embodiment, TP-ABC compositions comprising TXA127 and PanCyte in the treatment of metabolic disorders and circulatory disease.

[00215] “Interleukin-7” and “IL-7,” which refers to the human interleukin (UniProt No. P13232 (26-177)) encoded by the *IL7* gene having the amino acid sequence DCDIEGKDGKQYESVLMVSIQQLDSMKEIGSNCLNNEFNFFKRHICDANKEGMFLFRAARKLR QFLKMNSTGDFDLHLLKVSEGTTILLNCTGQVKGRKPAALGEAQPTKSLEENKSLKEQKKLNDL CFLKRLQLQEIKTCWNKILMGTKEH, and recombinant and synthetic versions and sequence variants thereof, having at least a portion of the biological activity of native IL-7. IL-7 stimulates the differentiation of multipotent (e.g., pluripotent) hematopoietic stem cells into lymphoid progenitor cells, including expansion of CD4/CD8 T cells. IL-7 limits the production of suppressor regulatory T cells and T cell anergy through TGF- β antagonism, and supports production of central memory T cells. IL-7 is considered for use in treating lymphopenia in HIV, oncology, transplant, HBV and HCV infection, as well as treating minimal residual disease or advanced tumors, and may have roles in immune reconstitution or enhancement of immunotherapy. As the reported half-life of IL-7 in humans is approximately 10 h (Sportès, C. et al. Phase I Study of Recombinant Human Interleukin-7 Administration in Subjects with Refractory Malignancy. Clin Cancer Res 2010;16:727-735), its therapeutic utility in

unmodified form may be limited. In one embodiment, TP-ABC compositions comprising IL-7 have utility in the treatment of immunological conditions and diseases, including infectious diseases.

[00216] “Fibroblast growth factor 18” or “FGF-18,” which refers to the human protein (UniProt No. O76093(28-207)) encoded by the *FGF18* gene, having the amino acid sequence EENVDFRIHVENQTRARDDVSRKQLRLYQLYSRTSGKHIQVLGRRISARGEDGDKYAQLLVETD TFGSQVRIKGGKETEFYLCMNRKKGKLVGKPDGTSKECVFIEKVLENNYTALMSAKYSGWYVVGFT KKGRPRKGPKTRENQQDVHFMKRYPKGQPELQKPFKYTTVTKRSSRRIRPHTPA and recombinant and synthetic versions and sequence variants thereof, having at least a portion of the biological activity of native FGF-18. FGF-18 is a protein member of the fibroblast growth factor (FGF) family. FGF family members possess broad mitogenic and cell survival activities, and are involved in a variety of biological processes, including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth, and invasion. It has been shown *in vitro* that this protein is able to induce neurite outgrowth in PC12 cells. FGF-18 stimulates the proliferation of chondrocyte and osteoblasts (cells that produce and maintain bone and cartilage), and its use is considered for the repair and generation of the cartilage, for example in the knee joints (Ellsworth JL. Fibroblast growth factor-18 is a trophic factor for mature chondrocytes and their progenitors. Osteoarthritis Cartilage (2002) 10:308-320). In one embodiment, TP-ABC compositions comprising FGF-18 are useful in the treatment of bone and tissue diseases and conditions.

[00217] “Alpha-melanocyte stimulating hormone” or “ α -MSH” refers to the 13-amino acid peptide generated as a proteolytic cleavage product from ACTH (1-13), which is in turn a cleavage product of proopiomelanocortin (POMC), having the sequence N-Ac-SYSMGFRWGLPV, and synthetic versions and sequence variants thereof, having at least a portion of the biological activity of native α -MSH. Alpha-MSH is a non-selective agonist of the melanocortin receptors MC1, MC3, MC4 & MC5 but not MC2. Alpha-MSH stimulates melanocytes to produce and release melanin which has a photo-protective effect; it signals the brain, which has effects on appetite and sexual arousal. It is considered for use in treating erythropoietic protoporphyria (EPP, intolerant to sun), nonsegmental vitilligo (skin discoloration), actinic keratosis (AK, solar keratosis, precursor to skin cancer), polymorphous light eruption (PLE/PMLE), post-surgery kidney damage, erectile dysfunction, and sexual dysfunction. Its half-life has been reported to be seconds, limiting its therapeutic utility in unmodified form. In one embodiment, TP-ABC compositions comprising alpha-melanocyte stimulating hormone are useful in the treatment of erythropoietic protoporphyria (EPP, intolerant to sun), nonsegmental vitilligo (skin discoloration), actinic keratosis (AK, solar keratosis, precursor to skin cancer), polymorphous light eruption (PLE/PMLE), post-surgery kidney damage, erectile dysfunction, and sexual dysfunction.

[00218] “Endostatin,” which refers to the naturally-occurring 20-kDa C-terminal fragment derived from type XVIII collagen (UniProt. No. P39060(1572-1754)) having the amino acid sequence HSHRDFQPVLHLVALNSPLSGGMRGIRGADFQCFQQAQARAVGLAGTFRAFLSSRLQDLYSIVRRA DRAAVPIVNLKDELLFPSWEALFSGSEGPLKPGARIFSFDDGKDVLRHPTWPQKSVWHGSDPNGR RLTESYCETWRTEAPSATGQASSLLGGRLLGQSAASCHHAYIVLCIENSFMTASK, and

recombinant and synthetic versions and sequence variants thereof, having at least a portion of the biological activity of native endostatin. Endostatin is an angiogenesis inhibitor and may interfere with the pro-angiogenic action of growth factors such as basic fibroblast growth factor (bFGF/FGF-2) and VEGF. It is considered for use in certain cancers. Its half-life is 13 h (Thomas, JP et al. Phase I Pharmacokinetic and Pharmacodynamic Study of Recombinant Human Endostatin in Patients With Advanced Solid Tumors. *J. Clin. Oncol.* (2003) 21:223-231), which may limit its therapeutic utility in unmodified form. In one embodiment, TP-ABC compositions comprising endostatin are useful in the treatment of cancer.

[00219] “Humanin,” which refers to the peptide (UniProt No. Q8IVG9(1-24)) encoded by the *MT-RNR2* gene, having the amino acid sequence MAPRGFSCLLLLTSEIDLPKRRA, and recombinant and synthetic versions and sequence variants thereof, having at least a portion of the biological activity of native humanin. Humanin has a role in neuro-protection against cell death associated with Alzheimer’s disease (AD), AD-specific insults, prion induced apoptosis, and chemically induced neuronal damage (Hashimoto, Y, A rescue factor abolishing neuronal cell death by a wide spectrum of familial Alzheimer’s disease genes and A β . *PNAS* (2001) 98:6336-6341). More recently, humanin was found to help improve insulin action and lower blood glucose levels (Muzumdar RH, Humanin: A Novel Central Regulator of Peripheral Insulin Action. *PLoS One* (2009) 4:e6334). In one embodiment, TP-ABC compositions comprising humanin are useful in treating Alzheimer’s disease, diabetes, vascular diseases, and cardiovascular diseases.

[00220] “Glucagon,” which refers to the human glucose regulating peptide having the amino acid sequence HSQGTFTSDYSKYLDSRRAQDFVQWLMNT, and recombinant and synthetic versions and sequence variants thereof, having at least a portion of the biological activity of native glucagon. The term “glucagon” as used herein also includes peptide mimetics of glucagon. Native glucagon is produced by the pancreas, released when blood glucose levels start to fall too low, causing the liver to convert stored glycogen into glucose and release it into the bloodstream. While the action of glucagon is opposite that of insulin, which signals the body’s cells to take in glucose from the blood, glucagon also stimulates the release of insulin, so that newly-available glucose in the bloodstream can be taken up and used by insulin-dependent tissues. Glucagon-containing polypeptides of the disclosure may find particular use in increasing blood glucose levels in individuals with extant hepatic glycogen stores and maintaining glucose homeostasis in diabetes. Glucagon has been cloned, as disclosed in U.S. Patent No. 4,826,763. The half-life of glucagon is very short, which can limit its therapeutic utility. In one embodiment, TP-ABC compositions comprising glucagon are useful in the treatment of hypoglycemia and other glucose-related disorders.

[00221] “Glucagon-like protein-1” or “GLP-1” refers to human glucagon like peptide-1 and sequence variants thereof having at least a portion of the biological activity of native GLP-1. The term “GLP-1” includes human GLP-1(1-37) having the amino acid sequence HDEFERHAEGTFTSDVSSTLEGQAALFIWLKGRG, GLP-1(7-37), and GLP-1(7-36)amide. GLP-1 stimulates insulin secretion, usually during periods of hyperglycemia. The safety of GLP-1

compared to insulin is enhanced by this property and by the observation that the amount of insulin secreted is proportional to the magnitude of the hyperglycemia. The biological half-life of GLP-1(7-37)OH has been reported to be 3 to 5 minutes (U.S. Patent No. 5,118,666), which can limit its therapeutic utility. In one embodiment, TP-ABC compositions comprising GLP-1 polypeptides find particular use in the treatment of diabetes and insulin-resistance disorders for glucose regulation. GLP-1 has been cloned and derivatives prepared, as described in U.S. Patent No. 5,118,666.

[00222] “Glucagon-like protein-2” or “GLP-2,” which, collectively herein, refers to human glucagon like peptide-2 having the sequence HADGSFSDEMNTILDNLAARDFINWLIQTKITD, species homologs of human GLP-2, and non-natural sequence variants having at least a portion of the biological activity of mature GLP-2 including variants such as, but not limited to, a variant with glycine substituted for alanine at position 2 of the mature sequence resulting in HGDGSFSDEMNTILDNLAARDFINWLIQTKITD (“2G”) as well as Val, Glu, Lys, Arg, Leu or Ile substituted for alanine at position 2. GLP-2 or sequence variants have been isolated, synthesized, characterized, or cloned, as described in U.S. Patent No. 5,789,379, U.S. Patent No. 5,834,428, U.S. Patent No. 5,990,077, U.S. Patent No. 5,994,500, U.S. Patent No. 6,184,201, U.S. Patent No. 7,186,683, U.S. Patent No. 7,563,770, U.S. Patent Application Publication No. 20020025933, and U.S. Patent Application Publication No. 20030162703. In one embodiment, TP-ABC compositions comprising GLP2 are useful in the treatment of certain gastrointestinal diseases and disorders, such as short-bowel syndrome, irritable bowel syndrome, Crohn's disease, and other diseases of the intestines.

[00223] “Factor XIII A chain”, “FXIIIa” or “F13A” refers to the coagulation protein (UniProt No. P00488(2-732)) having the amino acid sequence SETSRTAFGRRRAVPPNNSNAAEDDLPTVELQGVVPRGVNLQEFLNVTSVHLFKERWDTNKVD HHTDKYENNKLVRRGQSFYVQIDFSRPYDPRRDLFRVEYVIGRYPQENKGTYPVPIVSELQSGK WGAKIVMREDRSVRLSIQSSPKCIVGKFRMYVAVWTPYGVLRTSRNPETDTYILFNPWCEDDAV YLDNEKEREEYVLNDIGVIFYGEVNDIKTRSWSYGQFEDGILDTCLYVMDRAQMDLSGRGNPIK VSRVGSAMVNAKDDEGVLVGSDNIYAYGVPPSAWTGSVDILLEYSSENVPVRYGQCWVFAG VFNTFLRCLGIPARIVTNYFSAHDNDANLQMDIFLEEDGNVNSKLTKDSVWNYHCWNEAWMTR PDLPGVFGGWQAVDSTPQENS DGM YRCGPASVQA IKGHVCFQFDAPFVFAEVNSDLIYITAKK DGTHVVENVDATHIGKLIVTKQIGGDGMMDITDTYKFQEGQEEERLALETALMYGAKKPLNTE GVMKSRSNVDMDFEVENAVLGKDFKLSITFRNNSHNRYTITAYLSANITFYTGVPKAEFKKETFD VTLEPLSFKKEAVLIQAGEYMGQLLEQASLHFFVTARINETRDVLAKQKSTVLTPEIIKVRGTQV VGSDMTVTVQFTNPLKETLRNVWVHLDGPGVTRPMKKMFREIRPNSTVQWEEVCRPWVSGHR KLIASMSSDSL RHVYGELDVQIQRRPSM, and recombinant and synthetic versions and sequence variants thereof, having at least a portion of the biological activity of native FXIIIa. Factor XIII is the last enzyme in the coagulation cascade and is involved in cross-linking fibrin molecules to each other in a newly formed blood clot. By forming intermolecular covalent bonds between fibrin monomers and by cross-linking alpha-2 antiplasmin, fibrinogen, fibronectin, collagen, and other proteins enhance the

mechanical strength of the fibrin clot, protect from proteolytic degradation, and provide stability to the extracellular matrix. Plasma FXIII circulates as a heterotetramer composed of 2 A subunits and 2 B subunits noncovalently linked together and bound to fibrinogen. The B subunit, which appears to stabilize the structure of the A subunit and to protect the A subunit from proteolysis, is normally present in excess in plasma as free FXIII-B subunit. Most patients with FXIII deficiency have mutations in the FXIII-A subunit; few cases of patients with FXIII-B subunit mutations have been reported (Mikkola, H, 1996, *Semin Thromb Hemost*, 22:393; Ichinose A, 1996, *Semin Thromb Hemost*, 22:385). FXIII is used or is considered for use in treating hemophilia and related coagulopathies, congenital FXIII deficiency, and acquired FXIII deficiency due to chronic liver disease, inflammatory bowel disease, and post-surgery bleeding. In one embodiment, TP-ABC compositions comprising factor VIII are useful in the treatment of factor VIII deficiencies, hemophilia, and bleeding disorders.

[00224] “Factor X” or “FX,” which refers to the coagulation protein (UniProt No. P00742(2-488)) having the amino acid sequence
 GRPLHLVLLSASLAGLLLLGESLFIRREQANNILARVTRANSFLEEMKKGHLERECMEETCSYEE
 AREVFEDSDKTNEFWNKYKDGQDQETSPCQNQGKCKDGLGEYTCTCLEGFEGKNCELFRKLC
 SLDNGDCDQFCHEEQNSVVCSCARGYTLADNGKACIPTGPYPCGKQTLERRKRSVAQATSSSGE
 APDSITWKPYDAADLDPTENPFDLLDFNQTPERGDNNLTRIVGGQECKDGECPWQALLINEEN
 EGFCGGTILSEFYILTAHCLYQAKRFKVRVGDRNTEQEEGGEAVHEVEVVIKHNRFTEKETYDF
 DIAVLRCLKTPITFRMNVAPACLPERDWAESTLMTQKTGIVSGFGRTHEKGRQSTRCLKMLEVPYV
 DRNSCKLSSSFITQNMFCAGYDTKQEDACQDSSGGPHVTRFKDTYFVTGIVSWGEGCARKGKY
 GIYTKVTAFLKWIDRSMKTRGLPKAKSHAPEVITSSPLK, and recombinant and synthetic versions and sequence variants thereof, having at least a portion of the biological activity of native FX. Factor X is activated into factor Xa by both factor IX (with its cofactor, factor VIII, to make a complex known as intrinsic Xase) and factor VII with its cofactor, tissue factor (to make a complex known as extrinsic Xase). Factor X is the first member of the final common (or thrombin) pathway. Factor X can be used to treat factor X deficiency, hemophilia A & B using bypass strategies due to FVIII and FIX patients developing inhibitory antibodies to FVIII and FIX replacement therapies), emergency treatment of patients with hemorrhages due to oral anticoagulants overdose or unknown causes of critical bleeding, and patients who develop acquired FX deficiency caused by lack of vitamin K, amyloidosis, severe liver disease & use of anticoagulants (e.g. warfarin). While the half-life of mature factor X is 40-45h, the plasma half-life of activated factor X (Fxa) is <1-2 min ((Bunce MW, 2008, *Blood*, 117:290), which can limit its therapeutic utility in unmodified form. In one embodiment, TP-ABC compositions comprising factor X have utility in the treatment of factor VIII and factor IX deficiencies, hemophilia and bleeding disorders.

8. Compositions of albumin binding conjugates with linked therapeutic drugs (TD-ABC)

[00225] In another aspect, the disclosure provides compositions of therapeutic drugs linked to albumin binding conjugates (hereinafter “TD-ABC”). It is contemplated that the subject conjugates and

compositions are designed such that they have enhanced properties or combinations of properties compared both to unmodified drugs or to drugs linked to carboxylic acids or polyethyleneglycol (PEG), including, but not limited to enhanced binding to human serum albumin, increased terminal half-life, tunable terminal half-life, enhanced solubility, lack of aggregation during recovery and in final product, and high thermal stability.

[00226] Non-limiting examples of functional classes of pharmacologically active payload agents for use in linking to an albumin binding conjugate of the disclosure may be any one or more of the following: hypnotics and sedatives, psychic energizers, tranquilizers, respiratory drugs, anticonvulsants, muscle relaxants, antiparkinson agents (dopamine antagonists), analgesics, anti-inflammatories, antianxiety drugs (anxiolytics), appetite suppressants, antimigraine agents, muscle contractants, anti-infectives (antibiotics, antivirals, antifungals, vaccines), antiarthritics, antimalarials, antiemetics, anepileptics, bronchodilators, anti-cancer agents, antithrombotic agents, antihypertensives, cardiovascular drugs, antiarrhythmics, antioxidants, anti-asthma agents, hormonal agents (including contraceptives), sympathomimetics, diuretics, lipid regulating agents, antiandrogenic agents, antiparasitics, anticoagulants, neoplastics, antineoplastics, hypoglycemics, nutritional agents and supplements, growth supplements, antienteritis agents, diagnostic agents, contrasting agents, and radioactive imaging agents.

[00227] For the TD-ABC compositions, it is contemplated that a drug can be a pharmacologically active agent that possesses a suitably reactive functional group, including, but not limited to a native amino group, a sulfhydryl group, a carboxyl group, an aldehyde group, a ketone group, an alkene group, an alkyne group, an azide group, an alcohol group, a heterocycle, or, alternatively, is modified to contain at least one of the foregoing reactive groups or a suitable cross-linker for coupling to an XTEN of the subject conjugates of the disclosure using a conjugation method described herein or are otherwise known to be useful in the art for conjugating such reactive groups. Specific functional moieties and their reactivities are described in Organic Chemistry, 2nd Ed. Thomas Sorrell, University Science Books, Herndon, VA (2005). Further, it will be understood that any drug containing a reactive group or that is modified to contain a suitable cross-linker can also contain a single atom residue as the point of attachment after conjugation to which the XTEN of the albumin binding conjugate reactant is linked.

[00228] In some embodiments, the drug for conjugation to the XTEN component of the TD-ABC is one or more agents described herein or selected from the drugs of Table 5, or a pharmaceutically acceptable salt, acid or derivative or agonist thereof. In one embodiment, the drug is derivatized to introduce a reactive group for conjugation to the XTEN. In another embodiment, the drug is a radionuclides such as ¹¹¹In and ⁹⁰Y, Lu¹⁷⁷, Bismuth²¹³, Californium²⁵², Iridium¹⁹² and Tungsten¹⁸⁸/Rhenium¹⁸⁸.

Table 5: Drugs for conjugation to albumin binding conjugate compositions

Drugs
Erlotinib; Bortezomib; Atrretinoin, Allopurinol, arsenic trioxide, clofarabine, dexrazoxane, Fulvestrant; Sunit (SUI1248), Letrozole; Imatinib mesylate; PTK787/ZK 222584; Bendamustine; Romidepsin; Pralatrexate; Cabazitaxel (XRP-6258); Everolimus (RAD-001); Abirateron; Oxaliplatin; 5-FU (5-fluorouracil), leucovorin, rapamycin; lapatinib; lonafarnib; sorafenib; gefitinib; cyclophosphamide; busulfan; improsulfan; piposulfan; benzodopa; carboquone; meturedopa; uredopa; altretamine; triethylenemelamine; triethylenephosphoramide; triethylenethiophosphoramide; trimethylmelamine; bullatacin; bullatacinone; camptothecin; topotecan; bryostatin; calystatin; CC-1065; adozelesin; calicheamicin; auristatin; monomethyl auristatin E (MMAE); monomethyl auristatin F (MMAF); (valine-citrulline-PAB)-monomethyl auristatin E; (valine-citrulline-PAB)-monomethyl auristatin F; carzelesin; bizelesin; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin; eleutherobin; pancratistatin; sarcodictyin; spongistatin; chlorambucil; chlornaphazine; cholophosphamide; estramustine; ifosfamide; mechlorethamine; mechlorethamine oxide hydrochloride; melphalan; novembichin; phenesterine; prednimustine; trofosfamide; uracil mustard; carmustine; chlorozotocin; fotemustine; lomustine; nimustine; ranimustine; calicheamicin; dynemicin; dynemicin A; clodronate; esperamicin; neocarzinostatin chromophore; aclacinomysins, actinomycin; anthramycin; azaserine; bleomycin; cactinomycin; carabacin; carminomycin; carzinophilin; chromomycin; dactinomycin; daunorubicin; detorubicin; 6-diazo-5-oxo-L-norleucine; doxorubicin; morpholino-doxorubicin; lenalidomide, cyanomorpholino-doxorubicin; (valine-citrulline-PAB)-doxorubicin; 2-pyrrolino-doxorubicin and deoxydoxorubicin; epirubicin; esorubicin; idarubicin; marcellomycin; mitomycin C; mycophenolic acid; nogalamycin; olivomycin; peplomycin; potfiromycin; puromycin; quelamycin; rodorubicin; streptonigrin; streptozocin; tubercidin; ubenimex; zinostatin; zorubicin; 5-fluorouracil (5-FU); fdenopterin; methotrexate; pteropterin; trimetrexate; fludarabine; 6-mercaptopurine; thiamiprine; ancitabine; azacitidine; 6-azauridine; carmofur; cytarabine; dideoxyuridine; doxifluridine; enocitabine; meclorothamine, floxuridine; calusterone; dromostanolone propionate; epitostanol; mepitiostane; testolactone; aminoglutethimide; trilostane; frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansine; ansamitocins; mitoguazone; mopidanmol; nitracrine; pentostatin; phenamet; pirarubicin; losoxantrone; methoxsalen, podophyllinic acid; 2-ethylhydrazide; procarbazine; razoxane; rhizoxin; ribavirin; zidovudine; acyclovir; gangcyclovir; vidarabine; idoxuridine; trifluridine; foscarnet; amantadine; rimantadine; saquinavir; indinavir; ritonavir; alpha-interferons and other interferons; AZT; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; T-2 toxin; verracurin A; roridin A; anguidine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids; epacitaxel; paclitaxel; docetaxel; doxetaxel; irinotecan; pemetrexed; chlorambucil; gemcitabine; 6-thioguanine; cisplatin; carboplatin; vinblastine; platinum; etoposide, VP-16; ifosfamide; mitoxantrone; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; capecitabine; mesna, lidocaine; bupivacaine; memantine; quinacrine, donepezil; rivastigmine; galantamine; morphine; oxycodone; hydromorphone; oxymorphone; metopon; apomorphine; normorphine; etorphine; buprenorphine; meperidine; lopermide; anileridine; ethoheptazine; piminidine; betaprodine; diphenoxylate; fentanyl; sufentanyl; alfentanil; remifentanyl; levorphanol; dextromethorphan; phenazocine; pentazocine; cyclazocine; methadone; isomethadone; propoxyphene; naloxone; naltrexone; treprostinil; N-methylnaloxone; 6-amino-14-hydroxy-17-allylnordesomorphine; naltrendol, N-methylnaloxone; nalbuphine; butorphanol; cyclazocine; pentazocine; nalmephe; naltrindole; nor-binaltorphimine; oxilorphan; 6-amino-6-desoxo-naloxone; pentazocine; levallorphanmethylnaloxone; buprenorphine; cyclorphan; levalorphan; cyclosporine; cyclosporine A; mycophenylate mofetil; sirolimus; tacrolimus; prednisone; azathioprine; cyclophosphamide; prednisone; aminocaproic acid; chloroquine; hydroxychloroquine; dexamethasone; chlorambucil; danazol; bromocriptine; Nilotinib (AMN107); Nelarabine, amifostine, amiodarone, aminocaproic acid, aminohippurate sodium, aminoglutethimide, aminolevulinic acid, aminosalicic acid, amsacrine, anagrelide, anastrozole, asparaginase, anthracyclines, bexarotene, bicalutamide, bleomycin, buserelin, busulfan, cabergoline, capecitabine, carboplatin, carmustine, chlorambucil, cilastatin sodium, cisplatin, cladribine, clodronate, cyclophosphamide, cyproterone, cytarabine, camptothecins, 13-cis retinoic acid, all trans retinoic acid; dacarbazine, dactinomycin, daunorubicin, deferroxamine, dexamethasone, diclofenac, diethylstilbestrol, docetaxel, doxorubicin, epirubicin, estramustine, etoposide, exemestane, fexofenadine, fludarabine, fludrocortisone, fluorouracil, fluoxymesterone, flutamide, gemcitabine, epinephrine, L-Dopa, hydroxyurea, idarubicin, ifosfamide, imatinib, irinotecan, itraconazole, goserelin acetate, letrozole, leucovorin, levamisole, lisinopril, lovathyroxine sodium, mechlorethamine, medroxyprogesterone, megestrol, melphalan, metaraminol bitartrate, metoclopramide, mexiletine, mitomycin, mitotane, naloxone, nicotine, nilutamide, octreotide, pamidronate, pilcamycin, porfimer, prednisone, prochlorperazine, ondansetron, raltitrexed, sirolimus, tacrolimus, tamoxifen, temozolomide, testosterone, tetrahydrocannabinol, thalidomide, thioguanine, topotecan, tretinoin, valrubicin, vincristine, vindesine, vinorelbine, dolasetron, granisetron; formoterol, fluticasone, leuprolide,

Drugs

midazolam, alprazolam, amphotericin B, podophylotoxins, nucleoside antivirals, aroyl hydrazones, sumatriptan; macrolides such as erythromycin, oleandomycin, troleandomycin, roxithromycin, clarithromycin, davercin, azithromycin, flurithromycin, dirithromycin, josamycin, spiramycin, midecamycin, leucomycin, miocamycin, rokitamycin, andazithromycin, and swinolide A; fluoroquinolones such as ciprofloxacin, ofloxacin, levofloxacin, trovafloxacin, alatrofloxacin, moxifloxacin, norfloxacin, enoxacin, grepafloxacin, sunitinib, gatifloxacin, lomefloxacin, sparfloxacin, temafloxacin, pefloxacin, amifloxacin, fleroxacin, tosufloxacin, prulifloxacin, irloxacin, pazufloxacin, clinafloxacin, and sitafloxacin; aminoglycosides such as gentamicin, netilmicin, paramycin, tobramycin, amikacin, kanamycin, neomycin, and streptomycin, vancomycin, teicoplanin, rampolanin, mideplanin, colistin, daptomycin, gramicidin, colistimethate; polymyxins such as polymixin B, capreomycin, bacitracin, penems; penicillins including penicillinase-sensitive agents like penicillin G, penicillin V; penicillinase-resistant agents like methicillin, oxacillin, cloxacillin, dicloxacillin, floxacillin, nafcillin; gram negative microorganism active agents like ampicillin, amoxicillin, and hetacillin, cillin, and galampicillin; antipseudomonal penicillins like carbenicillin, ticarcillin, azlocillin, mezlocillin, and piperacillin; cephalosporins like cefpodoxime, cefprozil, ceftbuten, ceftizoxime, ceftriaxone, cephalothin, cephalirin, cephalixin, cephradine, cefoxitin, cefamandole, cefazolin, cephaloridine, cefaclor, cefadroxil, cephaloglycin, cefuroxime, ceforanide, cefotaxime, cefatrizine, cephaetrile, cefepime, cefixime, cefonicid, cefoperazone, cefotetan, cefinazidole, ceftazidime, loracarbef, and moxalactam, monobactams like aztreonam; and carbapenems such as imipenem, meropenem, pentamidine isethiouate, albuterol sulfate, lidocaine, metaproterenol sulfate, beclomethasone dipropionate, triamcinolone acetamide, budesonide acetamide, fluticasone, ipratropium bromide, flunisolide, cromolyn sodium, and ergotamine tartrate; taxanes such as paclitaxel; SN-38, tyrphostins, 20-epi-1,25 dihydroxy vitaminD3, 5-ethynyluracil, abiraterone, Acivicin, Aclarubicin, Acodazole Hydrochloride, AcrQnine, acylfulvene, adecypenol, adramycin, Aldesleukin, ALL-TK antagonists, ambamustine, amidox, Ambomycin, Ametantrone Acetate, amrubicin, andrographolide, angiogenesis inhibitors, antagonist D, antagonist G, antarelix, anti-androgen, anti-dorselizing morphogenetic protein-1, anti-estrogen, antimetabolites, anti-neoplaston, anti-oestrogens, anti-sense oligonucleotides, anti-venom, aphidicolinglycinate, apoptosis gene modulators, apoptosis regulators, apurinic acid, ara-CDP-DL-PTBA, arginine deaminase, Asperlin, asulacrine, atamestane, atrimustine, atrisacrine, axinastatin 1, axinastatin 2, axinastatin 3, azasetron, azatoxin, azatyrosine, Aztepa:Azotomycin, baccatin III derivatives, balanol, Batimastat, BCR/ABLantagonists, benzochlorins, Benzodepa, benzoylstauroporine, stauroporine, beta-alethine, betaclamycin B, betalactamderivatives, betamethasone, betulinic acid, bFGFinhibitor, Bicalutamide, Bisantrone Hydrochloride, bisaziridinylspermine, bisnafide, Bisnafide Dimesylate, bistrateneA, Bleomycin Sulfate, breflate, Brequinar Sodium, bromine epiandrosterone, Bropirimine, budotitane, buthionine sulfoximine, calcipotriol, calphostin C, camptothecin derivatives, canarypox IL-2, capedtabine, Caracemide, Carbetimer, carboxamide-amino-triazole:carboxyamidotriazole, CaRestM3, CARN700, cartilage derived inhibitor, Carubicin Hydrochloride, casein kinase inhibitors(ICOS), castanospermine, cecropin B, Cedefingol, cetorelix, chlorins, chloroquinoxaline sulfonamide, chlorotrianisene, cicaprost, Cirolemycin, cis-porphyrin, clomifene analogues, clotrimazole, collismycin A, collismycin B, combretastatin A4, combretastatin analogue, conagenin, crambescidin 816, crisnatol, Crisnatol Mesylate, cryptophycin 8, cryptophycin A derivatives, curacin A, cyclopentantraquinones, cycloplatam, cypemycin, cytarabineocfosfate, cytolyticfactor, cytostatin, cytotoxic agents, Daunorubicin Hydrochloride, Decitabine, dehydridemnin B, deslorelin, dexifosfamide, Dexormaplatin, dexrmzoxane, dextrapamil, Dezaguanine, Dezaguanine Mesylate, DHEA, diaziquorie, dicarbazine, didemnin 13, didox, diethylnorspermine, dihydro-5-azacytidine: dihydrotaxol, 9-, dioxamycin, diphenylspiromustine, docosanol, Doxorubicin Hydrochloride, Droloxifene, Droloxifene Citrate, Dromostanolone Propionate, dronabinol, Duazomycin, duocannycin SA, ebselen, ecorustine, edelfosine, edrocolomab, Eflornithine Hydrochloride, eflornithine, elemene, Elsamitrucin, emitefur, Enloplatin, Enpromate, epiandrosterone, Epiripidine, Epirubicin Hydrochloride, epristeride, Erbulozole, erythrocyte gene therapy, Esorubicin Hydrochloride, estramustine analogue, estrogen agonists, estrogen antagonists, Etanidazole, ethinyloestradiol, Ethiodized Oil I131, Etoposide Phosphate, Etoprine, fadrozole, Fadrozole Hydrochloride, Fazarabine, fazarabine, fenretinide, Fenretinide:Floxuridine, finasteride, flavopiridol, flezelastine, Fludarabine Phosphate, fluorodaunorubicin hydrochloride, Flurocitabine, forfenimex, formestane, Fosquidone, fostriecin, Fostriecin Sodium, gadoliniumtexaphyrin, galocitabine, ganirelix, gelatinase inhibitors:gemicitabine, Gemicitabine Hydrochloride, glutathione inhibitors, Gold Au198, goserelin, hepsulfam, heregulin, hexamethylenebisacetamide, hexamethylmelamine, human chorionic gonadotrophin:monophosphoryl lipid A + myobacterium cell walls k, hypericin, ibandronic acid, Idarubicin Hydrochloride, idoxifene, idramantone, Ilmofofine, ilomastat, imidazoacridones, imiquimod, immuno stimulant peptides:insulin-like growth factor-1 receptor inhibitor, interferon agonists, Interferon Alfa-2a, Interferon Alfa-2b, Interferon Alfa-n1, Interferon Alfa-n3, Interferon Beta-1a, Interferon Gamma-1b, iobenguane, iododoxorubicin, ipomeanol, Iproplatin, Irinotecan Hydrochloride, iroplact, irsogladine, isobengazole, isohomohalicondrin B, itasetron, jasplakinolide, kahalalide F, lamellarin-Nriacetate, lanreotide, Lanreotide Acetate, leinamycin, lenograstim, lentinan sulfate, leptolstatin, leukemia inhibiting factor, leukocyte alpha interferon, Leuprolide Acetate, leuprolide+estrogen+progesterone, leuprorelin, liarozole, Liarozole Hydrochloride, linear polyamine analogue, lipophilic disaccharide peptide,

Drugs

lipophilic platinum compounds, lissoclinamide7, lobaplatin, lombricine, lometrexol, Lometrexol Sodium, lonidamine, Losoxantrone Hydrochloride, lovastatin, loxoribine, luprolide, lurtotecan, lutetlumtexaphyrin, lysofylline, lyticpeptides, maitansine, mannostatin A, marimastat, Masoprocol, maspin, matrilysin inhibitors, matrix metallo proteinase inhibitors, mecaptopurine, Mechlorethamine Hydrochloride, Megestrol Acetate, Melengestrol Acetate, Menogaril, merbarone, meterelin, methioninase, methlorethamine, Metoprime, Meturedopa, microalgal, mifepristone, MIFinhibitor, miltefosine, mirimostim, mismatched double stranded RNA, Mitindomide, Mitocarcin, Mitocromin, Mitogillin, mitoguazone, Mitomalcin, mitomycin analogues, mitonafide, Mitosper, mitotoxin fibroblast growth factor-saporin, mofarotene, molgramostim, monoclonal antibody, multiple drug resistance gene inhibitor, multiple tumor suppressor1-based therapy, mustard anticancer agent, mutamycin, mycaperoxide B, mycobacterial cell wall extract, Mycobacterium bovis, myriaporone, N-acetyldinaline:N-substitutedbenzamides, nafarelin, nagrestip, naloxone+pentazocine, napavin, naphterpin, nartograstim, nedaplatin, nemorubicin, neridronic acid, neutral endo peptidase, nisamycin, nitric oxide modulators, nitrogen mustard derivatives, nitroxide antioxidant, nitrullin, Nocodazole:Nogalamycin, O6-benzylguanine, oestradiol, okicenone, oligonucleotides, onapristone:ondansetron, ondansetron, oracin, oral cytokine inducer, Ormaplatin, osaterone, oxaunomycin, Oxisuran, palauamine, palmitoylrizoxin, pamidronic acid, panaxytriol, panomifene, parabactin, pazelliptine:pegaspargase, Pegaspargase, peldesine:pentosanpolysulfatesodium, Peliomycin, Pentamustine, pentozole, Peplomycin Sulfate, perflubron, Perfosfamide, perillyl alcohol, phenazinomycin, phenylacetate, phosphatase inhibitors, picibanil, pilocarpine hydrochloride, piritrexim, Piroxantrone Hydrochloride, placetin A, placetin B, plasminogen activator inhibitor, platinum complex, platinum compounds, platinum-triamine complex, Plicamycin, Plomestane, Porfimer Sodium, Procarbazine Hydrochloride, propylbis-acridone, prostaglandin J2, prostatic carcinoma, proteasome inhibitors, protein A-based immune modulator, protein kinase C inhibitor, protein tyrosine phosphatase inhibitors, purine nucleoside phosphorylase inhibitors, Puromycin Hydrochloride, purpurins, Pyrazofurin, pyrazoloacridine, pyridoxylated hemoglobin polyoxyethylene conjugate, raf antagonists, ramosetron, rasfarnesyl protein transferase inhibitors, ras inhibitors:ras-GAP inhibitor, retelliptine demethylated, rhenium Re186 etidronate, Riboprime, ribozymes, RII retinamide, RM-131 (ghrelin agonist), RM-493 (agonis for melanocortin type 4 receptor), Rogletimide, rohitumine, romurtide, roquinimex, rubiginone B1, ruboxyl, Safingol Hydrochloride, Safingol, saintopin: Sar CNU, sarcophytol A, sargramostim, Sdi1 mimetics, Semustine, senescence derived inhibitor 1, sense oligonucleotides, signal transduction inhibitors, signal transduction modulators, Simtrazene, single chain antigen binding protein, sobuzoxane, sodium borocaptate, sodium phenylacetate, solverol, somatomedin binding protein, sonermin, Sparfosate Sodium, sparfosic acid, Sparsomycin, Spirogermanium Hydrochloride, Spiromustine, spiromustine:splenopentin, Spiroplatin, splcamycin D, squalamine, stem cell inhibitor, stem-cell division inhibitors, stipiamide, stromelysin inhibitors, Strontium Chloride Sr89, sulfmosine, Sulofenur, superactive vasoactive intestinal peptide antagonist, suradista, suramin, swainsonine, synthetic glycosamino glycans, Talisomycin, tallimustine, tamoxifen methiodide, taumustine, tazarotene, Tecogalan Sodium, Tegafur, tellurapyrylium, telnoporfir, telomerase inhibitors, Teloxantrone Hydrochloride, Temoporfir, ternoazolomide, Teroxirone, tetrachlorodecaoxide, tetrazomine, textotere, thallblastine, thiocoraline, thrombopoietin mimetic, thymalfasin, thymopoietin receptor agonist, thymotrinan, thyroid stimulating hormone, Tiazofurin, tinethylotiopurpurin, Tirapazamine, titanocene dichloride, Topotecan Hydrochloride, topsentin, toremifene, Toremifene Citrate, totipotent stem cell factor, translation inhibitors, Trestolone Acetate, triacetyluridine, triciribine, Triciribine Phosphate:Trimetrexate, Trimetrexate Glucuronate, Triptorelin, triptorelin:tropisetron, tubulazole hydrochloride, turosteride, tyrosine kinase inhibitors, UBC inhibitors, urodepa, urogenital sinus-derived growth inhibitory factor, urokinase receptor antagonists, vaporeotide, variolin B, vector system, velaresol, venom, veramine, verdins, Verleporfin, verteporfir, Vinblastine Sulfate, vincristine sulfate, vindesine, Vindesine Sulfate, Vinepidine Sulfate, Vinglycinatate Sulfate, Vinleurosine Sulfate, vinorelbine tartrate, vinrosidine sulfate, vinxaltine, Vinzolidine Sulfate, vitaxin, Vorozole, zanoterone, Zeniplatin, zilascorb, zinostatin stimalamer, Zorubicin Hydrochloride, Bovine pancreatic RNase, Human pancreatic RNase, Mammalian pancreatic RNase, onconase, ranpirnase, pokeweed antiviral protein, rachelmycin, ricin-A chain, gelonin, everolimus, carfilzomib, tubulyisin, tubulyisin B, tubulyisin M, maytansinoid DM1, maytansinoid DM4, triptolide, SJG-136, apaziquone, irofulven, illudin S, tomaymycin, zoledronate

9. Compositions of albumin binding conjugates with linked nucleic acids (NA-ABC)

[00229] In another aspect, the disclosure provides compositions of nucleic acids linked to albumin binding conjugate (hereinafter “NA-ABC”). It is contemplated that the subject compositions are designed such that they have enhanced properties or combinations of properties compared both to unmodified nucleic acids or to nucleic acids linked directly to carboxylic acids or polyethyleneglycol (PEG), including, but not limited to enhanced binding to human serum albumin, increased terminal half-life,

tunable terminal half-life, enhanced solubility, lack of aggregation during recovery and in final product, and high thermal stability.

[00230] Examples of nucleic acids used in NA-ABC compositions as therapeutic agents include, but are not limited to, aptamers, antisense DNA, antisense RNA, small interfering RNA (siRNA), exon skipping oligonucleotides, RNA editing, microRNA therapeutic inhibitors (antimiR) and mimics (promiR), long non-coding RNA modulators and mRNA. NA-ABC compositions can be used, for example, in gene therapy.

10. Cross-linker reactants for conjugation

[00231] In another aspect, the disclosure relates to therapeutic protein or therapeutic drug conjugation to XTEN using a suitable cross-linker. In particular, the herein-described cross-linkers are useful for conjugation to the protein, drug and XTEN reactants bearing at least one thiol, amino, aminooxy, carboxyl, aldehyde, alcohol, azide, alkyne or any other reactive group available and suitable, as known in the art, for reaction between the components described herein.

[00232] In one embodiment, the disclosure provides TP-ABC or compositions thereof comprising an XTEN conjugated to a therapeutic protein by a cross-linker, wherein the cross-linker is selected from reactive homobifunctional or heterobifunctional cross-linkers. In another embodiment, the disclosure provides TD-ABC or compositions thereof comprising an XTEN conjugated to a therapeutic drug by a cross-linker, wherein the cross-linker is selected from reactive homobifunctional or heterobifunctional cross-linkers. In another embodiment, the disclosure provides albumin binding conjugates or compositions thereof comprising an XTEN conjugated to a therapeutic protein and also to a therapeutic drug by a cross-linker, wherein the cross-linker is selected from reactive homobifunctional or heterobifunctional cross-linkers. Cross-linking generally refers to a process of chemically linking two or more molecules by a covalent bond. The process is also called conjugation or bioconjugation with reference to its use with proteins and other biomolecules. For example, proteins can be modified to alter N- and C-termini, and amino acid side chains on proteins and peptides in order to block or expose reactive binding sites, inactivate functions, or change functional groups to create new targets for cross-linking.

[00233] Examples of cross-linkers useful for such conjugation of the therapeutic proteins or drugs to XTEN include, but are not limited to azidoacetic acid NHS ester, succinamidyl iodoacetic acid (SIA), and SPDP (succinimidyl 3-(2-pyridyldithio)propionate).

III). PHARMACEUTICAL COMPOSITIONS OF THE ALBUMIN BINDING CONJUGATES

[00234] In another aspect, the disclosure provides pharmaceutical compositions comprising TP-ABC and TD-ABC. In one embodiment, the pharmaceutical composition comprises the TP-ABC or TD-ABC and at least one pharmaceutically acceptable carrier. In another embodiment, the pharmaceutical composition comprises the TP-ABC or TD-ABC and optionally, suitable formulations of carrier, stabilizers and/or excipients. The pharmaceutical compositions of the present disclosure can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the

subject conjugate or composition is combined in admixture with a pharmaceutically acceptable carrier vehicle, such as aqueous solutions or buffers, pharmaceutically acceptable suspensions and emulsions. Examples of non-aqueous solvents include, but are not limited to, propyl ethylene glycol, polyethylene glycol and vegetable oils. Therapeutic formulations can be prepared for storage by mixing the active ingredient having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers, as described in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980), in the form of lyophilized formulations or aqueous solutions. In addition, the pharmaceutical compositions can also contain other pharmaceutically active compounds or a plurality of compositions of the disclosure.

[00235] The pharmaceutical compositions may be administered to a subject for therapy by any suitable route including parenteral (including subcutaneous, subcutaneous by infusion pump, intramuscular, intravenous and intradermal), intravitreal, and pulmonary. It will also be appreciated that the preferred route will vary with the condition and age of the recipient, and the disease being treated.

[00236] In preferred embodiments, the pharmaceutical composition is administered parenterally. In one embodiment, the composition may be supplied as a lyophilized powder to be reconstituted prior to administration. In another embodiment, the composition may also be supplied in a liquid form, which can be administered directly to a patient. In one embodiment, the pharmaceutical composition is supplied as a liquid in a pre-filled syringe for a single injection.

[00237] The conjugates and compositions of the disclosure may be formulated using a variety of excipients. Suitable excipients include microcrystalline cellulose (e.g. Avicel PH102, Avicel PH101), polymethacrylate, poly(ethyl acrylate, methyl methacrylate, trimethylammonioethyl methacrylate chloride) (such as Eudragit RS-30D), hydroxypropyl methylcellulose (Methocel K100M, Premium CR Methocel K100M, Methocel E5, Opadry®), magnesium stearate, talc, triethyl citrate, aqueous ethylcellulose dispersion (Surelease®), and protamine sulfate. The conjugates and compositions of the disclosure may be formulated for slow release, e.g., with slow release agents. The slow release agent may also comprise a carrier, which can comprise, for example, solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents. Pharmaceutically acceptable salts can also be used in these slow release agents, for example, mineral salts such as hydrochlorides, hydrobromides, phosphates, or sulfates, as well as the salts of organic acids such as acetates, propionates, malonates, or benzoates. The composition may also contain liquids, such as water, saline, glycerol, and ethanol, as well as substances such as wetting agents, emulsifying agents, and pH buffering agents. Liposomes may also be used as a carrier.

[00238] It is an object of the disclosure that the pharmaceutical compositions comprising the subject TP-ABC, TD-ABC or NA-ABC or compositions thereof can be formulated at a high concentration, yet have a low level of viscosity to enhance the ability to administer the pharmaceutical composition to a subject.

[00239] For liquid formulations, a desired property is that the formulation be supplied in a form that can pass through a 25, 26, 27, 28, 29, 30, 31, or 32 gauge needle for intravenous, intramuscular, intraarticular, intraocular, or subcutaneous administration. In one embodiment, the pharmaceutical composition is formulated in a saline buffer solution at a concentration of at least at least 1 mM, or at least 2 mM, or at least 3 mM, or at least 4 mM, or at least 5 mM, or at least 6 mM, or at least 7 mM, or at least 8 mM, or at least 9 mM, or at least 10 mM and the resulting solution can be passed through a 25, 26, 27, 28, 29, 30, 31, or 32 gauge needle for intravenous, intramuscular, intraarticular, or subcutaneous administration. In another embodiment, the pharmaceutical composition is formulated in a saline buffer solution at a concentration of at least at least 1 mM, or at least 2 mM, or at least 3 mM, or at least 4 mM, or at least 5 mM, or at least 6 mM, or at least 7 mM, or at least 8 mM, or at least 9 mM, or at least 10 mM and has a viscosity of less than 10 cP, or less than 15 cP, or less than 20 cP, or less than 25 cP, or less than 30 cP.

[00240] Syringe pumps may also be used to deliver the pharmaceutical compositions of the invention. Such devices are described in U.S. Patent No. 4,976,696, U.S. Patent No. 4,933,185, U.S. Patent No. 5,017,378, U.S. Patent No. 6,309,370, U.S. Patent No. 6,254,573, U.S. Patent No. 4,435,173, U.S. Patent No. 4,398,908, U.S. Patent No. 6,572,585, U.S. Patent No. 5,298,022, U.S. Patent No. 5,176,502, U.S. Patent No. 5,492,534, U.S. Patent No. 5,318,540, and U.S. Patent No. 4,988,337, the contents of which are incorporated herein by reference. One skilled in the art, considering both the present disclosure and the disclosures of the patents referenced herein could produce a syringe pump for the extended release of the compositions of the present disclosure.

IV). METHODS OF USE OF THERAPEUTIC PROTEIN-ALBUMIN BINDING CONJUGATES

[00241] In one aspect, the disclosure provides TP-ABC and TD-ABC and compositions thereof for use in the treatment and prevention of diseases.

[00242] In some embodiments, the disclosure provides a method of treating a disease in a subject, comprising administering to the subject an effective amount of a TP-ABC or a TD-ABC or an NA-ABC or a composition thereof to a subject in need thereof. In one embodiment, the TP-ABC comprises a single type of therapeutic protein selected from Table 4, or an active fragment or sequence variant thereof. In another embodiment, the TD-ABC comprises a single type of drug selected from Table 5 or an active analog thereof. In another embodiment, the TP-ABC comprises two different therapeutic proteins selected from Table 4. In another embodiment, the TP-ABC comprises a therapeutic protein selected from Table 4 and a therapeutic drug selected from Table 5. In another embodiment, the TD-ABC comprises two types of drugs selected from Table 5. In another embodiment, the TP-ABC comprises a scFv derived from an antibody selected from Table 4 and a drug selected from Table 5.

[00243] In the method, the therapeutic protein or therapeutic drug is one that is known in the art to have a beneficial effect or has affinity to a disease target when administered to a subject with a particular disease or condition. In the one embodiment, the method is useful in treating or ameliorating or

preventing a disease selected from cancer, cancer supportive care, neoplasms, cardiovascular disease, central nervous system disease, congenital deficiency disease, endocrine disease, gastrointestinal disease, genitourinary disease, hematological disease, HIV infection, hormonal system disease, inflammation, autoimmune disease, infectious disease, metabolic disease, musculoskeletal disease, nephrology disorders, ophthalmologic disease, pulmonary disease, pain, renal disease, respiratory disease, urogenital disease, immune disorders, nervous system disease, skin and connective tissue disease, and wound disease.

[00244] In another embodiment, the method comprises administering to a human patient with a disease at least two therapeutically effective bodyweight adjusted bolus doses of a pharmaceutical composition comprising a conjugate provided herein, wherein said therapeutically effective bodyweight adjusted bolus dose is at least about 0.05 mg/kg, at least about 0.1 mg/kg, at least about 0.2 mg/kg, at least about 0.4 mg/kg, at least about 0.8 mg/kg, at least about 1.0 mg/kg, at least about 1.2 mg/kg, at least about 1.4 mg/kg, at least about 1.6 mg/kg, at least about 1.8 mg/kg, at least about 2.0 mg/kg, at least about 2.2 mg/kg, at least about 2.4 mg/kg, at least about 2.6 mg/kg, at least about 2.7 mg/kg, at least about 2.8 mg/kg, at least 3.0 mg/kg, at least 4.0 mg/kg, at least about 5.0 mg/kg, at least about 6.0 mg/kg, at least about 7.0 mg/kg, at least about 10 mg/kg, or at least about 15 mg/kg, or at least about 20 mg/kg, or at least about 30 mg/kg, or at least about 50 mg/kg.

[00245] In some embodiments of the method of treatment, the composition can be administered to a subject subcutaneously, intramuscularly, intraocularly, or intravenously. In one embodiment, the composition is administered in a therapeutically effective amount. In one embodiment, administration of two or more consecutive doses of the therapeutically effective amount of the composition results in a gain in time spent within a therapeutic window for the composition compared to the therapeutic protein or a therapeutic drug (e.g., payload) not linked to the conjugates provided herein and administered using comparable doses to a subject. The gain in time spent within the therapeutic window can be at least three-fold longer than unmodified payload, or alternatively, at least four-fold, or five-fold, or six-fold, or seven-fold, or eight-fold, or nine-fold, or at least 10-fold, or at least 20-fold, or at least about 30-fold, or at least about 50-fold, or at least about 100-fold longer than the therapeutic payload not linked to the composition.

[00246] In another aspect, the disclosure provides a regimen for treating a subject with a disease, said regimen comprising a pharmaceutical composition comprising any of the TP-ABC, TD-ABC, or NA-ABC embodiments described herein. In one embodiment of the regimen, the regimen further comprises the steps of determining the amount of pharmaceutical composition needed to achieve a therapeutic effect in the patient and then administering the pharmaceutical composition.

[00247] The disclosure provides a treatment regimen for a diseased subject comprising administering a pharmaceutical composition comprising a TP-ABC, TD-ABC, or NA-ABC of any of the embodiments described herein in two or more successive doses administered at an effective amount, wherein the administration results in the improvement of at least one parameter associated with the disease.

[00248] In another embodiment, the disclosure provides a TP-ABC composition for use in the preparation of a medicament for use in treating a disease in a subject. In another embodiment, the disclosure provides a TD-ABC composition for use in the preparation of a medicament for use in treating a disease in a subject. In the foregoing embodiment, the disease is selected from the group consisting of cancer, cancer supportive care, neoplasms, cardiovascular disease, central nervous system disease, congenital deficiency disease, endocrine disease, gastrointestinal disease, genitourinary disease, hematological disease, HIV infection, hormonal system disease, inflammation, autoimmune disease, infectious disease, metabolic disease, musculoskeletal disease, nephrology disorders, ophthalmologic disease, pulmonary disease, pain, renal disease, respiratory disease, urogenital disease, immune disorders, nervous system disease, skin and connective tissue disease, and wound disease.

V). PHARMACEUTICAL KITS

[00249] In another aspect, the disclosure provides a kit to facilitate the use of the TP-ABC, TD-ABC, and NA-ABC compositions. The kit comprises a pharmaceutical composition comprising TP-ABC or TD-ABC or NA-ABC provided herein, a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc., formed from a variety of materials such as glass or plastic. The container holds a pharmaceutical composition as a formulation that is effective for treating a subject and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The package insert lists the approved indications for the drug, instructions for the reconstitution and/or administration of the drug for the use for the approved indication, appropriate dosage and safety information, and information identifying the lot and expiration of the drug. In another embodiment of the foregoing, the kit can comprise a second container that can carry a suitable diluent for the pharmaceutical composition, the use of which will provide the user with the appropriate concentration to be delivered to the subject. In another embodiment, the kit comprises, a first container comprising an amount of a TP-ABC or TD-ABC drug sufficient to administer in treatment of a subject with a disease; an amount of a pharmaceutically acceptable carrier; a second container that can carry a suitable diluent for the subject composition, which will provide the user with the appropriate concentration of the pharmaceutical composition to be delivered to the subject; a label identifying the drug and storage and handling conditions; and/or a sheet of the approved indications for the drug and instructions for the reconstitution and/or administration of the drug for the use for the treatment of an approved indication, appropriate dosage and safety information, and information identifying the lot and expiration of the drug.

[00250] In another embodiment, the disclosure provides a pre-filled syringe comprising a pharmaceutical composition comprising a therapeutically effective amount of the TP-ABC for administration to a subject in need thereof. In another embodiment, the disclosure provides a pre-filled syringe comprising a pharmaceutical composition comprising a therapeutically effective amount of the TD-ABC for administration to a subject in need thereof. In one embodiment, the syringe is used for the subcutaneous administration of the pharmaceutical composition. In another embodiment, the syringe is

used for the intramuscular administration of the pharmaceutical composition. In another embodiment, the syringe is used for the intravenous administration of the pharmaceutical composition.

VI). NUCLEIC ACID SEQUENCES

[00251] The present disclosure provides isolated polynucleic acids encoding the polypeptide components of the conjugates and compositions thereof and sequences complementary to polynucleic acid molecules encoding the polypeptide components of the conjugates and compositions. In one embodiment, the disclosure provides polynucleic acids encoding the XTEN of any of the albumin binding conjugate embodiments described herein, or the complement of the polynucleic acid. In another embodiment, the disclosure provides polynucleic acids encoding the therapeutic protein of any of the TP-ABC embodiments described herein, or the complement of the polynucleic acid.

[00252] In one embodiment, the disclosure encompasses methods to produce polynucleic acids encoding the polypeptide components of the subject compositions, or sequences complementary to the polynucleic acids, including homologous variants thereof. In general, the methods include producing a polynucleotide sequence coding for the polypeptide components of the subject conjugates and compositions and expressing the resulting gene product and assembling nucleotides encoding the components, ligating the components in frame, incorporating the encoding gene into an expression vector appropriate for a host cell, transforming the appropriate host cell with the expression vector, and culturing the host cell under conditions causing or permitting the resulting fusion protein to be expressed in the transformed host cell, thereby producing the polypeptide, which is recovered by methods described herein or by standard protein purification methods known in the art. In one embodiment of the foregoing, the host cell is a prokaryote cell. In another embodiment, the host cell is *E. coli*. In another embodiment, the host cell is a eukaryote cell. Standard recombinant techniques in molecular biology can be used to make the polynucleotides and expression vectors of the present disclosure.

[00253] In accordance with the disclosure, nucleic acid sequences that encode the polypeptides of the subject conjugates or compositions (or its complement) are used to generate recombinant DNA molecules that direct the expression in appropriate host cells. Several cloning strategies are suitable for performing the present disclosure, many of which are used to generate a construct that comprises a gene coding for a conjugate or composition of the present disclosure, or its complement. In one embodiment, the cloning strategy is used to create a gene that encodes a polypeptide of a subject conjugate or composition that comprises nucleotides encoding the polypeptide that is used to transform a host cell for expression of the polypeptide. In another embodiment, the cloning strategy is used to create a gene that encodes a therapeutic protein payload that comprises nucleotides encoding the therapeutic protein that is used to transform a host cell for expression of the protein for conjugation to the XTEN of the TP-ABC composition.

[00254] In one approach, a construct is first prepared containing the DNA sequence corresponding to a polypeptide of the subject conjugate or composition. Exemplary methods for the preparation of such constructs are described in the Examples. The construct is then used to create an expression vector

suitable for transforming a host cell, such as a prokaryotic host cell (e.g., *E. coli*) for the expression and recovery of the protein. Exemplary methods for the creation of expression vectors, the transformation of host cells and the expression and recovery of the polypeptides of the subject conjugates or compositions are described in the Examples.

[00255] The gene encoding a polypeptide of the subject conjugate or composition can be made in one or more steps, either fully synthetically or by synthesis combined with enzymatic processes, such as restriction enzyme-mediated cloning, PCR and overlap extension, including methods more fully described in the Examples. The methods disclosed herein can be used, for example, to ligate short sequences of polynucleotides encoding the individual component genes of a desired sequence. Genes encoding polypeptides of the subject conjugates or compositions can be assembled from oligonucleotides using standard techniques of gene synthesis. The gene design can be performed using algorithms that optimize codon usage and amino acid composition. The resulting assembled genes encoding the polypeptide of the subject conjugate or composition, and the resulting genes used to transform a host cell and produce and recover the polypeptide for incorporation into the TP-ABC for evaluation of its properties, as described herein.

[00256] The following are examples of conjugates, compositions, methods, and treatment regimens of the disclosure. It is understood that various other embodiments may be practiced, given the general description provided above.

EXAMPLES

[00257] The following examples are given for the purpose of illustrating various embodiments of the disclosure and are not meant to limit the present disclosure in any fashion. The present examples are exemplary and are not intended as limitations on the scope of the disclosure. Changes therein and other uses will occur to those skilled in the art.

[00258] Example 1: Synthesis and recovery of cysteine-containing XTEN LX-40.

[00259] To generate the coding sequence for LX-40, the amino acid sequence of an extended polypeptide, XTEN_AE144, was modified by replacement of three amino acid residues with three cysteine residues, at amino acid positions 36, 72 and 108, and each of the three cysteines was flanked by two glycine residues as “GCG”. The coding DNA sequence was codon-optimized and the gene was synthesized by the company GenScript. The synthesized DNA fragment was digested with the restriction enzymes BsaI/AgeI and the band of the right size was gel-purified. The DNA purified from this band was used as the insert for plasmid construction (e.g., ligation of vector and insert).

The plasmid pNL0322 was digested with BsaI/AgeI and purified by agarose gel electrophoresis.

[00260] The plasmid pNL0322 (with T7 promotor) was digested with BsaI/AgeI and the linearized plasmid was purified by agarose gel electrophoresis. The XTEN-encoding fragment was ligated into the linearized expression vector. DH5α competent cells were transformed with the ligation mixture and grown on an agar plate with antibiotic selection. Several bacterial colonies were selected to produce and purify the XTEN-encoding plasmids. The correct plasmids encoding the protein HD2-R-XTEN_AE144

(C36, C72, C108)-R-H8 were identified and confirmed by DNA sequencing. The resulting DNA sequence and protein sequences of LX-40 are provided in Table 6.

[00261] Expression of LX-40

[00262] *E. coli* strain AmE098 transformed with the expression vector was grown in high-density fermentation under the control of promoter sequence of T7 RNA polymerase. Briefly, the transformed cells were grown in LB media until an OD600 of 2.5 was reached and the culture was transferred into a 10-liter fermenter containing a rich medium with 2.1 g/L glucose. After batch feed was exhausted, 70% w/v glucose was added based on a pre-programmed glucose limited profile. The culture was induced with IPTG at 40-50 OD600 and then cultured another 18-24 hours before harvest. The cells were pelleted by centrifugation and frozen at -80 °C.

[00263] Purification of LX-40

[00264] 1) Heat and acid clarification step

[00265] 3.5 kilograms of fermentation cell paste was resuspended in 7 L of 50 mM citrate buffer pH 4.0. The mixture was heat-treated at 80 °C for 15 minutes and then cooled down to <10°C. The pH of the suspension was adjusted to 3.0 with concentrated hydrochloric acid, and the mixture was stirred overnight at 4°C. The next day, the mixture was divided into 500 mL centrifuge bottles (70% full) and centrifuged at 7,000 rpm for 40 minutes. The supernatant was then harvested and 0.1% Celite (Supelco, Cat. #: 13360-U) was added before passing through a depth filter and then a 0.2 µm filter.

[00266] 2) Cation exchange capture step

[00267] One liter Capto SP ImpRes resin (GE Healthcare) was packed in BPG100 column housing, sanitized with 0.5 M NaOH and 1 M NaCl, thoroughly rinsed with distilled water and equilibrated with citrate-phosphate buffer pH 3.0, 100 mM NaCl. Seven liters of clarified lysate (pH 3.0, conductivity 12.4 mS/cm) was loaded onto the column at 100 mL/min and chased with 2 column volumes of equilibration buffer. 20 mM citric acid pH 2.5 was used as Buffer A and 40 mM sodium phosphate dibasic pH 9.0 was used as Buffer B. Protein was step eluted with 34% Buffer A/66% Buffer B. The column was then stripped using 20 mM phosphate, 500 mM NaCl pH 7.0. Fractions were analyzed by non-reducing 4-12% Bis-Tris SDS-PAGE and Coomassie staining.

[00268] 3) Trypsin digestion, trypsin inactivation, and in-process TFF

[00269] Seven milliliters (mL) of 40% w/w sodium hydroxide was added to the 5.6 L of Capto SP pool to increase the pH to >7.5. Forty two microliters (µL) of 71 mg/mL trypsin (Roche) was added for the enzymatic cleavage of the helper domain HD2 and His-tag (H8) of the expression product. The solution was incubated at room temperature overnight with gentle stirring. The remaining trypsin activity was inactivated by adding EDTA and DTT to final concentrations of 2 mM and 20 mM, respectively, and heating to 80 °C. The mixture was then cooled down in an ice bath. The chilled solution was filtered, concentrated to 1 liter and buffer-exchanged into 20 mM MES, pH 5.5 by Tangential Flow Filtration (TFF) using Pellicon cassette (EMD Millipore).

[00270] 4) Anion exchange polishing step

[00271] One liter of Capto Q ImpRes resin (GE Healthcare) was packed in BPG100 column housing, sanitized with 0.5 M NaOH and 1 M NaCl, rinsed with distilled water and equilibrated with Buffer A (20 mM MES, pH 5.5). Trypsin-digested reaction mixture was loaded onto the column and chased with 1 column volume of Buffer A. A two-step gradient elution was applied, first using 0-40% Buffer B (20 mM MES, pH 5.5, 500 mM NaCl) in 10 column volumes and then 40-60% Buffer B in 1 column volume. The column was stripped with 100% Buffer B. Fractions were analyzed by non-reducing Bis-Tris SDS-PAGE and visualized by Stains-All (Sigma-Aldrich).

[00272] 5) Formulation and characterization

[00273] The fractions selected in Step 4) were pooled and buffer exchanged into 20 mM MES pH 5.5 using TFF cassette, concentrated, aliquoted, and stored frozen at -80 °C as purified LX-40 XTEN with 144 amino acid residues, the protein sequence of which is presented in Table 6.

Table 6: DNA and amino acid sequence for CXTEN (1xAmino, 3-Thio-XTEN144) LL-40

Sequence Name	DNA Sequence	Amino Acid Sequence
N-term-HD2-R-AE144_3Cys-R-H8	ATGAAAAACCCAGAGCAAGCAGAAGAACAAGCTGAAGAACAG CGCGAAGAAACAagegcgtctcgtGGGTCTCCAggtgcAGGT AGCCCAGCTGGTAGCCCAACCTCTACCGAAGAAGGTACCTCT GAATCCGCTACTCCAGAATCCGGTCCTGGTACTAGCACTGAG GGCtgcGGTGAATCTGCTCCAGGCTCCCCGGCAGGTAGCCCT ACCTCTACCGAAGAGGGCACTAGCACCGAACCATCTGAGGGT TCCGCTCCTGGCACCTCCACTGAAGGtgcGGCGAAAGTGCT CCGGGTACTTCCGAAAGCGCAACTCCGGAATCCGGCCCTGGT TCTGAGCCTGCTACTTCCGGCTCTGAAACTCCAGGTAGCGAG CCAGCGGGCtgcGGTCTGAAACTCCAGGTTACCGGCGGGT AGCCCCGACGAGCACGGAGGAAGGTACCTCTGAGTCGGCCACT CCTGAGTCCGGTCCGGGCACGGAAagegcctctcgtccgca GAACAGAACTGATCTCTGAAGAGGATCTGtctccggtacc ggtCATCACCATCACCACCATCATCTACTAA	MKNPEQAEEQAEEQREETS ASRGSPGAGSPAGSPTSTE EGTSESATPESGPGTSTEG CGESAPGSPAGSPTSTEEG TSTEPSEGSAPGTSTEGCG ESAPGTSESATPESGPGSE PATSGSETPGSEPAGCGSE TPGSPAGSPTSTEEGTSES ATPESGPGTESASRSAEQK LISEEDLSPATGHHHHHHH H
LX-40		GSPGAGSPAGSPTSTEEGT SESATPESGPGTSTEGCGE SAPGSPAGSPTSTEEGTST EPSEGSAPGTSTEGCGESA PGTSESATPESGPGSEPAT SGSETPGSEPAGCGSETPG SPAGSPTSTEEGTSESATP ESGPGTESASR

[00274] **Example 2: Synthesis and recovery of cysteine-containing XTEN LX-31.**

[00275] To generate the gene coding for LX-31, the amino acid sequence of an extended polypeptide, XTEN_AE144, was modified by replacement of three amino acid residues with three cysteine-containing sequences, at amino acid positions 31, 82 and 134, the cysteine-containing sequences having three cysteines was flanked by two glycine residues as “GCG”. The DNA fragment coding gene LX-31, the XTEN_AE144 containing three cysteines, was obtained by PCR on the codon-optimized DNA sequence of an extended polypeptide, XTEN_AE864 template, with primers designed to introduce three “GCG” sequences at residues 31, 82 and 134. The band having the right size PCR fragment was gel-purified and digested with the restriction enzymes BsaI/NotI. The digested DNA was used as the insert for plasmid construction (e.g., ligation of vector and insert).

[00276] The plasmid pYS0066 (with T7 promotor) was digested with BsaI/NotI and the digestion reaction with the linearized plasmid was purified by agarose gel electrophoresis. The XTEN-encoding fragment was ligated into the linearized expression vector. DH5 α competent cells were transformed with the ligation mixture and grown on an agar plate with antibiotic selection. Several bacterial colonies were selected to produce and purify the XTEN-encoding plasmids. The correct plasmids encoding the protein HD2-R-XTEN_AE144 (C31, C82, C134)-R-H8 were identified and confirmed by DNA sequencing to identify a clone (pYS0071). The resulting DNA sequence and protein sequences of LX-32 are provided in Table 7.

[00277] Expression of LX-31

[00278] *E. coli* strain AmE098 transformed with the expression vector was grown in high-density fermentation under the control of promoter sequence of T7 RNA polymerase. Briefly, the transformed cells were grown in LB media until an OD600 of 2.5 was reached and the culture was transferred into a 10-liter fermenter containing a rich medium with 2.1 g/L glucose. After batch feed was exhausted, 70% w/v glucose was added based on a pre-programmed glucose limited profile. The culture was induced with IPTG at 40-50 OD600 and then cultured another 18-24 hours before harvest. The cells were pelleted by centrifugation and frozen at -80 °C.

[00279] Purification of LX-31

[00280] 1) Heat and acid clarification step

[00281] 3.5 kilograms of fermentation cell paste was resuspended in 7 L of 50 mM citrate buffer pH 4.0. The mixture was heat-treated at 80 °C for 15 minutes and then cooled down to <10 °C. The pH of the suspension was adjusted to 3.0 with concentrated hydrochloric acid, and the mixture was stirred overnight at 4 °C. The next day, the mixture was divided into 500 mL centrifuge bottles (70% full) and centrifuged at 7,000 rpm for 40 minutes. The supernatant was then harvested and 0.1% Celite (Supelco, Cat. #: 13360-U) was added before passing through a depth filter and then a 0.2 μ m filter.

[00282] 2) Cation exchange capture step

[00283] One liter Capto SP ImpRes resin (GE Healthcare) was packed in BPG100 column housing, sanitized with 0.5 M NaOH and 1 M NaCl, thoroughly rinsed with distilled water and equilibrated with citrate-phosphate buffer pH 3.0, 100 mM NaCl. Seven liters of clarified lysate (pH 3.0, conductivity 12.4 mS/cm) was loaded onto the column at 100 mL/min and chased with 2 column volumes of equilibration buffer. 20 mM citric acid pH 2.5 was used as Buffer A and 40 mM sodium phosphate dibasic pH 9.0 was used as Buffer B. Protein was step eluted with 34% Buffer A/66% Buffer B. The column was then stripped using 20 mM phosphate, 500 mM NaCl pH 7.0. Fractions were analyzed by non-reducing 4-12% Bis-Tris SDS-PAGE and Coomassie staining.

[00284] 3) Trypsin digestion, trypsin inactivation, and in-process TFF

[00285] Seven milliliters (mL) of 40% w/w sodium hydroxide was added to the 5.6 L of Capto SP pool to increase the pH to >7.5. Forty two microliters (μ L) of 71 mg/mL trypsin (Roche) was added for the enzymatic cleavage of the helper domain HD2 and His-tag (H8) of the expression product. The solution

was incubated at room temperature overnight with gentle stirring. The remaining trypsin activity was inactivated by adding EDTA and DTT to final concentrations of 2 mM and 20 mM, respectively, and heating to 80 °C. The mixture was then cooled down in an ice bath. The chilled solution was filtered, concentrated to 1 liter and buffer-exchanged into 20 mM MES, pH 5.5 by Tangential Flow Filtration (TFF) using Pellicon cassette (EMD Millipore).

[00286] 4) Anion exchange polishing step

[00287] One liter Capto Q ImpRes resin (GE Healthcare) was packed in BPG100 column housing, sanitized with 0.5 M NaOH and 1 M NaCl, rinsed with distilled water and equilibrated with Buffer A (20 mM MES, pH 5.5). Trypsin-digested reaction mixture was loaded onto the column and chased with 1 column volume of Buffer A. A two-step gradient elution was applied, first using 0-40% Buffer B (20 mM MES, pH 5.5, 500 mM NaCl) in 10 column volumes and then 40-60% Buffer B in 1 column volume. The column was stripped with 100% Buffer B. Fractions were analyzed by non-reducing Bis-Tris SDS-PAGE and visualized by Stains-All (Sigma-Aldrich).

[00288] 5) Formulation and characterization

[00289] The fractions selected in Step 4) were pooled and buffer exchanged into 20 mM MES pH 5.5 using TFF cassette, concentrated, aliquoted, and stored frozen at -80 °C as purified LX-31 XTEN with 144 amino acid residues, the protein sequence of which is presented in Table 7.

Table 7: DNA and amino acid sequence for CXTEN (1xAmino, 3-Thio-XTEN144) LX-31

Sequence Name	DNA Sequence	Amino Acid Sequence
N-term-HD2-R-AE144_3Cys-R-H8	ATGAAAAACCCAGAGCAAGCAGAAGAACAAGCTGAAGAACAGCGCGAAGAAACAagecgctctcggttccgctGGGTCTCCAacgGAAGGTACTAGCACGGAGCCGAGCGAGGGTAGTGCTCCGGGTACGAGCGAGAGCACGGCAGAAGCCGCTGGCtgcGGTACTGCTGAAGCGGCAACCCCTGAGAGCGGCCAGGTACTTCTGAGAGCGCCACTCCTGAATCCGGCCCTGGTAGCGAGCCGGCAACCTCCGGCTCAGAACTCCTGGTTCGGAACCGAGCGACCGGTACC GCTGAAGCCGCAGGTtgtGGCACTGCGGAAGCTGCAACCGAAGAGGGTACCAGCACGGAACCGAGCGAGGGTCTGCCCCGGGTACTTCCACCGAACCATCGGAGGGCTCTGCACCTGGTAGCGAACCTGCGACGTCTGGTCTGAAACGCCGACTGCAGAAGCGGCTGGTtgtGGCACCGCCGAAGCAGCTagcgctctcgctcgeaCATCACCATCACCACCATCATCACTAA	MKNPEQAEQAEQREETSASRSAGSPTEGTSTEPSEG SAPGTSESTAEAGCGTAE AATPESGPGTSESATPESG PGSEPATSGSETPGSEPAT SGTAEEAGCGTAEAAATEEG TSTEPSEGSAPGTSTEPSE GSAPGSEPATSGSETPTAE AAGCGTAEAAASASRAHHH HHHHH
LX-31		SAGSPTEGTSTEPSEGSAPGTSESTAEAGCGTAEAAATPESGPGTSESATPESGPGS EPATSGSETPGSEPATSGT AEAAGCGTAEAAATEEGTST EPSEGSAPGTSTEPSEGSAPGSEPATSGSETPTAEAAAGCGTAEAAASASR

[00290] Example 3: Small-scale synthesis of UHLX-31

[00291] In order to produce albumin binding conjugates, the structure referred to in Formula XXXI (FTA_01-IA) was used to derivatize cysteine-containing XTEN (LX-31, with 3 cysteine residues and 144 total amino acid residues). A molar excess of the FTA_01-IA reagent over the number of cysteine groups on the XTEN polymer was added in a buffered solution. Analysis of the conjugation reaction was

performed by reversed phase high performance liquid chromatography (RP-HPLC). A reaction mixture containing 10 µg of XTEN was injected onto a C18 RP-HPLC column (Phenomenex, product number #00F-4053-E0, 4.6 mm × 150 mm, 5 µm particle size) and eluted by a gradient of 5-50% Buffer B (0.1% trifluoroacetic acid, TFA, in acetonitrile) in Buffer A (0.1% TFA in water) over 22.5 minutes at a flow rate of 1 ml/min. Absorbance was monitored at 215 nm in order to detect the amide bonds on the XTEN and/or FTA_01-IA. The albumin binding subunit described here, when attached to a cysteine group of one of the XTENs, will also be referred to as FTA_01 (Formula LIII). Thus an “FTA_01 conjugate” can be used to refer to the materials produced in this way.

[00292] To optimize the conditions for producing UHLX-31, diagrammed in FIG. 14, reaction mixtures containing 4, 5, or 6 molar equivalents of FTA_01-IA relative to the precursor XTEN LX-31 were prepared (1.3, 1.6 and 2 molar equivalents relative to the number of cysteine groups) and these reactions were analyzed by RP-HPLC. In the conditions tested, the majority of the material was triply conjugated, as shown in FIG. 15. Peak 1 represents FTA_01-IA. Peak 2 corresponds to the presumptive doubly conjugated byproduct and peak 3 corresponds to the desired triply conjugated product. In this analysis, the unmodified material was not clearly visible (severely broadened with low peak height when run alone), likely due to oxidation during analysis. However, it is expected that little, if any, unconjugated material is present in the reaction mixtures. The observation that the area under the curve (AUC) of the doubly and triply conjugated material is similar to what would be expected for an XTEN polymer suggests that most of the material is accounted for by these conjugates and that little, if any, is fully unconjugated.

[00293] In all cases, conditions were chosen in which the smallest amount of FTA_01-IA produced the largest amount of product. After performing the optimization reactions, the reaction was scaled up to produce a sufficient amount of material for pharmacokinetic analysis *in vivo*.

[00294] The triple FTA_01 conjugate produced above precipitated from solution upon acidification at high concentration, making further purification by preparative reverse phase HPLC impractical. Since the purity of this compound was already acceptable for the purposes of the intended pharmacokinetic evaluation (e.g., minimal amounts of unmodified material detected), the compound was buffer exchanged into 20 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) with 50 mM NaCl pH 7 using centrifugal ultrafiltration.

[00295] Example 4: Pharmacokinetic analysis of XTEN-FTA_01 conjugates in rats

[00296] A number of albumin binding conjugates, listed in Table 8, were prepared from the XTEN of Table 1, covering a range of XTEN with lengths from 144 to 288 amino acids. In two cases, the albumin binding subunit was conjugated to XTEN of the same amino acid length (i.e., 288 residues) but to cysteines in different positions on the XTEN. In one of the cases, an albumin binding conjugate was prepared with three albumin binding subunits and the length of this XTEN in amino acids (i.e., 144 residues) was identical to that of two other albumin binding conjugates having only one or two linked albumin binding subunits. In order to assay the plasma concentration of these albumin binding conjugates

in plasma, chelated lanthanide metals were used. The albumin binding conjugates described in Table 6 were labeled with the indicated metal by conjugation of DOTA chelator to the amino terminus, and then by the coordination of the lanthanide metal. XTEN polymers were conjugated through their amino terminus to the DOTA chelator (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) using the amine reactive DOTA NHS ester (Macrocyclics Cat. No. B-280, Dallas, TX). In one case (AE144 C73/K144, UHLX-36), there was two sites of conjugation for the DOTA chelator (the C-terminal lysine residue, K144 in addition to the N-terminal amino group), meaning that each molecule may contain up to 2 chelators and metals.

Table 8: XTEN-FTA 01 conjugates

Compound Name	XTEN Length (amino acids)	Cysteine (C) and Lysine (K) locations in XTEN	Incorporated Metal Label
UHLX-31	144	C31, C82, C134	Europium (Eu)
UHLX-34	216	C109	Terbium (Tb)
UHLX-32	288	C283	Thulium (Tm)
UHLX-35	288	C145	Holmium (Ho)
UHLX-36	144	C73/K144	Praseodymium (Pr)
UHLX-33	144	C139	Lutetium (Lu)

[00297] The compound of interest was treated with a molar excess of the DOTA NHS ester reagent, sufficient to conjugate the chelator to the majority of the amino reactive groups on the albumin binding conjugate. Conversion was observed using Matrix-assisted laser desorption/ionization – time of flight mass spectrometry (MALDI-TOF MS). After conjugation and removal of the DOTA chelator by desalting and/or ultrafiltration/diafiltration (UF/DF) in a centrifugal concentrator, an approximate five-fold molar excess of a water soluble salt of the lanthanide metal ion of interest was used. This solution was then desalted using a desalting column and then further by UF/DF in a centrifugal concentrator.

[00298] The concentration of these metals can be determined using inductively coupled plasma mass spectrometry (ICP-MS) analysis, which quantifies metals in complex mixtures. Lanthanide series metals are particularly suitable since they have one or two different isotopes with substantial natural abundance, and these common natural isotopes generally do not overlap in atomic weight with those of the other metals in this study. Additionally, lanthanide series metals are not commonly found in biological tissues, meaning that interference from the biological matrix is not expected.

[00299] A mixture of the labeled albumin binding conjugates was prepared with each compound at an approximate concentration of 2 mg/ml. These were injected intravenously into a group of female Sprague Dawley rats (n = 3) weighing about 225 g each. Before injection, and at various points after injection (3, 8, 18, 24, 48, 72, 120, 192, and 240 h), blood samples of approximately 200 µl were collected from these animals into lithium heparin treated microtubes. Fluid was replaced by the addition of saline and the whole blood samples were centrifuged to yield plasma, which was stored at approximately -80°C until analysis.

[00300] These plasma samples and a sample of the dosing solution were subjected to ICP-MS analysis in order to quantify the concentration of the metal labels in the plasma samples. The concentration of each metal could be converted to the concentration of the XTEN FTA_01 conjugate since the molar labeling ratio was known in all cases. Graphs of plasma concentration vs. time after injection are shown in FIG. 16. Analysis of these data using the software package PK Solutions 2.0 yielded commonly used pharmacokinetic parameters, and the elimination half-lives of the conjugates are shown in Table 9.

Table 9: Pharmacokinetic analysis of ABC compositions in rats

Composition Name	T _{1/2} (hr)
UHLX-31	58
UHLX-34	28
UHLX-32	35
UHLX-35	38
UHLX-36	20
UHLX-33	25

[00301] Most strikingly, the UHLX-31 with three cysteine residues in the XTEN and three linked albumin binding subunits had a longer half-life than any of the other conjugates tested, despite being similar in molecular weight to the other albumin binding conjugates having XTEN with 144 amino acids. These observations suggest a reversible interaction with albumin that is greatly strengthened by multivalency of the additional albumin binding subunits. Examining the albumin binding conjugates of varying XTEN polymer lengths reveals a trend that the molecular weight of the XTEN portion of the conjugate greatly affects the half-life, suggesting that albumin binding from even a single carboxylic acid provides some benefit in plasma half-life which is most likely augmented by the hindrance to renal filtration of a larger polymer.

[00302] Example 5: Large scale synthesis and purification of UHLX-40

[00303] In order to produce the UHLX-40 product shown in FIG. 4, 2 grams of the 144 amino acid XTEN precursor LX-40 (shown in Table 1), was treated with between 4 and 4.5 molar equivalents of FTA_01-IA (dissolved in anhydrous dimethylformamide, DMF), buffered to pH 8 with 100 mM HEPES, overnight at 25°C. Analysis of the conjugation reaction was performed by the analytical reverse phase HPLC method described in Example 3. A reaction mixture containing 10 µg XTEN was injected onto a C18 RP-HPLC column (Phenomenex, P/N #00F-4053-E0, 4.6 mm × 150 mm, 5 µm particle size) and analyzed using a method of 5 - 50% Buffer B (0.1% TFA in acetonitrile) in Buffer A (0.1% TFA in water) over 22.5 minutes at a flow rate of 1 mL/min. Absorbance was monitored at 215 nm in order to detect the amide bond(s) on the XTEN and FTA_01-IA. The HPLC analysis of this reaction is shown in FIG. 17.

[00304] An 800 mg aliquot of this crude reaction material was prepared for purification by UF/DF against 20 mM 2-(N-morpholino)ethanesulfonic acid (MES) pH 6.35. A tangential flow filtration (TFF) UF/DF system and two 5 kDa polyethersulfone membranes (Millipore P2B005A01) were sanitized with 5

hold-up volumes of 0.5 M NaOH. This NaOH solution was recirculated for 120 minutes, and the UF/DF system was subsequently equilibrated with 5 hold-up volumes 20 mM MES pH 6.35. This procedure was expected to reduce the fraction of DMF solvent in the reaction mixture to less than 1%.

[00305] Concurrently, a chromatography system and Capto Q Impres anion exchange (AEX) column (16 mm diameter, 200 mm length, 40.2 mL column volume) were sanitized with 3 column volumes (CV) of 0.5 M NaOH. The chromatography system and column were incubated in 0.5 M NaOH for 120 minutes then neutralized with AEX Mobile Phase B (20 mM MES, 200 mM NaCl, pH 6.35) and subsequently equilibrated with 5 CV AEX Mobile Phase A (20 mM MES, pH 6.35).

[00306] A 40 mL aliquot of crude reaction was diluted 10 fold with 20 mM MES pH 6.35 to a total volume of 400 mL then concentrated to 150 mL (minimum system holdup volume) via the sanitized, equilibrated TFF system. The dilution to 400 mL and concentration to 150 mL cycle was repeated two more times, and an offline sample of the permeate was measured to ensure the permeate pH and conductivity matched the diafiltration buffer within ± 0.2 pH units and ± 2 mS/cm, respectively.

[00307] The diafiltered sample was loaded onto the sanitized, equilibrated AEX column at 150 cm/hr (approximately 5 mL/min in this example) and chased with Mobile Phase A. UV light absorbance at 215 nm was monitored throughout the loading and elution processes. The flow-through was collected into 1 CV fractions. The elution was effected with a linear gradient from 0-100% B over 20 CV, and the elution was collected into 1 CV fractions. The column was then treated with 20 mM MES, 200 mM NaCl, pH 6.35. AEX elution fractions were loaded onto RP-HPLC for analysis. Samples containing less than 5% impurities, as determined by RP-HPLC were pooled. The concentration of this pool was 0.9 mg/mL and the endotoxin content was 1.2 EU/mg. Chromatographic analysis of the purification is shown in FIG. 18A. RP-HPLC analyses of the load material and the pooled fractions containing the desired product are shown in FIG. 18B and FIG. 18C, respectively.

[00308] Example 6: Viscosity analysis of UHLX-40, PEG40K Branched, and Human Serum Albumin

[00309] Many therapeutic biopolymers can be dosed via the subcutaneous route. In such cases, formulation viscosity is one important parameter that can limit the dosing that can be achieved. Generally, a reasonable dose for the therapeutic must be administered in a fixed volume (approximately 1 ml or less). This dose is limited in concentration either by the solubility limit of the therapeutic or by the viscosity of the formulation. Generally, a viscosity of 20 cP or less is desirable since this value allows injection through a narrow gauge needle for patient comfort. To evaluate the viscosity of formulations comprising conjugates of the present disclosure, solutions of UHLX-40, branched 40 kDa PEG, and human serum albumin in the low millimolar range were prepared for comparison studies.

[00310] Human serum albumin was obtained from Sigma Aldrich as a lyophilized powder. PEG40K Branched was obtained from JenKem Technology (SKU: Y-NH2-40K) as a lyophilized powder. Between 90 – 100 mg of UHLX-40 previously formulated in 20 mM HEPES pH 7 buffer was exchanged into 10 mM ammonium bicarbonate buffer using a PD-10 desalting column (GE Healthcare, Cat No. 17-0851-01)

before being dried under reduced pressure in a centrifugal vacuum concentrator. For analysis, the powders of UHLX-40, PEG40K Branched, and human serum albumin were dissolved at various molar concentrations between 2 mM – 10 mM in Dulbecco's Phosphate Buffered Saline (ThermoFisher, Cat Number 14190136) or 20 mM histidine 154 mM NaCl pH 5.5.

[00311] For viscosity measurements, samples of UHLX-40, human serum albumin, and PEG40K Branched were prepared at concentrations of 2.2 mM and 5 mM in 20 mM histidine 154 mM NaCl pH 5.5 buffer as described above. Preparation of a solution of human serum albumin at 5 mM was not possible to achieve. The concentrations of the UHLX-40 samples were confirmed by RP-HPLC quantitation, the concentration of human serum albumin was confirmed by 280 nm absorbance, and the concentrations of PEG40K Branched were confirmed by SEC-HPLC / Refractive Index measurement.

[00312] Viscosity studies were performed using a thermostatted Rheosense microVISC™ viscometer equipped with a microVISC chip, 0~100 cP. The temperature of the instrument was maintained at 25 °C for the analyses. The instrument was first checked for performance using a viscosity standard (Rheosense Water Based Calibration Fluid, 2 cP) prior to analysis of samples. Neat samples were loaded in the positive displacement pipettes (Rheosense) and, after ensuring there were no visible air bubbles, mounted in the instrument. Measurements were made and an average of two readings is reported. The results of this experiment are shown in FIG. 19, and demonstrate that the albumin binding conjugate composition with 3 albumin binding subunits (UHLX-40) was 9- to 10-fold less viscous than comparable concentrations of branched 40 kDa PEG, supporting the conclusion that solutions of therapeutic proteins conjugated to albumin binding conjugates provided herein would be less viscous than solutions of PEGylated therapeutic proteins at comparable concentrations.

[00313] Example 7: DLS analysis of UHLX-40

[00314] A high concentration sample of UHLX-40 was prepared in the same manner as in Example 5.

[00315] Dynamic light scattering (DLS) studies were performed using a Malvern Zetasizer Nano S instrument. The instrument was first checked for performance using a 60 nm polystyrene standard. Samples were then loaded in a cuvette and three measurements made for each sample. A refractive index value of 1.450 was used for the analyses. Water was chosen as the dispersant and samples were equilibrated at 25 °C for two minutes prior to measurements. Data was acquired by Malvern Zetasizer software and the model 'protein analysis' was used for analysis. The result of this analysis on a 5 mM sample of UHLX-40 showed no signs of aggregation and a volume distribution profile that matches what would be expected for an XTEN of 144 amino acids. A plot volume distribution profile of UHLX-40 is shown in FIG. 20.

[00316] Example 8: Human Serum Albumin association with albumin binding conjugates (UHLX-30, 34, 35, and 40) measured by size exclusion chromatography

[00317] To demonstrate the albumin binding strength of conjugates provided herein, mixtures of conjugates and human serum albumin (HSA) were prepared. The conjugates UHLX-40, UHLX-30 (864 amino acid XTEN with one FTA_01 moiety conjugated), UHLX-35, and UHLX-34 were incubated with

the following molar equivalents of HSA: 0.1, 0.2, 0.33, 1 (stoichiometric), 5, and 10. The concentration of HSA was held constant in each mixture at 1 mg/ml. These mixtures were buffered with Dulbecco's phosphate-buffered saline and incubated for 1 hour at 25°C. Following this incubation, the mixtures were subjected to Size Exclusion Chromatography (SEC) and Multi-Angle Light Scattering (MALS) for analysis.

[00318] A sample from each mixture containing 10 µg of HSA was injected on a Yarra SEC column (Phenomenex Cat# 00H-4514-K0 Yarra 3µ SEC 4000, 7.8 mm x 300 mm). 10 µg samples of UHLX-40, UHLX-30, UHLX-35, UHLX-34, and HSA were also analyzed. The column was run at a 0.5 ml/min flow rate for 30 minutes in a mobile phase of 50 mM sodium phosphate and 300 mM sodium chloride. Absorbance chromatograms were collected at the wavelengths of 215 nm and 280 nm.

[00319] XTEN molecules absorb 215 nm light but since they do not contain aromatic residues, the XTEN molecules will have a negligible extinction coefficient at 280 nm. HSA, however, absorbs a significant amount of both 215 nm and 280 nm light. Example SEC analyses are shown in FIG. 21A, including the SEC traces for UHLX-40 at 215 nm, HSA at 215 nm, a 1:1 mixture of UHLX-40 and HSA at 215 nm, and a 1:1 mixture of UHLX-40 and HSA at 280 nm. In the example shown, the uncomplexed UHLX-40 and HSA elute at similar retention times. In the mixture of the two, however, a peak with an earlier retention time (corresponding to a higher molecular weight) is observed, demonstrating the formation of a complex between UHLX-40 and HSA. In this case, the stoichiometric ratio between UHLX-40 and HSA resulted in a complete conversion of UHLX-40 and HSA to complex together. Such high levels of conversion were not observed for UHLX-30, UHLX-35, and UHLX-34 at stoichiometric ratios. Uncomplexed XTEN and HSA remain in these traces, suggesting weaker binding of these conjugates bearing single albumin binding subunits. The complex formation percentage for UHLX-40, UHLX-30, UHLX-35, and UHLX-34 is quantified in FIG. 21B. This value is calculated as the area under the curve (AUC) of the complex peak at 280 nm divided by the total AUC of the trace at 280 nm.

[00320] MALS analysis on the UHLX-40 and HSA mixture was used to determine the stoichiometry. The molecular weight of the complex observed in the stoichiometric 1:1 mixture of the two components is approximately 80 kDa, as shown in FIG. 22A, which corresponds to the expected mass between one molecule of UHLX-40 and one molecule of HSA. Even with an approximately 10-fold excess of HSA, the molecular weight of the complex observed by MALS is 80 kDa (FIG. 22B). Thus, despite containing three albumin binding subunits, the results support the conclusion that this compound binds HSA in 1:1 stoichiometry, suggesting that the additional albumin binding subunits strengthen the complexation to a single molecule of HSA through increased avidity and do not bind multiple molecules of HSA.

[00321] Example 9: Human Serum Albumin Association with an albumin binding peptide

[00322] Peptides conjugated to a carboxylic acid are capable of binding to HSA and can thus display an extended *in vivo* half-life relative to the corresponding unconjugated peptide. In order to compare the binding strength of such a conjugate to albumin to that of one of the albumin binding conjugate compositions in this invention, one would perform size exclusion chromatography analysis. In this case,

the peptide – carboxylic acid conjugate is expected to elute at a considerably lower retention time relative to the albumin. To evaluate the binding strength, a 1:1 stoichiometric mixture of the peptide and albumin can be mixed and incubated for 1 h at room temperature as described above in Example 8. Since the association of the peptide would not significantly affect the retention time of the albumin by SEC analysis, the depletion in the area under the curve (AUC) of peptide – carboxylic acid conjugate relative to an injection of the same amount of material in the absence of albumin can be used to determine the amount of this material that associated with HSA.

[00323] Example 10: Determination of binding parameters using a Surface Plasmon Resonance (SPR) measurements

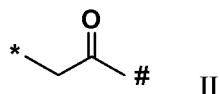
[00324] Binding analysis utilizing a Surface Plasmon Resonance (SPR) measurement can allow for the determination of the kinetic and thermodynamic binding parameters between the albumin binding conjugates described in this disclosure and human serum albumin. In such an experiment, for example using a BIAcore instrument, the analyte conjugate are labeled with biotin and immobilized onto a streptavidin functionalized chip (Sensor Chip SA) designed for use with this instrument. An increase in SPR signal is used to verify that material is successfully immobilized on the surface. After immobilization, varying concentrations of HSA are injected into the flow cell for binding analysis. Binding is observed using the SPR signal, which would be processed to provide binding parameters, including K_d , k_{off} , and k_{on} .

CLAIMS

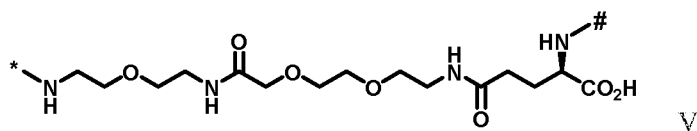
1. An albumin binding conjugate comprising:

a) an extended polypeptide (XTEN) having three cysteine residues, wherein the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity, or is identical to a sequence set forth in Table 1, when optimally aligned;

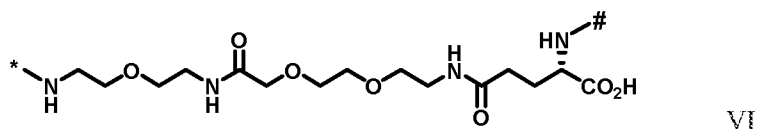
b) three linker moieties, each of which has the structure of formula II



c) three soluble bridge moieties, each of which has the structure of formula V

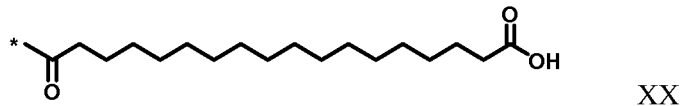


or formula VI



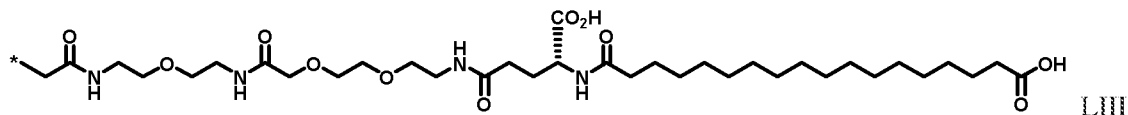
and

d) three carboxylic acid moieties, each of which has the structure of formula XX



wherein the XTEN, three linker moieties, three soluble bridge moieties, and three carboxylic acid moieties are configured according to the configuration set forth in FIG. 1B.

2. The albumin binding conjugate of claim 1, wherein the albumin binding conjugate comprises three albumin binding subunits comprising the linker moiety, the soluble bridge moiety, and the carboxylic moiety, with each albumin binding subunit having the structure of formula LIII



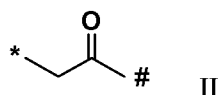
wherein each of the albumin binding subunits is linked to a thiol group of a cysteine residue of the XTEN.

3. An albumin binding conjugate, comprising:

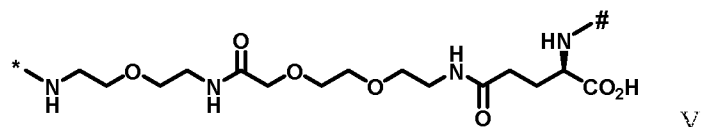
a) an extended polypeptide (XTEN) comprising at least two cysteine residues, wherein the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least

96%, at least 97%, at least 98%, at least 99% sequence identity, or is identical to a sequence set forth in Table 1, when optimally aligned;

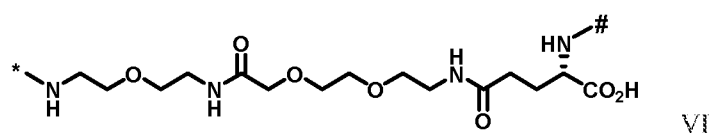
b) at least two linker moieties, each of which has the structure of formula II



c) at least two soluble bridge moieties, each of which has the structure of formula V

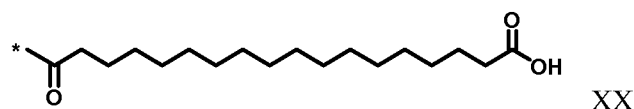


or formula VI



and

d) at least two carboxylic acid moieties, each of which has the structure of formula XX

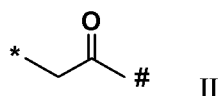


wherein the XTEN, two linker moieties, two soluble bridge moieties, and two carboxylic acid moieties are configured according to the configuration set forth in FIG. 1A.

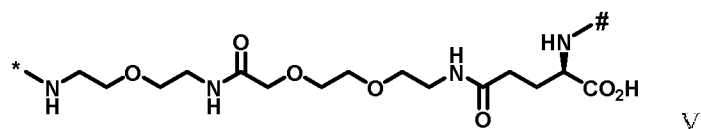
4. An albumin binding conjugate, comprising:

a) an extended polypeptide (XTEN) comprising four cysteine residues, wherein the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity or is identical to a sequence set forth in Table 1, when optimally aligned;

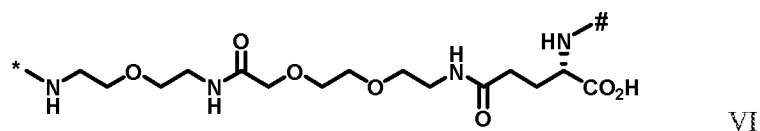
b) at least four linker moieties, each of which has the structure of formula II



c) at least four soluble bridge moieties, each of which has the structure of formula V

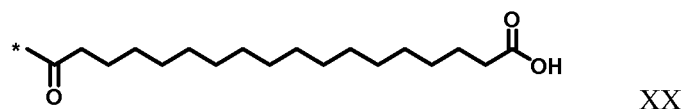


or formula VI



and

d) at least four carboxylic acid moieties, each of which has the structure of formula XX



wherein the XTEN, four linker moieties, four soluble bridge moieties, and four carboxylic acid moieties are configured according to the configuration set forth in FIG. 1C.

5. The albumin binding conjugate of any one of claims 1-4, wherein the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity, or is identical to sequence LX-31 or LX-40 set forth in Table 1.

6. The albumin binding conjugate of claim 5, wherein the XTEN is LX-40 and the composition is configured according to the structure set forth in FIG. 4.

7. The albumin binding conjugate of claim 5, wherein the XTEN is LX-31 and the composition is configured according to the structure set forth in FIG. 14.

8. The albumin binding conjugate of any one of the preceding claims, further comprising a single atom residue attached to the N-terminus of the XTEN, wherein the single atom residue is carbon, nitrogen, sulfur or oxygen.

9. The albumin binding conjugate of any one of the preceding claims, comprising a first therapeutic protein.

10. The albumin binding conjugate of claim 9, wherein the first therapeutic protein is selected from the group consisting of cytokines, interleukins, growth factors, growth hormones, endocrine hormones, exocrine hormones, coagulation factors, glucose-regulating peptides, enzymes, receptor agonists, receptor antagonists, kinases, antibodies, antibody fragments and toxins.

11. The albumin binding conjugate of claim 9, wherein the first therapeutic protein is selected from the group consisting of the therapeutic proteins of Table 4.

12. The albumin binding conjugate of any one of claims 1-8, comprising a first therapeutic drug.

13. The albumin binding conjugate of claim 12, wherein the first therapeutic drug is selected from the group consisting of hypnotics, sedatives, psychic energizers, tranquilizers, respiratory drugs, anticonvulsants, muscle relaxants, antiparkinson agents, analgesics, anti-inflammatories, antianxiety drugs, anxiolytics, appetite suppressants, antimigraine agents, muscle contractants, anti-infectives, antiarthritics, antimalarials, antiemetics, anepileptics, bronchodilators, anti-cancer agents, antithrombotic agents, antihypertensives, cardiovascular drugs, antiarrhythmics, antioxidants, anti-asthma agents,

hormonal agents, sympathomimetics, diuretics, lipid regulating agents, antiandrogenic agents, antiparasitics, anticoagulants, neoplastics, antineoplastics, hypoglycemics, antienteritis agents, diagnostic agents, contrasting agents, and radioactive imaging agents.

14. The albumin binding conjugate of claim 12, wherein the first therapeutic drug is selected from the group consisting of the drugs of Table 5.

15. The albumin binding conjugate of any one of claims 9-11 and 12-14 wherein the albumin binding conjugate has a terminal half-life when administered to a subject that is at least 2-fold, or at least 3-fold, or at least 4-fold, or at least 5-fold, or at least 6-fold, or at least 7-fold, or at least 8-fold, or at least 9-fold, or at least 10-fold longer compared to an unmodified first therapeutic protein or an unmodified first therapeutic drug when administered to a subject at a comparable molar dose.

16. The albumin binding conjugate of any one of claims 9-11 and 12-14, wherein the albumin binding conjugate has a terminal half-life when administered to a subject that is at least 12 h, or at least 24 h, or at least 36 h, or at least 48 h, or at least 72 h, or at least 96 h, or at least 120 h, or at least 144 h, or at least 7 days, or at least 10 days, or at least 14 days, or at least 21 days.

17. The albumin binding conjugate of any one of the preceding claims, wherein the albumin binding conjugate binds to human serum albumin in an in vitro assay with a K_d of less than 1×10^{-4} M, or a K_d of less than 3.3×10^{-4} M, or a K_d of less than 1×10^{-5} M, or a K_d of less than 3.3×10^{-5} M, or a K_d of less than 1×10^{-6} M, or a K_d of less than 3.3×10^{-6} M, or a K_d of less than 1×10^{-7} M, or a K_d of less than 3.3×10^{-7} M, or a K_d of less than 1×10^{-8} M, or a K_d of less than 3.3×10^{-8} M, or a K_d of less than 1×10^{-9} M, or a K_d of less than 1×10^{-10} M.

18. The albumin binding conjugate of any one of claims 9-11 and 12-14 wherein the albumin binding conjugate binds to human serum albumin in an in vitro assay with at least 2-fold, or at least 3-fold, or at least 4-fold, or at least 5-fold, or at least 6-fold, or at least 7-fold, or at least 8-fold, or at least 9-fold, or at least 10-fold, or at least 20-fold, or at least 50-fold, or at least 100-fold greater affinity compared to a binding affinity of a first therapeutic protein or a first therapeutic drug conjugated to a single carboxylic acid moiety that is comparable to a carboxylic acid moiety incorporated into the albumin binding conjugate.

19. The albumin binding conjugate of any one of claims 9-11 and 12-14, wherein the conjugate comprising the first therapeutic protein or the first therapeutic drug binds to human serum albumin with a K_d of 10^{-1} M or less, or a K_d of 10^{-2} M or less, or a K_d of 10^{-3} M or less, in an in vitro assay compared to a K_d of a first therapeutic protein or a first therapeutic drug conjugated to a single carboxylic acid moiety comparable to the carboxylic acid moieties incorporated into the albumin binding conjugate.

20. The albumin binding conjugate of any one of claims 9-11 and 12-14, wherein the conjugate is capable of being formulated in a saline or buffer solution at a molar concentration that is at least 10%, or at least 20%, or at least 30%, or at least 40%, or at least 50% higher than that which can be achieved for a first therapeutic protein or a first therapeutic drug conjugated to a single carboxylic acid moiety comparable to the carboxylic acid moieties incorporated into the albumin binding conjugate.

21. A pharmaceutical composition comprising the albumin binding conjugate of any one of claims 9-11 and 12-14, and optionally, suitable formulations of carrier, stabilizers and/or excipients.
22. The pharmaceutical composition of claim 21, wherein the composition is suitable for subcutaneous, intravenous, or intramuscular administration.
23. The pharmaceutical composition of claim 22, wherein the composition is in a liquid form.
24. The pharmaceutical composition of claim 23, wherein the composition is in a pre-filled syringe for a single injection.
25. The pharmaceutical composition of claim 23, wherein the composition is formulated in a saline or buffer solution at a concentration of at least 1 mM, or at least 2 mM, or at least 3 mM, or at least 4 mM, or at least 5 mM, or at least 6 mM, or at least 7 mM, or at least 8 mM, or at least 9 mM, or at least 10 mM and wherein the saline or buffer solution comprising the composition can be passed through a 25, 26, 27, 28, 29, 30, 31, or 32 gauge needle for intravenous, intramuscular, intraarticular, or subcutaneous administration.
26. The pharmaceutical composition of claim 23, wherein the composition is formulated in a saline or buffer solution at a concentration of at least 1 mM, or at least 2 mM, or at least 3 mM, or at least 4 mM, or at least 5 mM, or at least 6 mM, or at least 7 mM, or at least 8 mM, or at least 9 mM, or at least 10 mM and has a viscosity of less than 10 cP, or less than 15 cP, or less than 20 cP, or less than 25 cP, or less than 30 cP.
27. Use of the albumin binding conjugate of any one of claims 9-11 and 12-14, in the preparation of a medicament for use in a subject in need thereof.
28. A method of treating a disease in a subject, comprising administering to a subject with a disease one or more therapeutically effective doses of the pharmaceutical composition of any one of claims 21-26.
29. The method of claim 28, wherein the therapeutically effective dose is administered every week, every two weeks, every three weeks, or monthly.
30. The method of claim 28 or 29, wherein the therapeutically effective dose is administered subcutaneously, intravenously, intraperitoneally, or intramuscularly.
31. A pharmaceutical composition for use in a treatment regimen for the treatment of a disease, comprising the pharmaceutical composition of any one of claims 21-26 for administration to a subject with the disease in two or more consecutive doses using a therapeutically effective dose.
32. A kit comprising the pharmaceutical composition of any one of claims 21-26, a container and a label or package insert on or associated with the container.
33. An extended polypeptide (XTEN) comprising at least 2, at least 3, or at least 4 cysteine residues, wherein the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity or is identical to a sequence set forth in Table 1, when optimally aligned.
34. An isolated nucleic acid comprising a polynucleotide sequence encoding the XTEN of

claim 33 or a complement thereof.

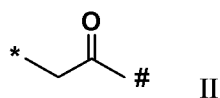
35. An expression vector comprising the nucleic acid of claim 34, wherein the vector further comprises a recombinant regulatory sequence operably linked to the nucleic acid.

36. An isolated prokaryotic host cell, comprising the expression vector of claim 35.

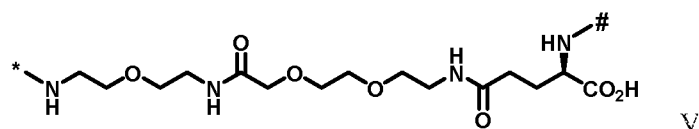
37. An albumin binding conjugate comprising:

a) an extended polypeptide (XTEN) having three cysteine residues, wherein the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity, or is identical to a sequence set forth in Table 1, when optimally aligned;

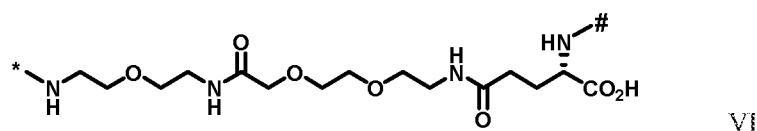
b) three linker moieties, each of which has the structure of formula II



c) three soluble bridge moieties, each of which has the structure of formula V

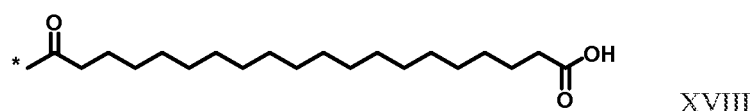


or formula VI

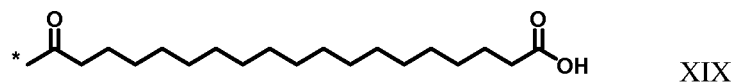


and

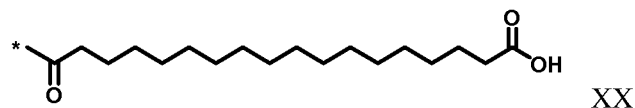
d) three carboxylic acid moieties, each of which has the structure of formula XVIII



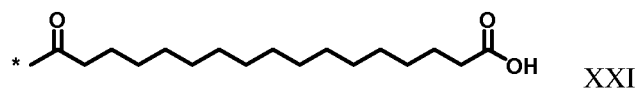
or formula XIX



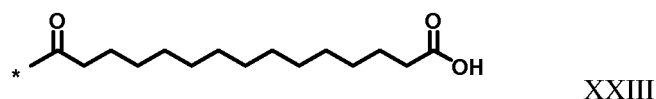
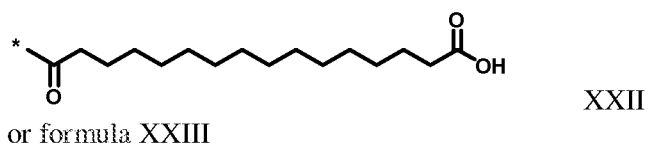
or formula XX



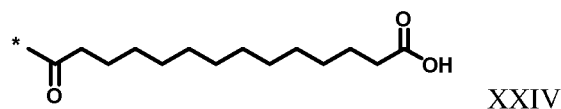
or formula XXI



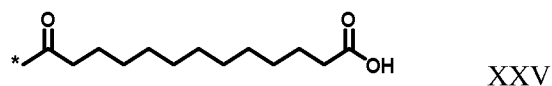
or formula XXII



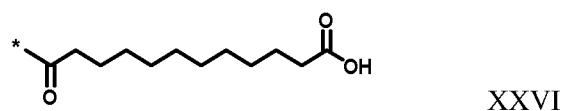
or formula XXIV



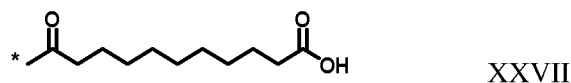
or formula XXV



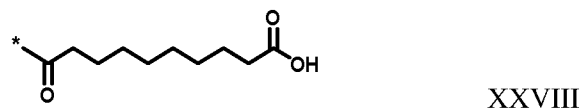
or formula XXVI



or formula XXVII



or formula XXVIII

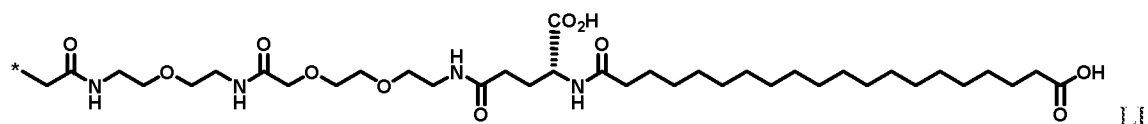


wherein the XTEN, three linker moieties, three soluble bridge moieties, and three carboxylic acid moieties are configured according the configuration set forth in FIG. 1B.

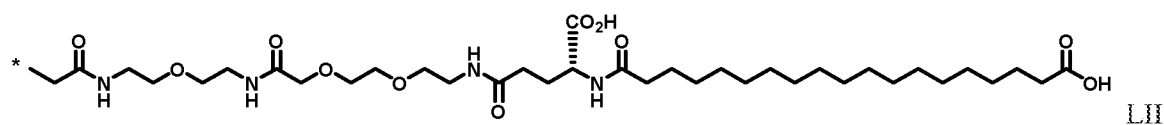
38. An albumin binding conjugate comprising:

a. an extended polypeptide (XTEN) having three cysteine residues, wherein the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity, or is identical to a sequence set forth in Table 1, when optimally aligned;

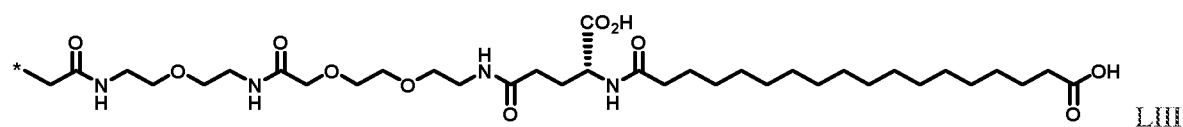
b. three albumin binding subunits, each of which has the structure of formula LI



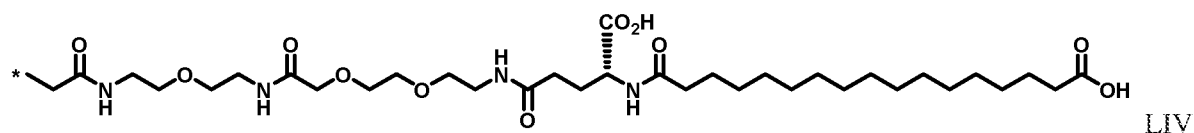
or formula LII



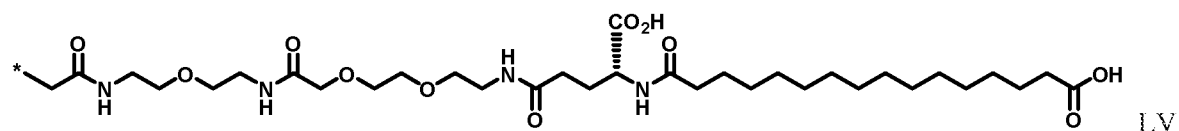
or formula LIII



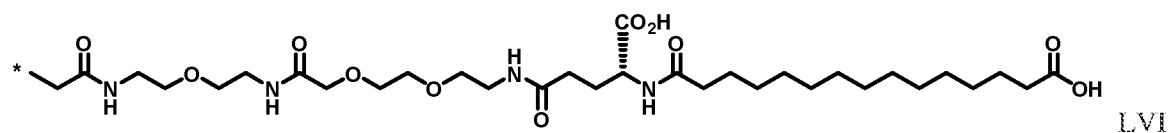
or formula LIV



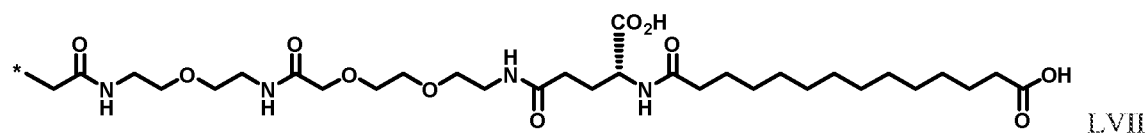
or formula LV



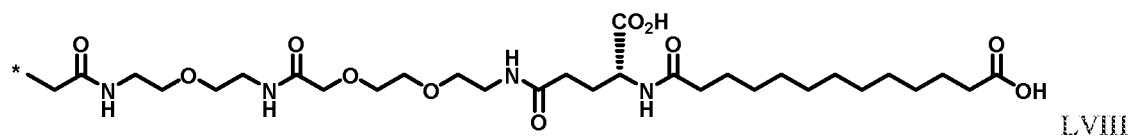
or formula LVI



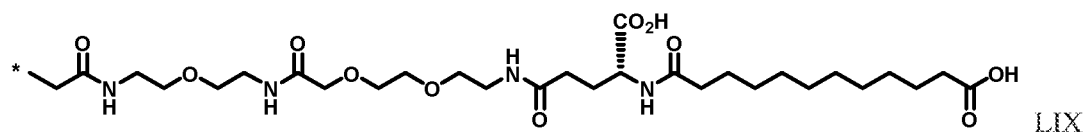
or formula LVII



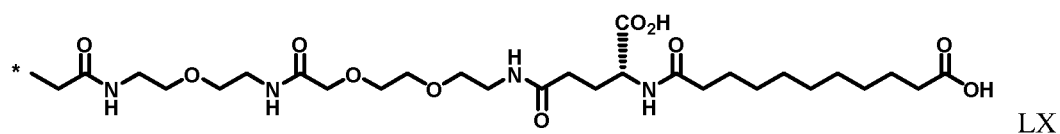
or formula LVIII



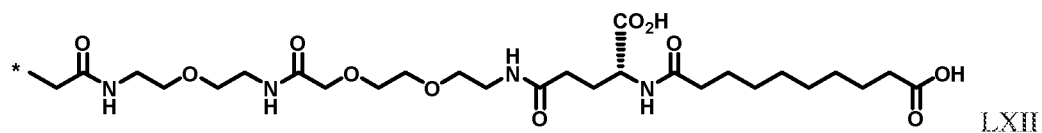
or formula LIX



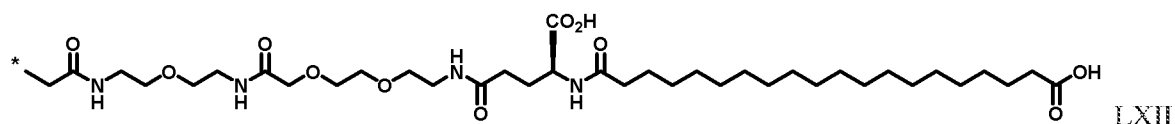
or formula LX



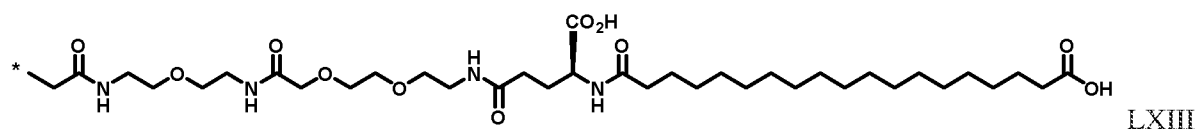
or formula LXI



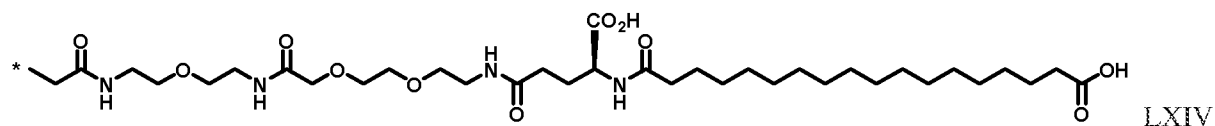
or formula LXII



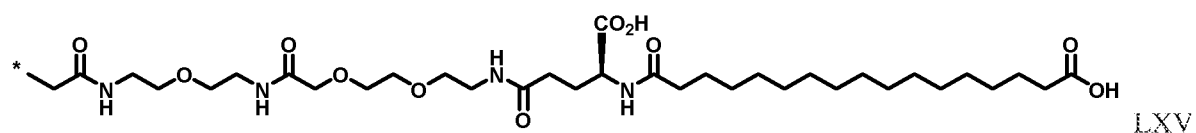
or formula LXIII



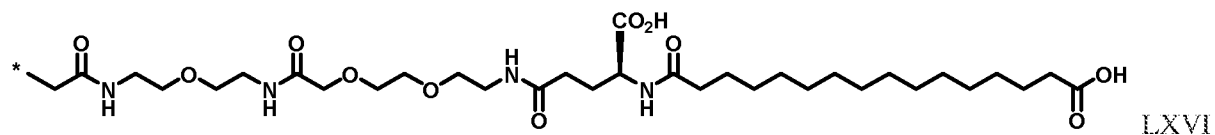
or formula LXIV



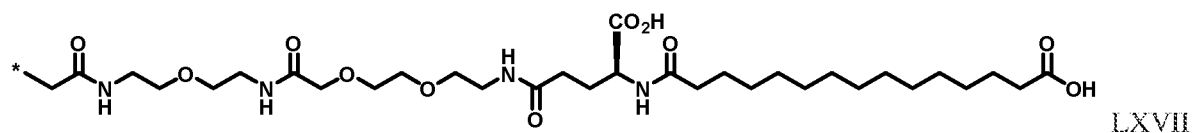
or formula LXV



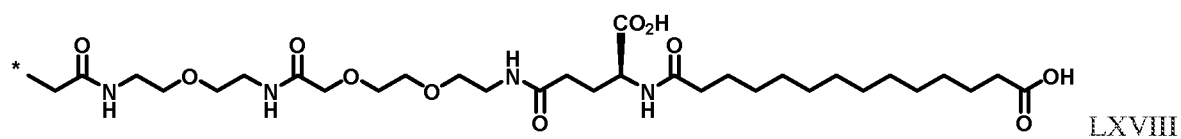
or formula LXVI



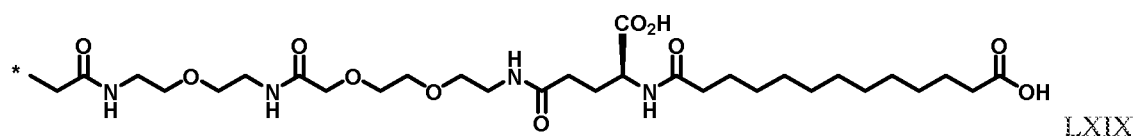
or formula LXVII



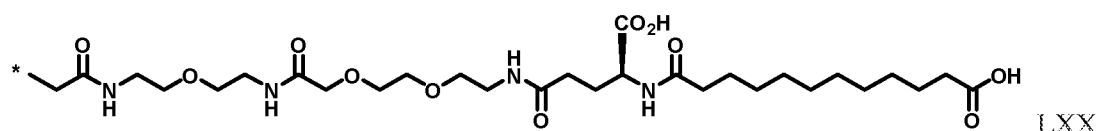
or formula LXVIII



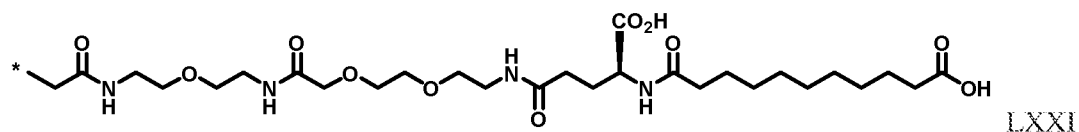
or formula LXIX



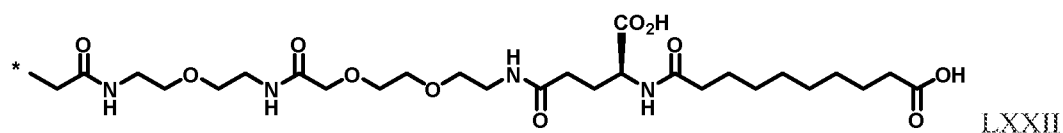
or formula LXX



or formula LXXI



or formula LXXII



wherein each individual albumin binding subunit is linked to a thiol group of a cysteine residue of the XTEN.

39. The albumin binding conjugate of claim 37 or claim 38, wherein the XTEN exhibits at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity, or is identical to sequence LX-31 or LX-40 set forth in Table 1.

40. The albumin binding conjugate of claim 39, wherein the XTEN is LX-40.

41. The albumin binding conjugate of claim 39, wherein the XTEN is LX-31.

42. The albumin binding conjugate of any one of claims 37-41, further comprising a single atom residue attached to the N-terminus of the XTEN, wherein the single atom residue is carbon, nitrogen, sulfur or oxygen.

43. The albumin binding conjugate of any one of claims 37-42, comprising a first therapeutic protein.

44. The albumin binding conjugate of claim 43, wherein the first therapeutic protein is selected from the group consisting of cytokines, interleukins, growth factors, growth hormones, endocrine hormones, exocrine hormones, coagulation factors, glucose-regulating peptides, enzymes, receptor agonists, receptor antagonists, kinases, antibodies, antibody fragments and toxins.

45. The albumin binding conjugate of claim 44, wherein the therapeutic protein is selected from the group consisting of the therapeutic proteins of Table 4.

46. The albumin binding conjugate of any one of claims 37-42, comprising a first therapeutic drug.

47. The albumin binding conjugate of claim 46, wherein the first therapeutic drug is selected from the group consisting of hypnotics, sedatives, psychic energizers, tranquilizers, respiratory drugs, anticonvulsants, muscle relaxants, antiparkinson agents, analgesics, anti-inflammatories, antianxiety

drugs, anxiolytics, appetite suppressants, antimigraine agents, muscle contractants, anti-infectives, antiarthritics, antimalarials, antiemetics, anepileptics, bronchodilators, anti-cancer agents, antithrombotic agents, antihypertensives, cardiovascular drugs, antiarrhythmics, antioxidants, anti-asthma agents, hormonal agents, sympathomimetics, diuretics, lipid regulating agents, antiandrogenic agents, antiparasitics, anticoagulants, neoplastics, antineoplastics, hypoglycemics, antienteritis agents, diagnostic agents, contrasting agents, and radioactive imaging agents.

48. The albumin binding conjugate of claim 46, wherein the first therapeutic drug is selected from the group consisting of the drugs of Table 5.

49. The albumin binding conjugate of any one of claims 43-45 and 46-48, wherein the albumin binding conjugate has a terminal half-life when administered to a subject that is at least 2-fold, or at least 3-fold, or at least 4-fold, or at least 5-fold, or at least 6-fold, or at least 7-fold, or at least 8-fold, or at least 9-fold, or at least 10-fold longer compared to an unmodified first therapeutic protein or an unmodified first therapeutic drug.

50. The albumin binding conjugate of any one of claims 43-45 and 46-48, wherein the conjugate has a terminal half-life when administered to a subject of at least 12 h, or at least 24 h, or at least 36 h, or at least 48 h, or at least 72 h, or at least 96 h, or at least 120 h, or at least 144 h, or at least 7 days, or at least 10 days, or at least 14 days, or at least 21 days.

51. The albumin binding conjugate of any one of the preceding claims, wherein the albumin binding conjugate binds to human serum albumin in an in vitro assay with a K_d of less than 1×10^{-4} M, or a K_d less than 3.3×10^{-4} M, or a K_d less than 1×10^{-5} M, or a K_d less than 3.3×10^{-5} M, or a K_d less than 1×10^{-6} M, or a K_d less than 3.3×10^{-6} M, or a K_d less than 1×10^{-7} M, or a K_d less than 3.3×10^{-7} M, or a K_d less than 1×10^{-8} M, or a K_d less than 3.3×10^{-8} M, or a K_d less than 1×10^{-9} M, or a K_d less than 1×10^{-10} M.

52. The albumin binding conjugate of any one of claims 43-45 and 46-48, wherein the albumin binding conjugate binds to human serum albumin in an in vitro assay with at least 2-fold, or at least 3-fold, or at least 4-fold, or at least 5-fold, or at least 6-fold, or at least 7-fold, or at least 8-fold, or at least 9-fold, or at least 10-fold, or at least 20-fold, or at least 50-fold, or at least 100-fold greater affinity compared to a first therapeutic protein or a first therapeutic drug conjugated to a single carboxylic acid moiety comparable to the carboxylic acid moieties incorporated into the albumin binding conjugate.

53. The albumin binding conjugate of any one of claims 43-45 and 46-48, wherein the conjugate comprising the first therapeutic protein or the first therapeutic drug binds to human serum albumin with a K_d of 10^{-1} M or less, or a K_d of at least 10^{-2} M or less, or a K_d of 10^{-3} M or less in an in vitro assay compared to a K_d of a first therapeutic protein or a first therapeutic drug conjugated to a single carboxylic acid moiety comparable to the carboxylic acid moieties incorporated into the albumin binding conjugate.

54. The albumin binding conjugate of any one of claims 43-45 and 46-48, wherein the conjugate is capable of being formulated in a saline or buffer solution at a molar concentration that is at least 10%, or at least 20%, or at least 30%, or at least 40%, or at least 50% higher than that which can be

achieved for a first therapeutic protein or a first therapeutic drug conjugated to a single carboxylic acid moiety comparable to the carboxylic acid moieties incorporated into the albumin binding conjugate.

55. A pharmaceutical composition comprising the albumin binding conjugate of any one of claims 43-45 and 46-48, and optionally, suitable formulations of carrier, stabilizers and/or excipients.

56. The pharmaceutical composition of claim 55, wherein the composition is suitable for subcutaneous, intravenous, or intramuscular administration.

57. The pharmaceutical composition of claim 56, wherein the composition is in a liquid form.

58. The pharmaceutical composition of claim 57, wherein the composition is in a pre-filled syringe for a single injection.

59. The pharmaceutical composition of claim 57, wherein the composition is formulated in a saline or buffer solution at a concentration of at least 1 mM, or at least 2 mM, or at least 3 mM, or at least 4 mM, or at least 5 mM, or at least 6 mM, or at least 7 mM, or at least 8 mM, or at least 9 mM, or at least 10 mM and wherein the saline buffer solution comprising the composition can be passed through a 25, 26, 27, 28, 29, 30, 31, or 32 gauge needle for intravenous, intramuscular, intraarticular, or subcutaneous administration.

60. The pharmaceutical composition of claim 57, wherein the composition is formulated in a saline or buffer solution at a concentration of at least 1 mM, or at least 2 mM, or at least 3 mM, or at least 4 mM, or at least 5 mM, or at least 6 mM, or at least 7 mM, or at least 8 mM, or at least 9 mM, or at least 10 mM and has a viscosity of less than 10 cP, or less than 15 cP, or less than 20 cP, or less than 25 cP, or less than 30 cP.

61. Use of the albumin binding conjugate of any one of claims 43-45 and 46-48, in the preparation of a medicament for use in a subject in need thereof.

62. A method of treating a disease in a subject, comprising administering to a subject with a disease one or more therapeutically effective doses of the pharmaceutical composition of any one of claims 55-60.

63. The method of claim 62, wherein the therapeutically effective dose is administered every week, every two weeks, every three weeks, or monthly.

64. The method of claim 62 or claim 63, wherein the therapeutically effective dose is administered subcutaneously, intravenously, intraperitoneally, or intramuscularly.

65. A pharmaceutical composition for use in a treatment regimen for the treatment of a disease, comprising the pharmaceutical composition of any one of claims 55-60 for administration to a subject with the disease in two or more consecutive doses using a therapeutically effective dose.

1/22

FIG.1A

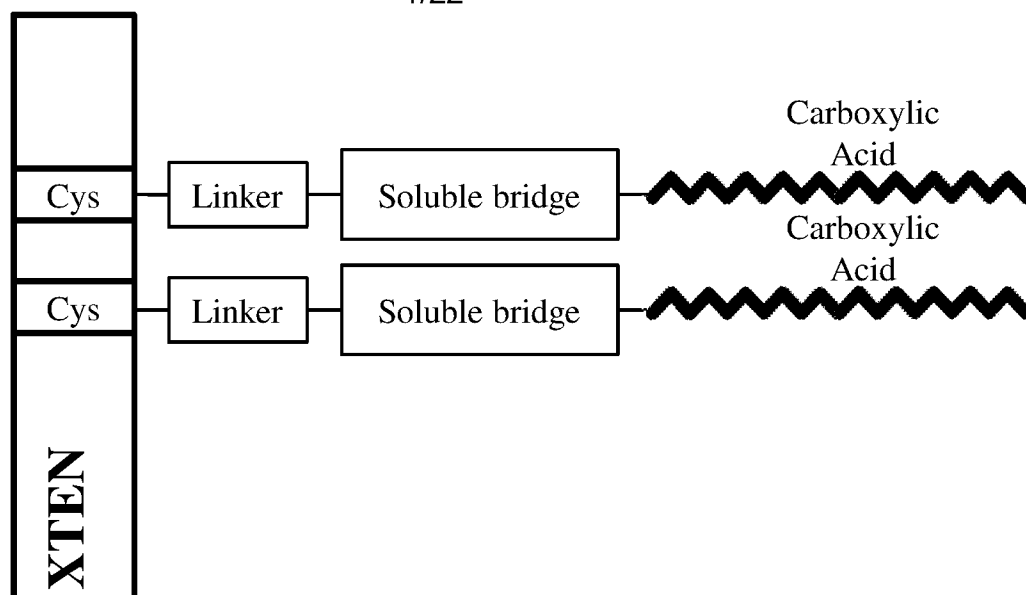


FIG.1B

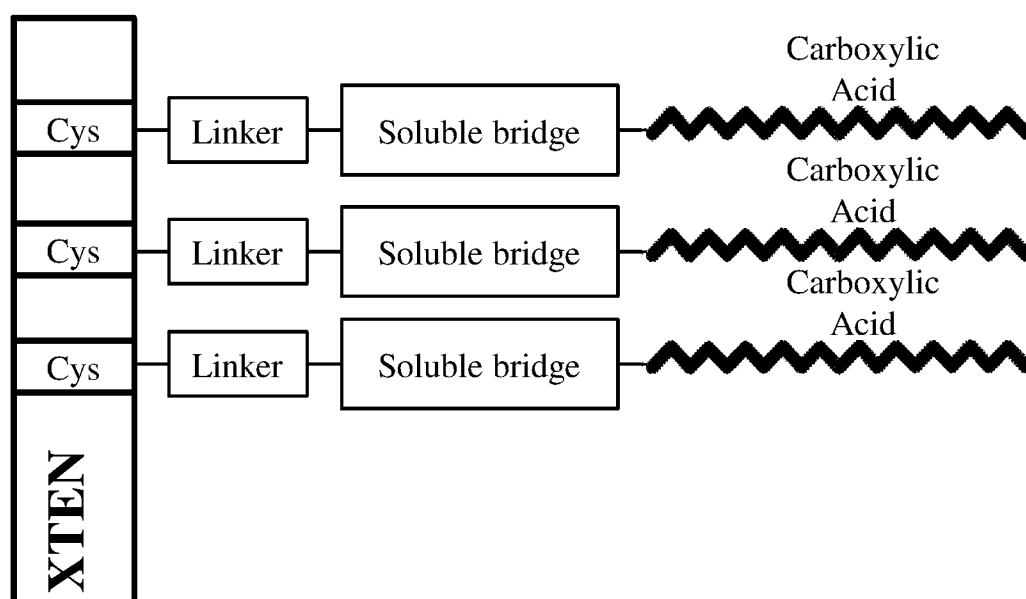


FIG.1C

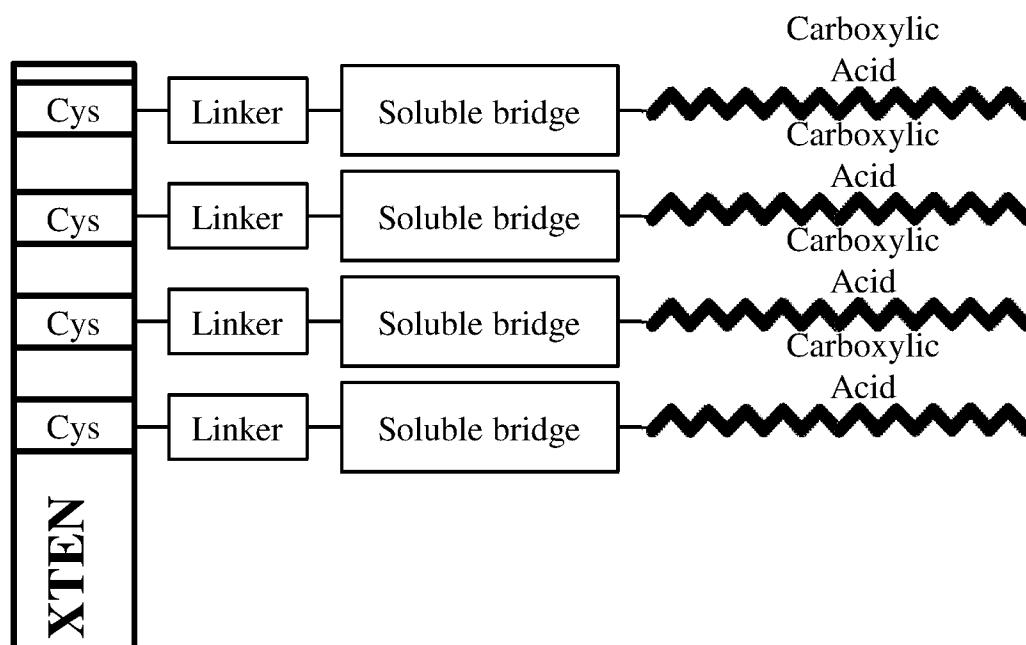


FIG. 1

2/22

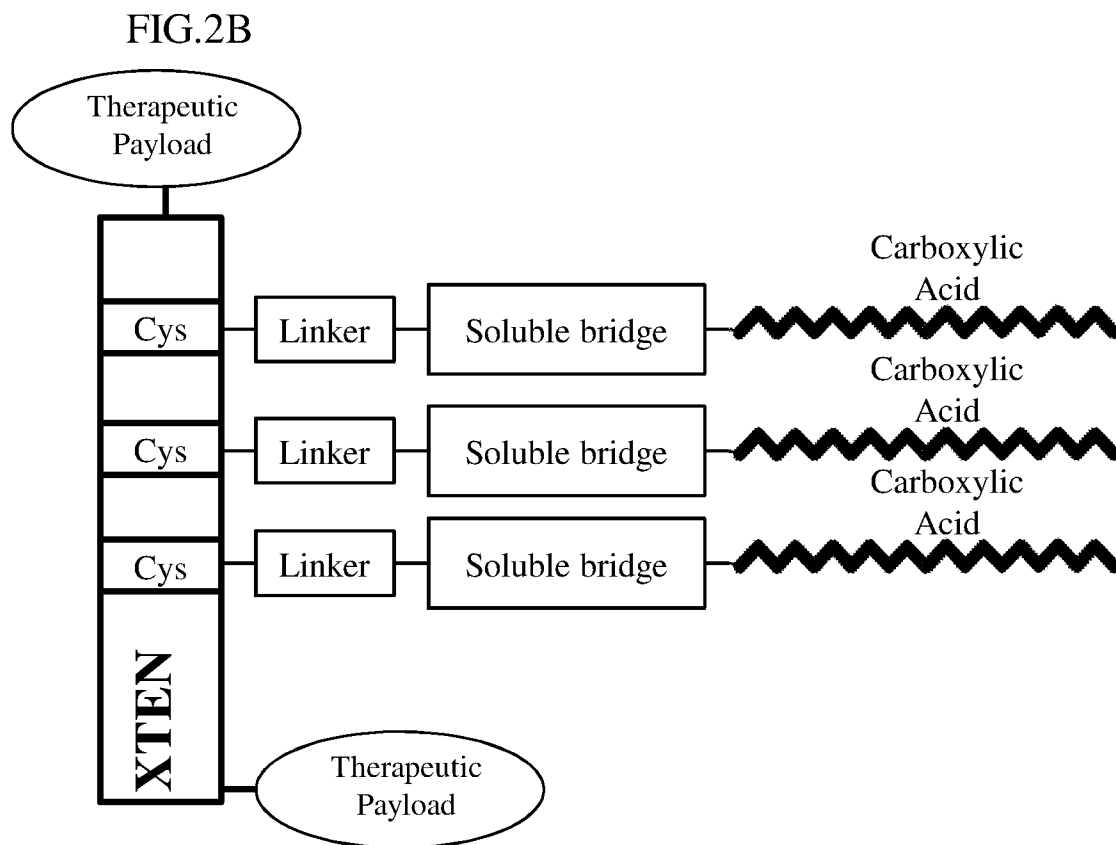
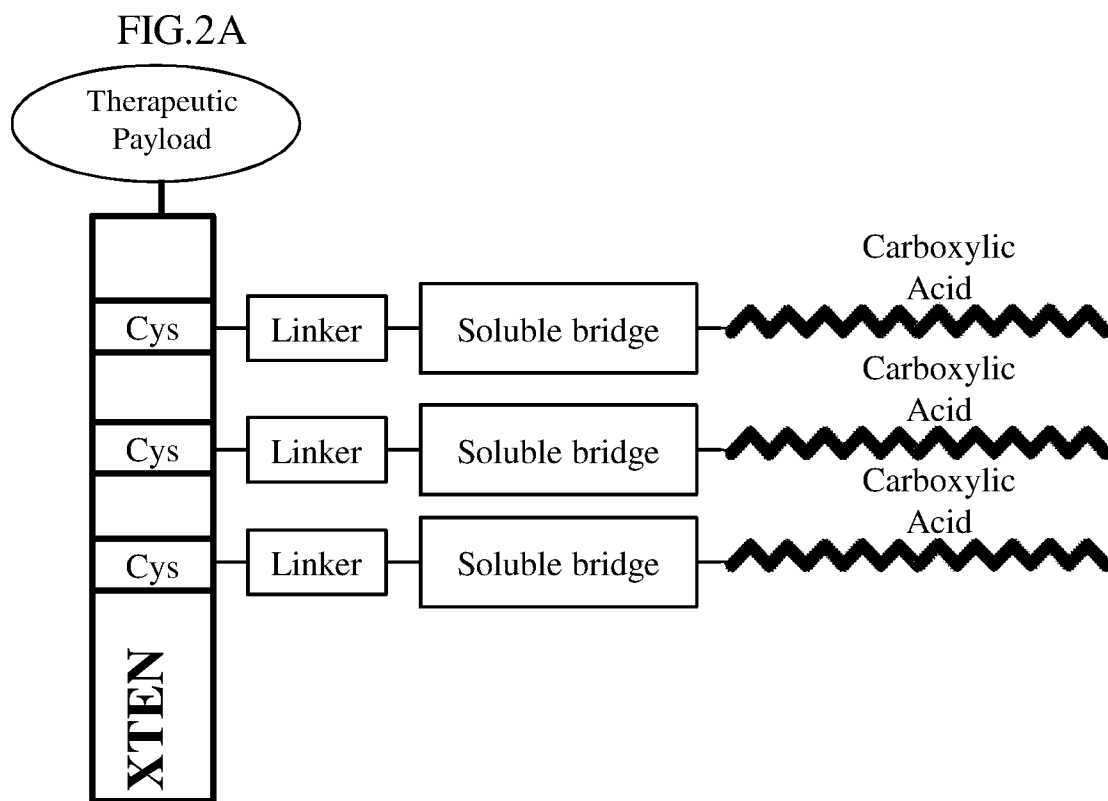


FIG. 2

3/22

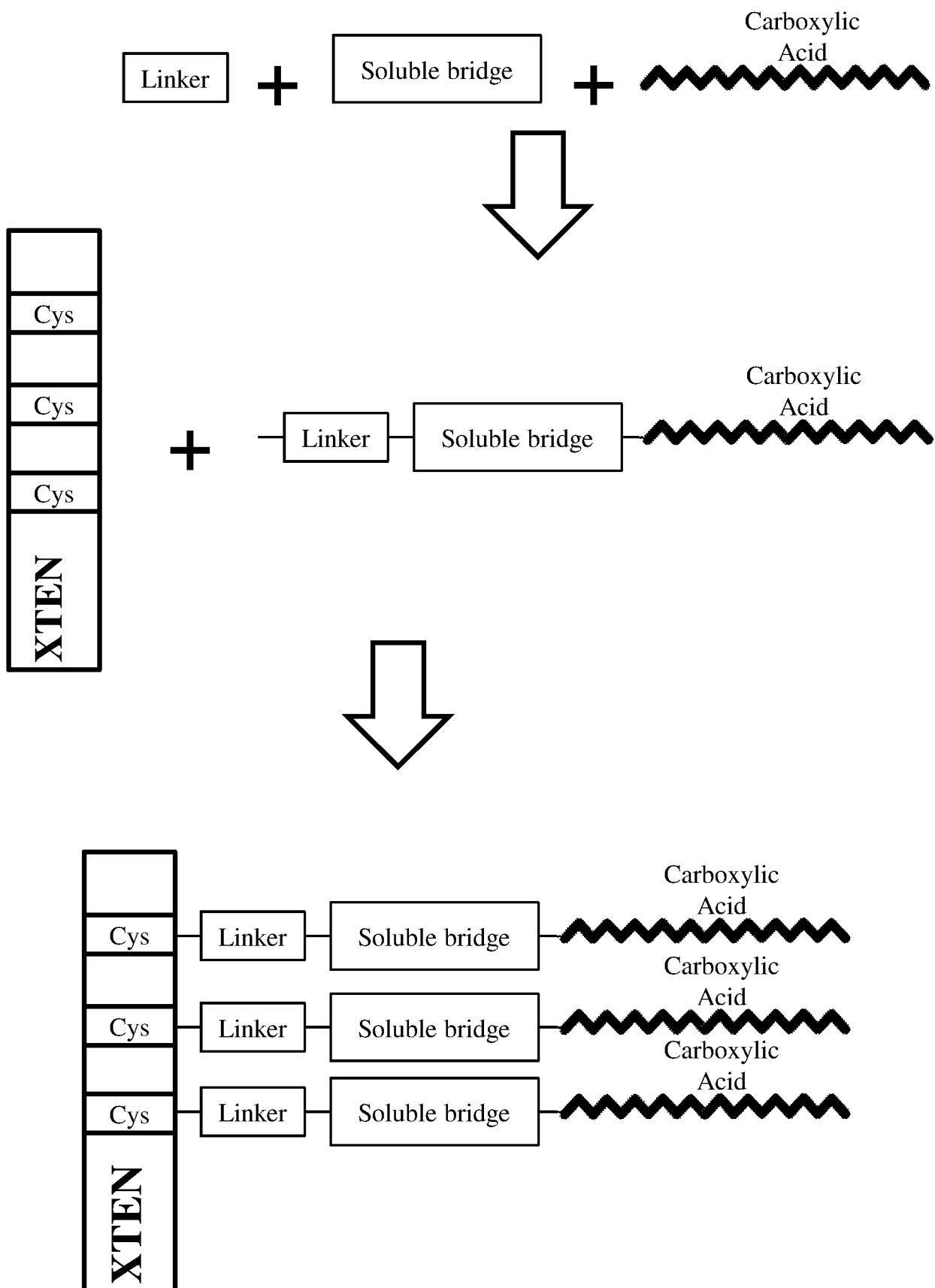
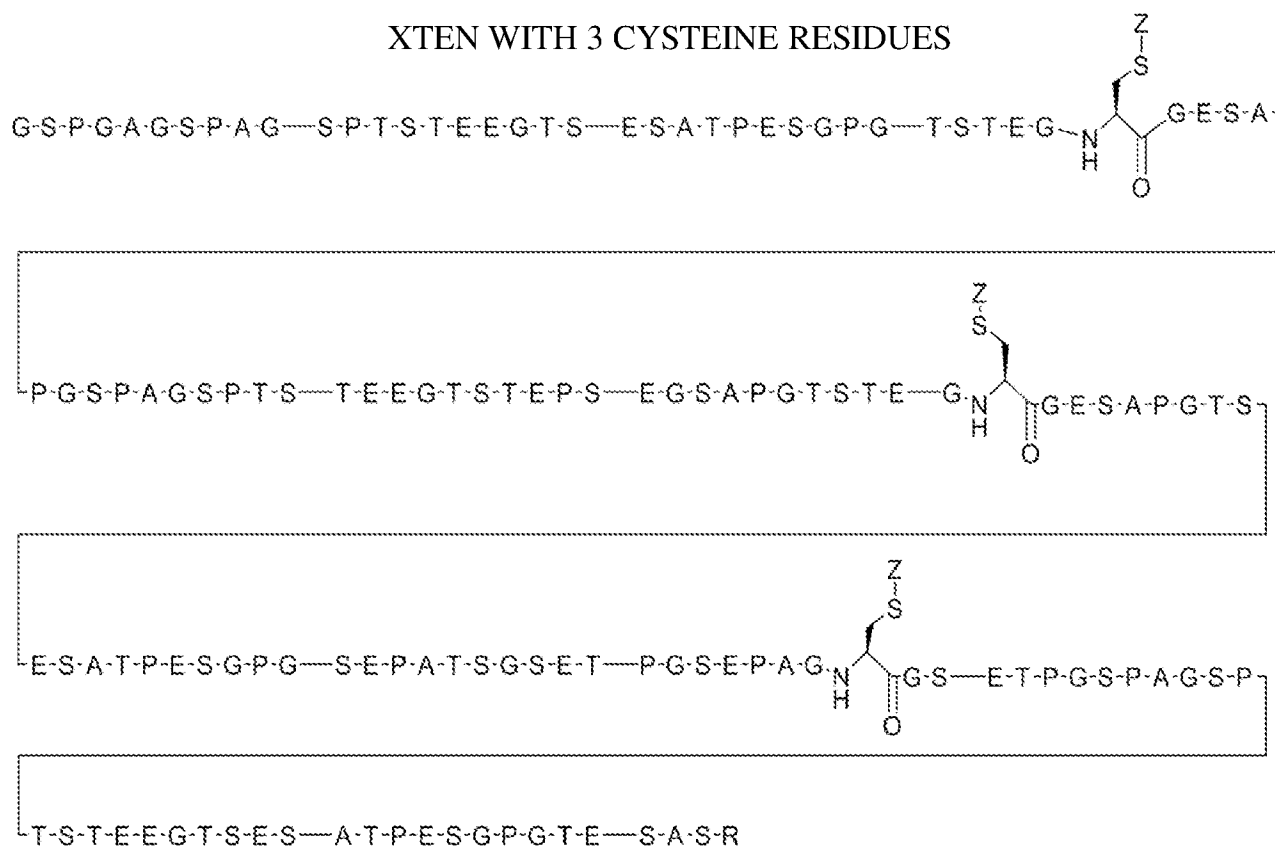
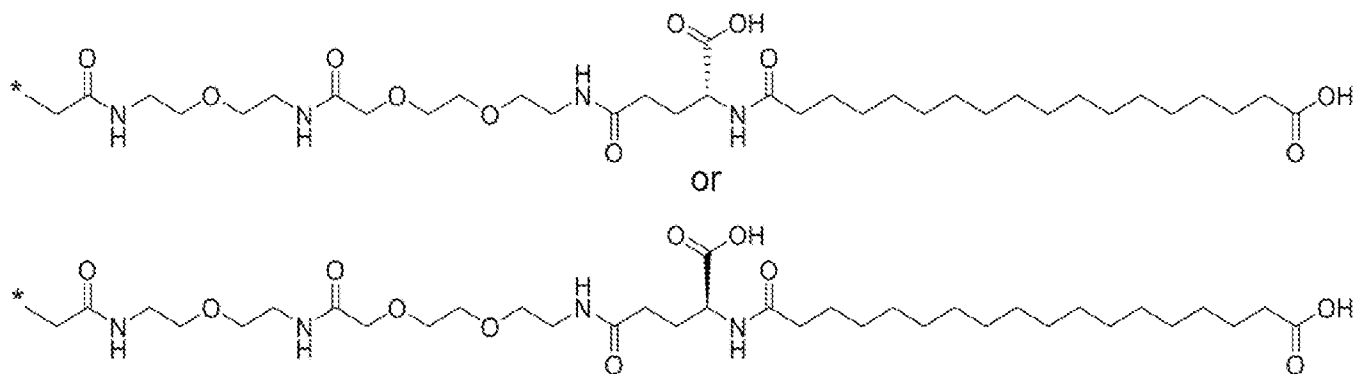


FIG. 3

XTEN WITH 3 CYSTEINE RESIDUES


$$Z =$$

Albumin binding subunit

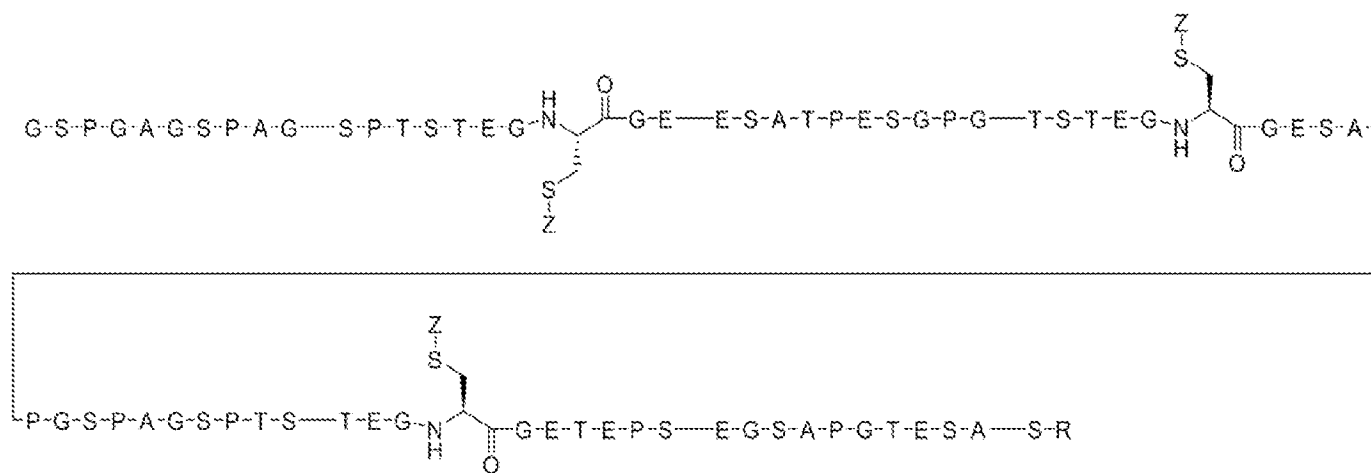


* = conjugation site of cysteine residue

FIG. 4

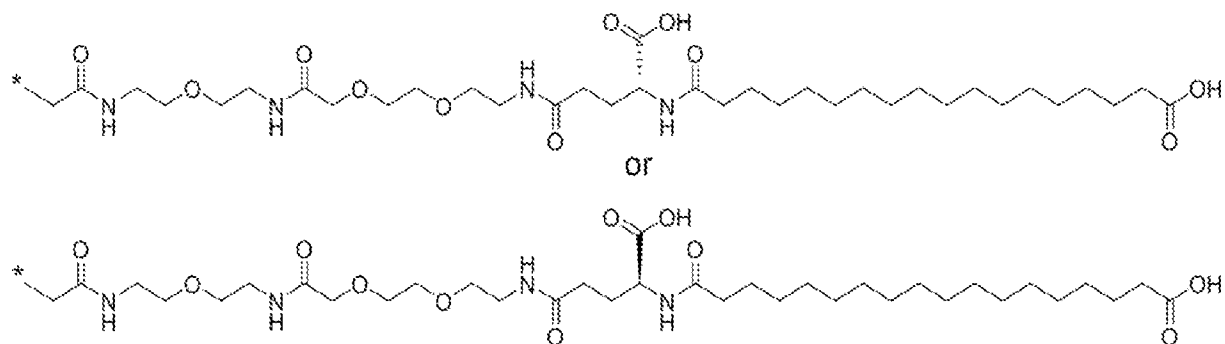
5/22

XTEN WITH 3 CYSTEINE RESIDUES



Z =

Albumin binding subunit



* = conjugation site of cysteine residue

FIG. 5

6/22

XTEN WITH 3 CYSTEINE RESIDUES

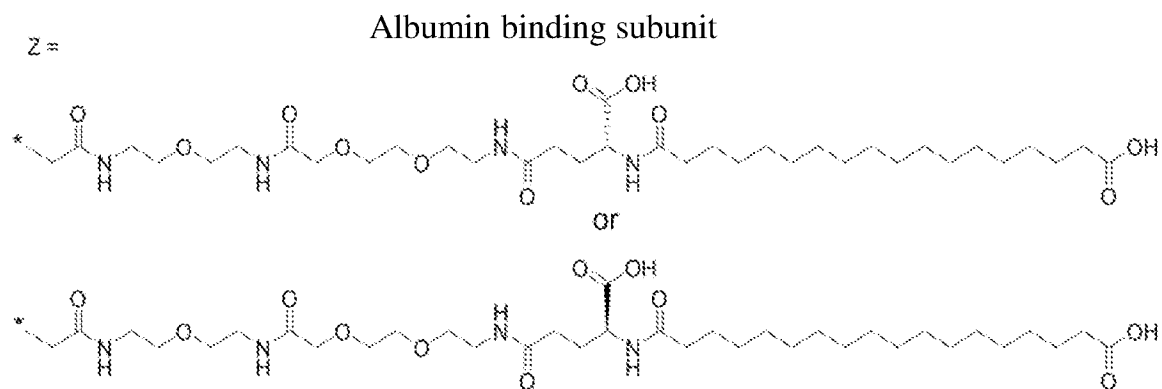
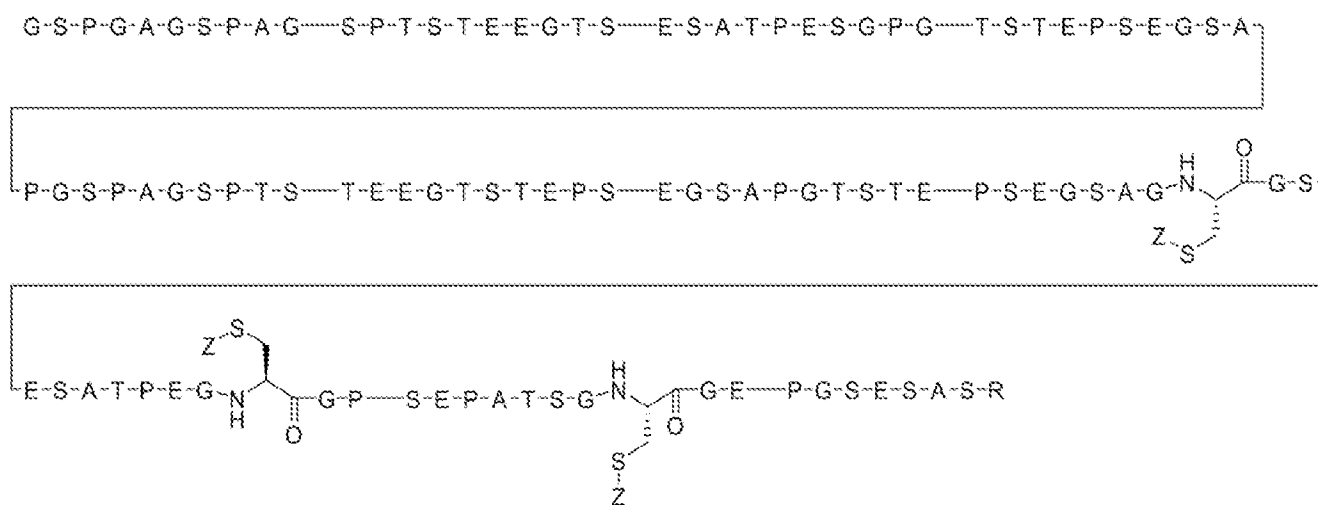


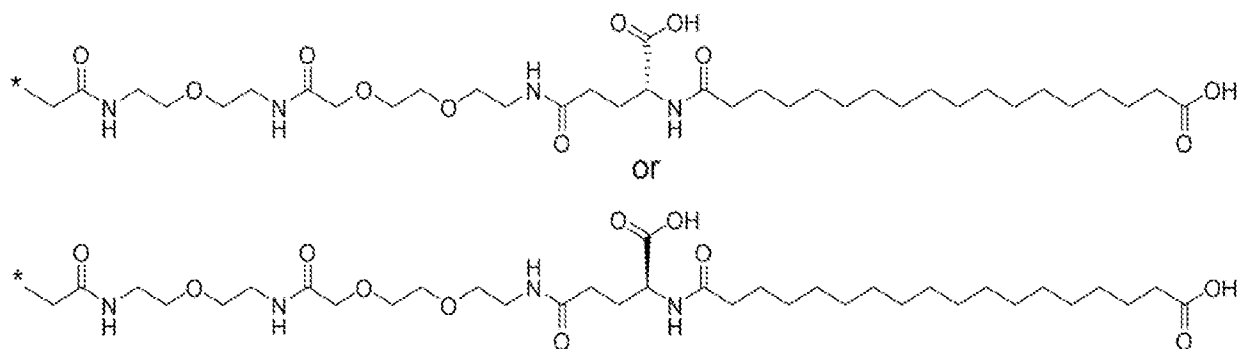
FIG. 6

8/22

XTEN WITH 3 CYSTEINE RESIDUES



Albumin binding subunit

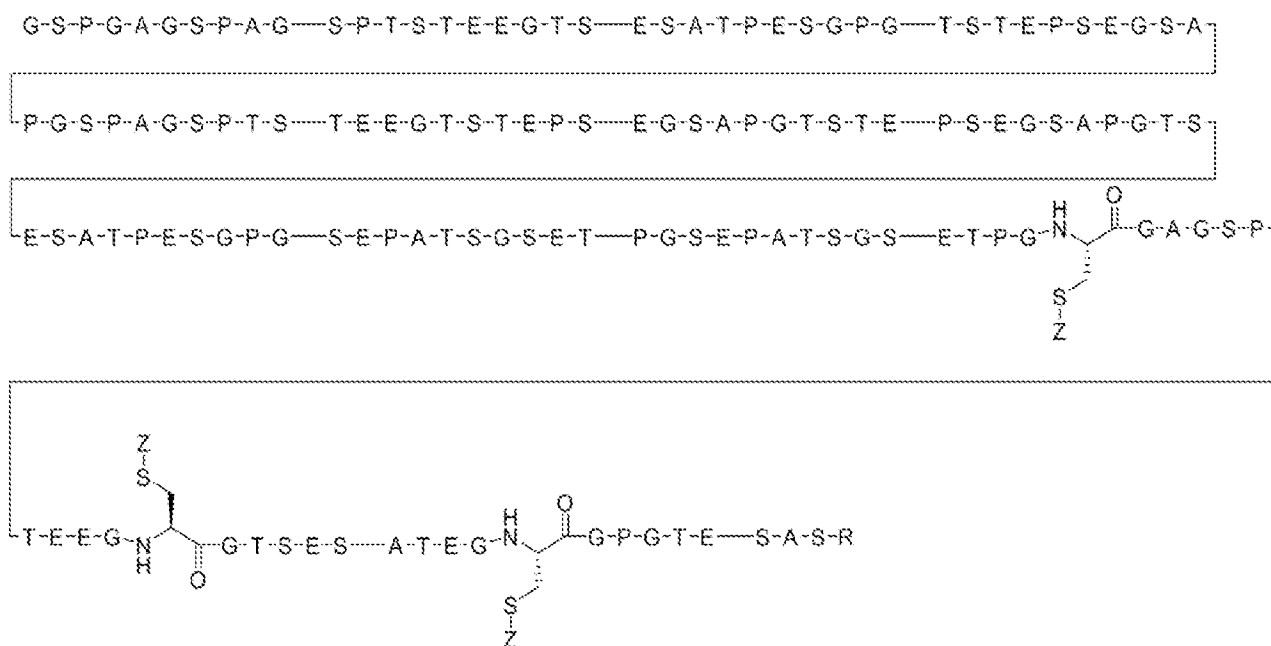
$$Z =$$


* = conjugation site of cysteine residue

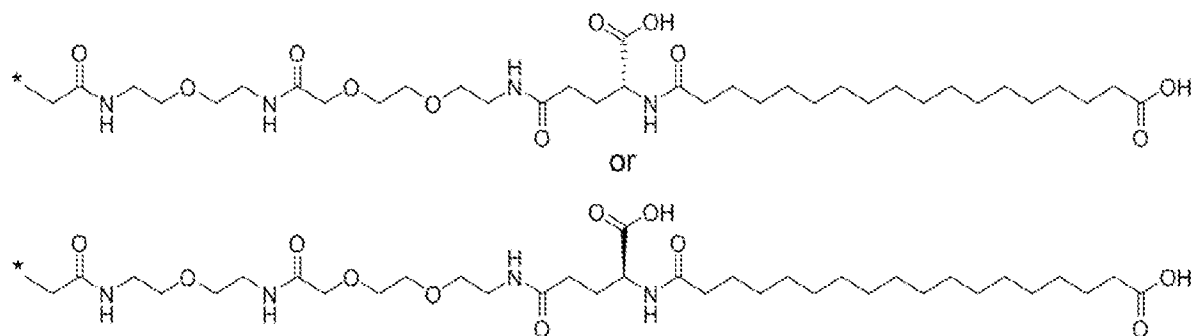
FIG. 8

9/22

XTEN WITH 3 CYSTEINE RESIDUES



Albumin binding subunit

 $z =$ 

* = conjugation site of cysteine residue

FIG. 9

XTEN WITH 3 CYSTEINE RESIDUES

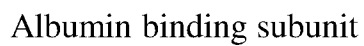
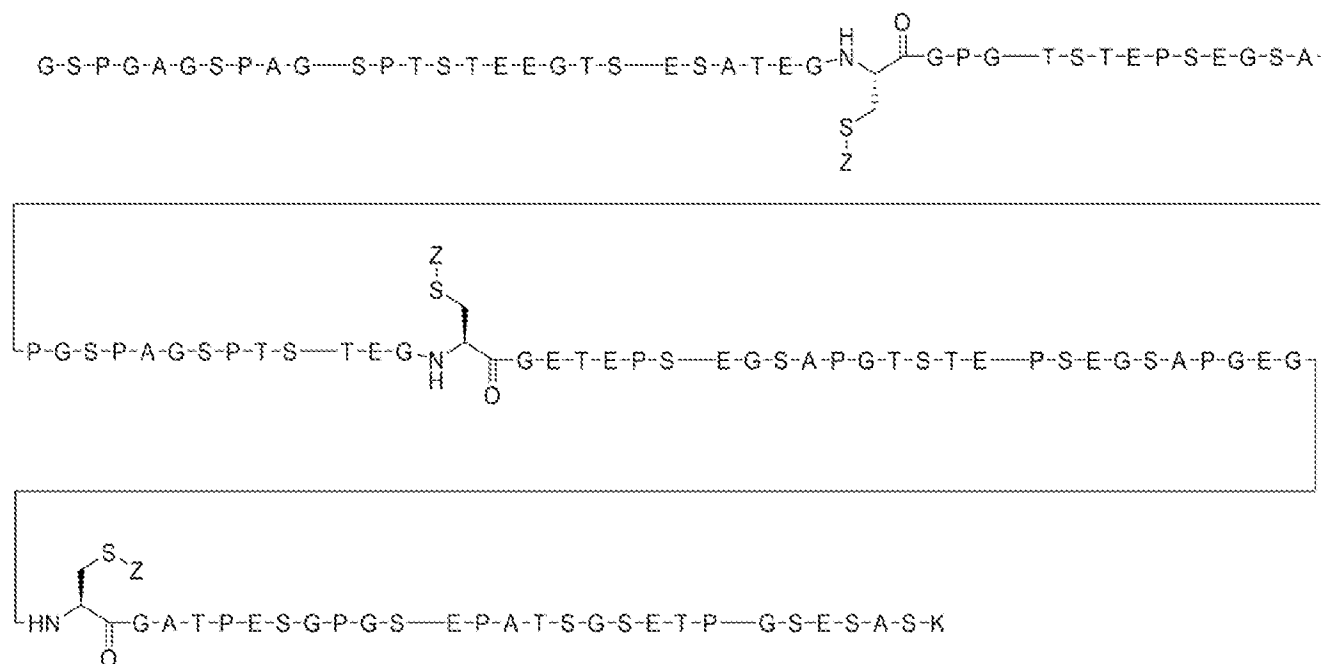




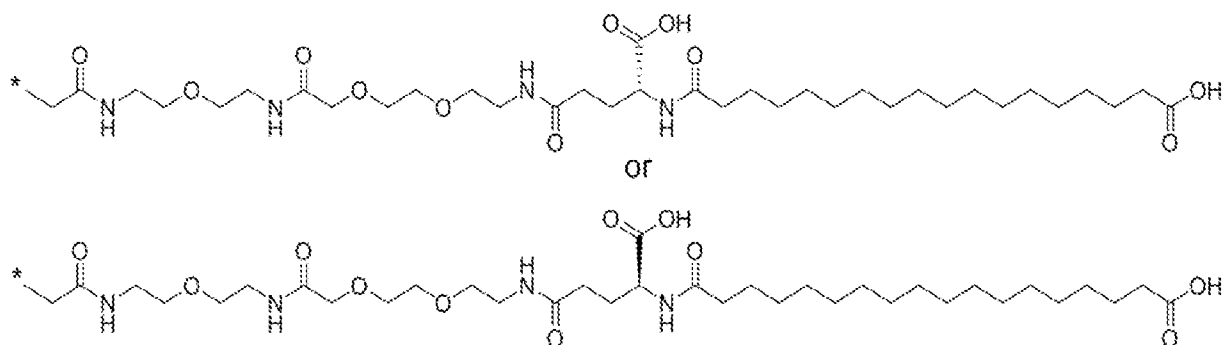
FIG. 10

11/22

XTEN WITH 3 CYSTEINE RESIDUES



Albumin binding subunit

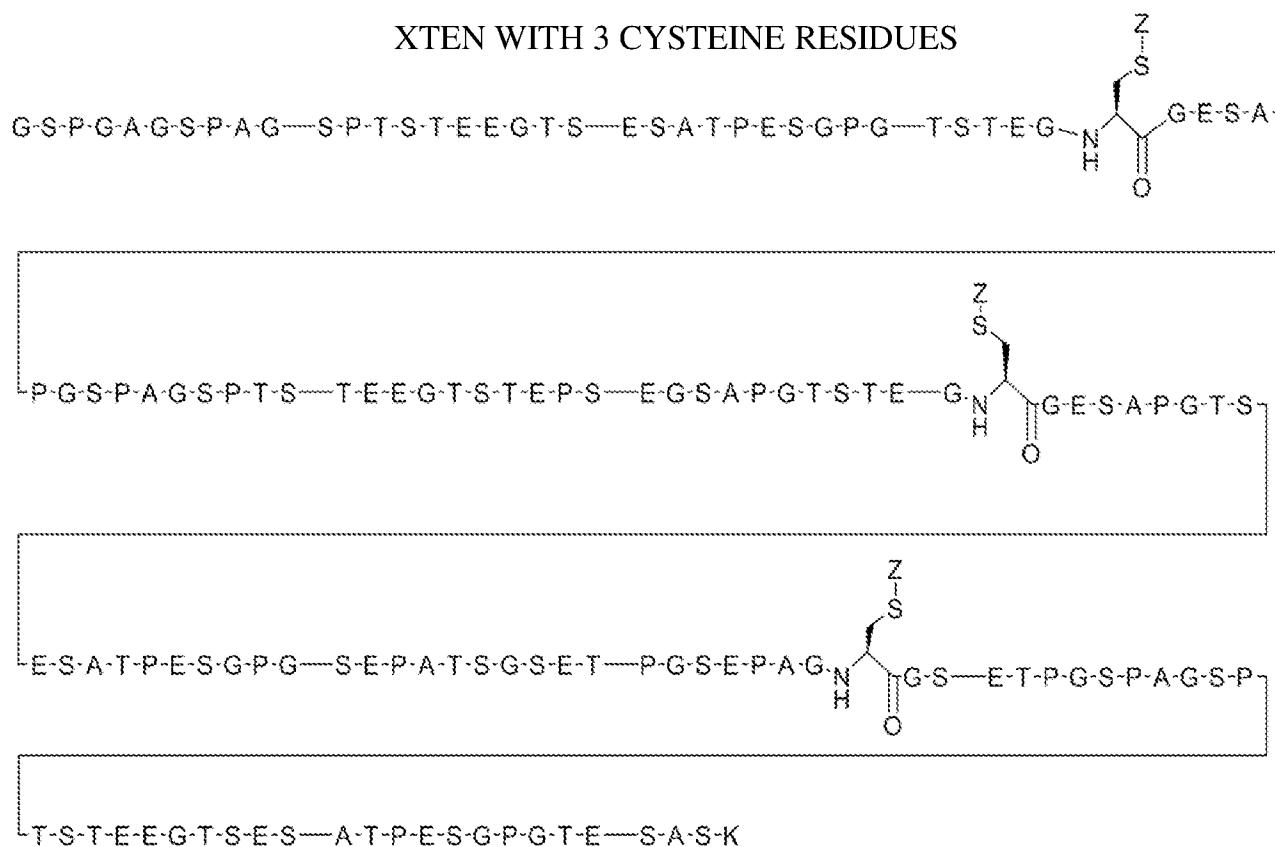
$$Z =$$


* = conjugation site of cysteine residue

FIG. 11

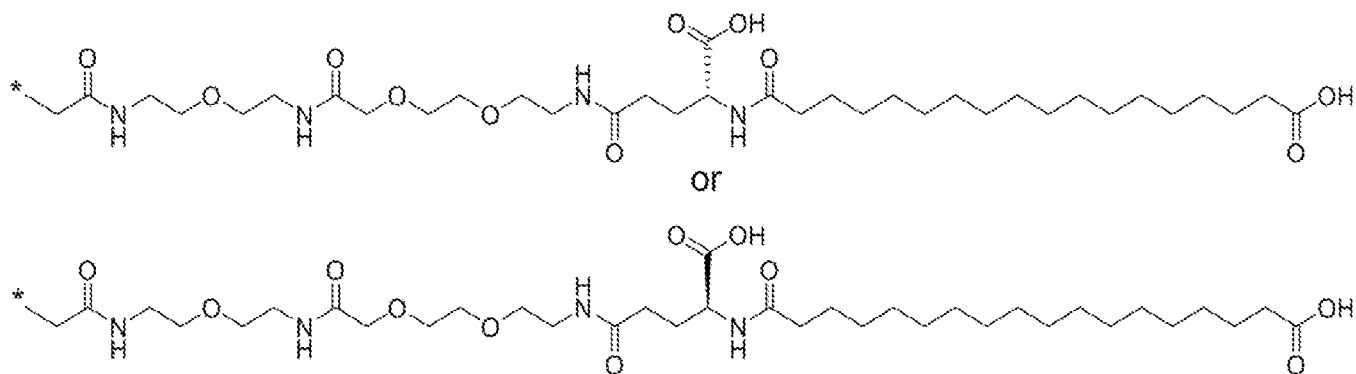
12/22

XTEN WITH 3 CYSTEINE RESIDUES



Z =

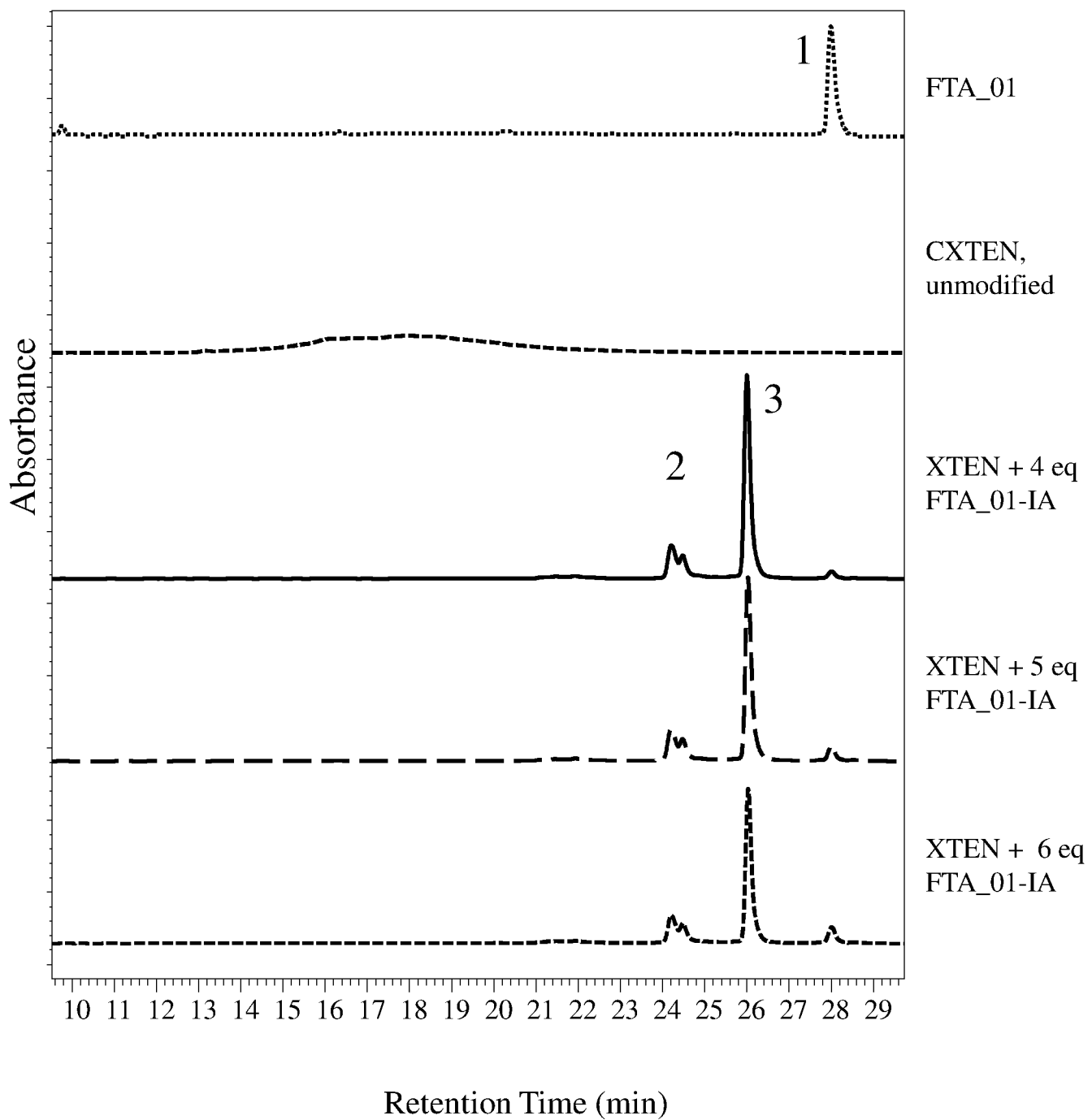
Albumin binding subunit



* = conjugation site of cysteine residue

FIG. 12

15/22

**FIG. 15**

16/22

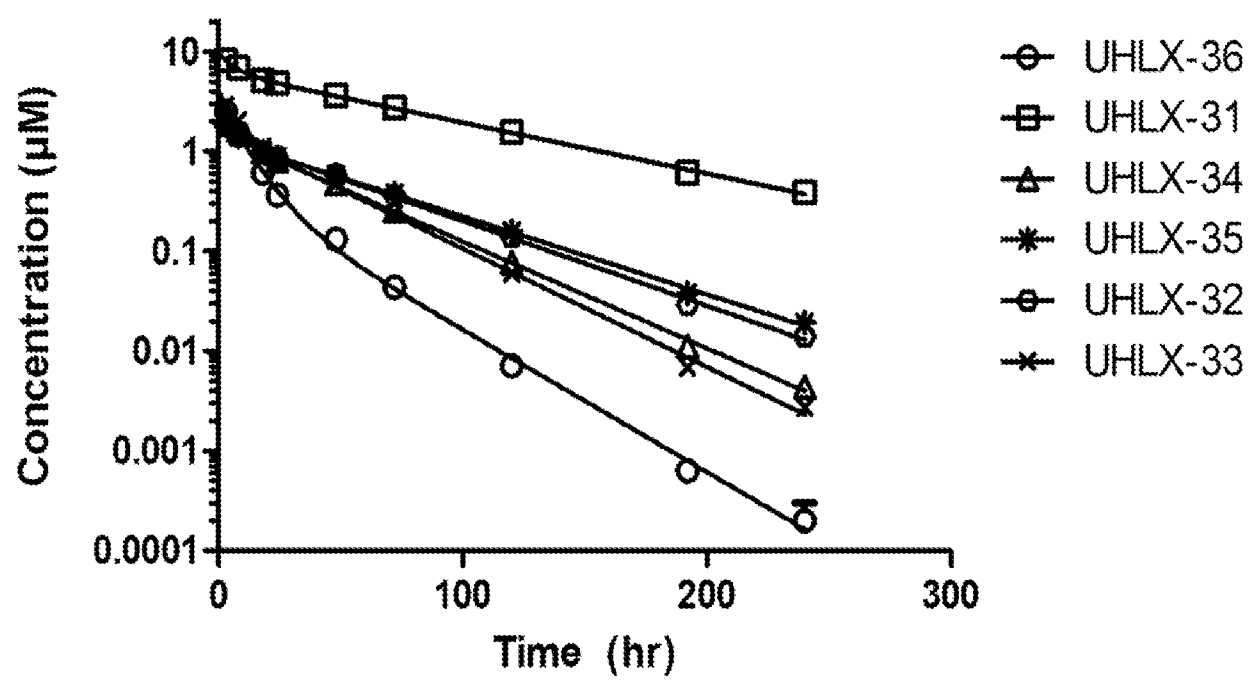
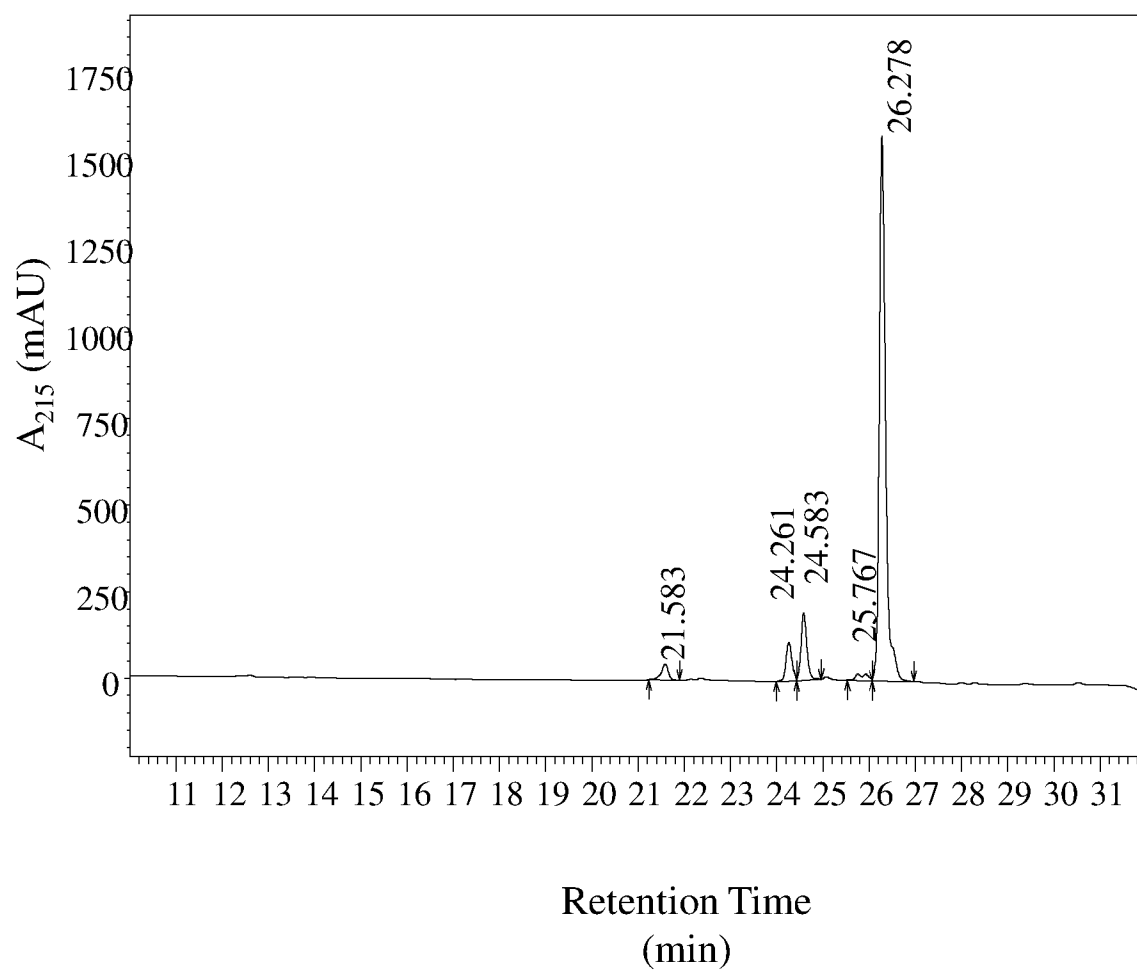


FIG. 16

17/22

**FIG. 17**

18/22

FIG. 18A

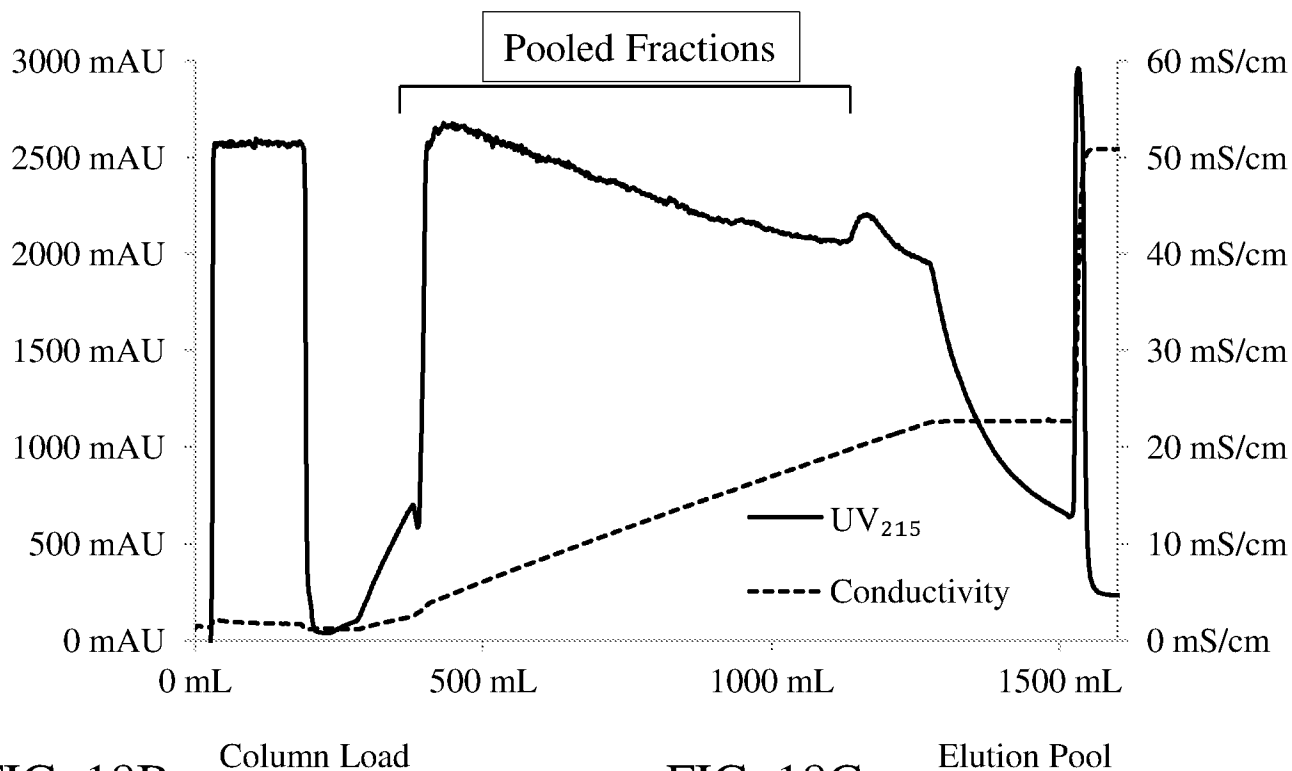


FIG. 18B

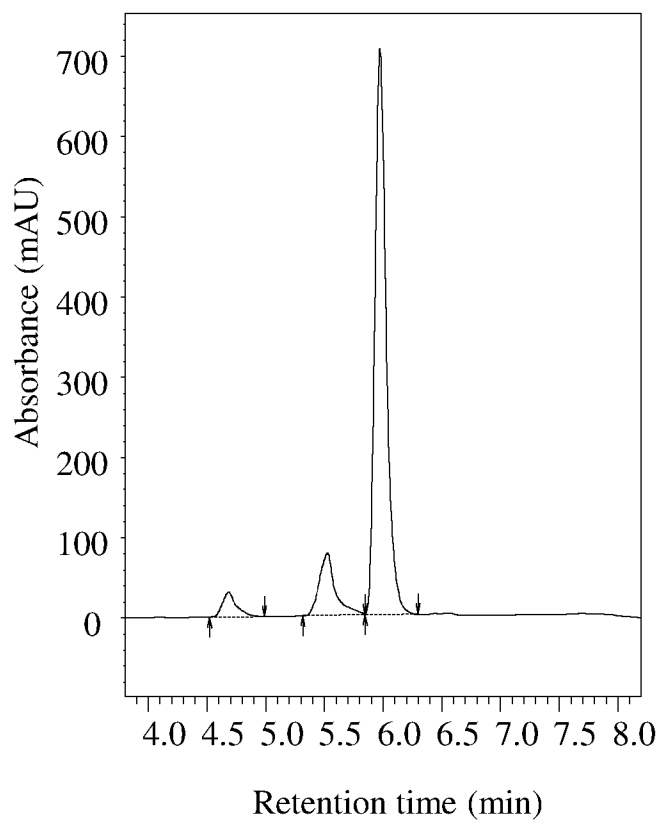


FIG. 18C

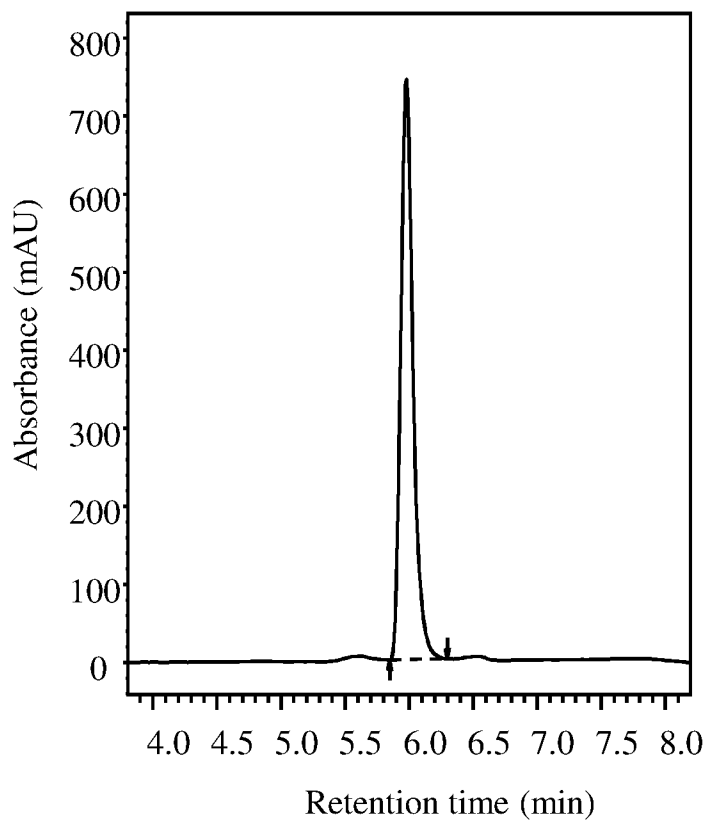
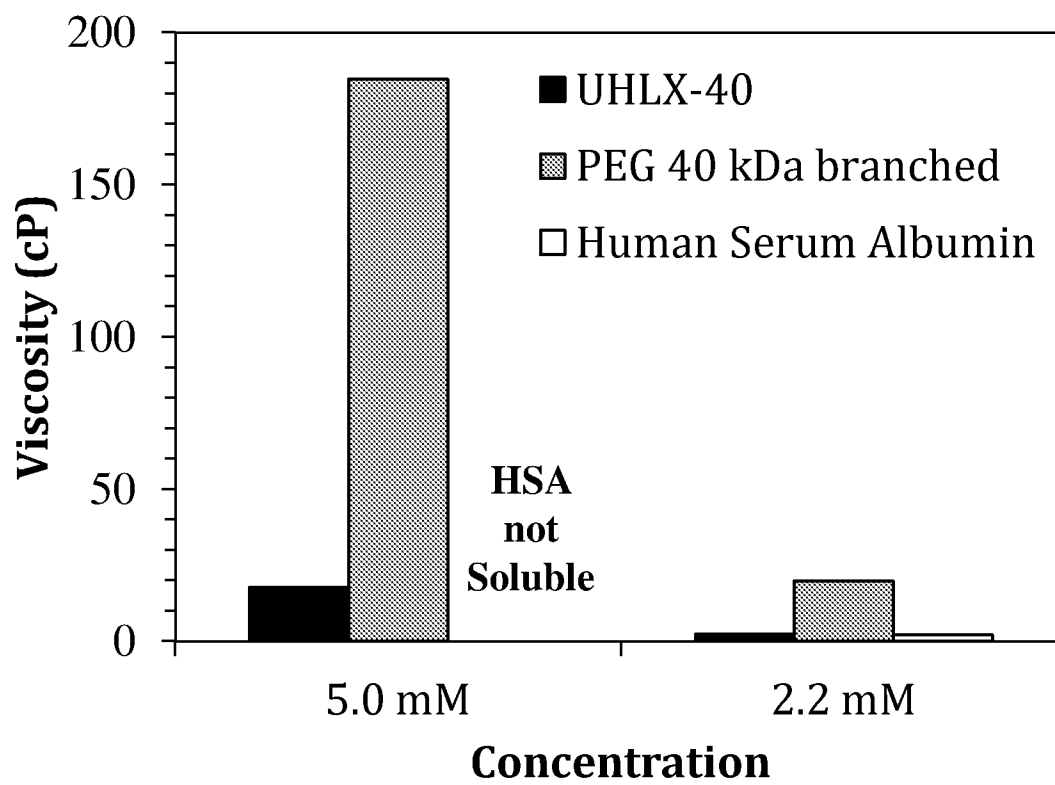


FIG. 18

19/22

**FIG. 19**

20/22

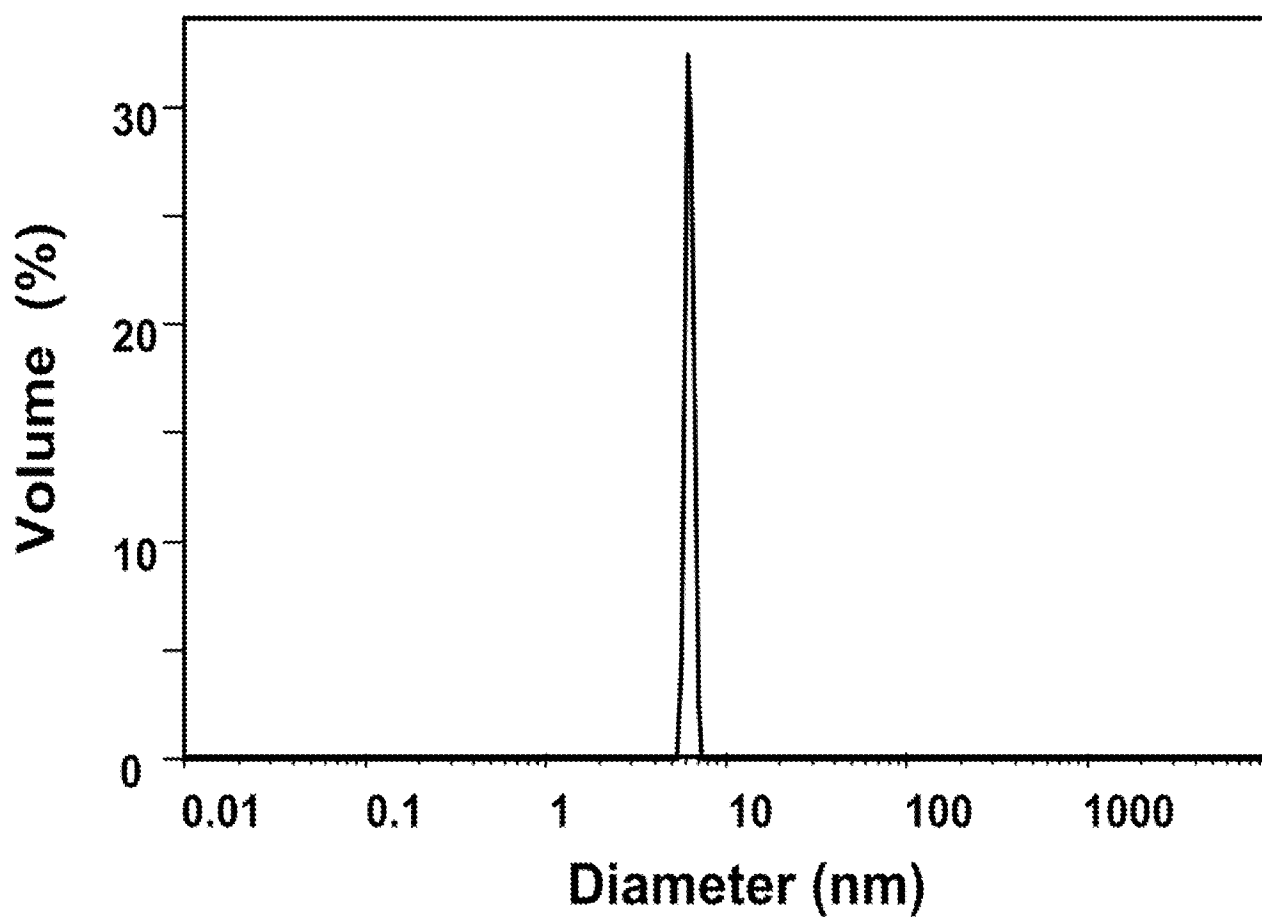


FIG. 20

FIG. 21A

21/22

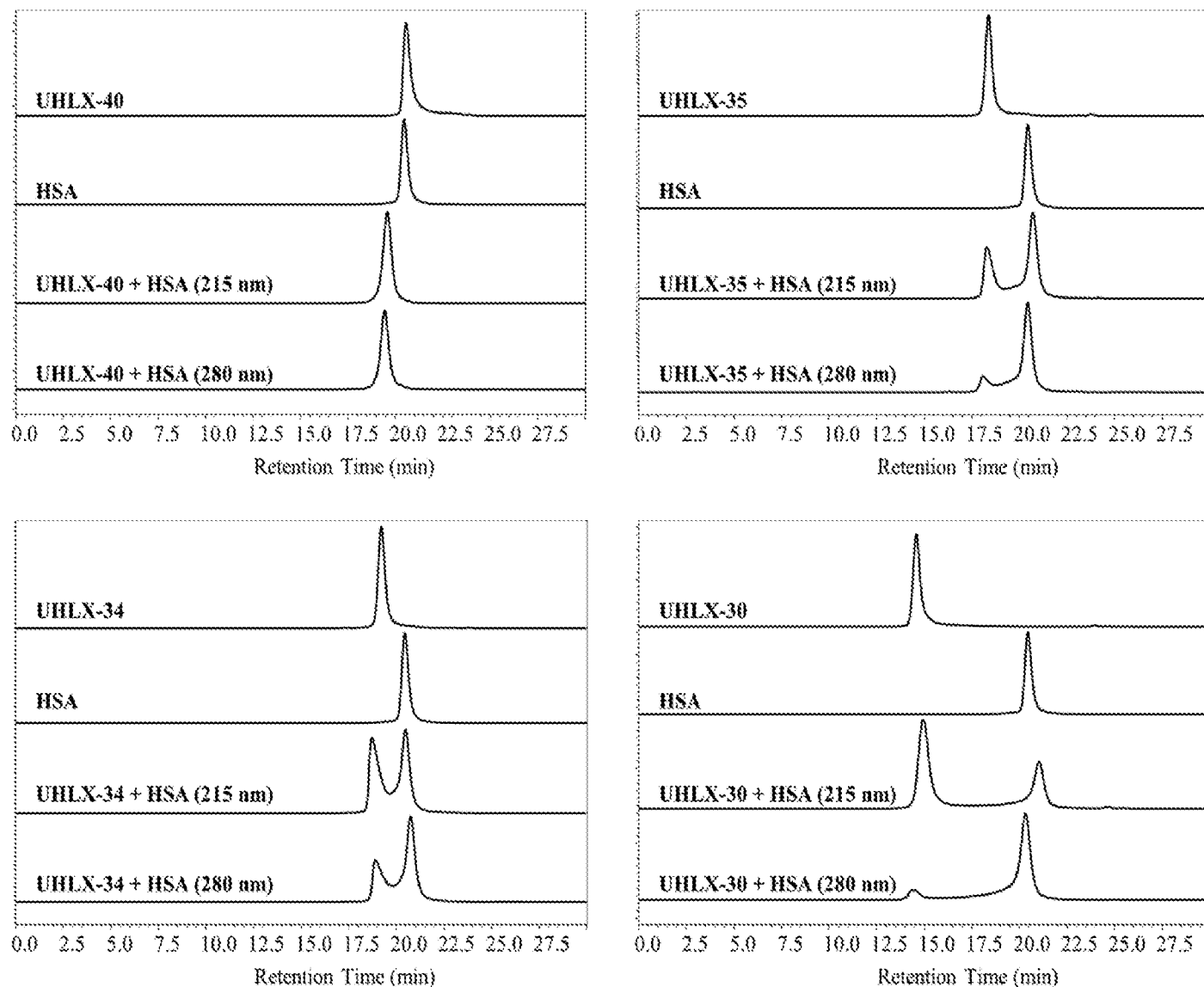


FIG. 21B

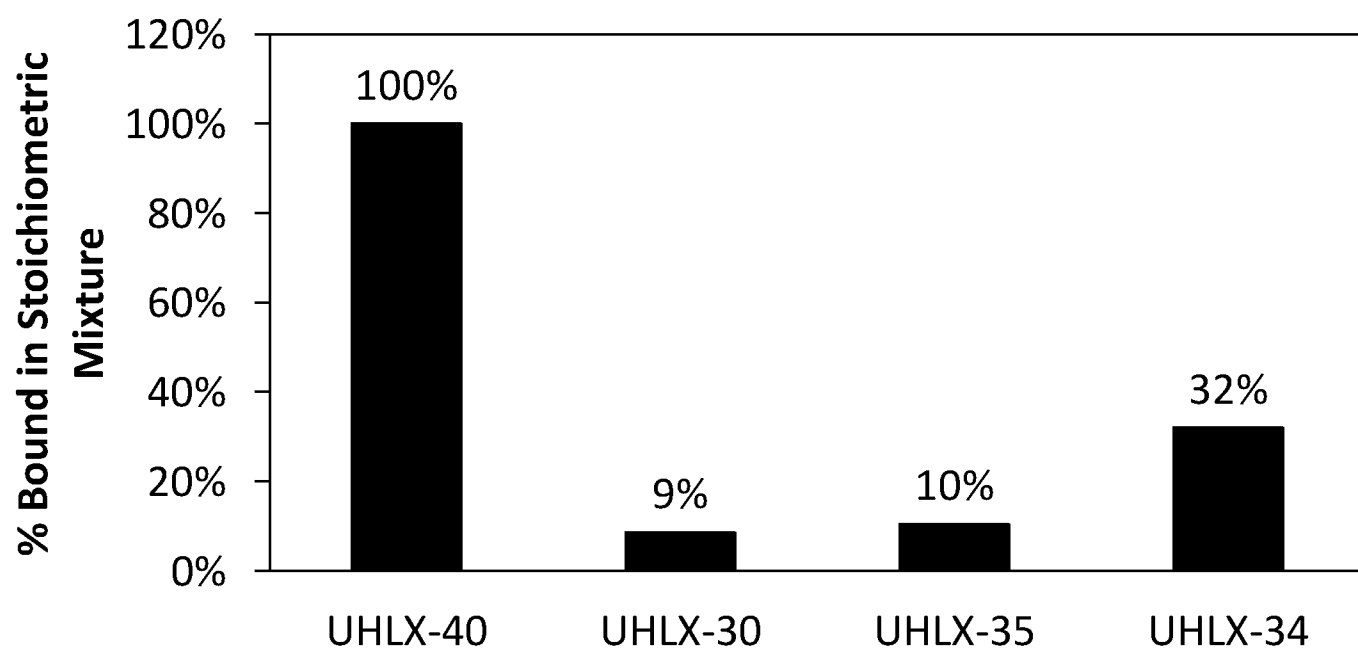


FIG. 21

22/22

FIG. 22A

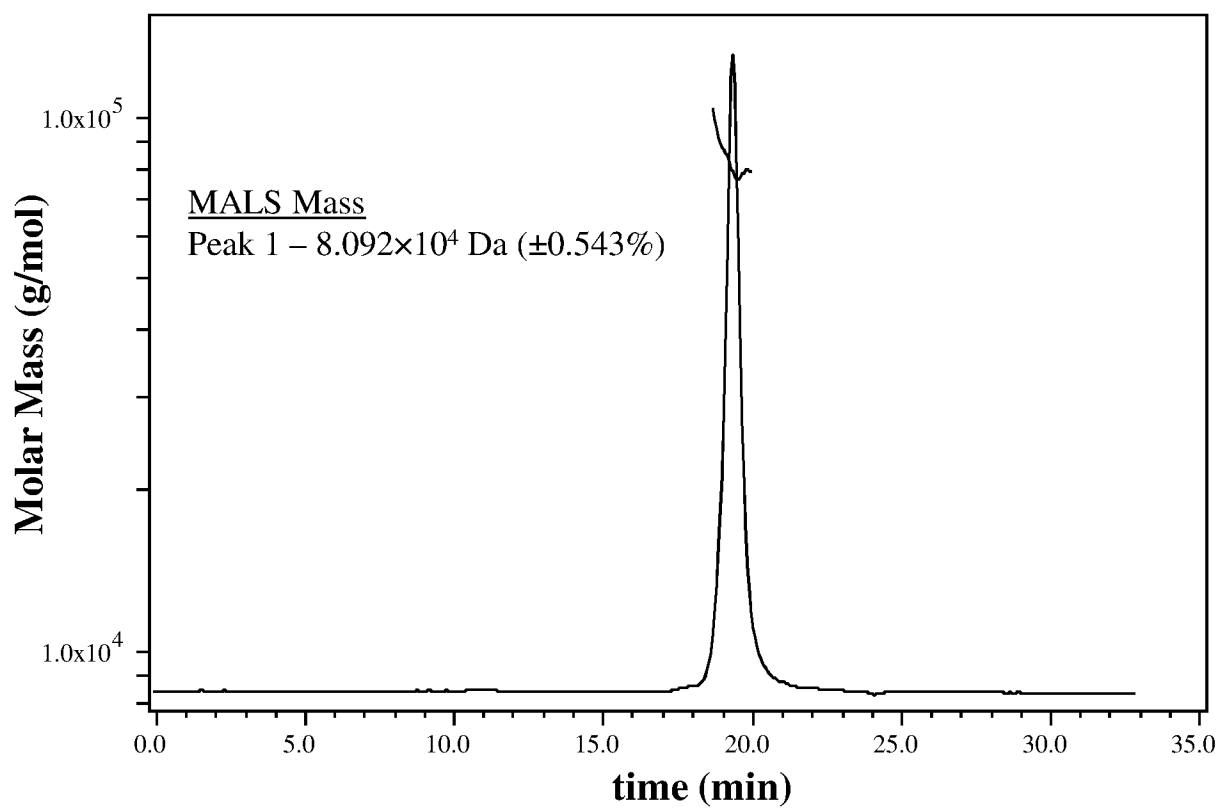


FIG. 22B

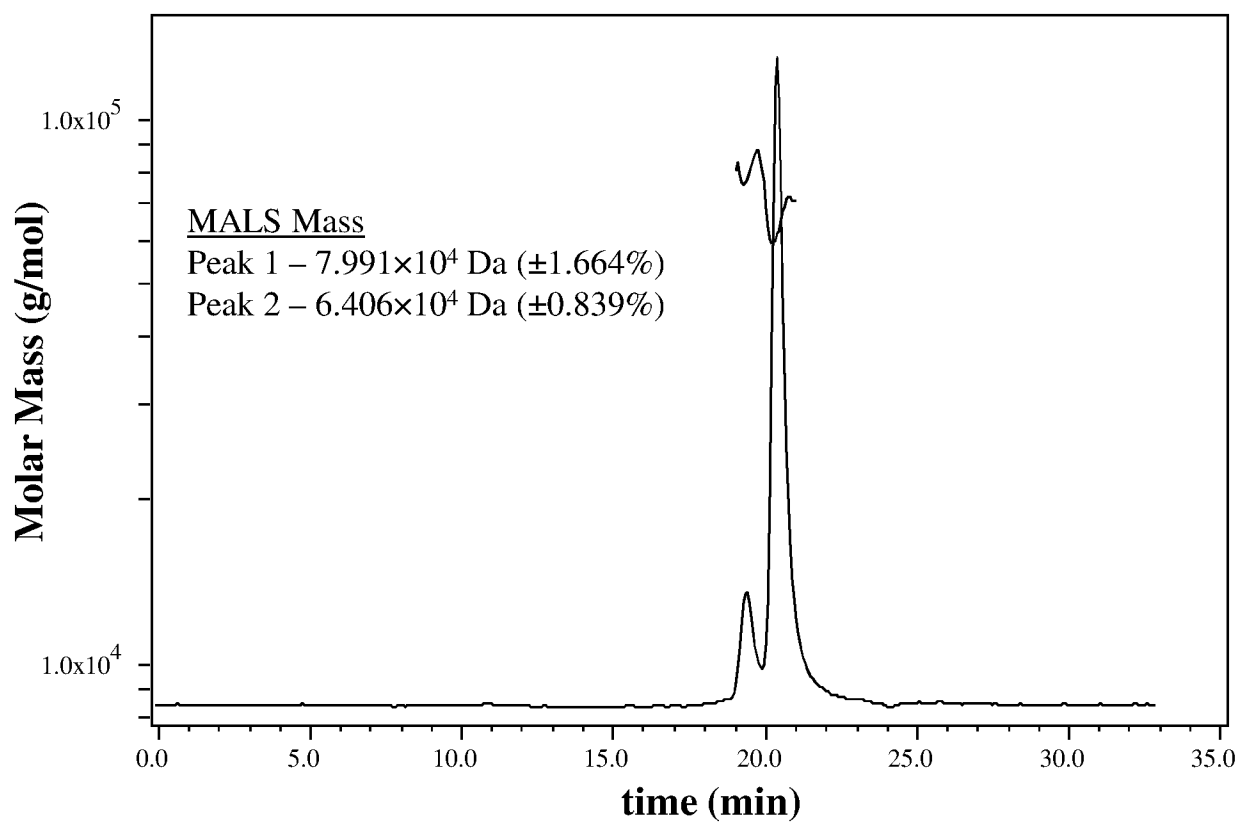


FIG. 22

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/32043

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

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

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

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

3. ☒ Claims Nos.: 8-32, 42-65
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US17/32043

A. CLASSIFICATION OF SUBJECT MATTER
 IPC - A61K 47/48, 38/24; C07K 14/755 (2017.01)
 CPC - A61K 47/48246, 38/24; C07K 14/755

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2013/130684 A1 (AMUNIX OPERATING INC.) 06 September 2013; paragraphs [00151], [00227]-[00229]	1-4, 5/1-4, 6/5/1-4, 7/5/1-4, 33-38, 39/37-38, 40/39/37-38, 41/39/37-38
A	US 2012/0178691 A1 (SCHELLENBERGER, V et al.) 12 July 2012; paragraphs [0006]-[0007], [0250]	1-4, 5/1-4, 6/5/1-4, 7/5/1-4, 33-38, 39/37-38, 40/39/37-38, 41/39/37-38
A	US 2015/0158929 (SCHELLENBERGER, V et al.) 11 June 2015; paragraphs [0010], [0242], [0509]	1-4, 5/1-4, 6/5/1-4, 7/5/1-4, 33-38, 39/37-38, 40/39/37-38, 41/39/37-38
A	WO 2015/106052 A1 (BIOGEN MA INC.) 16 July 2015; paragraphs [0006]-[0007], [0010], [0196], [0211]	1-4, 5/1-4, 6/5/1-4, 7/5/1-4, 33-38, 39/37-38, 40/39/37-38, 41/39/37-38

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

* Special categories of cited documents:	"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
"A" document defining the general state of the art which is not considered to be of particular relevance	"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
"E" earlier application or patent but published on or after the international filing date	"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
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"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	

Date of the actual completion of the international search

14 July 2017 (14.07.2017)

Date of mailing of the international search report

01 AUG 2017

Name and mailing address of the ISA/

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 Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

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 PCT OSP: 571-272-7774