

DECREASED RETENTION OF MINIPROTEINS IN KIDNEY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 63/425,263, filed on November 14, 2022, the disclosure of which is incorporated by reference herein in their entirety for all purposes.

BACKGROUND

[0002] Cancer is a leading cause of death worldwide. Classical cancer therapies such as radiotherapy, chemotherapy, and surgical procedures can be accompanied by severe side effects, due to killing of healthy non-cancerous cells. Newer therapeutics enhance the targeting of cytotoxic drugs to tumor cells, relative to earlier therapies, including those that use biologics conjugates. However, often times, following administration, such therapeutics rapidly accumulate in the kidney and/or are cleared.

SUMMARY

[0003] In various aspects, provided is a composition represented by the formula selected from M-L-C-R, M-L-C, M-C-R, M-L-R, M-C, M-L, and M-R, wherein M comprises a miniprotein (M), L comprises a linear, branched, or enzymatically cleavable linker (L), C comprises a chelator (C), and R comprises a radionuclide (R) wherein the composition is characterized in its reduced kidney uptake. In some embodiments, the composition comprises a linear polypeptide, a folded polypeptide (e.g., covalently linked polypeptide, non-covalently linked polypeptide, or polypeptide include a di-sulfide linkage), cysteine-dense peptide, a knottin peptide, a binder, an affibody, an engineered Kunitz domain, a monobody, an anticalin, a designed ankyrin repeat domain (DARPin), or an avimer. In various embodiments, M comprises an amino acid sequence that shares at least 90% identity to any one of SEQ ID NOs: 1-68 in Tables 4A and 4B. In some embodiments, M comprises an amino acid sequence that shares 100% identity to any one of SEQ ID NOs: 1-68. In some embodiments, M comprises an amino acid sequence that shares 100% identity to any one of SEQ ID NOs: 1-3, 5-15, and 47-67. In some embodiments, M comprises an amino acid sequence that shares 100% identity to SEQ ID NO: 16.

[0004] In some facets, provided is a composition comprising or consisting essentially of decharged molecules. In some embodiments, the composition comprises an amino acid sequence comprising a percentage of charged amino acids between 1-5% of the total amino acid sequence, wherein the charged amino acids are selected from Lys, Arg, or His. In

certain embodiments, the composition comprises an amino acid sequence comprising a percentage of charged amino acids between 5-10% of the total amino acid sequence, wherein the charged amino acids are selected from Lys, Arg, or His. In additional embodiments, the composition comprises an amino acid sequence comprising a percentage of charged amino acids between 10-15% of the total amino acid sequence, wherein the charged amino acids are selected from Lys, Arg, His, or non-canonical amino acids such as trimethyllysine. In further embodiments, the composition comprises an amino acid sequence comprising a percentage of charged amino acids between 1-5% of the total amino acid sequence, wherein the charged amino acids are selected from Asp or Glu. In additional embodiments, the composition comprises an amino acid sequence comprising a percentage of charged amino acids between 5-10% of the total amino acid sequence, wherein the charged amino acids are selected from Asp or Glu. In yet other embodiments, the composition comprises an amino acid sequence comprising a percentage of charged amino acids between 10-15% of the total amino acid sequence, wherein the charged amino acids are selected from Asp or Glu. Preferably, the composition is characterized to exhibit reduced kidney uptake in a subject. Accordingly, in various embodiments, the composition wherein M comprises an amino acid sequence comprising a desired percentage of charged amino acids is characterized to exhibit reduced kidney uptake. In some embodiments, M targets any one of the target proteins in Table 10.

[0005] In some embodiments, the composition is characterized to exhibit 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, or greater than 10% ID/g in a tumor 24 hours post administration. In preferred embodiments, the composition is characterized to exhibit greater than 5% ID/g in a tumor 24 hours post administration. In other embodiments, the composition is characterized to exhibit less than 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or less than 20% ID/g in a kidney 4 hours post administration. In preferred embodiments, the composition is characterized to exhibit less than 5% ID/g in a kidney 4 hours post administration. In certain aspects, the composition is characterized to exhibit an adherence in a tumor and normal, non-ablatable tissues at a ratio of greater than 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1 or 10:1 after 24 hours.

[0006] In alternative aspects, provided is a composition of a miniprotein (M) and a linker (L), wherein L is linked to the C-terminus or N-terminus of M cysteine, and L comprises an enzymatically cleavable linker of the formula $L = R1-X1-X2-Lys$, wherein:

R1 = H, PEG(4-36), 4-aminomethyl-phenylacetic acid (AmPA),
Aminomethylbenzoyl (AmBz), or (succinic acid-PEG(4-36) and

X1, or X2, = Met, Leu, norleu (Nle), Ile, Glu, Methoxinine, Phe, Tyr, beta-Ala, MWK or MVK, dTyr-Gly-Phe (yGF), dArg-Gly-Phe (rGF), Gly(1-10), Citrulline, or Sarcosine.

[0007] In various related aspects, provided is a composition of a miniprotein (M) and a linker (L), wherein L is linked via a succinic acid derivative to the Nε-of Lysine of M and L comprises an enzymatically cleavable linker of the formula L= R1-X1-X2-Lys, wherein:

R1= H, PEG(4-36), 4-aminomethyl-phenylacetic acid (AmPA),

Aminomethylbenzoyl (AmBz), or (succinic acid-PEG(4-36) and

X1, or X2, = Met, Leu, norleu (Nle), Ile, Glu, Methoxinine, Phe, Tyr, beta Ala, MWK or MVK, yGF, rGF, Gly(1-10), Citrulline, or Sarcosine.

[0008] In various related aspects, provided is a composition that is cleaved by one or more proteases in the kidney. In some embodiments, the composition is cleaved by cathepsin B or a neutral endopeptidase, metalloprotease, or dipeptidyl peptidase in a kidney brush border membrane.

[0009] Additionally disclosed herein is a method of treating cancer in a patient, the method comprising administering a composition disclosed herein to the patient. Additionally disclosed herein is a method of co-administering any of the compositions disclosed herein and a peptide comprising an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NOs: 16-52, or SEQ ID NO: 68.

[0010] In various embodiments, the method reduces uptake of M, L, C, and R, and combinations thereof in a kidney.

[0011] In various embodiments, co-administering any one of the compositions disclosed herein and a peptide comprising an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NOs: 16-52, or SEQ ID NO: 68 results in competitive inhibition of the peptide.

[0012] In certain embodiments, the composition is administered intravenously or subcutaneously.

[0013] In various embodiments, any one of the compositions disclosed herein and the peptide comprising an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NOs: 16-52, or SEQ ID NO: 68 are co-formulated with a pharmaceutically acceptable buffer.

[0014] In various embodiments, the method produces a treatment characterized to exhibit reduced uptake of M in a kidney.

[0015] Additionally disclosed herein is a composition represented by the formula selected from one or more of M-L-C-R, M-L-C, M-C-R, M-L-R, M-C, and M-L, wherein M comprises a miniprotein (M), L comprises a linear, branched, or enzymatically cleavable linker (L), C comprises a chelator (C), and R comprises a radionuclide (R), and a peptide, wherein the peptide is characterized as competitively inhibiting the target for uptake in a kidney.

[0016] In various embodiments, the peptide comprises an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NOs: 16-52, or SEQ ID NO: 68, and wherein the peptide is present at a concentration of 10-1000X relative to the concentration of M-L-C-R or M-L-C.

[0017] In various embodiments, the peptide comprises an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NOs: 16-52, or SEQ ID NO: 68, and wherein the peptide is present at a concentration of 100X or greater than 100X relative to the concentration of M.

[0018] In various embodiments, the peptide comprises an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NOs: 16-52, or SEQ ID NO: 68, and wherein the peptide is present at a concentration of 10X-100X, 100X-300X, and 300X-1000X relative to the concentration of M.

[0019] In various embodiments, the peptide comprises an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NOs: 16-52, or SEQ ID NO: 68, and wherein the peptide is present at a concentration of 100X or greater than 100X relative to the concentration of M.

[0020] Additionally disclosed herein is a composition represented by the formula selected from one or more of, M-L-C, M-C, M-L, and M, wherein M comprises a miniprotein (M) comprising an amino acid selected from SEQ ID NO: 4 or SEQ ID NO: 7, L comprises a linear, branched, or enzymatically cleavable linker (L), C comprises a chelator (C), and a peptide comprising an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NOs: 16-52, or SEQ ID NO: 68, wherein the peptide is characterized as competitively inhibiting the target for uptake in a kidney.

[0021] In various embodiments, the peptide is present at a concentration of 10-1000X 10-1000X relative to the concentration of M.

[0022] In various embodiments, the peptide is present at a concentration of 100X or greater than 100X relative to the concentration of M.

[0023] In various embodiments, the peptide reduces the uptake of M-L-C-R, M-L-C, or R in a kidney.

[0024] In certain aspects, the composition comprises one or more chelators selected from DOTA, NOPO, Crown, or Macropa.

[0025] In other aspects, the composition comprises one or more radionuclides selected from Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211.

[0026] Provided herein is a pharmaceutical composition comprising (M)-L-(C)-R and at least one drug moiety.

[0027] In various aspects, the composition binds to a target expressed on tumor cell with an affinity of 1 pM to 100 nM as measured by an in vitro binding assay.

[0028] In other aspects, the composition is characterized by peptide solubility of 1-100 mg/mL in an assay formulation and peptide stability of 80-95% at 75°C for at least 1 hour.

[0029] In some preferred embodiments, the composition is cleared from a kidney at or near the glomerular filtration rate of a subject. In other preferred embodiments, the composition is characterized to transit or passage through one or more kidneys (e.g., a kidney, a liver, a bone marrow, or a spleen) at slower rates than glomerular filtration wherein uptake or reuptake is minimized, systemic exposures are increased, and tumor targeting is similar or superior.

[0030] Preferably, the composition is directed to cells where the expression of the target is higher in a cancer cell than in a non-cancer cell.

[0031] In various aspects, provided is a method treating one or more conditions or disorders selected from breast cancer, ovarian cancer, melanoma, pancreatic cancer, peripheral neuroma, glioblastoma, adrenocortical carcinoma, AIDS-related lymphoma, anal cancer, bladder cancer, meningioma, glioma, astrocytoma, cervical cancer, chronic myeloproliferative disorders, colon cancer, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extracranial germ cell tumors, extrahepatic bile duct cancer, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumors, gestational trophoblastic tumors, hairy cell leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, hypopharyngeal cancer, islet cell carcinoma, Kaposi sarcoma, laryngeal cancer, leukemia, lip cancer, oral cavity cancer, liver cancer, male breast cancer, malignant mesothelioma, medulloblastoma, Merkel cell carcinoma, metastatic squamous neck cell carcinoma, multiple myeloma and other plasma cell neoplasms, mycosis fungoides and the Sezary syndrome, myelodysplastic syndromes, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, small cell

lung cancer, head and neck cancer, skin cancer, oropharyngeal cancer, bone cancers, including osteosarcoma and malignant fibrous histiocytoma of bone, paranasal sinus cancer, parathyroid cancer, penile cancer, pheochromocytoma, pituitary tumors, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, small intestine cancer, soft tissue sarcoma, supratentorial primitive neuroectodermal tumors, pineoblastoma, testicular cancer, thymoma, thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis and ureter, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Wilm's tumor and other childhood kidney tumors.

[0032] Additionally disclosed herein is a use of any of the compositions disclosed herein to treat cancer in a subject.

[0033] The present disclosure provides technologies such as compositions and methods of use and manufacture thereof to address needs in the field of cancer. For example, in contrast to classical cancer diagnostics or therapies, targeted molecules can be designed to increase specificity and decrease toxicity of, *e.g.*, imaging or therapeutic modalities. For instance, delivery of a therapeutic such as a chelator and/or radionuclide (*e.g.*, alpha emitter) using a polypeptide to specifically target the therapeutic to the tumor microenvironment provides focused treatment to tumor cells and avoids or reduces risk of toxicity to surrounding healthy tissues by the therapeutic targeted to, *e.g.*, a tumor.

[0034] In contrast to classical cancer therapies, radionuclide therapies are more targeted and less toxic. For instance, delivery of a radionuclide specifically to a tumor microenvironment allows for selective radiation of tumor tissue, effectively killing malignant cells while preserving the surrounding healthy tissue. For example, a radionuclide can employ a targeting molecule that specifically binds to target protein expressed at an increased level and/or density on the surface of tumor cells relative to non-tumor cells. Binding of the radionuclide to the target-positive tumor cells targets radiation to those cells without targeting healthy tissue. Full-length antibodies have previously been evaluated as targeting moieties; however, due to considerations such as their large size, full length antibodies can have several challenges such as having poor tumor tissue penetration and a long circulating half-life leading to irradiation of normal tissues. A key drawback of radionuclide therapies is the potential for systemic exposure or renal toxicity in radionuclide therapy. Accordingly, a need remains for new approaches to reduce undesirable effects of radionuclide therapy, while maintaining therapeutic effectiveness by specifically targeting tumors, and particularly solid tumors. The present disclosure provides technologies that meet this and other needs.

[0035] Among other things, the present disclosure provides conjugates comprising polypeptides (*e.g.*, miniproteins) that target tumor microenvironments and/or tumor cells conjugated to one or more additional components including, for example, a linker, chelator, and/or radionuclide (*e.g.*, an alpha emitter). In some embodiments, such conjugates are used in treatment of cells expressing a target (*e.g.*, any one of the target proteins in Table 10). In some such embodiments, the cells are cancer cells.

[0036] In some embodiments, a composition comprises a linker and a chelator.

[0037] In some embodiments, a composition comprises a linker, chelator, and radionuclide.

[0038] In some embodiments, a composition comprises a miniprotein, an optional linker, a chelator and/or a radionuclide.

[0039] In some embodiments, a composition is represented by the formula selected from one or more of M-L-C-R, M-L-C, M-C-R, M-L-R, M-C, M-L, and M-R, wherein M comprises a miniprotein (M), L comprises a linker (L), C comprises a chelator (C), and R comprises a radionuclide (R).

[0040] In some embodiments, a polypeptide in accordance with the present disclosure is manufactured using solid phase peptide synthesis methods. In some embodiments, the polypeptide is recombinant. In some embodiments, the polypeptide is a folded polypeptide held together by disulfide bonds, covalent, or non-covalent interactions. In some embodiments, the polypeptide comprises or consists of a miniprotein. In some embodiments, the miniprotein comprises or consists of a linear polypeptide, a folded polypeptide (*e.g.*, covalently linked polypeptide, non-covalently linked polypeptide, or polypeptide include a di-sulfide linkage), cysteine-dense peptide, a knottin peptide, a binder, an affibody, an engineered Kunitz domain, a monobody, an anticalin, a designed ankyrin repeat domain (DARPin), or an avimer. In some embodiments, the miniprotein comprises or consists of approximately 100 amino acids or less. In some embodiments, the miniprotein is a cysteine-dense protein. In some embodiments, the miniprotein comprises at least one cysteine-dense region. In some embodiments, the miniprotein comprises one or more disulfide bridges. In some embodiments, the miniprotein comprises or consists of a non-disulfide-containing amino acid sequences. In some embodiments, the miniprotein comprises three alpha helices with 58 amino acids and has a molar mass of about 6 kDa. In some embodiments, the miniprotein is stable at high temperatures and under acidic or alkaline conditions. In some embodiments, the miniprotein comprises an engineered protein derived from a lipocalin. In some embodiments, the miniprotein comprises an eight-stranded β -barrel. In some

embodiments, the miniprotein displays (i) high structural plasticity as a consequence of sequence variation and (ii) elevated conformational flexibility, allowing induced fit to targets with differing shape. In some embodiments, the miniprotein comprises a class of antibody mimetics which consist of two or more peptide sequences of 30 to 70 amino acids each, which are connected by linker peptides. In some embodiments, the miniprotein comprises a peptide derived from Ankyrin. In some embodiments, the miniprotein comprises an ankyrin repeat, a 33 residue motif consisting of two alpha-helices and a beta-turn. In some embodiments, the miniprotein comprises a peptide derived from the Kunitz domain of a Kunitz-type protease inhibitor such as bovine pancreatic trypsin inhibitor (BPTI), amyloid precursor protein (APP) or tissue factor pathway inhibitor (TFPI).

[0041] In some embodiments, the present disclosure provides compositions that have various advantages over compositions comprising a full-length protein. For example, in some embodiments, a composition of the present disclosure comprises one or more improved properties selected from increased miniprotein expression, increased thermostability, increased receptor binding specificity and/or affinity, increased chemical stability, increased tolerance to acidic pH, increased tolerance to proteolytic activity (*e.g.*, reduced sensitivity to proteolysis), reduced aggregation, increased solubility, and/or reduced immunogenicity.

[0042] In some embodiments, a composition of the present disclosure specifically binds to at least one epitope of a target in, on, or near a cell. In some embodiments, the target is any one of the target proteins in Table 10. In some embodiments, the cell is a tumor cell (which may or may not have properties of having cancer, but be derived from a tumor sample). In some embodiments, the cell is a cancer cell. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a primary cell. In some embodiments, the primary cell is taken from a sample from a subject (*e.g.*, from a biopsy, *e.g.*, from a tumor). In some embodiments, the cell is from a cell line.

[0043] In some embodiments, a miniprotein or conjugate thereof selectively binds to a target. In some embodiments, the target is a protein or portion thereof expressed on the surface of a cell. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a human cell. In some embodiments, the cell is a cancer cell. In some such embodiments, the cancer cell is in (*i.e.*, part of) or near a tumor. In some embodiments, the cancer cell is a circulating cell.

[0044] In some embodiments, a target is a cell adhesion receptor, a cytokine receptor, a chemokine receptor, a growth factor receptor, an immune cell receptor, or a tumor associated

extracellular matrix polypeptide. In some such embodiments the target comprises or consists of 5T4, ADAM9, AG-7, AGS-16/ENPP3, ALPV, ASCT2, AXL, B7H3/CD276, B7H4, BCMA, C4.4a, CA6, CA9, CAIX, CCR2, CCR7, CD123, CD138, CD142, CD166, CD19, CD20, CD205, CD22, CD228, CD25, CD30, CD33, CD352, CD37, CD38, CD44v6, CD45, CD46, CD47, CD48, CD5, CD51, CD56, CD7, CD70, CD71, CD74, CD79B, CDH6, CEACAM5, Cholecystokinin 2 receptor, cKIT, CLDN18, CLDN18.2, CLDN6, CLDN6+CLDN9, CLL-1, cMET, cMET+EGFR, Cripto, CXCR4, DLL3, DPEP3, EFNA4, EGFR, EGFR+HER3, EGFR+MUC1, EGFRvIII, EPHA2, ETBR, FAP, FAPI, FCRH5, FGFR2, FGFR3, FLT3, FR α , GCC, GD2, GD3, Globo H, GPC3, GPCR5D, gpNMB, GPR20, HER2, HER2+HER3, IGF1R, IL13Ra, IL-4R, Integrin β -6, KAAG1, L1CAM, LAMP1, Lewis Y Ag, LHRH receptor, LIV1, LIV1A, LRRC15, LY6E, Ly75/CD205, MC1R, MELTF, Mesothelin, MSLN, MT1-MMP, MUC1, MUC16/CA-125, NaPi-2b, Nectin-4, Neurokinin 1 receptor, NKG2D, Norepinephrine transporter, NOTCH3, NTSR1, P-Cadherin, PDL1, PRLR, PSMA, PTK7, RNF43, ROR1, ROR2, SEZ6, SLAMF7, SLC44A4, SLITRK6, SS2R, STEAP1, TF, TIM1, TNFSF9, Trop-2, or a portion thereof..

[0045] In related aspects, the present disclosure provides pharmaceutical compositions comprising a conjugate. In some embodiments, a conjugate comprises one or more of a miniprotein, linker, chelator, and radionuclide.

[0046] In some embodiments, one or more components of a conjugate are provided using a vector, *e.g.*, a recombinant expression vector comprising a nucleic acid encoding a polypeptide.

[0047] In some embodiments the present disclosure provides a host cell comprising a vector encoding one or more components of a conjugate as provided herein.

[0048] In some embodiments, the present disclosure provides a composition that is capable of binding to, modulating, and/or inhibiting any one of the target proteins in Table 10. In some embodiments, the target protein selected from Table 10 is the human isoform. In some embodiments, a composition of the present disclosure is or comprises, peptide therapy, peptide receptor radionuclide therapy,

[0049] In some embodiments a composition of the present disclosure is formulated for administration to a subject in need thereof (*e.g.*, a pharmaceutical composition). In some embodiments, a composition is administered for the treatment of one or more diseases, disorders, or conditions. In some embodiments, a disease, disorder or condition is cancer. In some embodiments, the treatment comprises providing a composition (*e.g.*, by administering, *e.g.*, by contacting a cell or population of cells, *e.g.*, a tumor), wherein the composition comprises a component that specifically binds to any one of the target proteins in Table 10. In some embodiments, the target proteins selected from Table 10 is expressed on a cell or population of cells. In some embodiments, providing the composition treats the disease, disorder or condition.

[0050] In some embodiments, the present disclosure provides methods for modulating any one of the target proteins in Table 10. That is, in some embodiments, a method of modulating biological activity of any one of the target proteins in Table 10 comprises providing (*e.g.*, by administering, *e.g.*, by contacting a cell or population of cells) a composition comprising one or more components in an amount effective to modulate any one of the target proteins in Table 10 and its activity. For example, in some embodiments, a composition comprises a miniprotein that specifically binds to any one of the target proteins in Table 10 and one or more of a linker, chelator, and/or radionuclide. In some such embodiments, the composition binds to cells expressing any one of the target proteins in Table 10 and targets one or more therapies (*e.g.*, a chelator, *e.g.*, a radionuclide) to a cell expressing the target protein selected from Table 10 such as, for example, by internalization of the composition or a component thereof into the cell.

[0051] In some embodiments, the present disclosure provides methods and compositions for use therein that are capable of activating or inhibiting immune cell response. In some embodiments, provided compositions are administered for the treatment of cancer. In some embodiments, the cancer is associated with expression or overexpression of any one of the target proteins in Table 10. In some embodiments, the cancer is selected from non-small-cell lung cancer (NSCLC), cutaneous squamous cell carcinoma, pancreatic cancer, primary hepatocellular carcinoma, colorectal carcinoma, clear cell renal carcinoma, breast cancer and prostate cancer.

[0052] In some embodiments, the present disclosure provides methods and compositions for use in detecting the presence or extent of a disease, disorder, or condition in a subject. In some embodiments, the disease, disorder, or condition is cancer. In some embodiments, the subject has been diagnosed as having cancer. In some embodiments, the subject is suspected

as having or at risk of having cancer. In some embodiments, the subject diagnosed with cancer has been treated with one or more treatments, such as a composition as provided herein. In some embodiments, a method of detecting (*e.g.*, monitoring/determining prognosis, diagnosing, *etc.*) comprises measuring a level of a target or a miniprotein that specifically binds to a target in a sample comprising one or more cells from a subject (*e.g.*, using a cell-based assay) or in a subject (*e.g.*, using an *in vivo* scan or measurement). In some such embodiments, a level of the target or of the miniprotein is used to determine presence and/or extent of cancer in the subject by comparing a level to a control level or to a level from the same patient at a different point in time.

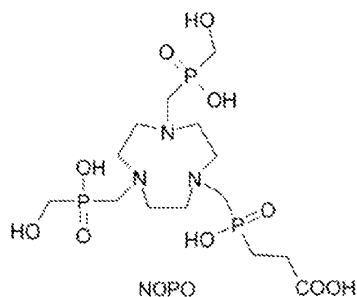
[0053] In some embodiments, the present disclosure provides kits. In some such embodiments, a kit comprises one or more components such as a miniprotein, linker, chelator, and/or radionuclide that may be combined in one or more methods for use in binding to a target (*e.g.*, any one of the target proteins in Table 10).

In some embodiments, the present disclosure provides compositions comprising a miniprotein (M), an optional linker (L), and one or both of a chelator (C) and a radionuclide (R), represented by a formula selected from one or more of M-L-C-R, M-L-C, M-C-R, M-L-R, M-C, M-L, and M-R. In some embodiments, M comprises or consists of a linear polypeptide, a folded polypeptide (*e.g.*, covalently linked polypeptide, non-covalently linked polypeptide, or polypeptide include a di-sulfide linkage), cysteine-dense peptide, a knottin peptide, a binder, an affibody, an engineered Kunitz domain, a monobody, an anticalin, a designed ankyrin repeat domain (DARPin), or an avimer. In some embodiments, the binder comprises or consists of a linear polypeptide, a folded polypeptide, and/or a non-disulfide sequence. In some embodiments, M is characterized in that it comprises 10-100 amino acids, and no more than 100 amino acids. In some preferred embodiments, M is characterized in that it comprises (i) no more than 100 amino acids and/or 12 kDa; (ii) at least one secondary structure elements; (iii) a sequestered hydrophobic core; and/or displays cooperative folding. In some embodiments, M comprises no more than about 100 amino acids or less, 90 amino acids, 85 amino acids, 80 amino acids, 75 amino acids, 70 amino acids, 65 amino acids, 60 amino acids, 55 amino acids, 50 amino acids, 45 amino acids, 40 amino acids, 35 amino acids, 30 amino acids, 25 amino acids, 20 amino acids, 15 amino acids, or 10 amino acids. In some embodiments, the miniprotein comprises at least one disulfide bridge. In some embodiments, the miniprotein comprises zero, one, two or more disulfide bonds.

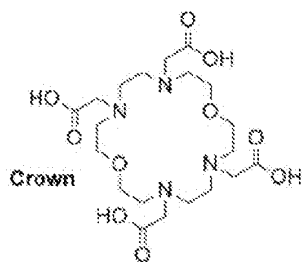
[0054] In some embodiments, the present disclosure provides compositions set forth as L-C, wherein L comprises or consists of a linker, C comprises or consists of a chelator, and wherein the linker is designed to be conjugated to a polypeptide.

[0055] In some embodiments, the present disclosure provides compositions set forth as L-C-R, wherein L comprises or consists of a linker, C comprises or consists of a chelator, and R comprises or consists of a radionuclide, and wherein the composition is capable of being conjugated to a miniprotein. In some embodiments, when L is present, L comprises or consists of a polyethylene glycol (PEG) linker of PEG2, PEG4, PEG6, PEG8, PEG12, PEG24, PEG36, an ester linker, an amide linker, a maleimide linker, a succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) linker, a propanoic acid linker, a caproic acid linker, or (Gly)_n-(γGlu)_n (SEQ ID NO: 79) or (PEG)_n, wherein n is from 1 to 10, (Gly)₁₋₁₀ (SEQ ID NO:80), or any fragment or combination via covalent bond thereof. In some embodiments, when C is present, C comprises or consists of:

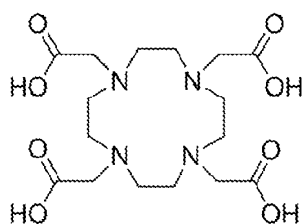
i) NOPO



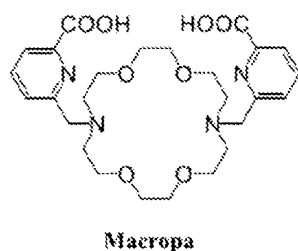
ii) Crown



iii) DOTA, or



iv) Macropa



[0056] In some embodiments, when C is present, C comprises or consists of derivative of NOPO, Crown, Macropa, or tetrazacyclododecane-1,4,7,10-tetraacetic acid (DOTA). In some embodiments, when L is present and C is absent, L is covalently attached to M. In some embodiments, when R is present, R comprises or consists of Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211. In some embodiments, a composition binds to the target with an affinity of 1 pM to 100 nM as measured by an in vitro binding assay. In some embodiments, when M is present the miniprotein binding to the target modulates biological function. In some embodiments, when M is present, M selectively binds to any one of the target proteins in Table 10 or a portion thereof.

[0057] In some embodiments, the present disclosure provides isolated constructs or pharmaceutically acceptable salts thereof comprising a miniprotein (M), optional linker (L), and at least one of a chelator (C) or radionuclide (R).

[0058] In some embodiments, the present disclosure provides pharmaceutical compositions comprising a miniprotein (M), wherein the miniprotein selectively binds to a target.

[0059] In some embodiments, the composition displays adherence in a tumor. In some embodiments, the composition displays passage through a kidney, a liver, a bone marrow, or a spleen.

[0060] In some embodiments, the pharmaceutical composition comprises a miniprotein (M), an optional linker (L), and one or both of a chelator (C) and radionuclide (R). In some embodiments, when C is present, C covalently attaches to M. In some embodiments, the chelation efficiency is > 90%. In some embodiments, the pharmaceutical composition further comprises a radionuclide R. In some embodiments, when R is present, it is an alpha-emitter. In some embodiments, R, when present, is Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211. In various embodiments, the radionuclide R is directly conjugated to M through a prosthetic group. In some embodiments, M specifically binds to a target. In some embodiments, the target is any one of the target proteins in Table 10. In some embodiments, the target protein selected from Table 10 is expressed on a cell. In some embodiments, the cell is a human cell. In some embodiments, the human cell is a tumor cell. In some embodiments, the tumor cell is a solid tumor cell. In some embodiments, the miniprotein comprises or consists of a linear polypeptide, a folded polypeptide (e.g., covalently linked polypeptide, non-covalently linked polypeptide, or polypeptide include a di-sulfide linkage), cysteine-dense peptide, a knottin peptide, a binder, an affibody, an engineered Kunitz domain, a monobody, an anticalin, a designed ankyrin repeat domain (DARPin), or an avimer. In some embodiments, the miniprotein comprises one or more disulfide bonds. In some embodiments, the miniprotein is characterized in that it has nM or sub-nM binding affinity on the target *in vivo* or in a cell-based assay. In some embodiments, the miniprotein has a binding affinity of 1 pM to 100 nM to any one of the target proteins in Table 10 on a cell line expressing human isoform of the target protein selected from Table 10. In some embodiments, the miniprotein has an amino acid sequence no more than about 100 amino acids or less, 90 amino acids, 85 amino acids, 80 amino acids, 75 amino acids, 70 amino acids, 65 amino acids, 60 amino acids, 55 amino acids, 50 amino acids, 45 amino acids, 40 amino acids, 35 amino acids, 30 amino acids, 25 amino acids, 20 amino acids, 15 amino acids, or 10 amino acids.

[0061] In some embodiments, administration of the pharmaceutical composition to a subject in need thereof does not elicit an immune response or wherein the immune response elicited is tolerable to the subject. In some embodiments, the composition comprises high tumor tissue penetration. In some embodiments, the composition is not taken up and/or retained in the kidney or liver. In some embodiments, the composition is internalized in a cell expressing human isoform of the target protein selected from Table 10.

[0062] In some embodiments, a composition comprises a miniprotein-drug conjugate, comprising a miniprotein and at least one drug moiety. In some embodiments, a

pharmaceutical composition comprises a miniprotein-drug conjugate, comprising a miniprotein and at least one drug moiety. In certain embodiments, the drug moiety includes but is not limited to a V-ATPase inhibitor, a pro-apoptotic agent, a Bcl2 inhibitor, an MCL1 inhibitor, a HSP90 inhibitor, an IAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an auristatin, a dolastatin, a maytansinoid, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of proteins CRM1, a DPP-IV inhibitor, proteasome inhibitors, inhibitors of phosphoryl transfer reactions in mitochondria, a protein synthesis inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder and a DHFR inhibitor.

[0063] In other embodiments, the drug moiety includes but is not limited to alkylating agents, such as a thioTEPA and cyclophosphamide (CYTOXAN™); alkyl sulfonates such as busulfan, improsulfan, and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide, and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); camptothecin (including the synthetic analogue topotecan); bryostatins; callystatin; CC-1065 (including its adozelesin, carzelesin, and bizelesin synthetic analogues) and derivatives thereof; cryptophycines (particularly cryptophycin 1 and cryptophycin 8); dolastatin, auristatins (including analogues monomethyl-auristatin E and monomethyl-auristatin F (see, *e.g.*, U.S. Published Application No. 2005-0238649, published Oct. 27, 2005, incorporated herein in its entirety); duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); eleutherobin; pancratistatin; sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gammaII and calicheamicin phill, see for example, Agnew, Chem. Intl. Ed. Engl. 33:183-186; dynemicin, including dynemicin A; bisphosphonates, such as clodronate; esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromomophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (Adriamycin™) (including morpholino-doxorubicin,

cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycine, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitio stanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenis her such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; democolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone, mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2®-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitabronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, *e.g.*, paclitaxel (TAXOL™, Bristol-Myers Squibb Oncology, Princeton, N.J.) and doxetaxel (TAXOTERE®, Rhone-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine (Gemzar™); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine (Navelbine™); novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids, or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including Nolvadex™), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston™); aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, megestrol acetate (Megace™), exemestane, formestane,

fadrozole, vorozole (Rivisor™), letrozole (Femara™), and anastrozole (Arimidex™); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids, or derivatives of any of the above.

[0064] In some embodiments, the pharmaceutical composition comprises one or more of an antioxidant molecule, wherein the antioxidant molecule neutralizes a free radical. In some embodiments, the pharmaceutical composition comprises a stabilizer. In further embodiments, the stabilizer comprises gentisic acid or salts thereof, ascorbic acid or salts thereof, methionine, N-acetyl cysteine, histidine, melatonin, ethanol, Se-methionine, or combinations thereof. In some embodiments, the pharmaceutical composition is characterized to exhibit an improved resistance to a peptidase, a protease, or heat.

[0065] In some embodiments, a method of producing a composition represented by the formula selected from one or more of M-L-C-R, M-L-C, M-C-R, M-L-R, M-C, M-L, and M-R, wherein M comprises a miniprotein (M), L comprises a linker (L), C comprises a chelator (C), and R comprises a radionuclide (R) comprises synthesizing a miniprotein (M) and/or linker (L), and optionally reacting a chelator (C) and/or a radionuclide (R), and conjugating one or more of the miniprotein (M) to the linker (L), one or more of the miniprotein (M) to the chelator (C), one or more of the miniprotein (M) to the radionuclide (R), one or more of the miniprotein (M) to the linker (L) to the chelator (C), one or more of the miniprotein (M) to the linker (L) to the chelator (C) and the radionuclide (R), one or more of the miniprotein (M) to the chelator (C) to the radionuclide (R), or one or more of the miniprotein (M) to the linker (L) to the radionuclide (R).

[0066] In some embodiments, the method involves reacting a chelator (C) and a radionuclide (R) at a temperature of between about 25°C and 75°C during an incubation period. In some embodiments, reacting a chelator (C) and a radionuclide (R) is performed during an incubation period of about 5 minutes to about 30 minutes. In further embodiments, reacting a chelator (C) and a radionuclide (R) is performed at a pH in the range of about 5.0 to 7.4. In some embodiments, reacting a chelator (C) and a radionuclide (R) is performed in an aqueous solution that is substantially free of alcohol.

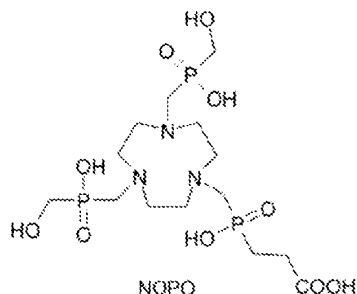
[0067] In some embodiments, a method of delivering a radionuclide to a selected location within a patient involves administering a composition represented by the formula selected from one or more of M-L-C-R, M-L-C, M-C-R, M-L-R, M-C, M-L, and M-R, wherein M comprises a miniprotein (M), L comprises a linker (L), C comprises a chelator (C), and R comprises a radionuclide (R). In some embodiments, the radionuclide is selected from Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-

211. In some embodiments, the method further comprises carrying out an imaging procedure to evaluate the localization of the radionuclide within the body, wherein the imaging procedure optionally comprises positron emission tomography (PET) imaging or single-photon emission computerized tomography (SPECT) imaging. In further embodiments, the imaging procedure allows for selecting patients. In certain embodiments, the imaging procedure allows for monitoring patients. In some embodiments, the imaging procedure allows for determining an appropriate dose for treating a patient in need of a pharmaceutical composition comprising one or more miniprotein.

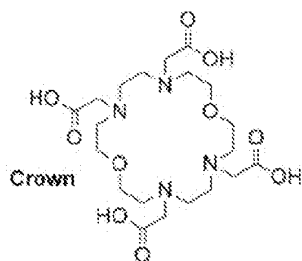
[0068] In some embodiments, the present disclosure provides methods of treating a subject in need thereof comprising administering a composition comprising a miniprotein (M), an optional linker (L), and one or both of a chelator (C) and a radionuclide (R).

[0069] In some embodiments, L comprises or consists of a polyethylene glycol (PEG) linker, an ester linker, an amide linker, a maleimide linker, a valine-citrulline linker, a hydrazone linker, a N-succinimidyl-4-(2-pyridyldithio)butyrate (SPDB) linker, a succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) linker, a vinylsulfone-based linker, a propanoic acid linker, a caproic acid linker, or any fragment or combination thereof. In some embodiments, C comprises or consists of:

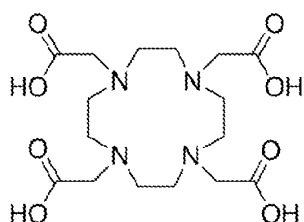
i) NOPO



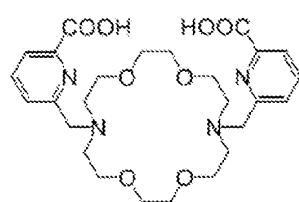
ii) Crown



iii) DOTA, or



iv) Macropa



Macropa

In some embodiments, R comprises or consists of Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211. In some embodiments, M comprises or consists of a linear polypeptide, a folded polypeptide (e.g., covalently linked polypeptide, non-covalently linked polypeptide, or polypeptide include a di-sulfide linkage), cysteine-dense peptide, a knottin peptide, a binder, an affibody, an engineered Kunitz domain, a monobody, an anticalin, a designed ankyrin repeat domain (DARPin), or an avimer. In some embodiments, M is characterized in that it comprises between 10-100 amino acids, and no more than 100 amino acids. In some preferred embodiments, M is characterized in that it comprises (i) no more than 100 amino acids and/or 12 kDa; (ii) at least two secondary structure elements; (iii) a sequestered hydrophobic core; and/or displays cooperative folding. In some embodiments, the composition comprises at least one additional component. In some embodiments, the composition can penetrate tumor tissue. In some embodiments, the miniprotein comprises no more than about 100 amino acids or less, 90 amino acids, 85 amino acids, 80 amino acids, 75 amino acids, 70 amino acids, 65 amino acids, 60 amino acids, 55 amino acids, 50 amino acids, 45 amino acids, 40 amino acids, 35 amino acids, 30 amino acids, 25 amino acids, 20 amino acids, 15 amino acids, or 10 amino acids. In some embodiments, the miniprotein comprises at least one disulfide bridge. In some embodiments, the miniprotein specifically binds to a target. In some embodiments, the

composition displays μM or nM binding affinity to the target in an *in vitro* assay. In some embodiments, the composition binds to the target with an affinity of 1 pM to 100 nM as measured by an *in vitro* binding assay. In some embodiments, the composition is characterized in that it has high tissue penetrating properties relative to a composition comprising a full-size protein that binds to the same target. In some embodiments, the miniprotein binding to the target modulates biological function. In some embodiments, administration of the composition to a subject in need thereof does not elicit an immune response or wherein the immune response elicited is tolerable to the subject. In some embodiments, the tolerable immune response includes a systemic immune response or a local immune response. In some embodiments, the subject is diagnosed as having cancer. In some embodiments, a cancer cell from the subject expresses any one of the target proteins in Table 10, or a portion thereof. In some embodiments, the expression of the target is higher in the cancer cell than in a non-cancer cell. In some embodiments, the cancer is selected from breast cancer, ovarian cancer, melanoma, pancreatic cancer, peripheral neuroma, glioblastoma, adrenocortical carcinoma, AIDS-related lymphoma, anal cancer, bladder cancer, meningioma, glioma, astrocytoma, cervical cancer, chronic myeloproliferative disorders, colon cancer, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extracranial germ cell tumors, extrahepatic bile duct cancer, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumors, gestational trophoblastic tumors, hairy cell leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, hypopharyngeal cancer, islet cell carcinoma, Kaposi sarcoma, laryngeal cancer, leukemia, lip cancer, oral cavity cancer, liver cancer, male breast cancer, malignant mesothelioma, medulloblastoma, Merkel cell carcinoma, metastatic squamous neck cell carcinoma, multiple myeloma and other plasma cell neoplasms, mycosis fungoides and the Sezary syndrome, myelodysplastic syndromes, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, small cell lung cancer, head and neck cancer, skin cancer, oropharyngeal cancer, bone cancers, including osteosarcoma and malignant fibrous histiocytoma of bone, paranasal sinus cancer, parathyroid cancer, penile cancer, pheochromocytoma, pituitary tumors, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, small intestine cancer, soft tissue sarcoma, supratentorial primitive neuroectodermal tumors, pineoblastoma, testicular cancer, thymoma, thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis and ureter, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Wilm's tumor and other childhood kidney tumors. In some embodiments,

the composition is administered intravenously or subcutaneously. In some embodiments, the cancer is treated after administration of the composition.

[0070] Additionally disclosed herein are uses of a composition disclosed herein for treating cancer in a subject. Additionally disclosed herein is an isolated polynucleotide comprising one or more nucleic acid sequences encoding a polypeptide selected from SEQ ID NO: 1-68; or a nucleic acid sequence encoding a polypeptide comprising at least 90%, 95%, 96%, 97%, 98%, 99% or greater identity to SEQ ID NO: 1-68. Additionally disclosed herein is a vector comprising an isolated polynucleotide disclosed herein. Additionally disclosed herein is a host cell transformed with an isolated polynucleotide disclosed herein or a vector disclosed herein.

[0071] Additionally disclosed herein is a method for characterizing kidney uptake of a composition, the method comprising: providing a plurality of kidney cells; contacting the plurality of kidney cells with a composition represented by the formula selected from one or more of M-L-C-R, M-L-C, M-C-R, M-L-R, M-C, M-L, and M-R, wherein M comprises a miniprotein (M), L comprises a linear, branched, or enzymatically cleavable linker (L), C comprises a chelator (C), and R comprises a radionuclide (R), wherein the composition further comprises a biotin group complexed with a fluorescently labeled streptavidin; and lysing the plurality of kidney cells and measuring kidney uptake of the composition by detecting fluorescence of the fluorescently labeled streptavidin. In various embodiments, the plurality of kidney cells are provided in a well. In various embodiments, M comprises an amino acid sequence that shares at least 90% identity to any one of SEQ ID NOs: 1-68. In various embodiments, M comprises an amino acid sequence that shares 100% identity to any one of SEQ ID NOs: 1-68.

[0072]

BRIEF DESCRIPTION OF FIGURES

[0073] FIG. 1 depicts analysis generated from SPECT/CT scans to quantify the injected dose per gram (%ID/g) of kidney tissue in mice treated with exemplary Nectin-4 charge variant conjugates (Compound ID NOs: C14, C45, C48, and C52).

[0074] FIG. 2 depicts analysis generated from SPECT/CT scans to quantify the injected dose per gram (%ID/g) of kidney tissue in mice. Co-administration of an exemplary decoy peptide (Compound ID NO: C15) reduces kidney uptake of an exemplary Nectin-4 targeting miniprotein conjugate (Compound ID NO: C14) scaffolds.

[0075] FIG. 3 depicts analysis generated from SPECT/CT scans to quantify the injected dose per gram (%ID/g) of kidney tissue in mice. Exemplary B7H3 charge variant conjugates

(Compound ID NOs: C1, C3, C5, C9, and C43) demonstrate reduced levels of kidney retention in mouse biodistribution.

[0076] FIG. 4 depicts analysis generated from SPECT/CT scans to quantify the injected dose per gram (%ID/g) of kidney tissue in mice. Co-administration of an exemplary decoy peptide (Compound ID NO: C7) reduces kidney uptake of an exemplary B7H3 targeting affibody conjugate (Compound ID NO: C9).

[0077] FIG. 5 depicts analysis generated from SPECT/CT scans to quantify the injected dose per gram (%ID/g) of kidney tissue in mice. Comparison of an exemplary conjugate with an added exemplary Version 1 cleavable linker (Compound ID NO: C64) and an exemplary Nectin-4 conjugate (Compound ID NO: C14) showed minimal alterations in kidney uptake and retention.

[0078] FIG. 6 depicts a DELFIA saturation binding curve-fitting of an exemplary conjugate (Compound ID NO: C34) for estimating K_D .

[0079] FIG. 7 depicts a DELFIA competitive binding curve of an exemplary conjugate (Compound ID NO: C40) for estimating K_i .

[0080] FIG. 8 depicts binding kinetics of an exemplary conjugate (Compound ID NO: C11) to immobilized ligand. The change in signal over time is proportional to peptide binding to the ligand, generating a sensorgram. Black lines are fitted by the 1:1 binding model to calculate a K_D (M). Concentrations correspond to labels on the diagram as follows: a: 25 nM; b: 12.5 nM; c: 6.25 nM; d: 3.13 nM; e: 3.13 nM; f: 1.56 nM; and g: 0.78 nM.

[0081] FIG. 9A depicts quantitative uptake of an exemplary AF647-labeled conjugate (Compound ID NO: C9) in the Opossum kidney proximal tubule cell (OK-PTC) uptake assay.

[0082] FIG. 9B depicts quantitative uptake in the Opossum kidney proximal tubule cell (OK-PTC) uptake assay. Co-treatment with an exemplary decoy peptide (Compound ID NO: C15) at 100X and 10X molar excess reduces the uptake of an exemplary AF647-labeled conjugate (Compound ID NO: C14).

[0083] FIG. 9C depicts quantitative uptake in the Opossum kidney proximal tubule cell (OK-PTC) uptake assay. Uptake of an exemplary AF647-labeled control conjugate (Compound ID NO: C14) is reduced in a dose-dependent manner by pre-treatment with a mixture of lysine and arginine amino acids.

[0084] FIG. 10 depicts quantitative uptake in the Opossum kidney proximal tubule cell (OK-PTC) uptake assay. Co-treatment with a 20-fold excess of exemplary decoys C79, C96, and C78 reduces the uptake of the test agents C132 – C139.

[0085] FIG. 11 depicts quantitative uptake in the Opossum kidney proximal tubule cell (OK-PTC) uptake assay. Co-treatment with 10X molar excess of exemplary decoy peptides (Compound ID NOs: C15, C80, and C83) reduces the uptake of an exemplary biotinylated test agent (Compound ID NO: C14)

[0086] FIG. 12 depicts analysis generated from SPECT/CT scans to quantify the injected dose per gram (%ID/g) of kidney tissue in mice. Co-administration of an exemplary decoy peptide (Compound ID NO: C79) reduces kidney uptake and retention of an exemplary Nectin-4 targeting miniprotein conjugate (Compound ID NO: C67) scaffolds.

[0087] FIG. 13 depicts analysis generated from SPECT/CT scans to quantify the injected dose per gram (%ID/g) of kidney tissue in mice. Comparison of an exemplary conjugate with an added exemplary Version 2 cleavable linker (Compound ID NO: C68) and an exemplary Nectin-4 conjugate (Compound ID NO: C67) showed no alterations in early kidney uptake and modest reduction in kidney retention at later timepoints.

[0088] FIG. 14 depicts analysis generated from SPECT/CT scans to quantify the injected dose per gram (%ID/g) of kidney tissue in mice. Comparison of an exemplary conjugate with an added albumin-binding motif (Compound ID NO: C69) and an exemplary Nectin-4 conjugate (Compound ID NO: C67) showed reduction in kidney uptake.

[0089] FIG. 15 depicts tumor volume measurements after single dose treatment of test articles in mouse efficacy studies. HT-1376 cells with exogenously expressed target were treated with either vehicle or ²²⁵Ac-labeled test article (Compound ID NO: C116) at X or 2X nCi and showed reduction in tumor volume.

[0090] FIG. 16 depicts body weight measurements after single dose treatment of test articles in mouse efficacy studies. HT-1376 cells with exogenously expressed target were treated with either vehicle or ²²⁵Ac-labeled test article (Compound ID NO: C116) at X or 2X nCi showed no change in body weight.

DETAILED DESCRIPTION

[0091] Among other things, the present disclosure provides compositions and methods of use thereof. In some embodiments, a composition selectively binds to a target. In some embodiments the composition comprises one or more therapeutic agents (*e.g.*, a chelator, a radionuclide), wherein the therapeutic agent is selectively targeted to a cell, *e.g.*, expressing any one of the target proteins in Table 10, such that the target-expressing cell is treated and cells not expressing the target are not treated. In various embodiments, the target protein is

any one of the target proteins in Table 10. In particular embodiments, the target protein is Nectin-4. The present disclosure recognizes that a source of a problem in treating cells expressing a target (*e.g.*, cancer cells) is that traditional therapies are not selective enough to specifically target cells and to deliver a therapeutic in a way that minimizes damage to surrounding cells. The present disclosure provides the insight that a combination of selective targeting with a specific therapeutic such as a chelator and/or radionuclide (*e.g.*, an alpha emitter) provides an advantage over previously used therapeutics (*e.g.*, antibodies, beta-emitters, *etc.*)

[0092] Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include the plural and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, biochemistry, enzymology, molecular and cellular biology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art.

[0093] The methods and techniques of the present disclosure are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, *e.g.*, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989); Ausubel *et al.*, *Current Protocols in Molecular Biology*, Greene Publishing Associates (1992, and Supplements to 2002); Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990); Wittrup and VanAntwerp, *Fine Affinity Discrimination by Yeast Surface Display and Flow Cytometry*, *Biotechnol. Prog.* 2002, (16) 31-37; C. Queen *et al.*, A humanized antibody that binds to the interleukin 2 receptor, *Proc. Natl. Acad. Sci. USA* 1989, 86 (24) 10029-10033; Scheinberg DA and McDevitt MR. *Actinium-225 in targeted alpha-particle therapeutic applications*. *Curr Radiopharm.* 2011;4(4):306-320.

[0094] All publications, patents, and other references mentioned herein are hereby incorporated by reference in their entireties. In case of conflict, the present specification, including definitions, will control. Materials, methods, and examples as disclosed herein are illustrative only and not intended to be limiting.

Definitions

[0095] 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 to which this present disclosure pertains. Further, unless otherwise required by context, singular terms shall include the plural and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, biochemistry, enzymology, molecular and cellular biology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art.

[0096] Throughout this specification and claims, the word “comprise” or variations such as “comprises” or “comprising,” will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

[0097] As used herein, ranges and amounts can be expressed as “about” a particular value or range. About also includes the exact amount. Hence “about 100 nucleotides” can mean “about 100 nucleotides” and also “100 nucleotides.” Generally, the term “about” as used herein includes an amount that would be expected to be within experimental error.

[0098] Unless otherwise indicated, and as an example for all sequences described herein under the general format “SEQ ID NO:”, “nucleic acid comprising SEQ ID NO: 1” refers to a nucleic acid, at least a portion of which has either (i) the sequence of SEQ ID NO: 1, or (ii) a sequence complementary to SEQ ID NO: 1. The choice between the two is dictated by the context. For instance, if the nucleic acid is used as a probe, the choice between the two is dictated by the requirement that the probe be complementary to the desired target.

[0099] As used herein, the term “administration” refers providing a composition to a subject or system. Administration to a subject may be by any appropriate route, dose and/or dose schedule.

[00100] As used herein, the term “affibody” refers to a subgenus of miniproteins. An affibody is a molecule derived from the Z-domain of staphylococcal protein A that consists of three alpha helices with 58 amino acids and has a molar mass of about 6 kDa. See, for exemplary details of affibody structures and uses, Orlova, A; Magnusson, M; Eriksson, T L; Nilsson, M; Larsson, B; Höiden-Guthenberg, I; Widström, C; Carlsson, J et al. (2006). “Tumor imaging using a picomolar affinity HER2 binding affibody molecule”, Cancer Res. 66 (8): 4339-48. Exemplary Affibody® Molecules are commercially available from Abcam Corp. Cambridge Mass. An affibody is stable at high temperatures and under acidic or alkaline conditions. Target specificity is obtained by randomization of 13 amino acids located

in two alpha-helices involved in the binding activity of the parent protein domain (Feldwisch J, Tolmachev V.: (2012) Methods Mol Biol. 899:103-26).

[00101] As used herein, the term “affinity maturation” generally refers to a process whereby successive changes to a sequence (*e.g.*, successive mutations) are made and selection of the polypeptide sequences are performed to choose one or more sequences with increased affinity relative to the “starting” sequence or another sequence with less affinity as compared to one with greater affinity.

[00102] As used herein, the terms “amino acid sequence” and “polypeptide” refer to a polymer of amino acids connected by one or more peptide bonds. A polypeptide of the present disclosure encompasses both naturally-occurring and non-naturally-occurring proteins, and any fragments, portions, peptides, mutants, derivatives, and analogs thereof. A polypeptide may be monomeric or polymeric. Further, a polypeptide may comprise a number of different domains each of which has one or more distinct activities. A polypeptide may be fully or partially synthetic or otherwise modified (*i.e.*, comprising one or more synthetically-produced amino acids and/or modifications thereof). The term “peptide” may be used to refer to a short polypeptide, such as one comprising fewer than about 70 amino acids (*e.g.*, between about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, or 70 amino acids).

[00103] As used herein, the term “anticalin” refers to a subgenus of miniproteins. An anticalin is an engineered protein derived from a lipocalin (Beste G, Schmidt FS, Stibora T, Skerra A. (1999) Proc Natl Acad Sci U S A. 96(5): 1898-903; Gebauer and Skerra (2009) Curr Opin in Chemical Biology 13:245-255). Anticalins possess an eight-stranded b-barrel which forms a highly conserved core unit among the lipocalins and naturally forms binding sites for ligands by means of four structurally variable loops at the open end. Anticalins, although not homologous to the IgG superfamily, show features that so far have been considered typical for the binding sites of antibodies: (i) high structural plasticity as a consequence of sequence variation and (ii) elevated conformational flexibility, allowing induced fit to targets with differing shape.

[00104] As used herein, a “compound” refers at least a miniprotein with an amino acid sequence. Compounds may include miniproteins with different modifications, such as a N-terminal modification or a C-terminal modification. In various embodiments, a “compound” can include a miniprotein and one or more additional elements, examples of which include a linker, a chelator, and/or a radionuclide. For example, a compound may include a miniprotein conjugated to a chelator and/or a radionuclide *e.g.*, via a linker. As denoted herein, compounds are identified with a specific compound number *e.g.*, “C1,” “C2,” “C3”,

etc. Different compounds may have different sequences. In various embodiments, different compounds may have the same sequence (e.g., assigned the same SEQ ID NO), but may have one or more of different modifications (e.g., different N-terminal or C-terminal modifications), different linkers, different chelators, and/or different radionuclides.

[00105] As used herein, the term “attenuate” as used herein generally refers to a functional deletion, including a mutation, partial or complete deletion, insertion, or other variation made to a gene sequence or a sequence controlling the transcription of a gene sequence, which reduces or inhibits production of the gene product, or renders the gene product non-functional. In some instances, a functional deletion is described as a knockout mutation. Attenuation also includes amino acid sequence changes by altering the nucleic acid sequence, placing the gene under the control of a less active promoter, down-regulation, expressing interfering RNA, ribozymes or antisense sequences that target the gene of interest, or through any other technique known in the art. In one example, the sensitivity of a particular enzyme to feedback inhibition or inhibition caused by a composition that is not a product or a reactant (non-pathway specific feedback) is lessened such that the enzyme activity is not impacted by the presence of a compound. In other instances, an enzyme that has been altered to be less active can be referred to as attenuated.

[00106] As used herein, the term “avimer” refers to a subgenus of miniproteins. An avimer is a class of antibody mimetics which consist of two or more peptide sequences of preferably 30 to 35 amino acids each, which are derived from A-domains of various membrane receptors and which are connected by linker peptides. Binding of target molecules occurs via the A-domain and domains with the desired binding specificity can be selected, for example, by phage display techniques. The binding specificity of the different A-domains contained in an avimer may, but does not have to be identical (Weidle UH, et al., (2013), *Cancer Genomics Proteomics*; 10(4): 155-68). For further details see *Nature Biotechnology* 23(12), 1556 - 1561 (2005) and *Expert Opinion on Investigational Drugs* 16(6), 909-917 (June 2007).

[00107] As used herein, the term “binder” refers to a subgenus of miniprotein. A binder is characterized in that it comprises or consists of a polypeptide (e.g., peptide) that is capable of binding or has known ability to engage and associate a target or a portion thereof. Binders generally comprise a cysteine-containing peptide comprising one or more disulfide bonds, though some binders do not comprise cysteine-residues and/or disulfide bonds. Binders are preferably cleared rapidly from circulation when administered systemically to a mammalian subject. As will be understood, given context, reference to a binder may be or include its nucleic acid sequence or amino acid sequence encoding it. A binder may be provided, for

instance, as a polynucleotide, polypeptide, using a vector, host cell, *etc.*, and/or any combination of modalities. A binder may be derived or manufactured using any method known to those of skill in the art. For instance, in some embodiments, a binder can be recombinant (*i.e.*, produced using recombinant nucleic acids encoding a polypeptide). In some embodiments, a binder can be synthetic (*e.g.*, synthesized such as using standard solid phase synthesis methods, such as solid phase peptide synthesis, known to those of skill in the art (*see, e.g.*, Palomo, J. *RSC Adv.*, 2014,4, 32658-32672) and described herein.

[00108] The term “chelator” as used herein refers to any molecule or moiety that is capable of forming a complex (*i.e.*, “chelates”) with a metal ion. Chelators generally have two or more unshared electron pairs that can be used to donate to a metal ion. Metal ions are usually coordinated to the chelator by two or more pairs of electrons.

[00109] As used herein, the term “conjugated” refers to the joining by covalent or noncovalent means of two compounds or agents. In some embodiments, a “conjugate” may refer to, for example, a peptide coupled to one or more of, *e.g.*, a linker, chelator, and/or radionuclide.

[00110] As used herein, a “conservative amino acid substitution” is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (*e.g.*, charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of homology may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. *See, e.g.*, Pearson, 1994, *Methods Mol. Biol.* 24:307-31 and 25:365-89 (herein incorporated by reference). The following six groups each contain amino acids that are conservative substitutions for one another: 1) Serine (S), Threonine (T); 2) Aspartic Acid (D), Glutamic Acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Alanine (A), Valine (V), and 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

[00111] As used herein, the terms “cysteine-dense peptide” and “CDP” are used interchangeably and refer to a subgenus of miniproteins that generally comprise at least two independent folding domains and a high density of cysteines. In some embodiments, the CDP comprises at least one, two, three, four, or more cysteine residues in a span of from about 10 to about 90 amino acid residues, preferably 13 to 80 amino acid residues.

(<https://pubmed.ncbi.nlm.nih.gov/29483648/>) In some embodiments, the CDP comprises a

constrained distribution of cysteines, Cys-X_[0-15]-Cys-X_[0-15]-Cys-X_[0-15]-Cys-X_[0-15]-Cys-X_[0-15]-Cys (wherein X represents any amino acid) (SEQ ID NO: 81).

[00112] As used herein, the term “deletion” generally refers to the removal of one or more nucleotides from a nucleic acid molecule or one or more amino acids from a protein, the regions on either side being joined together.

[00113] As used herein, the phrase “degenerate variant” of a reference nucleic acid sequence encompasses nucleic acid sequences that can be translated, according to the standard genetic code, to provide an amino acid sequence identical to that translated from the reference nucleic acid sequence. The term “degenerate oligonucleotide” or “degenerate primer” is used to signify an oligonucleotide capable of hybridizing with target nucleic acid sequences that are not necessarily identical in sequence but that are homologous to one another within one or more particular segments.

[00114] As used herein, the term “derived from,” with reference to a nucleic acid sequence refers to a nucleic acid sequence that has at least 85% sequence identity to a reference naturally occurring nucleic acid sequence from which it is derived. The term “derived from,” with reference to an amino acid sequence refers to an amino acid sequence that has at least 85% sequence identity to a reference naturally occurring amino acid sequence from which it is derived. The term “derived from” as used herein does not denote any specific process or method for obtaining the nucleic acid or amino acid sequence. For example, the nucleic acid or amino acid sequence can be chemically synthesized.

[00115] As used herein, the term “designed ankyrin repeat domain (DARPin)” refers to a subgenus of miniproteins. A DARPin is a peptide derived from Ankyrin which is a family of proteins that mediate attachment of integral membrane proteins to the cytoskeleton. A single ankyrin repeat is preferably a 33 residue motif consisting of two alpha-helices and a beta-turn. They can be engineered to bind different targets by randomizing residues in the first alpha-helix and a beta-turn of each repeat. Their binding interface can be increased by increasing the number of modules (a method of affinity maturation). For further details see J. Mol. Biol. 332, 489-503 (2003), PNAS 100(4), 1700-1705 (2003) and J. Mol. Biol. 369, 1015-1028 (2007) and US20040132028A1. DARPins typically provide a rigid interface and lack structural flexibility (Gebauer and Skerra, 2009).

[00116] As used herein, the term “domain” as used herein refers to a structure of a biomolecule that contributes to a known or suspected function of the biomolecule. Domains may be co-extensive with regions or portions thereof; domains may also include distinct, non-contiguous regions of a biomolecule. Examples of protein domains include, but are not

limited to, an Ig domain, an extracellular domain, a transmembrane domain, and a cytoplasmic domain.

[00117] As used herein, the term “engineered Kunitz domain” refers to a subgenus of miniproteins. An engineered Kunitz domain is preferably a peptide derived from the Kunitz domain of a Kunitz-type protease inhibitor such as bovine pancreatic trypsin inhibitor (BPTI), amyloid precursor protein (APP) or tissue factor pathway inhibitor (TFPI). Kunitz domains have a molecular weight of approximately 6 kDA and domains with the required target specificity can be selected by display techniques such as phage display (Weidle et al., (2013), *Cancer Genomics Proteomics*; 10(4): 155-68).

[00118] As used herein, the term “expression control sequence” as used herein refers to polynucleotide sequences which are necessary to affect the expression of coding sequences to which they are operatively linked. Expression control sequences are sequences which control the transcription, post-transcriptional events and translation of nucleic acid sequences. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (*e.g.*, ribosome binding sites); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence. The term “control sequences” is intended to include, at a minimum, all components whose presence is essential for expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

[00119] As used herein, the term “fusion protein” refers to a polypeptide comprising a polypeptide or fragment coupled to heterologous amino acid sequences. Fusion proteins are useful because they can be constructed to contain two or more desired functional elements from two or more different proteins. A fusion protein comprises at least 10 contiguous amino acids from a polypeptide of interest, more preferably at least 20 or 30 amino acids, even more preferably at least 40, 50 or 60 amino acids, yet more preferably at least 75, 100 or 125 amino acids. Fusions that include the entirety of the proteins of the present disclosure have particular utility. The heterologous polypeptide included within the fusion protein of the present disclosure is at least 6 amino acids in length, often at least 8 amino acids in length, and usefully at least 15, 20, and 25 amino acids in length. Fusions that include larger polypeptides, such as an IgG Fc region, and even entire proteins, such as the green

fluorescent protein ("GFP") chromophore-containing proteins, have particular utility. Fusion proteins can be produced recombinantly by constructing a nucleic acid sequence which encodes the polypeptide or a fragment thereof in frame with a nucleic acid sequence encoding a different protein or peptide and then expressing the fusion protein. Alternatively, a fusion protein can be produced chemically by crosslinking the polypeptide or a fragment thereof to another protein.

[00120] As used herein, when referring to a protein, "homology" to a second protein can exist if the nucleic acid sequence that encodes the protein has a similar sequence to the nucleic acid sequence that encodes the second protein. Alternatively, a protein has homology to a second protein if the two proteins have "similar" amino acid sequences. (Thus, the term "homologous proteins" is defined to mean that the two proteins have similar amino acid sequences.) Homology between two regions of amino acid sequences (especially with respect to predicted structural similarities) can be interpreted as implying similarity in function. Homologous proteins or peptides with residue positions that are not identical are often recognized to differ by conservative amino acid substitutions.

[00121] As used herein the term "identical" refers to a nucleic acid sequence or amino acid sequence refers to at least two nucleic acid or at least two amino acid sequences or subsequences that have a specified percentage of nucleotides or amino acids, respectively, that are the same, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm or by visual inspection. For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. A length of sequence identity comparison may be over a stretch of any number of nucleotides or amino acids. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters. A number of algorithms are known in the art. Non-limiting examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in, *e.g.*, Altschul *et al.* (1990) J. Mol. Biol. 215: 403-410 and Altschul *et al.* (1977) Nucleic Acids Res. 25: 3389-3402, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. Additionally or alternatively sequences can be compared using FASTA, Gap or Bestfit, which are programs in Wisconsin Package Version 10.0, Genetics Computer Group (GCG),

Madison, Wis. FASTA provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences. Pearson, *Methods Enzymol.* 183:63-98 (1990) (hereby incorporated by reference in its entirety). For instance, percent sequence identity can be determined using FASTA with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) or using Gap with its default parameters as provided in GCG Version 6.1, herein incorporated by reference.

[00122] As used herein, the term “isolated” polynucleotide or polypeptide is one which is substantially separated from other cellular components that naturally accompany the native polynucleotide in its natural host cell, *e.g.*, ribosomes, polymerases and genomic sequences with which it is naturally associated. For instance, an isolated molecule is one that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) exists in a purity not found in nature, where purity can be adjudged with respect to the presence of other cellular material (*e.g.*, is free of other proteins from the same species) (3) is expressed by a cell from a different species, or (4) does not occur in nature (*e.g.*, it is a fragment of a polynucleotide or polypeptide found in nature or it includes amino acid analogs or derivatives not found in nature or linkages other than standard peptide bonds). Thus, a polynucleotide or polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be “isolated” from its naturally associated components. A polynucleotide or polypeptide may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art. As thus defined, “isolated” does not necessarily require that any molecule so described has been physically removed from its native environment. In some embodiments, as used in reference to an isolated construct, isolated means in the absence of a pharmaceutically acceptable salt.

[00123] As used herein, the term “ K_D ” or “Kd” refers to the dissociation equilibrium constant for a particular targeting molecule-targeting protein interaction. Typically, the antibody of the present disclosure binds to any one of the target proteins in Table 10 with a dissociation equilibrium constant (K_D) of less than about 10^{-7} M, such as less than about 10^{-8} M, 10^{-9} M or 10^{-10} M or less, for example, as determined using surface plasmon resonance (SPR) techniques in a BIACORE instrument.

[00124] As used herein, the term “knock out” generally refers to a gene whose level of expression or activity has been reduced to zero. In some examples, a gene is knocked out via deletion of some or all of its coding sequence. In other examples, a gene is knocked out via

introduction of one or more nucleotides into its open reading frame, which results in translation of a nonsense or otherwise nonfunctional protein product.

[00125] The term “knottin” as used herein refers to a structural motif of a miniprotein containing three disulfide bridges.

[00126] The term “knottin peptide” as used herein refers to a subgenus of miniproteins that comprises at least one knottin.

[00127] The term “linker” as used herein refers to a moiety that is used to conjugate a miniprotein to a chelator.

[00128] As used herein, the term “miniprotein” refers to short proteins of 10 to 100 amino acids with well-defined folds comprising two or more secondary structure elements, a sequestered hydrophobic core, and/or cooperative folding. CDPs, knottins, affibodies, engineered Kunitz domains, monobodies (adnectins), anticalins, designed ankyrin repeat domains (DARPs), avimers, and binders as disclosed herein are all examples of miniproteins.

[00129] As used herein, the term “modification,” with reference to a nucleic acid sequence, refers to a nucleic acid sequence that comprises at least one substitution, alteration, inversion, addition, or deletion of nucleotide compared to a reference nucleic acid sequence. As used herein, the term “modification,” with reference to an amino acid sequence refers to an amino acid sequence that comprises at least one substitution, alteration, inversion, addition, or deletion of an amino acid residue compared to a reference nucleic acid sequence.

[00130] As used herein, the term “modified derivative” refers to polypeptides or fragments thereof that are substantially homologous in primary structural sequence but which include, *e.g.*, in vivo or in vitro chemical and biochemical modifications or which incorporate amino acids that are not found in the native polypeptide. Such modifications include, for example, acetylation, carboxylation, phosphorylation, glycosylation, ubiquitination, labeling, *e.g.*, with radionuclides, and various enzymatic modifications, as will be readily appreciated by those skilled in the art. A variety of methods for labeling polypeptides and of substituents or labels useful for such purposes are well known in the art, and include radioactive isotopes such as ¹²⁵I, ³²P, ³⁵S, and ³H, ligands which bind to labeled antiligands (*e.g.*, antibodies), fluorophores, chemiluminescent agents, enzymes, and antiligands which can serve as specific binding pair members for a labeled ligand. The choice of label depends on the sensitivity required, ease of conjugation with the primer, stability requirements, and available instrumentation. Methods for labeling polypeptides are well known in the art. See, *e.g.*,

Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates (1992, and Supplements to 2002) (hereby incorporated by reference).

[00131] As used herein, the term “molecule” means any compound, including, but not limited to, a small molecule, peptide, protein, sugar, nucleotide, nucleic acid, lipid, *etc.*, and such a compound can be natural or synthetic.

[00132] As used herein, the term “monobody” or “adnectin” are used interchangeably and refer to a subgenus of miniproteins. A monobody relates to a molecule, preferably based on the 10th extracellular domain of human fibronectin III (10Fn3), which adopts an Ig-like b-sandwich fold of preferably 94 residues with 2 to 3 exposed loops, but lacks the central disulfide bridge (Gebauer and Skerra (2009) Curr Opinion in Chemical Biology 13:245-255). Adnectins with the desired target specificity can be genetically engineered by introducing modifications in specific loops of the protein.

[00133] As used herein, the term “mutein” or “mutant protein” or “variant” means a protein comprising an amino acid sequence with at least one variation (*e.g.*, an insertion, a deletion, or a substitution, which can be a conservative or non-conservative substitution) compared to a reference sequence. When applied to sequences (*e.g.*, nucleic acid sequences, amino acid sequences) “mutated” means that nucleotides in a nucleic acid sequence or amino acids in an amino acid sequences may be inserted, deleted or changed compared to a reference sequence. A single alteration may be made at a locus (a point mutation) or multiple nucleotides or amino acids may be inserted, deleted or changed at a single locus. In addition, one or more alterations may be made at any number of loci within a nucleic acid or amino acid sequence. A nucleic acid or amino acid sequence may be mutated by any method known in the art including but not limited to mutagenesis techniques such as “error-prone PCR” (a process for performing PCR under conditions where the copying fidelity of the DNA polymerase is low, such that a high rate of point mutations is obtained along the entire length of the PCR product; see, *e.g.*, Leung et al., Technique, 1:11-15 (1989) and Caldwell and Joyce, PCR Methods Applic. 2:28-33 (1992)); “oligonucleotide-directed mutagenesis” (a process which enables the generation of site-specific mutations in any cloned DNA segment of interest; see, *e.g.*, Reidhaar-Olson and Sauer, Science 241:53-57 (1988)); directed evolution (*e.g.*, exposing a polypeptide to differing sets of conditions resulting in production of different polypeptides with one or more amino acid changes that may or may not confer greater fitness upon the polypeptide); and site-directed mutagenesis (*e.g.*, specifically directed changes in a sequence).

[00134] As used herein, the term “non-disulfide sequence” refers to an amino acid sequence encoding a polypeptide that does not comprise more than one cysteine residue and/or disulfide bonds in its folded and active form. In some embodiments, miniprotein may comprise or consist of a non-disulfide sequence.

[00135] As used herein, the term “non-peptide analog” refers to a compound with properties that are analogous to those of a reference polypeptide. A non-peptide compound may also be termed a “peptide mimetic” or a “peptidomimetic.” See, *e.g.*, Jones, *Amino Acid and Peptide Synthesis*, Oxford University Press (1992); Jung, *Combinatorial Peptide and Nonpeptide Libraries: A Handbook*, John Wiley (1997); Bodanszky et al., *Peptide Chemistry - A Practical Textbook*, Springer Verlag (1993); *Synthetic Peptides: A Users Guide*, (Grant, ed., W. H. Freeman and Co., 1992); Evans et al., *J. Med. Chem.* 30:1229 (1987); Fauchere, J. *Adv. Drug Res.* 15:29 (1986); Veber and Freidinger, *Trends Neurosci.*, 8:392-396 (1985); and references cited in each of the above, which are incorporated herein by reference. Such compounds are often developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to useful peptides of the present disclosure may be used to produce an equivalent effect and are therefore envisioned to be part of the present disclosure.

[00136] As used herein, the terms “nucleic acid sequence” and “polynucleotide” are used interchangeably to refer to a polymer of nucleotides. The term includes DNA molecules (*e.g.*, cDNA or genomic or synthetic DNA) and RNA molecules (*e.g.*, mRNA or synthetic RNA), as well as analogs of DNA or RNA containing non-natural nucleotide analogs, non-native internucleoside bonds, or both. The nucleic acid can be in any topological conformation. For instance, the nucleic acid can be single-stranded, double-stranded, triple-stranded, quadruplexed, partially double-stranded, branched, hairpinned, circular, or in a padlocked conformation. The nucleic acid sequence can contain natural, non-natural, or altered nucleotides; and contain a natural, non-natural, or altered internucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified nucleic acid sequence. Nucleic acid sequences include, but are not limited to, all nucleic acid sequences which are obtained by any means available in the art, including, without limitation, recombinant means, *e.g.*, the cloning of nucleic acid sequences from a recombinant library or a cell genome, using ordinary cloning technology and polymerase chain reaction, and the like, and by synthetic means. Polynucleotides of the present disclosure may include both sense and antisense strands of RNA, cDNA, genomic DNA, and synthetic forms and mixed polymers of the above. They

may be modified chemically or biochemically or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those of skill in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages (*e.g.*, methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, *etc.*), charged linkages (*e.g.*, phosphorothioates, phosphorodithioates, *etc.*), pendent moieties (*e.g.*, polypeptides), intercalators (*e.g.*, acridine, psoralen, *etc.*), chelators, alkylators, and modified linkages (*e.g.*, alpha anomeric nucleic acids, *etc.*) Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule. Other modifications can include, for example, analogs in which the ribose ring contains a bridging moiety or other structure such as the modifications found in “locked” nucleic acids.

[00137] As used herein, the terms “operatively linked” or “operably linked” expression control sequences refers to a linkage in which the expression control sequence is contiguous with the gene of interest to control the gene of interest, as well as expression control sequences that act in trans or at a distance to control the gene of interest.

[00138] As used herein, the terms “polypeptide mutant” or “mutein” refers to a polypeptide whose sequence contains an insertion, duplication, deletion, rearrangement or substitution of one or more amino acids compared to the amino acid sequence of a native or wild-type protein. A mutein may have one or more amino acid point substitutions, in which a single amino acid at a position has been changed to another amino acid, one or more insertions and/or deletions, in which one or more amino acids are inserted or deleted, respectively, in the sequence of the naturally-occurring protein, and/or truncations of the amino acid sequence at either or both the amino or carboxy termini. A mutein may have the same but preferably has a different biological activity compared to the naturally-occurring protein. A mutein has at least 85% overall sequence homology to its wild-type counterpart. Even more preferred are muteins having at least 90% overall sequence homology to the wild-type protein. In an even more preferred embodiment, a mutein exhibits at least 95% sequence identity, even more preferably 98%, even more preferably 99% and even more preferably 99.9% overall sequence identity. Sequence homology may be measured by any common sequence analysis algorithm, such as Gap or Bestfit. Amino acid substitutions can include those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3)

alter binding affinity for forming protein complexes, (4) alter binding affinity or enzymatic activity, and (5) confer or modify other physicochemical or functional properties of such analogs.

[00139] As used herein, the term “polypeptide fragment” as used herein refers to a polypeptide that has a deletion, *e.g.*, an amino-terminal and/or carboxy-terminal deletion compared to a full-length polypeptide. In a preferred embodiment, the polypeptide fragment is a contiguous sequence in which the amino acid sequence of the fragment is identical to the corresponding positions in the naturally-occurring sequence. Fragments typically are at least 5, 6, 7, 8, 9 or 10 amino acids long, preferably at least 12, 14, 16 or 18 amino acids long, more preferably at least 20 amino acids long, more preferably at least 25, 30, 35, 40 or 45, amino acids, even more preferably at least 50 or 60 amino acids long, and even more preferably at least 70 amino acids long.

[00140] As used herein, the term “radionuclide” as used herein refers to an atom capable of undergoing radioactive decay.

[00141] As used herein, the term “recombinant” refers to a biomolecule, *e.g.*, a gene or protein, that (1) has been removed from its naturally occurring environment, (2) is not associated with all or a portion of a polynucleotide in which the gene is found in nature, (3) is operatively linked to a polynucleotide which it is not linked to in nature, and/or (4) does not occur in nature. The term “recombinant” can be used in reference to cloned DNA isolates, chemically synthesized polynucleotide analogs, or polynucleotide analogs that are biologically synthesized by heterologous systems, as well as proteins and/or mRNAs encoded by such nucleic acids. As used herein, an endogenous nucleic acid sequence in the genome of an organism (or the encoded protein product of that sequence) is deemed “recombinant” herein if a heterologous sequence is placed adjacent to the endogenous nucleic acid sequence, such that the expression of this endogenous nucleic acid sequence is altered. In this context, a heterologous sequence is a sequence that is not naturally adjacent to the endogenous nucleic acid sequence, whether or not the heterologous sequence is itself endogenous (originating from the same host cell or progeny thereof) or exogenous (originating from a different host cell or progeny thereof). By way of example, a promoter sequence can be substituted (*e.g.*, by homologous recombination) for the native promoter of a gene in the genome of a host cell, such that this gene has an altered expression pattern. This gene would now become “recombinant” because it is separated from at least some of the sequences that naturally flank it. A nucleic acid is also considered “recombinant” if it contains any modifications that do not naturally occur to the corresponding nucleic acid in a genome. For instance, an endogenous

coding sequence is considered “recombinant” if it contains an insertion, deletion or a point mutation introduced artificially, *e.g.*, by human intervention. A “recombinant nucleic acid” also includes a nucleic acid integrated into a host cell chromosome at a heterologous site and a nucleic acid construct present as an episome.

[00142] As used herein, the term “recombinant host cell” (or simply “host cell”), as used herein, is intended to refer to a cell into which a recombinant vector has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term “host cell” as used herein. A recombinant host cell may be an isolated cell or cell line grown in culture or may be a cell which resides in a living tissue or organism.

[00143] As used herein, the term “region” as used herein refers to a physically contiguous portion of the primary structure of a biomolecule. In the case of proteins, a region is defined by a contiguous portion of the amino acid sequence of that protein.

[00144] As used herein, “sequence homology” for polypeptides, also referred to as “percent sequence identity,” is typically measured using sequence analysis software. See, *e.g.*, the Sequence Analysis Software Package of the Genetics Computer Group (GCG), University of Wisconsin Biotechnology Center, 910 University Avenue, Madison, Wis. 53705. Protein analysis software matches similar sequences using a measure of homology assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG contains programs such as “Gap” and “Bestfit” which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild-type protein and a mutein thereof. See, *e.g.*, GCG Version 6.1. A preferred algorithm when comparing a particular polypeptide sequence to a database containing a large number of sequences from different organisms is the computer program BLAST (Altschul et al., J. Mol. Biol. 215:403-410 (1990); Gish and States, Nature Genet. 3:266-272 (1993); Madden et al., Meth. Enzymol. 266:131-141 (1996); Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997); Zhang and Madden, Genome Res. 7:649-656 (1997)), especially blastp or tblastn (Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997)). Preferred parameters for BLASTp are: Expectation value: 10 (default); Filter: seg (default); Cost to open a gap: 11 (default); Cost to extend a gap: 1 (default); Max. alignments: 100 (default); Word size: 11 (default); No. of descriptions: 100 (default); Penalty Matrix:

BLOWSUM62. The length of polypeptide sequences compared for homology will generally be at least about 16 amino acid residues, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues, and preferably more than about 35 residues. When searching a database containing sequences from a large number of different organisms, it is preferable to compare amino acid sequences. Database searching using amino acid sequences can be measured by algorithms other than blastp known in the art. For instance, polypeptide sequences can be compared using FASTA, a program in GCG Version 6.1. FASTA provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences. Pearson, *Methods Enzymol.* 183:63-98 (1990) (incorporated by reference herein). For example, percent sequence identity between amino acid sequences can be determined using FASTA with its default parameters (a word size of 2 and the PAM250 scoring matrix), as provided in GCG Version 6.1, herein incorporated by reference.

[00145] As used herein, the term “specificity” generally refers to a sequence that, when in a conformation that can bind, selectively or “specifically” binds to a specific target. As used herein, “specifically binds” means that the binding of a polynucleotide, polypeptide, or protein is selective for a specified target and can be discriminated from unwanted or non-specific interactions. For example, the ability of a protein (*e.g.*, cysteine-dense peptides) to bind to a specific antigenic determinant can be measured techniques familiar to one of skill in the art, for example through an enzyme-linked immunosorbent assay (ELISA) or surface plasmon resonance. Between two molecules, “specific binding” refers to the ability of two molecules to bind to each other in preference to binding to other molecules in the environment. Typically, “specific binding” discriminates over adventitious binding in a reaction by at least two-fold, more typically by at least 10-fold, often at least 100-fold. Typically, the affinity or avidity of a specific binding reaction, as quantified by a dissociation constant, is about 10^{-7} M or stronger (*e.g.*, about 10^{-8} M, 10^{-9} M or even stronger). Specific-binding requires specificity of a particular first entity (*e.g.*, a polypeptide) for a particular second entity (*e.g.*, an antigen binding sequence).

[00146] As used herein, “stringent hybridization conditions” and “stringent wash conditions” in the context of nucleic acid hybridization experiments depend upon a number of different physical parameters. Nucleic acid hybridization will be affected by such conditions as salt concentration, temperature, solvents, the base composition of the hybridizing species, length of the complementary regions, and the number of nucleotide base mismatches between the hybridizing nucleic acids, as will be readily appreciated by those

skilled in the art. One having ordinary skill in the art knows how to vary these parameters to achieve a particular stringency of hybridization. In general, “stringent hybridization” is performed at about 25°C below the thermal melting point (T_m) for the specific DNA hybrid under a particular set of conditions. “Stringent washing” is performed at temperatures about 5°C lower than the T_m for the specific DNA hybrid under a particular set of conditions. The T_m is the temperature at which 50% of the target sequence hybridizes to a perfectly matched probe. See Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), page 9.51, hereby incorporated by reference. For purposes herein, “stringent conditions” are defined for solution phase hybridization as aqueous hybridization (*i.e.*, free of formamide) in 6xSSC (where 20xSSC contains 3.0 M NaCl and 0.3 M sodium citrate), 1% SDS at 65°C for 8-12 hours, followed by two washes in 0.2xSSC, 0.1% SDS at 65°C for 20 minutes. It will be appreciated by the skilled worker that hybridization at 65°C will occur at different rates depending on a number of factors including the length and percent identity of the sequences which are hybridizing.

[00147] As used herein, the term “synthetic” is used to refer to an entity that is made is lab-created and not naturally produced or isolated, without modification, from a naturally occurring source. A recombinant polymer, such as a recombinant polynucleotide or polypeptide, may be synthetic. Synthetic polymers such as polynucleotides or polypeptides may be produced by any method known to those of skill in the art, including but not limited to solid phase synthesis, solution phase synthesis, biological synthesis by, *e.g.*, host cells, *etc.*

[00148] As used herein, the term “subject” is a mammal. A subject may be a human or non-human mammal. Given context, a subject may be used interchangeably with patient, individual, donor, *etc.*

[00149] As used herein, the terms “substantial homology” or “substantial similarity,” when referring to a polynucleotide or polypeptide, indicate that, when optimally aligned with appropriate nucleotide or amino acid insertions or deletions with another reference molecule (or its complementary strand when appropriate), there is sequence identity in at least about 70%, 75%, 80%, 85%, preferably at least about 90%, and more preferably at least about 95%, 96%, 97%, 98% or 99% or more of the nucleic acid or amino acid residues, as measured by any well-known algorithm of sequence identity, such as, *e.g.*, FASTA, BLAST, Gap, *etc.*. Alternatively or additionally, substantial homology or similarity exists when, for example, a nucleic acid or fragment thereof hybridizes to another nucleic acid, to a strand of another nucleic acid, or to the complementary strand thereof, under stringent hybridization conditions.

[00150] As used herein, the term “target” refers to a protein or functional portion or variant thereof. A target may be or comprise a binding region, such as an epitope, to which a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) of the present disclosure binds. Further, the term “antigen” refers to a protein or functional portion or variant thereof to which an antibody or variant thereof binds to. A target or antigen may be expressed on the surface of a particular cell (a “target cell”) or expressed within (*e.g.*, on the surfaces of) cells in a population of cells. A target or antigen may have a certain percent identity to a reference protein and still be referred to as a target by a particular name (*e.g.*, any one of the target proteins in Table 10). A target or antigen may also refer to a protein in a pathway related to another protein. For example, if a target or antigen is any one of the target proteins in Table 10, a target or antigen may also be a protein in a pathway that is necessary for activity of the target protein selected from Table 10.

[00151] As used herein, the term “treatment” (as well as “treat” or “treating”) refers to partial or complete alleviation, amelioration, mitigation, prevention, reduction in risk of onset, relief, inhibition, delay in onset of, reduction in severity of, reduction in frequency or incidence of one or more causes, features, and/or symptoms of or associated with a particular disease, disorder, and/or condition.

[00152] As used herein, the term “vector” as used herein is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a “plasmid,” which generally refers to a circular double stranded DNA loop into which additional DNA segments may be ligated, but also includes linear double-stranded molecules such as those resulting from amplification by the polymerase chain reaction (PCR) or from treatment of a circular plasmid with a restriction enzyme. Other vectors include cosmids, bacterial artificial chromosomes (BAC) and yeast artificial chromosomes (YAC). Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome (discussed in more detail below). Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, vectors having an origin of replication which functions in the host cell). Other vectors can be integrated into the genome of a host cell upon introduction into the host cell, and are thereby replicated along with the host genome. Moreover, certain preferred vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as “recombinant expression vectors” (or simply “expression vectors”).

[00153] As used herein, the term “decharged molecules” refers to molecules that have been modified to contain fewer positively charged or polar features, greater negatively charged features, and/or both.

[00154] As used herein, the term “surface charge” refers to the electrostatic charge present at the surface of a protein (e.g., such as a miniprotein). In various embodiments, the surface charge of a miniprotein can influence the kidney uptake of the miniprotein (e.g., increase the kidney uptake of the miniprotein or reduce the kidney uptake of the miniprotein).

[00155] As used herein, the term “surface patch” refers to regions on the surface of a protein (e.g., a miniprotein) with surface characterizations. For example, a surface patch can be defined according to surface charges and/or surface hydrophobicities, that may influence the kidney uptake of the protein (e.g., miniprotein).

[00156] As used herein, the term “cleavable linker” refers to a linker that can be cleaved. A cleavable linker contains a cleavable bond that is cleaved in vivo, for example: by an acidic pH (pH less than 7, typically about 4 to 6), by glutathione, or where there is up-regulation of enzymes such as matrix proteases or peptidases from the proximal tubule. Examples of cleavable linkers are linkers that contain hydrazine, or disulfide bonds, or enzymatically cleavable peptide sequences.

[00157] As used herein, the term “decoy peptides” refers to molecules specifically designed to mimic the role of a certain receptor protein and interact with certain target entities.

Compositions

[00158] Provided herein are novel compositions comprising one or more of a polypeptide, linker, chelator, and/or radionuclide. In some embodiments, a composition comprises a linker and a chelator. In some embodiments, a composition comprises a linker, chelator, and radionuclide. In some embodiments, a composition comprises or consists of a polypeptide (*i.e.*, miniprotein), an optional linker, and a chelator and/or radionuclide. In some embodiments, a chelator and/or radionuclide are conjugated to a miniprotein via a linker. In various embodiments, compositions disclosed herein comprise a compound. In various embodiments, a compound, as described herein, can include a miniprotein. In various embodiments, a compound can include one or more additional elements, examples of which include a linker, a chelator, and/or a radionuclide.

[00159] In various embodiments, a compound includes a miniprotein comprising a sequence disclosed in Table 3 (in the column entitled “Sequence”). In various embodiments, a compound includes a miniprotein comprising a sequence disclosed in Table 3 (in the

column entitled “Sequence”) and further includes a linker disclosed in Table 3 (in the column entitled “Sequence”). In various embodiments, a compound includes a miniprotein comprising a sequence disclosed in Table 3 (in the column entitled “Sequence”) and further includes a radionuclide disclosed herein. In various embodiments, a compound includes a miniprotein comprising a sequence disclosed in Table 3 (in the column entitled “Sequence”) and further includes a chelator disclosed herein. In various embodiments, a compound includes a miniprotein comprising a sequence disclosed in Table 3 (in the column entitled “Sequence”) and further includes a linker disclosed in Table 3 (in the column entitled “Sequence”), and a chelator disclosed herein. In various embodiments, a compound includes a miniprotein comprising a sequence disclosed in Table 3 (in the column entitled “Sequence”) and further includes a linker disclosed in Table 3 (in the column entitled “Sequence”), and a radionuclide disclosed herein. In various embodiments, a compound includes a miniprotein comprising a sequence disclosed in Table 3 (in the column entitled “Sequence”) and further includes a linker disclosed in Table 3 (in the column entitled “Sequence”), a chelator disclosed herein, and a radionuclide disclosed herein.

[00160] In some embodiments, a miniprotein of the present disclosure comprises or consists of a CDP, a knottin, a binder, an affibody, an engineered Kunitz domain, a monobody, an anticalin, a designed ankyrin repeat domain (DARPin), and/or an avimer. In some embodiments, the miniprotein comprises or consists of a CDP. In some such embodiments, the miniprotein comprises or consists of a knottin. In some such embodiments, the miniprotein comprises or consists of a binder. In some such embodiments, the miniprotein comprises or consists of an affibody. In some such embodiments, the miniprotein comprises or consists of an engineered Kunitz domain. In some such embodiments, the miniprotein comprises or consists of a monobody. In some such embodiments, the miniprotein comprises or consists of an anticalin. In some such embodiments, the miniprotein comprises or consists of a designed ankyrin repeat domain (DARPin). In some such embodiments, the miniprotein comprises or consists of an avimer. In some embodiments the miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) is designed to be linked to one or more other components. For example, in some embodiments, a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) may be linked (conjugated) to another component such as a chelator and/or a radionuclide. In some embodiments, a radionuclide of the present disclosure is an alpha

emitter. In some such embodiments, a chelator and/or radionuclide are conjugated to a miniprotein via a linker.

[00161] Without wishing to be bound by any particular theory, the present disclosure contemplates that compositions of the present disclosure are more effective than previously described compositions (*e.g.*, such as those comprising antibodies and/or beta-emitter radionuclides). For example, while miniproteins (*e.g.*, to be used in compositions as provided herein) have several key features of antibody-based therapeutics (*e.g.*, affinity, potency, specificity, and ability to disrupt protein:protein interactions), they can avoid undesirable limitations such as, *e.g.*, large size, expensive manufacturing, and the necessity of chimerization or humanization. For instance, in some embodiments, a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) of the present disclosure is no more than about 100 amino acids in length. In some embodiments, such a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) may be or comprise a cysteine dense peptide. In some embodiments, a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) comprises one or more disulfide bridges. In some embodiments, a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) comprises multiple cysteine residues that crosslink to maintain a very stable, folded state for a peptide of its length (*e.g.*, relative to a peptide of the same length without as many cysteine residues). Without being bound by any particular theory, the present disclosure contemplates that in some embodiments, a miniprotein does not comprise multiple cysteine residues such as, for example, a miniprotein comprising a single cysteine residue. The present disclosure contemplates that stability conferred by crosslinked cysteines contributes to reduced immunogenicity of miniproteins or comprising such miniproteins. In some embodiments, such stability may also confer resistance to harsher conditions provided for efficient chelation (*e.g.*, high temperature, low pH incubations, *etc.*), while continuing to retain biological activity (*e.g.*, capability of binding a target).

[00162] In some embodiments, miniproteins as provided herein function as targeting moieties, *e.g.*, specifically binding to a target expressed on the surface of a tumor cell. In some such embodiments, a miniprotein is designed such that it may be joined to one or more additional components. For example, without being bound by any particular theory, miniproteins of the present disclosure may be formulated such that they are combined with

other components such as a therapeutic molecule (*e.g.*, chelator compositions and/or radionuclide) and/or a detectable agent (*e.g.*, a visualizable agent, *e.g.*, a metabolizable and visualizable agent, *etc.*). In some such embodiments, such miniproteins conjugated to one or more additional components may be used, for example, in diagnosis, prognosis, monitoring and/or treatment of one or more diseases, disorders or conditions such as those with expression of particular targets on particular populations of cells.

[00163] In some embodiments, a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) has low immunogenicity relative to a larger protein. In some such embodiments, the lower immunogenicity increases amenability to harsher environmental conditions (*e.g.*, high temperature and low pH incubations) while retaining biological activity. Thus, in some embodiments, a conjugate comprising a miniprotein has lower immunogenicity than a composition comprising a larger protein or different targeting moiety (*i.e.*, other than a miniprotein).

[00164] In some embodiments, a composition comprising a linker, chelator, and/or radionuclide can efficiently penetrate a tumor.

[00165] In some embodiments, miniproteins (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) have superior penetration efficiency relative to larger proteins. That is, in some embodiments, a miniprotein or composition comprising a miniprotein can penetrate a solid tumor better than a larger protein or composition comprising a protein larger than a miniprotein. For example, in some embodiments, a binder has superior tumor penetration efficiency with a hydrodynamic radius on the order of about 1 nm – 25 nm. In some embodiments, the hydrodynamic radius is between about 1 nm -5 nm. In some embodiments, the hydrodynamic radius is between about 1 nm – 3 nm. In some embodiments, the hydrodynamic radius is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nm.

[00166] As described herein, miniproteins (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer), are conjugated to a chelator. In some embodiments, the chelator binds a radionuclide (*e.g.*, an alpha-emitter radionuclide, *e.g.*, actinium). In some such embodiments, such radionuclide conjugates combine specific-binding capabilities and properties of a miniprotein (*e.g.*, CPD, knotting, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) with a radionuclide. That is, without being bound by any

particular theory, the present disclosure provides a conjugate wherein, in some embodiments, a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) targets a radioisotope to which it's conjugated to a cell expressing a target. In some embodiments, the target is expressed on the surface of a cell. In some embodiments, the target is any one of the target proteins in Table 10. In some embodiments, the cell is a tumor cell. In some embodiments, the conjugate binds to any one of the target proteins in Table 10 on the surface of the tumor cell. In some such embodiments, the radionuclide is targeted to the tumor cell. In some embodiments, the radionuclide is an alpha-emitter radionuclide and when internalized, serves to specifically target (*e.g.*, without damaging surrounding tissue/cells) the tumor cell.

Targets

[00167] Any cell expressing a target may be targeted by a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) as provided herein.

[00168] In some embodiments, a cell is a mammalian cell. In some embodiments, a cell is a human cell. In some embodiments, a cell is from a cell line. In some embodiments, a cell is a primary cell. In some embodiments, a primary cell is from a sample from a subject such as from a tumor or from corresponding tissue without a tumor (*e.g.*, from another area of an organ or from a healthy donor). In some embodiments, a cell is *in vitro* (*e.g.*, a primary cell, a cell line, *etc.*). In some embodiments, a cell is *in vivo* (*e.g.*, in a subject, *e.g.*, in a human subject, *e.g.*, in a tumor of a human subject.) In some embodiments, a cell expresses or has been induced to express (*e.g.*, via recombinant technology) a target. In some embodiments, the target is expressed on the surface of a cell. In some embodiments, a cell is contacted by a composition binding to a target expressed on its surface. In some embodiments, upon binding (*e.g.*, upon binding of a miniprotein provided by the present disclosure), a target and any bound proteins and/or payloads is/are internalized into the cell. In some embodiments, a cell is killed by a payload (*e.g.*, a radionuclide and/or chelator, *etc.*) after internalization.

[00169] In some embodiments, a target is a protein or portion thereof that is upregulated or overexpressed on cancer cells as compared to non-cancer cells. That is, in some embodiments, a target is expressed or overexpressed in a tumor or in a tumor microenvironment relative to a level of the target in non-diseased tissue (*e.g.*, tissue without a tumor or tumor microenvironment). In some such embodiments, the target is absent or non-detectable in non-diseased (*e.g.*, healthy) tissue. In some embodiments, a target is a

biomarker for cancer (*e.g.*, for cancer cells, for a tumor). In particular embodiments, a target is any one of the targets identified in Table 10.

[00170] In some embodiments, a target may be related to a protein such as, for example, a protein in a pathway activated or acted upon by another protein. For instance, in some embodiments, a protein may be expressed on the surface of a cancer cell and a target may be a pathway that the surface-cell protein acts upon. In some embodiments, a protein may be expressed on a cancer cell and a target may be a protein on a different cell that causing a cancer cell to proliferate or otherwise be refractory to a treatment. In some embodiments, a tumor-associated cell surface molecule or tumor-specific cell surface molecule may be targeted by a miniprotein or composition comprising a miniprotein as provided herein.

[00171] In some embodiments, the miniprotein or composition comprising a miniprotein specifically binds a target expressed on the surface of a cell. In some embodiments, a target is cleaved from a cell surface. In some such embodiments, if the target is in an organism, cleavage of the target results in circulation of the target throughout the system of the organism. In some such embodiments, a target is found at a particular level in, *e.g.*, blood, serum, plasma, *etc.* In some embodiments, however, a substantial portion of expressed target is localized to cell surfaces; thus, in some embodiments, measurements of a level of a target may not accurately reflect the amount of target in a population of cells (*e.g.*, a tumor). In some embodiments, a target is a secreted protein. In some such embodiments, a target is found at a particular level in, *e.g.*, blood, serum, plasma, *etc.* In some such embodiments, the miniprotein binds to a region of a target such as, for example, an epitope. In some embodiments, a miniprotein or composition comprising a miniprotein specifically binds a target expressed on the surface of a cancer cell. In some embodiments, the cancer cell is in, on, or near a solid tumor. In some embodiments, the cancer cell is a circulating cancer cell. In some embodiments, a miniprotein or composition comprising a miniprotein specifically binds a target or expressed at a higher level on a cancer cell than a reference cell. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a human cell.

[00172] In some embodiments, the miniprotein or composition comprising a miniprotein specifically binds to any one of the target proteins in Table 10. In some embodiments, the target comprises or consists of any one of the target proteins in Table 10. In some embodiments, the miniprotein specifically binds to a target comprising an amino acid sequence or portion thereof as set forth in Table 6.

Miniproteins

[00173] Provided herein are novel polypeptides (*i.e.*, miniproteins) and methods of use thereof. In some embodiments, a polypeptide comprises or consists of a miniprotein. In some such embodiments, the miniprotein comprises or consists of a CDP, knottin, and/or binder. In some embodiments the miniprotein is designed to be linked to one or more other components. For example, in some embodiments, a miniprotein may be linked (conjugated) to another component such as a chelator and/or a radionuclide. In some embodiments, conjugation is via a lysine or cysteine residue. For example, in some embodiments, a miniprotein is engineered to remove all lysine residues except for one, which is, in some embodiments, used for conjugation. In some embodiments, conjugation occurs via an optional linker. In some embodiments, conjugation between a miniprotein and a chelator and/or radionuclide is direct.

[00174] Without wishing to be bound by any particular theory, the present disclosure contemplates that therapeutics comprising compositions provided by the present disclosure are characterized by several features relative to other (*e.g.*, antibody-based) therapeutics. For example, in some embodiments, miniproteins display several key features of antibody-based therapeutics (*e.g.*, affinity, potency, specificity, and ability to disrupt protein:protein interactions) but also have several advantages as compared to antibody-based therapeutics such as smaller size, cheaper manufacturing, and elimination of need to chimerize or humanize the proteins. In addition, the size and specificity of binding increases tumor penetrance and uptake into cells expressing the target of the miniprotein or composition (*e.g.*, conjugate) comprising a miniprotein.

[00175] In some embodiments, a miniprotein of the present disclosure is no more than about 100 amino acids in length. In some embodiments, a miniprotein is about 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or more amino acids in lengths. In some such embodiments, however, a miniprotein of the present disclosure does not exceed about 100 amino acids in length. In some embodiments, a miniprotein is between about 20 to about 40, about 30 to about 50, about 40 to about 60, about 45 to about 65, about 50 to about 70, about 55 to about 75, about 65 to about 85 or more amino acids in length, but not exceeding about 100 amino acids in length. In some preferred embodiments, a miniprotein is about 65 amino acids or less. In some preferred embodiments, a miniprotein is about 50 amino acids or less.

[00176] In some embodiments, a miniprotein of the present disclosure is not larger than about 12 kDa. In some embodiments, a miniprotein of the present disclosure is about 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5 or more kDa. In some such embodiments, however, a miniprotein of the present disclosure does not exceed about 12 kDa.

[00177] In some embodiments, a miniprotein of the present disclosure comprises or consists of a linear polypeptide, a folded polypeptide (e.g., covalently linked polypeptide, non-covalently linked polypeptide, or polypeptide include a di-sulfide linkage), cysteine-dense peptide, a knottin peptide, a binder, an affibody, an engineered Kunitz domain, a monobody, an anticalin, a designed ankyrin repeat domain (DARPin), or an avimer.

[00178] In some embodiments, a miniprotein comprises one or more disulfide bridges. In some embodiments, a miniprotein comprises multiple cysteine residues. In some such embodiments, cysteine residues crosslink to maintain a very stable, folded state for a peptide of its length (e.g., relative to a peptide of the same length without as many cysteine residues). The present disclosure contemplates that such crosslinking confers improved stability with reduced (i.e., very low to no) immunogenicity and/or sustains or improves ability to maintain biological activity in harsh but efficient chelation conditions (e.g., high temperature and low pH).

[00179] In some embodiments, a miniprotein or composition comprising a miniprotein (e.g., a radionuclide conjugate) has low immunogenicity relative to a larger protein or composition comprising or consisting of a larger protein (e.g., an antibody).

[00180] In some embodiments, miniproteins (e.g., CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) have superior penetration efficiency relative to larger proteins. That is, in some embodiments, a miniprotein or composition comprising a miniprotein can penetrate a solid tumor better than a larger protein or composition comprising a protein larger than a miniprotein. For example, in some such embodiments, a miniprotein or composition comprising a miniprotein has a hydrodynamic radius of about 1 to about 25 nm. In some embodiments, a hydrodynamic radius is in a range of about 1-25 nm, 10-20 nm, 5-15 nm, 1-5 nm, 2-4 nm, or 1-3 nm. In some embodiments, hydrodynamic radius is measured using light scatter methods known to those of skill in the art.

[00181] In some embodiments, a miniprotein of the present disclosure is characterized in that it has one or more properties relative to a protein larger than 100 amino acids like an antibody, antibody fragment, VHH domain, single chain antibody or other protein or binder

greater than 12 kDA. In some embodiments, a property is selected from increased protein expression, increased thermoactivity, increased thermostability, increased pH activity, increased stability, increased activity, increased receptor binding specificity and/or affinity, increased specific activity, increased resistance to substrate and/or end-product inhibition, increased chemical stability, improved chemoselectivity, improved solvent stability, increased tolerance to acidic pH, increased tolerance to proteolytic activity (*i.e.*, reduced sensitivity to proteolysis), reduced aggregation, increased solubility, reduced immunogenicity, and altered temperature profile, increased resistance to liver uptake, kidney uptake or healthy tissue binding, increased tumor penetration, and/or increased volume of distribution.

[00182] In some embodiments, a miniprotein or composition comprising a miniprotein (*e.g.*, conjugate, *e.g.*, radionuclide conjugate) provided by the present disclosure exhibits binding affinity to any one of the target proteins in Table 10. In some embodiments, the target proteins selected from Table 10 is the human isoform. In some embodiments, the human isoform of any one of the target proteins in Table 10 is on a cell. In some embodiments, the cell is a cell line, a primary cell, or a cell in a human (*e.g.*, in a tumor).

[00183] In some embodiments, a miniprotein or composition comprising a miniprotein (*e.g.*, conjugate, *e.g.*, radionuclide conjugate) displays nM or sub-nM binding affinity to any one of the target proteins in Table 10. In some embodiments, the affinity is measured in an in vitro assay. In some embodiments, the in vitro assay is a cell-based assay. In some embodiments, affinity is measured in an in vivo assay (*e.g.*, a PET scan) or using a sample from a subject (*e.g.*, an in vitro assay using a biological specimen such as blood or a cell biopsy from a subject).

[00184] In some embodiments, a miniprotein or conjugate thereof displays a binding affinity to any one of the target proteins in Table 10. In some embodiments, the binding affinity of a miniprotein or conjugate thereof to the human isoform of any one of the target proteins in Table 10 is about 500 nM. In some embodiments, the miniprotein comprises picomolar binding affinity. In some embodiments, the miniprotein or conjugate thereof comprises a binding affinity characterized by a dissociation constant ranging from about 900 nM to about 1 nM, *e.g.*, 900, 800, 700, 600, 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, .9, .8, .7, .6, .5, .4 nM or less binding affinity to the human isoform of any one of the target proteins in Table 10. In some embodiments, the binding is selective to the human isoform of any one of the target proteins in Table 10 and, not, *e.g.*, non-human target proteins selected from Table 10.

[00185] In some embodiments, a miniprotein or conjugate thereof provided by the present disclosure has high affinity for any one of the target proteins in Table 10. In some such embodiments, the target protein selected from Table 10 is the human isoform of the selected protein from Table 10. In some embodiments, a miniprotein of the present disclosure is stable, including in the presence of one or more additional molecules (*e.g.*, a cytotoxic molecule, *e.g.*, radiation).

[00186] In some embodiments, binding ability of a miniprotein or conjugate thereof to a target is improved by one or more modifications. For example, in some embodiments, the binding ability of a miniprotein or conjugate thereof as provided herein to any one of the target proteins in Table 10, is improved using chemical crosslinking. In some embodiments, binding may be enhanced by using one or more of lysine residues, fusion proteins, non-natural amino acids, or other chemical moieties to enhance binding and/or functional activity.

[00187] In some embodiments, to ensure proper folding and connectivity, selected cysteine pairs can be replaced with selenocysteines. It is contemplated that, in some embodiments, diselenide crosslinks form more readily than disulfide crosslinks due to their lower redox potential and such a replacement may cross-couple remaining cysteine residues.

[00188] In some embodiments, a miniprotein of the present disclosure comprises or consists of an antigen for use in generating an antibody that specifically binds to at least one epitope on any one of the target proteins in Table 10. In some embodiments, such an antibody may be used for, *e.g.*, diagnostic purposes, blocking (*e.g.*, antagonism), *etc.*

[00189] In some embodiments, the miniprotein comprises one or more disulfide bridges.

[00190] In some embodiments, a miniprotein or conjugate thereof as provided herein does not comprises one or more cysteine residues. In some embodiments, the miniprotein does not comprise one or more disulfide bridges.

[00191] In some embodiments, a miniprotein or conjugate thereof as provided herein is specific for a target. In some embodiments, a miniprotein is specific for any one of the target proteins in Table 10 or a fragment thereof.

[00192] In some embodiments, a target is represented by a polypeptide or a portion thereof as set forth in any one of SEQ ID NOS: 69-72 as set forth in Table 6.

[00193] In some embodiments, a miniprotein or conjugate thereof as provided herein comprises or consists of a specific amino acid sequence.

[00194] In some embodiments, miniproteins or compositions comprising miniproteins (*e.g.*, radionuclide conjugates) are conjugated to a chelator that optionally binds a radionuclide (*e.g.*, actinium). In some embodiments, the conjugation is via a linker. In some

embodiments, conjugation is direct conjugation. In some embodiments, such radionuclide conjugates combine and synergize to provide target specificity (*e.g.*, via the miniprotein) and superior treatment (*e.g.*, via directed radioisotope delivery to the cell expressing the target).

[00195] As used herein and known to those of skill in the art, the twenty conventional amino acids and their abbreviations follow conventional usage. See Immunology-A Synthesis (Golub and Gren eds., Sinauer Associates, Sunderland, Mass., 2nd ed. 1991), which is incorporated herein by reference. In some embodiments, an amino acid of the present disclosure may be a stereoisomer (*e.g.*, D-amino acids) of the twenty conventional amino acids. In some embodiments, an amino acid in a polypeptide of the present disclosure may be a non-natural amino acid. For example, amino acids such as α -, α -disubstituted amino acids, N-alkyl amino acids, and other unconventional amino acids may also be suitable components for polypeptides of the present disclosure. Examples of unconventional amino acids include: 4-hydroxyproline, γ -carboxyglutamate, ϵ -N,N,N-trimethyllysine, ϵ -N-acetyllysine, O-phosphoserine, N-acetyls erine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, N-methylarginine, and other similar amino acids and imino acids (*e.g.*, 4-hydroxyproline). Arrangements of polypeptide sequence notations used herein have a left-side end corresponding to the amino terminal and a right-side end corresponding to the carboxy-terminal end, in accordance with standard usage and convention.

[00196] In some embodiments, a miniprotein as provided herein is specific for a polypeptide or portion thereof having an amino acid sequence or portion thereof as set forth in Tables 3, 4A, and 4B.

[00197] In some embodiments, a miniprotein comprises or consists of a specific amino acid sequence. In some embodiments, a miniprotein has an amino acid sequence that is 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or 100% identical to the amino acid sequence set forth in any of SEQ ID NOs: 1-68.

[00198] As used herein and known to those of skill in the art, the twenty conventional amino acids and their abbreviations follow conventional usage. See Immunology-A Synthesis (Golub and Gren eds., Sinauer Associates, Sunderland, Mass., 2nd ed. 1991), which is incorporated herein by reference. In some embodiments, an amino acid of the present disclosure may be a stereoisomer (*e.g.*, D-amino acids) of the twenty conventional amino acids. In some embodiments, an amino acid in a polypeptide of the present disclosure may be a non-natural amino acid. For example, amino acids such as α -, α -disubstituted amino acids, N-alkyl amino acids, and other unconventional amino acids may also be suitable components

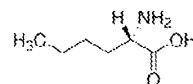
for polypeptides of the present disclosure. Examples of unconventional amino acids include: 4-hydroxyproline, γ -carboxyglutamate, ϵ -N,N,N-trimethyllysine, ϵ -N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, N-methylarginine, and other similar amino acids and imino acids (*e.g.*, 4-hydroxyproline). Arrangements of polypeptide sequence notations used herein have a left-side end corresponding to the amino terminal and a right-side end corresponding to the carboxy-terminal end, in accordance with standard usage and convention.

DOTA-PEG4: α -(1,4,7,10-tetraazacyclododecan-1,4,7,10-tetraacetate)-4(ethylene glycol)

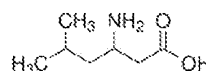
Biotin-PEG4:

FITC-PEG4 : Fluorescein isothiocyanate-4(ethylene glycol)

Norleucine: (S)-(+)-2-Aminohexanoic acid, (S)-2-Aminocaproic acid



Homoleucine: (S)-3-Amino-5-methylhexanoic acid hydrochloride



[00199] In some embodiments, a miniprotein of the present disclosure has glutamine, tryptophan, leucine, phenylalanine, alanine, 3-Pyridyl Alanine, substituted tryptophan, or substituted phenylalanine. In some embodiments, a miniprotein of the present disclosure has asparagine, aspartic acid, serine, lysine, glutamine, glutamic acid, leucine, alanine, norleucine, homo-leucine, homo serine, or substituted phenylalanine. In some embodiments, a miniprotein of the present disclosure has glycine, alanine, lysine, glutamic acid, leucine, serine, proline, phenylalanine, norleucine, homo-leucine, homo serine, or substituted phenylalanine. In some embodiments, a miniprotein of the present disclosure has glutamic acid, leucine, aspartic acid, methionine, glutamine, tyrosine, phenylalanine, norleucine, homo-leucine, homo serine, or substituted phenylalanine.

[00200] In some embodiments, a miniprotein of the present disclosure exhibits binding specificity to the human isoform of any one of the target proteins in Table 10. For example, in some embodiments a miniprotein provided by the present disclosure, such as, for example, those represented by any one of SEQ ID NOs: 1-68, demonstrates binding when expressed on the surface of yeast and binding to any one of the target proteins in Table 10 tested by flow cytometry. In some embodiments, a miniprotein provided by the present disclosure, such as, for example, those represented by any one of SEQ ID NOs: 1-68, demonstrates binding

specificity via flow cytometry when, for example, such as miniprotein of any one of the target proteins in Table 10 (*e.g.*, as represented by any of SEQ ID NOs: 1-68) only binds to the selected target protein from Table 10 and not to other target proteins.

[00201] In some embodiments, a miniprotein of the present disclosure such as, for example, any of those represented by SEQ ID NOs 1-68, shows greater than 10 nM potency. In some embodiments, a miniprotein shows potency greater than 1, 2, 3, 4, 5, 6, 7, 8, 9 nM or more.

CDPs

[00202] In some embodiments, miniproteins of the present disclosure comprise or consist of a cysteine-dense peptides (CDPs). In some embodiments, conjugates provided herein comprise a CDP. In some embodiments, a CDP functions as a targeting moiety, *e.g.*, specifically binding to a protein target expressed on the surface of a target tumor cell. In some embodiments, a CDP comprises or consists of at least two independent folding domains and a high density of cysteines. In some embodiments, the CDP comprises at least one, two, three, four, five, six, or more than six cysteine residues in a span of from about 10 to about 90 amino acid residues, preferably 13 to 80 amino acid residues. (See, *e.g.*, Correnti et al., Nat Struct Mol Biol. 2018 Mar;25(3):270-278, for exemplary CDPs and characteristics thereof).

Knottins

[00203] In some a embodiments, miniproteins of the present disclosure comprise or consist of knottin peptides. In some embodiments, conjugates provided herein comprise a knottin peptide. In some embodiments, a knottin peptide functions as a targeting moiety, *e.g.*, specifically binding to a target protein expressed on the surface of a target tumor cell. In some embodiments, a knottin comprises at least three disulfide bonds connected in an arrangement that generates the so-called “cysteine-knot” for which knottins are named. (See, *e.g.*, Kintzing & Cochran et al., Curr Opin Chem Biol. 2016 Oct;34:143-150.). In some embodiments, knottins have high stability (*e.g.*, thermal, proteolytic, chemical, *etc.*). In some embodiments, a knottin can be further engineered to modify binding, folding, and/or related properties.

[00204] In some embodiments, a given knottin is highly specific for a given target. In some embodiments, a knottin specifically binds to a target. In some embodiments, the target is located in, on, or near a cell. In some embodiments, the knottin specifically binds to any one of the target proteins in Table 10 or a fragment thereof. In some embodiments, a knottin is conjugated to a chelator and/or radionuclide. In some embodiments, conjugation is via a linker. It will be understood by those of skill in the art, that in some embodiments, the

particular knottin employed in a conjugate of the present disclosure may vary depending on the target protein of interest.

[00205] In some embodiments, folded structures of miniproteins (*e.g.*, CDPs, knottins, binders, affibodies, engineered Kunitz domains, monobodies, anticalins, designed ankyrin repeat domains (DARPin)s, avimers) make them rigid, providing for very tight and potent binding to the target protein or antigen (relative to less structured peptides). In some such embodiments, a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) exhibits extraordinary stability with resistance to heat, peptidase cleavage, and pH.

Binders

[00206] In some embodiments, a miniprotein of the present disclosure comprises or consists of a binder. In some embodiments, the binder functions as a targeting moiety, *e.g.*, specifically binding to a target expressed on the surface of a tumor cell.

[00207] In some embodiments, a binder has certain structural features; for example, in some embodiments, a binder may be rich in alpha-helices, such as a helix-helix-helix structure (see, *e.g.*, Crook et al., Nat Commun. (2017) 8, 2244; Berger et al, Elife (2016) 5, e20352; and Procko et al., Cell (2014), 157, 1644-1656). In some embodiments, a binder comprises sufficient surface to functionalize the molecule on a disparate surface to a binding surface. In some embodiments, a binder comprises a sequestered hydrophobic core. In some embodiments, a binder displays cooperative folding. In some embodiments, a binder has two or more of the following features: (i) represented by an amino acid sequence of 100 amino acids or fewer; (ii) at least two secondary structure elements; (iii) a sequestered hydrophobic core; and/or (iv) cooperative folding.

[00208] In some embodiments, a given binder is highly specific for a given target. In some embodiments, a binder specifically binds to a target. In some embodiments, the target is located in, on, or near a cell. In some embodiments, the binder specifically binds to any one of the target proteins in Table 10 or a fragment thereof. In some embodiments, a binder is conjugated to a chelator and/or radionuclide. In some embodiments, conjugation is via a linker. It will be understood by those of skill in the art, that in some embodiments, the particular binder employed in a conjugate of the present disclosure may vary depending on the target protein or antigen of interest.

Affibodies

[00209] In some embodiments, miniproteins of the present disclosure comprise or consist of affibodies. In some embodiments, conjugates provided herein comprise an affibody. In

some embodiments, an affibody functions as a targeting moiety, *e.g.*, specifically binding to a protein target or antigen expressed on the surface of a target tumor cell. In some embodiments, an affibody comprises or consists of no more than 100 amino acids, 90 amino acids, 80 amino acids, 70 amino acids, 60 amino acids, 50 amino acids, 40 amino acids, 30 amino acids, 20 amino acids, or 10 amino acids. In some embodiments, an affibody comprises or consists of at least three alpha helices with 58 amino acids. In some embodiments, the affibody comprises target specificity that is obtained by randomization of 13 amino acids located in two alpha-helices involved in the binding activity of the parent protein domain (Feldwisch J, Tolmachev V.; (2012) *Methods Mol Biol.* 899:103-26). In some embodiments, an affibody can be further engineered to modify binding, folding, and/or related properties.

[00210] In some embodiments, an affibody specifically binds to a target. In some embodiments, the target is located in, on, or near a cell. In some embodiments, the affibody specifically binds to any one of the target proteins in Table 10 or a fragment thereof. In some embodiments, an affibody is conjugated to a chelator and/or radionuclide. In some embodiments, conjugation is via a linker. It will be understood by those of skill in the art, that in some embodiments, the particular affibody employed in a conjugate of the present disclosure may vary depending on the target protein or antigen of interest.

Engineered Kunitz Domains

[00211] In some embodiments, miniproteins of the present disclosure comprise or consist of engineered Kunitz domains. In some embodiments, conjugates provided herein comprise an engineered Kunitz domain. In some embodiments, an engineered Kunitz domain functions as a targeting moiety, *e.g.*, specifically binding to a protein target expressed on the surface of a target tumor cell. In some embodiments, an engineered Kunitz domain comprises or consists of at least one peptide derived from the Kunitz domain of a Kunitz-type protease inhibitor such as bovine pancreatic trypsin inhibitor (BPTI), amyloid precursor protein (APP) or tissue factor pathway inhibitor (TFPI). In some embodiments, an engineered Kunitz domain can be further engineered to modify binding, folding, and/or related properties.

[00212] In some embodiments, an engineered Kunitz domain specifically binds to a target. In some embodiments, the target is located in, on, or near a cell. In some embodiments, the engineered Kunitz domain specifically binds to any one of the target proteins in Table 10 or a fragment thereof. In some embodiments, an engineered Kunitz domain is conjugated to a chelator and/or radionuclide. In some embodiments, conjugation is via a linker. It will be

understood by those of skill in the art, that in some embodiments, the particular engineered Kunitz domain employed in a conjugate of the present disclosure may vary depending on the target protein of interest.

Monobodies

[00213] In some embodiments, miniproteins of the present disclosure comprise or consist of monobodies. In some embodiments, conjugates provided herein comprise an antibody. In some embodiments, an antibody functions as a targeting moiety, *e.g.*, specifically binding to a protein target expressed on the surface of a target tumor cell. In some embodiments, an antibody comprises or consists of a molecule based on the 10th extracellular domain of human fibronectin III (10Fn3), which adopts an Ig-like b-sandwich fold of about 94 residues with 2 to 3 exposed loops, but lacks the central disulphide bridge. In some embodiments, an antibody can be further engineered to modify binding, folding, and/or related properties.

[00214] In some embodiments, an antibody specifically binds to a target. In some embodiments, the target is located in, on, or near a cell. In some embodiments, the antibody specifically binds to any one of the target proteins in Table 10 or a fragment thereof. In some embodiments, an antibody is conjugated to a chelator and/or radionuclide. In some embodiments, conjugation is via a linker. It will be understood by those of skill in the art, that in some embodiments, the particular antibody employed in a conjugate of the present disclosure may vary depending on the target protein of interest.

Anticalins

[00215] In some embodiments, miniproteins of the present disclosure comprise or consist of anticalins. In some embodiments, conjugates provided herein comprise an anticalin. In some embodiments, an anticalin functions as a targeting moiety, *e.g.*, specifically binding to a protein target expressed on the surface of a target tumor cell. In some embodiments, an anticalin comprises or consists of an eight-stranded β -barrel which forms a highly conserved core unit among the lipocalins and naturally forms binding sites for ligands by means of four structurally variable loops at the open end. In some embodiments, an anticalin can be further engineered to modify binding, folding, and/or related properties.

[00216] In some embodiments, an anticalin specifically binds to a target. In some embodiments, the target is located in, on, or near a cell. In some embodiments, the anticalin specifically binds to any one of the target proteins in Table 10 or a fragment thereof. In some embodiments, an anticalin is conjugated to a chelator and/or radionuclide. In some

embodiments, conjugation is via a linker. It will be understood by those of skill in the art, that in some embodiments, the particular anticalin employed in a conjugate of the present disclosure may vary depending on the target protein of interest.

Designed Ankyrin Repeat Domains

[00217] In some embodiments, miniproteins of the present disclosure comprise or consist of designed Ankyrin repeat domains. In some embodiments, conjugates provided herein comprise a designed Ankyrin repeat domain. In some embodiments, a designed Ankyrin repeat domain functions as a targeting moiety, *e.g.*, specifically binding to a protein target expressed on the surface of a target tumor cell. In some embodiments, a designed Ankyrin repeat domain comprises a peptide derived from Ankyrin. In some embodiments, a designed Ankyrin repeat domain comprises a single ankyrin repeat, preferably comprising a 33 residue motif comprising two alpha-helices and a beta-turn. In some embodiments, a designed Ankyrin repeat domain provides a rigid interface and lacks structural flexibility. In some embodiments, a designed Ankyrin repeat domain can be further engineered to modify binding, folding, and/or related properties.

[00218] In some embodiments, a designed Ankyrin repeat domain specifically binds to a target. In some embodiments, the target is located in, on, or near a cell. In some embodiments, the designed Ankyrin repeat domain specifically binds to any one of the target proteins in Table 10 or a fragment thereof. In some embodiments, a designed Ankyrin repeat domain is conjugated to a chelator and/or radionuclide. In some embodiments, conjugation is via a linker. It will be understood by those of skill in the art, that in some embodiments, the particular designed Ankyrin repeat domain employed in a conjugate of the present disclosure may vary depending on the target protein of interest.

Avimers

[00219] In some embodiments, miniproteins of the present disclosure comprise or consist of avimers. In some embodiments, conjugates provided herein comprise an avimer. In some embodiments, an avimer functions as a targeting moiety, *e.g.*, specifically binding to a protein target expressed on the surface of a target tumor cell. In some embodiments, an avimer comprises a peptide of about 10 amino acids, 20 amino acids, 30 amino acids, 40 amino acids, 50 amino acids, 60 amino acids, 70 amino acids, 80 amino acids, 90 amino acids, or 100 amino acids. In some embodiments, an avimer comprises at least one peptide sequence of about 30 to 35 amino acids. In some embodiments, an avimer comprises two or more of two peptide sequences of about 30 to 35 amino acids. In some embodiments, an avimer comprises one or more peptide sequences derived from A-domains of various membrane receptors.

(Weidle UH, et al., (2013), Cancer Genomics Proteomics; 10(4): 155-68). For further details see Nature Biotechnology 23(12), 1556 - 1561 (2005) and Expert Opinion on Investigational Drugs 16(6), 909-917 (June 2007). In some embodiments, an avimer can be further engineered to modify binding, folding, and/or related properties.

[00220] In some embodiments, an avimer specifically binds to a target. In some embodiments, the target is located in, on, or near a cell. In some embodiments, the avimer specifically binds to any one of the target proteins in Table 10 or a fragment thereof. In some embodiments, an avimer is conjugated to a chelator and/or radionuclide. In some embodiments, conjugation is via a linker. It will be understood by those of skill in the art that in some embodiments, the particular avimer employed in a conjugate of the present disclosure may vary depending on the target protein of interest.

Linkers

[00221] In some embodiments, the present disclosure provides linkers for use in one or more conjugates. For example, in some embodiments, a linker is linked to a chelator. In some embodiments, a linker is linked to a chelator, which itself is coupled to a radionuclide. In some embodiments, a miniprotein is conjugated to a chelator and/or radionuclide. In some embodiments, a miniprotein is conjugated to a chelator, optionally, through a linker. In some embodiments, a composition as provided herein comprises one or more linkers.

[00222] As described herein, in some embodiments, a miniprotein conjugate comprises a linker. In some embodiments, the linker functions to connect the chelator to miniprotein. In some embodiments, a linker is non-cleavable. In some embodiments, a linker is cleavable. In some embodiments, selection and placement of one or more linkers and chelators on a miniprotein aids to maintain desired potency and receptor engagement profile, enhance binder affinity and optimize physicochemical and pharmacokinetic properties of a miniprotein or conjugate thereof. Any suitable linker known in the art can be utilized. Exemplary linkers include, but are not limited to polyethylene glycol (PEG) linkers, an ester linker, an amide linker, a maleimide linker, a valine-citrulline linker, a hydrazone linker, a N-succinimidyl-4-(2-pyridyldithio)butyrate (SPDB) linker, a succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) linker, a vinylsulfone-based linker, a propanoic acid linker, a caproic acid linker, or a linker including any combination thereof. One or more additional linkers may be contemplated as will be known to those of skill in the art and chosen given the context and components of a given composition. In some embodiments, the linker is a PEG linker. In some embodiments, the linker is a non-cleavable PEG linker. In some embodiments, the PEG linker is PEG (4-24).

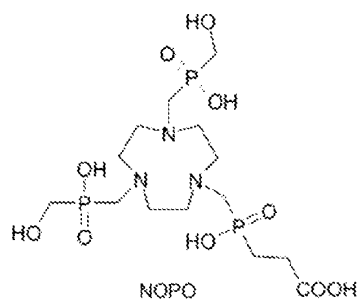
[00223] In some embodiments, linkers are used to assess lead polypeptide sequences binding to a target, a target expressed on cells, and target selectivity and/or affinity. For instance, in some embodiments, confirmation of in vitro on-target binding and affinity for lead polypeptide sequences and lead polypeptide sequences-linker-fluorophore reagent can be assessed using Biacore. In some embodiments, other linkers such as a fast clear linker or a halogen linker are also contemplated.

Chelators

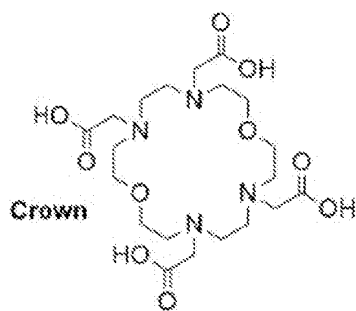
[00224] In some embodiments, a composition (*e.g.*, conjugate) as provided herein comprises a linker. In some embodiments, a composition comprises a linker and a chelator. In some embodiments, a composition comprises a linker, a chelator, and a radionuclide. In some embodiments, a composition comprises a miniprotein, optional linker, chelator, and/or radionuclide. In some embodiments, a chelator is covalently attached to a miniprotein. In some embodiments, a chelator binds to a radionuclide. In some embodiments, a chelator refers to any molecule or moiety that “binds” to a metal ion, in solution (effectively collecting/binding up metal ions so that they may, *e.g.*, no longer participate in one or more cellular activities or processes). In some embodiments, a chelator chelates one or more components of a metabolic pathway in a cell (*e.g.*, metal ions, *e.g.*, copper, iron, zinc, *etc.*). In some such embodiments, a chelator disrupts a life-cycle of a cancer cell and may, in some embodiments, reduce its viability, function, and/or ability to grow or proliferate. In some embodiments, a chelator chelates one or more toxins that are produced as a result of targeted radiotherapy (*e.g.*, to reduce toxicity of the therapy).

[00225] In some embodiments, a chelator comprises or consists of, but is not limited to diethylenetriamine pentaacetic acid (DTPA), tetrazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), ethylenediaminetetraacetic acid (EDTA), 1,4,7-triazacyclononane-N,N',N''-triacetic acid (NOTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), 1,4,7- triazacyclononane-N,N',N''-triacetic acid (NOTA), ({4-[2- (bis-carboxymethyl-amino)-ethyl]-7-carboxymethyl-[1,4,7] triazonan-1-yl}acetic acid (NETA), Macropa, and p-bromoacetamidobenzyl-tetraethylaminetetraacetic acid (TETA), porphyrins, polyamines, crown ethers, bis-thiosemicarbazones, polyoximes. In some embodiments, the chelator is 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA). In some embodiments, the chelator is Macropa. In some embodiments, a chelator comprises or consists of:

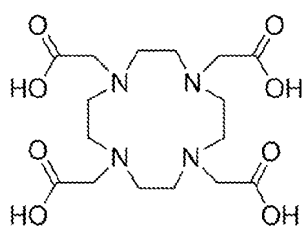
(i) NOPO:



(ii) CROWN

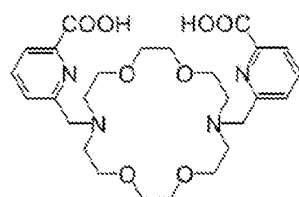


(iii) DOTA



; or

(iv) Macropa



Macropa

[00226] In additional embodiments, the chelation conditions are optimized using methods known to those of skill in the art (*see, e.g.*, J Nucl Med. 1998 Dec;39(12):2105-10). In some embodiments, chelation efficiency is about > 99%, > 98%, > 97%, > 96%, > 95%, > 94%, > 93%, > 92%, > 91%, > 90%, > 89%, > 88%, > 87%, > 86%, > 85%, > 84%, > 83%, > 82%, > 81%, or > 80%.

[00227] In some embodiments, a chelator for use in a composition as described herein is chosen based on if and which radionuclide is present. As provided herein, in some embodiments, a chelator is DOTA, NOPO, Crown, or Macropa. In some embodiments, DOTA is the chelator and the radionuclide is Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211. In some embodiments, Crown is the chelator and the radionuclide is Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211. In some embodiments, NOPO is the chelator, and the radionuclide is Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211. In some embodiments, Macropa is the chelator, and the radionuclide is Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211.

[00228] In some embodiments, a particular chelator or type of chelator may be chosen for certain applications. For instance, in some embodiments, NOPO is used in diagnostic or theranostic applications. In some embodiments, Crown is used for therapeutic applications. In some embodiments, DOTA is used for diagnostic, theranostic, and/or therapeutic applications. In some embodiments, Macropa is used for diagnostic, theranostic, and/or therapeutic applications.

[00229] It is recognized that screening chelators for certain characteristics is within the scope of this disclosure and methods for such screening are known to those of skill in the art. For example, in some embodiments, chelators are screened for their ability to bind

radionuclides (*e.g.*, Ga68, Ac225 and daughter(s) of Ac225 (Bi213)) and display serum stability.

[00230] In some embodiments, a miniprotein conjugate described herein comprises a chelator. Any suitable chelator known in the art can be utilized. In some embodiments the chelator is directly conjugated to the miniprotein. In some embodiments, the chelator is indirectly connected to the miniprotein through a linker. In some embodiments, the chelator is indirectly connected to the miniprotein through a linker (*e.g.*, a linker described herein).

Radionuclides

[00231] In some embodiments, the present disclosure provides one or more radionuclides for use in a composition (*e.g.*, conjugate).

[00232] In some embodiments, miniprotein conjugates comprise a radionuclide bound to a chelator. As will be understood to those of skill in the art, any suitable radionuclide known in the art may be used. In some embodiments, a radionuclide is selected for imaging of a tumor with in a human having cancer. In some embodiments, a radionuclide is selected for its inability to kill cells *in vivo*. In some embodiments, the radionuclide is selected for its ability to kill cells *in vivo*.

[00233] In some embodiments, a composition of the present disclosure comprises one or more cytotoxic payloads including particle-emitting isotopes such as alpha-, beta-particles, and Auger electrons in radiotherapeutic applications. In some embodiments, a radionuclide of the present disclosure is an alpha emitter. As will be known to those of skill in the art, in some embodiments, an alpha emitter has a more localized area of impact such that when internalized into a cell it will act to, *e.g.*, kill a cancer cell, but will spare surrounding tissue from extensive damage such as could occur with use of a beta or gamma emitter.

[00234] Studies have evaluated alpha nuclide therapy versus beta nuclide therapy with the stronger clinical results pointing to alpha nuclides. In some embodiments, a benefit of alpha therapy is that the short path length means patients do not have to physically distance themselves from family and health care providers making treatment more tolerable. Further, in some embodiments, alpha therapy exhibits better cell killing potency due to its ability to induce double stranded DNA breaks.

[00235] In some embodiments, a composition comprises a linker, chelator, and radionuclide. In some embodiments, a composition comprises a miniprotein, optional linker, chelator, and a radionuclide. Without being bound by any particular theory, the present disclosure contemplates that a wide variety of radionuclides can be used in the pharmaceutical composition or as a diagnostic. Exemplary radionuclides, include but are not

limited to, Actinium-225, Astatine-211, Bismuth-212, Bismuth-213, Cesium-137, Chromium-51, Cobalt-60, Copper-64, Dysprosium-165, Erbium-169, Fermium-255, Fluor-18, Gallium-67, Gallium-68, Gold-198, Holmium-166, Indium-111, Iodine-123, Iodine-124, Iodine-125, Iodine-131, Iridium-192, Iron-59, Lead-212, Lutetium-177, Molybdenum-99, Palladium-103, Phosphorus-32, Potassium-42, Rhenium-186, Rhenium-188, Samarium-153, Technetium-99m, Radium-223, Ruthenium-106, Sodium-24, Strontium-89, Terbium-149, Thorium-227, Xenon-133, Ytterbium-169, Ytterbium-177, Yttrium-90, and Zirconium-89. Accordingly, in some embodiments, a radionuclide is selected from: iodine (131I or 125I), yttrium (90Y), lutetium (177Lu), actinium (225Ac), praseodymium, astatine (211At), rhenium (186Re), bismuth (212Bi or 213Bi), indium (111In), technetium (99Tc), phosphorus (32P), rhodium (188Rh), sulfur (35S), carbon (14C), tritium (3H), chromium (51Cr), chlorine (36Cl), cobalt (57Co or 58Co), iron (59Fe), selenium (75Se), or gallium (67Ga) or (68Ga). In some embodiments, the present disclosure contemplates that certain radioisotopes may be useful in or as therapeutic agents including but not limited to yttrium (90Y), lutetium (177Lu), actinium (225Ac), praseodymium, astatine (211At), rhenium (186Re), bismuth (212Bi or 213Bi), and rhodium (188Rh). In some embodiments, radioisotopes are useful as labels, *e.g.*, for use in diagnostics. In some such embodiments, such radioisotopes may include but are not limited to iodine (131I or 125I), indium (111In), technetium (99Tc), phosphorus (32P), carbon (14C), lead (212Pb) or tritium (3H). See, *e.g.*, US Patent No. 7514078.

[00236] In some embodiments, radionuclides are conjugated to different complexing agents and chelators. In some embodiments, chelators are identified and attached/bound to miniproteins through a linker or by acyclic, cyclic and macrocyclic chelates such as, for example, 1,4,7,10,13,16-hexaazacyclohexadecane-N,N',N'',N''',N''',N''''-hexaacetic acid (HEHA), 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA), NOPO, Crown, *etc.* In some embodiments, certain chelators may be preferred for certain radionuclides such as, for example, Ac-225 with DOTA or Crown, Ga-68 with NOPO, *etc.* In some embodiments, preferred combinations of chelators and radionuclides comprise one or more of the following: DOTA and Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211; Crown and Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211; NOPO and Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211; and/or Macropa and Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211.

[00237] Preferably, in some embodiments, a preferred radionuclide complex comprises Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211. In some such embodiments, such a complex with desired stability is selected. That is, in some embodiments, a complex comprising Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211 is characterized as having better stability *in vivo* in comparison to other complexes. Without being bound by any particular theory, the present disclosure contemplates that, in some embodiments, a radionuclide complex comprising a miniprotein forms with the miniprotein target (*e.g.*, any one of the target proteins in Table 10 or a fragment thereof). In some such embodiments, such a complex is internalized in the target cell.

[00238] In some embodiments, a radionuclide complex forms with a chelator (*e.g.*, DOTA, NOPO, Crown, Macropa, *etc.*) and is considerably more stable *in vivo*. In some embodiments, a miniprotein forms internalizing complexes with targets (*e.g.*, any one of the target proteins in Table 10).

[00239] In some embodiments, a composition provided by the present disclosure comprises Actinium-225. In some embodiments, a composition provided by the present disclosure comprises gallium (Ga-68). In some embodiments, a composition provided by the present disclosure comprises copper (Cu-64). In some embodiments, a composition provided by the present disclosure comprises indium (In-111). In some embodiments, a composition provided by the present disclosure comprises lutetium (Lu-177). In some embodiments, a composition provided by the present disclosure comprises lead (Pb-212). In some embodiments, a composition provided by the present disclosure comprises copper (Cu-67). In some embodiments, a composition provided by the present disclosure comprises lutetium (Lu-177). In some embodiments, a composition provided by the present disclosure comprises lanthanum (La-132). In some embodiments, a composition provided by the present disclosure comprises lanthanum (La-135). In some embodiments, a composition provided by the present disclosure comprises indium (In-111). In some embodiments, a composition provided by the present disclosure comprises cerium (Ce-134). For example, in some embodiments, radioimmunotherapy comprising Ac-225 may provide i) limited range in tissue of a few cell diameters; ii) high linear energy transfer leading to dense radiation damage along each alpha track; iii) a 10 day half-life; and/or iv) four net alpha particles emitted per decay (*see, e.g.*, as described in Scheinberg, David A, and Michael R McDevitt. "Actinium-225 in targeted alpha-particle therapeutic applications." *Current radiopharmaceuticals* vol. 4,4 (2011): 306-20).

[00240] In some embodiments, targeting constructs (*e.g.*, 225-Ac-drug constructs, *e.g.*, 68-Ga-constructs) have potential for use in cancer. For example, in some such embodiments, such constructs may be used in the treatment of cancer, such as, for example 225-Ac-drug constructs. In some embodiments, such constructs may be used in imaging, such as for prognostics, diagnostics, and/or monitoring, such as Ga-68 or Cu-64-based constructs.

[00241] In some embodiments, Ac-225 is conjugated to a miniprotein as provided herein. In some embodiments, the actinium is conjugated onto a chelator and may include an optional linker to link it to a miniprotein, which miniprotein targets the conjugate to a cell expressing the target (*e.g.*, any one of the target proteins in Table 10).

[00242] In some embodiments, Ga-68 is conjugated to a miniprotein as provided herein. In some embodiments, the gallium is conjugated onto a chelator and may include an optional linker to link it to a miniprotein, which miniprotein targets the conjugate to a cell expressing the target (*e.g.*, any one of the target proteins in Table 10).

[00243] In some embodiments, Cu-64 is conjugated to a miniprotein as provided herein. In some embodiments, the copper is conjugated onto a chelator and may include an optional linker to link it to a miniprotein, which miniprotein targets the conjugate to a cell expressing the target (*e.g.*, any one of the target proteins in Table 10).

[00244] In some embodiments, In-111 is conjugated to a miniprotein as provided herein. In some embodiments, the indium is conjugated onto a chelator and may include an optional linker to link it to a miniprotein, which miniprotein targets the conjugate to a cell expressing the target (*e.g.*, any one of the target proteins in Table 10).

[00245] In some embodiments, Lu-177 is conjugated to a miniprotein as provided herein. In some embodiments, the lutetium is conjugated onto a chelator and may include an optional linker to link it to a miniprotein, which miniprotein targets the conjugate to a cell expressing the target (*e.g.*, any one of the target proteins in Table 10).

[00246] In some embodiments, Pb-212 is conjugated to a miniprotein as provided herein. In some embodiments, the lead is conjugated onto a chelator and may include an optional linker to link it to a miniprotein, which miniprotein targets the conjugate to a cell expressing the target (*e.g.*, any one of the target proteins in Table 10).

[00247] In some embodiments, Cu-67 is conjugated to a miniprotein as provided herein. In some embodiments, the lead is conjugated onto a chelator and may include an optional linker to link it to a miniprotein, which miniprotein targets the conjugate to a cell expressing the target (*e.g.*, any one of the target proteins in Table 10).

[00248] In some embodiments, La-132 is conjugated to a miniprotein as provided herein. In some embodiments, the lead is conjugated onto a chelator and may include an optional linker to link it to a miniprotein, which miniprotein targets the conjugate to a cell expressing the target (*e.g.*, any one of the target proteins in Table 10).

[00249] In some embodiments, La-135 is conjugated to a miniprotein as provided herein. In some embodiments, the lead is conjugated onto a chelator and may include an optional linker to link it to a miniprotein, which miniprotein targets the conjugate to a cell expressing the target (*e.g.*, any one of the target proteins in Table 10).

[00250] In some embodiments, In-111 is conjugated to a miniprotein as provided herein. In some embodiments, the lead is conjugated onto a chelator and may include an optional linker to link it to a miniprotein, which miniprotein targets the conjugate to a cell expressing the target (*e.g.*, any one of the target proteins in Table 10).

[00251] In some embodiments, Ce-134 is conjugated to a miniprotein as provided herein. In some embodiments, the lead is conjugated onto a chelator and may include an optional linker to link it to a miniprotein, which miniprotein targets the conjugate to a cell expressing the target (*e.g.*, any one of the target proteins in Table 10).

[00252] In some embodiments, alpha particles (*e.g.*, of Actinium-225, *etc.*) are positively charged. In some such embodiments, the range of penetration in tissue varies between 5 and 10 cell diameters (40 to 100 μm) depending on their energy (Radiobiologic principles in radionuclide therapy. Kassis AI, Adelstein SJ J Nucl Med. 2005 Jan; 46 Suppl 1():4S-12S). In some such embodiments, such penetration allows for localized irradiation of target cells with minimal toxicity on surrounding normal cells, and internalization by cancer cells with as few as 1–3 tracks across the cell nucleus resulting in cell death (Humm 1987; Macklis et al 1988; Humm and Chin 1993; Couturier et al 2005) causing single- and double-stranded DNA breaks. See, *e.g.*, Sofou S. Radionuclide carriers for targeting of cancer. Int J Nanomedicine. 2008;3(2):181-199. doi:10.2147/ijn.s2736.

Dose Calculation

[00253] In some embodiments, a dose of a radiotherapeutic is calculated. In some such embodiments, calculation of an absorbed dose (D) is necessary to quantitatively correlate tumor response to a particular radiotherapeutic modality and to project on the potential effect of other radiotherapeutic modalities or administration strategies. That is, in some embodiments, the absorbed dose from a target site is defined as the energy (E) absorbed by a particular mass of tissue, normalized by the tissue mass (M): $D = E/M$ (Sgouros 2005). The absorbed energy is defined as a function of three parameters: the number of disintegrations

within the particular volume of interest (δ), the energy emitted per disintegration (ϵ), and the fraction of emitted energy that is absorbed by the particular volume of interest (the target mass) (f): $E = \delta \times \epsilon \times f$. For the relatively long range beta emitters, the dose evaluation at a target site includes not only the energy emitted by radionuclides localized within the target volume, but also the energy emitted by radionuclides accumulated in neighboring organs or areas whose emissions cross along their path the target volume of interest (Kolbert et al 2003). In other words, in some embodiments, the calculated total absorbed dose is the sum of the dose contributions from all regions containing radionuclides that act as secondary sources. In some embodiments, the adsorbed dose due to photon emissions is usually calculated separately and added to the dose due to alpha or beta particles. In some embodiments, where a composition comprises an alpha particle emitter, such cross organ absorbed doses may be of no significance due to their short recoil distances. In some embodiments, given appropriate context, at the micron-scale and at distances comparable to a few cells, microdosimetric evaluations are used to evaluate dose or 'hits' acquired by cancer cells within micrometastatic clusters (Palm et al 2002).

[00254] In some embodiments, a miniprotein conjugate comprising a radionuclide displays binding specificity to the human isoform of any one of the target proteins in Table 10. In some embodiments, the miniprotein comprises a binding affinity characterized by a dissociation constant ranging from about 500 nM to about 1 pM, *e.g.*, 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 nM, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 pM binding affinity to the human isoform of any one of the target proteins in Table 10. Without being bound by any particular theory, the present disclosure contemplates that, in some embodiments, a preferred dissociation constant of a miniprotein is about 10 nM or less, about 7.5 nM, about 5 nM or less, about 2.5 nM or less, about 1 nM or less (*i.e.*, in the picomolar range).

[00255] In some embodiments, compositions as provided herein are characterized for one or more of absorbed dose, dose rate, tumor penetration profile of radionuclides, intracellular localization profiles of radionuclides of shorter range, and tumor radiosensitivity (see, *e.g.*, Sofou S. Radionuclide carriers for targeting of cancer. *Int J Nanomedicine*. 2008;3(2):181-199).

[00256] As is known to those of skill in the art, due to toxicity of radionuclides, dose needs to be carefully controlled and considered. Accordingly, in some embodiments, compositions comprising radionuclides of the present disclosure address dose-limiting toxicity of

compositions such that radionuclides do not accumulate significantly (*e.g.*, in a toxicity-limiting manner) in vital organs.

[00257] In some embodiments, alpha particle-emitting isotopes engage in on-target cell killing while minimizing toxic effects (*e.g.*, to surrounding tissue, *e.g.*, as compared to, *e.g.*, beta emitters, *etc.*).

[00258] In some embodiments, compositions provided herein (comprising a radionuclide) are administered in a single step such as, *e.g.*, using a ligand, *e.g.*, a miniprotein resulting in improved biodistributions (*e.g.*, specific targeting), pK with partial and acceptable damage or no damage to normal tissues, enhanced penetration of the pharmaceutical composition into the tumor heterogeneous interstitial space.

[00259] In some embodiments, one or more radionuclides is conjugated to a miniprotein. Relatedly, in some embodiments, radiolabeling efficiency of a miniprotein is optimized to radiolabel a desired number of radionuclides. In some embodiments, a ratio of radionuclides conjugated to a miniprotein is 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1. In some embodiments, radionuclides conjugated to a miniprotein does not present toxicity. In some embodiments, a composition comprising a miniprotein and radionuclide does not accumulate in the liver, spleen, and the pancreas and is cleared rapidly when administered to a subject. For instance, in some embodiments, after administration to a subject, biodistribution and $t_{1/2}$ in the kidney is >10% of the injected dose (ID) in tumors at 24 hrs and tumors is >3% ID at 24 hrs.

Radionuclides and Chelation

[00260] A radionuclide can be bound to a chelator through any method known in the art. In some embodiments, chelation methods may differ based on the radionuclide and chelator selected. For example, in some embodiments, chelation can be carried out in one step by incubating the miniprotein-chelator conjugate with the radionuclide for a predetermined period at a predetermined temperature to achieve a sufficient amount of chelation. In some embodiments, a miniprotein-chelator conjugate comprises a chelator or variant thereof as provided herein (*e.g.*, DOTA, *e.g.*, NOPO, *e.g.*, Crown, *e.g.*, Macropa, *etc.*). In some embodiments, miniprotein-chelator conjugates can be chelated to a radionuclide (*e.g.*, Actinium-225, Gallium-68, Copper-64, Lutetium-177, Indium-111, Lead-212, *etc.*) by incubation with the radionuclide for about 1 hour at 70°C. In some embodiments, miniprotein-chelator conjugates can be chelated to a radionuclide (*e.g.*, Actinium-225, Gallium-68, Copper-64, Lutetium-177, Indium-111, Lead-212, *etc.*) by incubation with the radionuclide for about 1 hour at 70°C.

[00261] In some embodiments, the chelation process yields a preparation in which at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the miniprotein-chelator is bound to a radionuclide. In some embodiments, the chelation process yields a preparation in which more than 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the miniprotein-chelator is bound to a radionuclide. Excess radionuclide can be removed from the preparation by purification methods known in the art.

Polypeptides

[00262] Among other things, the present disclosure provides polypeptides. In some embodiments, a polypeptide is assembled using solid phase synthesis methods. In some embodiments, a polypeptide is recombinant. In some embodiments, a polypeptide comprises or consists of a miniprotein. In some such embodiments, a miniprotein comprises or consists of a binder. In some embodiments, polypeptides of the present disclosure (including muteins, allelic variants, fragments, derivatives, and analogs) are encoded by polynucleotides as described and provided herein.

[00263] In some embodiments, a miniprotein of the present disclosure comprises or consists of a polypeptide capable of binding to target as shown in Tables 3, 4A, and 4B.

[00264] In some embodiments, the present disclosure provides binders comprising or consisting of a fragment of a polypeptide as provided herein. In some such embodiments, fragments include at least 20 contiguous amino acids, more preferably at least 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or more contiguous amino acids.

[00265] In some embodiments, miniproteins of the present disclosure can also include fusions or conjugates with one or more other components, such as heterologous polypeptides. For example, in some embodiments, heterologous sequences can comprise or consist of sequences designed to facilitate purification, *e.g.* histidine tags, and/or visualization of recombinantly-expressed proteins. Other non-limiting examples of such fusions or conjugates include those that permit display of the encoded protein on the surface of a phage or a cell, including any detectable or visualizable component such as, *e.g.*, green fluorescent protein (GFP), and fusions to the IgG Fc region.

[00266] In some embodiments, a miniprotein comprises or consists of a specific amino acid sequence. In some embodiments, a miniprotein has an amino acid sequence that is 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or 100% identical to the amino acid sequence set forth in any of SEQ ID NOs: 1-68.

[00267] As used herein and known to those of skill in the art, the twenty conventional amino acids and their abbreviations follow conventional usage. See Immunology-A Synthesis (Golub and Gren eds., Sinauer Associates, Sunderland, Mass., 2nd ed. 1991), which is incorporated herein by reference. In some embodiments, an amino acid of the present disclosure may be a stereoisomer (*e.g.*, D-amino acids) of the twenty conventional amino acids. In some embodiments, an amino acid in a polypeptide of the present disclosure may be a non-natural amino acid. For example, amino acids such as α -, α -disubstituted amino acids, N-alkyl amino acids, and other unconventional amino acids may also be suitable components for polypeptides of the present disclosure. Examples of unconventional amino acids include: 4-hydroxyproline, γ -carboxyglutamate, ϵ -N,N,N-trimethyllysine, ϵ -N-acetyllysine, O-phosphoserine, N-acetyls erine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, N-methylarginine, and other similar amino acids and imino acids (*e.g.*, 4-hydroxyproline). Arrangements of polypeptide sequence notations used herein have a left-side end corresponding to the amino terminal and a right-side end corresponding to the carboxy-terminal end, in accordance with standard usage and convention.

[00268] In some embodiments, miniproteins of the present disclosure comprising two or more cysteine residues, such as those set forth in SEQ ID NOs: 1-84, have cysteine residues connected via disulfide bridges (*e.g.*, via natural folding).

Nucleic Acids

[00269] Among other things, the present disclosure provides herein polynucleotides and methods of use thereof. In some embodiments, all or a portion of the polynucleotides encode a polypeptide (*e.g.*, a miniprotein) that specifically binds to any one of the target proteins in Table 10. In some embodiments, the target proteins selected from Table 10 is murine or the human isoform of the target protein selected from Table 10. In some embodiments, the nucleic acid sequence has a specific sequence. In some embodiments, a polynucleotide of the present disclosure is codon-optimized (*i.e.*, the nucleic acid sequence is codon optimized).

[00270] In some embodiments, a polynucleotide of the present disclosure comprises or consists of a nucleic acid sequence encoding a polypeptide that is or comprises a miniprotein that specifically binds to any one of the target proteins in Table 10, or any portion, fragment, or variant thereof.

[00271] In some embodiments, a miniprotein is represented by a nucleic acid molecule encoding an amino acid that, when folded, comprises one or more disulfide bridges.

[00272] In some embodiments, for example, a nucleic acid molecule (*i.e.*, a polynucleotide) may be non-identical to a reference sequence as provided herein, but still encode a binder as

provided by the present disclosure. In some such embodiments, such as a provided polynucleotide (*i.e.*, encoding a miniprotein or analog thereto) hybridizes under stringent conditions as disclosed herein.

[00273] In some embodiments, the present disclosure provides nucleic acid molecules comprising a fragment of any polynucleotide as provided herein. In some embodiments, a polynucleotide fragment comprises or consists of a portion of contiguous nucleic acid residues. For instance, in some embodiments, a polynucleotide fragment comprises or consists of 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 100 or more nucleic acid residues.

[00274] In some embodiments, fragments of the present disclosure display utility in a variety of systems and methods. For example, the fragments may be used as probes in various assays. For instance, in some embodiments, fragments may be used in hybridization techniques. Depending on the method, the target nucleic acid sequences may be either DNA or RNA. The target nucleic acid sequences may be fractionated (*e.g.*, by gel electrophoresis) prior to the hybridization, or the hybridization may be performed on samples *in situ*. One of skill in the art will appreciate that nucleic acid probes of known sequence find utility in determining chromosomal structure (*e.g.*, by Southern blotting) and in measuring gene expression (*e.g.*, by Northern blotting). In such experiments, the sequence fragments are preferably detectably labeled, so that their specific hybridization to target sequences can be detected and optionally quantified. In some embodiments, fragments may be used as probes, *e.g.*, such as when immobilized on a microarray. Methods for creating microarrays by deposition and fixation of nucleic acids onto support substrates are well known in the art. Reviewed in DNA Microarrays: A Practical Approach (Practical Approach Series), Schena (ed.), Oxford University Press (1999) (ISBN: 0199637768); Nature Genet. 21(1)(suppl):1-60 (1999); Microarray Biochip: Tools and Technology, Schena (ed.), Eaton Publishing Company/BioTechniques Books Division (2000) (ISBN: 1881299376), the disclosures of which are incorporated herein by reference in their entireties. Analysis of, for example, gene expression using microarrays comprising nucleic acid sequence fragments, such as the nucleic acid sequence fragments disclosed herein, is a well-established utility for sequence fragments in the field of cell and molecular biology. Other uses for sequence fragments immobilized on microarrays are described in Gerhold et al., Trends Biochem. Sci. 24:168-173 (1999) and Zweiger, Trends Biotechnol. 17:429-436 (1999); DNA Microarrays: A Practical Approach (Practical Approach Series), Schena (ed.), Oxford University Press (1999) (ISBN: 0199637768); Nature Genet. 21(1)(suppl):1-60 (1999); Microarray Biochip:

Tools and Technology, Schena (ed.), Eaton Publishing Company/BioTechniques Books Division (2000) (ISBN: 1881299376).

[00275] In some embodiments, a polynucleotide of the present disclosure comprises or consists of a nucleic acid sequence encoding a polypeptide that is or comprises a miniprotein that binds to any one of the target proteins in Table 10, or any portion, fragment, or variant thereof. In some embodiments, the polynucleotide encodes an polypeptide that comprises or consists of the amino acid sequence set forth in any one of SEQ ID NOs: 1-68. In some embodiments, the polynucleotide encodes a polypeptide sequence having at least 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or more identity to the amino acid sequences provided in Tables 3, 4A, and 4B.

[00276] In some embodiments, a miniprotein comprises one or more disulfide bridges. In some embodiments, a miniprotein is represented by a nucleic acid sequence encoding a polypeptide that, when folded, comprises one or more disulfide bridges.

[00277] In some embodiments, for example, a nucleic acid molecule (*i.e.*, a polynucleotide) may be non-identical to a reference sequence as provided herein, but still encode a miniprotein as provided by the present disclosure (*e.g.*, a miniprotein in accordance with any one of SEQ ID NOs: 1-68 or a close analog as provided for herein). In some such embodiments, such as provided polynucleotide (*i.e.*, encoding a miniprotein or analog thereto) hybridizes under stringent conditions as disclosed herein.

In some embodiments, the present disclosure provides nucleic acid molecules comprising a fragment of any polynucleotide as provided herein. In some embodiments, a polynucleotide fragment comprises or consists of a portion of contiguous nucleic acid residues identical to that of a polynucleotide of any of SEQ ID NOs: 1-68. For instance, in some embodiments, a polynucleotide fragment comprises or consists of 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 100 or more nucleic acid residues encoding some or all of a polypeptide or fragment thereof as set forth in any one of SEQ ID NOs: 1-68.

[00278] One of skill in the art will appreciate that the nucleic acid fragments of the present disclosure may be used in a wide variety of techniques capture and/or detection techniques not specifically described herein.

Vectors

[00279] Also provided herein are vectors, including expression vectors, which comprise, among other things, nucleic acids comprising or consisting of sequences encoding miniproteins that specifically bind to any one of the target proteins in Table 10. In some

embodiments, a vector is used to produce a polypeptide encoding a binder that binds to any one of the target proteins in Table 10. In some embodiments, the target protein selected from Table 10 is murine or a human isoform of any one of the target proteins in Table 10. In some embodiments, given appropriate contexts, a miniprotein is represented by an amino acid sequence with a corresponding nucleic acid sequence that has been codon optimized. In some such embodiments, one of skill in the art is capable of designing and optimizing polynucleotides that correspond to amino acids of miniproteins that bind to a target (*e.g.*, any one of the target proteins in Table 10), for which exemplary amino acid sequences are set forth in Tables 3, 4A, and 4B. In some embodiments, a vector comprises a nucleic acid sequence that comprises or consists of a sequence encoding any one of the target proteins in Table 10.

[00280] In some embodiments, the vector comprises a nucleic acid sequence encoding any one of the target proteins in Table 10 or a fragment or variant thereof, wherein the polynucleotide is codon-optimized (*i.e.*, the nucleic acid sequence is codon optimized). In some embodiments, the vector comprises a nucleic acid sequence encoding up to 100 amino acids. In some embodiments, a vector of the present disclosure comprises or consists of a nucleic acid sequence that encodes an amino acid sequence of a miniprotein. In some embodiments, the vectors of the present disclosure further comprise a nucleic acid sequence as provided herein operably linked to one or more expression control sequences.

[00281] Also provided herein are vectors, including expression vectors, which comprise, among other things, nucleic acids comprising or consisting those described herein. In some embodiments, a vector is used to produce a polypeptide encoding a miniprotein that binds to any one of the target proteins in Table 10. In some embodiments, the target protein selected from Table 10 is murine or a human isoform of any one of the target proteins in Table 10. In some embodiments, given appropriate contexts, a miniprotein of any one of the target proteins in Table 10 (*e.g.*, as provided in Tables 3, 4A, and 4B) has a corresponding nucleic acid sequence that has been codon optimized. In some such embodiments, one of skill in the art is capable of designing and optimizing polynucleotides that correspond to amino acids of particular miniproteins of any one of the target proteins in Table 10 such as, for example, polynucleotides comprising nucleic acid sequences that correspond to amino acid sequences of Tables 3, 4A and 4B. In some embodiments, a vector comprises a nucleic acid sequence that comprises or consists of a sequence having 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 98.1%, 98.2%, 98.3%, 98.4%, 98.5%, 98.6%, 98.7%, 98.8%, 98.9%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, 99.9%, or more identity to wild type

isform of any one of the target proteins in Table 10. In some embodiments, the vector comprises a nucleic acid sequence encoding any one of the target proteins in Table 10 or a fragment or variant thereof, wherein the polynucleotide is codon-optimized (*i.e.*, the nucleic acid sequence is codon optimized). In some embodiments, the vector comprises a nucleic acid sequence encoding up to 100 amino acids of any one of SEQ ID NOs: 1-68. In some embodiments, a vector of the present disclosure comprises or consists of a nucleic acid sequence, wherein the nucleic acid sequence encodes an amino acid sequence as set forth in Tables 3, 4A, and 4B. In some embodiments, the nucleic acid sequence is a codon optimized nucleic acid sequence. In some embodiments, the vectors of the present disclosure further comprise a nucleic acid sequence as provided herein operably linked to one or more expression control sequences.

Kidney Retention

[00282] In various aspects, provided are novel methods and compositions to address undesirable effects of radionuclide therapy upon transit through the kidney. Accordingly, disclosed herein are compositions characterized to exhibit reduced kidney retention. Kidney retention of peptide-based radiopharmaceuticals may be attributed as a result of target expression in the kidney (*e.g.*, PSMA) or protein conservation from ultrafiltration and reuptake into the proximal tubule cells. Reuptake in the proximal tubule cells occurs through protein or peptide cleavable by the kidney brush border peptidases and reuptake of short peptides, as well as receptor mediated reuptake. In receptor mediated reuptake, the peptide-based radiopharmaceutical binds to the megalin/cubulin receptor complex, undergoes receptor mediated endocytosis, and is degraded and retained in lysosomes, resulting in extended retention of radioactivity in the proximal tubules of the kidneys and dose-limiting toxicity of the radiopharmaceutical. Geenen et al., Nucl. Med. Biol, 2021, 102-103: 1-11.

Decharged Molecules

[00283] In exemplary aspects to reduce kidney retention, in some embodiments, the composition comprises a decharged molecule or molecules that have fewer charges that enable rapid clearance through the kidney. In some embodiments, a reduction in positively charged or polar molecules, an increase in negatively charged molecules, and/or both results in reduced kidney retention.

[00284] In some embodiments, the composition comprising a decharged molecule or molecules comprises an amino acid sequence comprising any one of SEQ ID NOs: 1-3, 8-11,

or 65-67. In some embodiments, the composition comprising a decharged molecule or molecules comprises a compound selected from any of C1-C6, C43-54, or C155-C157.

[00285] In some embodiments, the composition wherein M comprises an amino acid sequence comprising a percentage of charged amino acids between 1-5% of the total amino acid sequence, wherein the charged amino acids are selected from Lys, Arg, or His, is characterized to exhibit reduced kidney uptake. In some embodiments, the composition wherein M comprises an amino acid sequence comprising a percentage of charged amino acids between 5-10% of the total amino acid sequence, wherein the charged amino acids are selected from Lys, Arg, or His, is characterized to exhibit reduced kidney uptake. In some embodiments, the composition wherein M comprises an amino acid sequence comprising a percentage of charged amino acids between 10-15% of the total amino acid sequence, wherein the charged amino acids are selected from Lys, Arg, or His, is characterized to exhibit reduced kidney uptake. In some embodiments, the composition wherein M comprises an amino acid sequence comprising a percentage of charged amino acids between 15-20% of the total amino acid sequence, wherein the charged amino acids are selected from Lys, Arg, or His, is characterized to exhibit reduced kidney uptake. In some embodiments, the composition wherein M comprises an amino acid sequence comprising a percentage of charged amino acids between 20-25% of the total amino acid sequence, wherein the charged amino acids are selected from Lys, Arg, or His, is characterized to exhibit reduced kidney uptake. In some embodiments, the composition wherein M comprises an amino acid sequence comprising a percentage of charged amino acids between 25-30% of the total amino acid sequence, wherein the charged amino acids are selected from Lys, Arg, or His, is characterized to exhibit reduced kidney uptake. In some embodiments, the reduced percentage of positively charged amino acids in M results in decreased reabsorption of M at the negatively charged membrane of the renal proximal tubule cells.

[00286] In some embodiments, the composition wherein M comprises wherein an amino acid sequence comprising a percentage of charged amino acids between 1-5% of the total amino acid sequence, wherein the charged amino acids are selected from Asp or Glu, is characterized to exhibit reduced kidney uptake. In some embodiments, the composition wherein M comprises wherein an amino acid sequence comprising a percentage of charged amino acids between 5-10% of the total amino acid sequence, wherein the charged amino acids are selected from Asp or Glu, is characterized to exhibit reduced kidney uptake. In some embodiments, the composition wherein M comprises wherein an amino acid sequence comprising a percentage of charged amino acids between 10-15% of the total amino acid

sequence, wherein the charged amino acids are selected from Asp or Glu, is characterized to exhibit reduced kidney uptake. In some embodiments, the composition wherein M comprises wherein an amino acid sequence comprising a percentage of charged amino acids between 15-20% of the total amino acid sequence, wherein the charged amino acids are selected from Asp or Glu, is characterized to exhibit reduced kidney uptake. In some embodiments, the composition wherein M comprises wherein an amino acid sequence comprising a percentage of charged amino acids between 20-25% of the total amino acid sequence, wherein the charged amino acids are selected from Asp or Glu, is characterized to exhibit reduced kidney uptake. In some embodiments, the composition wherein M comprises wherein an amino acid sequence comprising a percentage of charged amino acids between 25-30% of the total amino acid sequence, wherein the charged amino acids are selected from Asp or Glu, is characterized to exhibit reduced kidney uptake. In some embodiments, the reduced percentage of polar amino acids in M results in decreased reabsorption of M at the negatively charged membrane of the renal proximal tubule cells.

[00287] Example 10 and Example 11 and FIG. 1 and FIG. 3 provide exemplary data on the level of kidney retention measured in mice treated with radioactively-labeled peptides and imaged via SPECT/CT. In some embodiments, the percentage of ID/g in the kidney at 4 hours is reduced by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% when the composition comprises a percentage of charged amino acids compared to a composition in which the percentage of charged amino acids has not been decreased. In some embodiments, the percentage of ID/g in the kidney at 24 hours is reduced by 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% when the composition comprises a percentage of charged amino acids compared to a composition in which the percentage of charged amino acids has not been increased.

Albumin binding domains

[00288] In exemplary aspects to reduce kidney retention, in some embodiments, the composition comprises amino acid sequences comprising albumin binding domains. Thus, such compositions can bind to albumin, which, in circulation, can extend the circulating half-life of the amino acid sequence and reduce kidney retention. In some embodiments, the composition comprising amino acid sequences comprising albumin binding domains comprises an amino acid sequence of any one of SEQ ID NOs: 7 or 13. In some embodiments, the composition comprising amino acid sequences comprising albumin binding

domains comprises a compound selected from any of C12, C23-C25, C29, C30, C32, C33, C66, C69, or C71.

Cleavable Linker

[00289] In other aspects to reduce kidney retention, in some embodiments, the composition comprises M-L-C-R, wherein L is a cleavable linker. In some embodiments, the cleavable linker is distal to the miniprotein to not interfere with tumor binding. In some embodiments, the cleavable linker is engineered to optimize physicochemical characteristics. In some embodiments, a cleavable linker has a short length. In some embodiments, the cleavable linker is selectively cleaved in the proximal tubule of the kidney. In some embodiments, selective cleavage of the cleavable linker in the proximal tubule of the kidney is due to the presence of peptidases in the proximal tubule. In some embodiments, cleavage of the cleavable linker in the proximal tubule releases chelator-radionuclide-miniprotein that filters into the bladder and avoids kidney reuptake. See Arano, Y. Nuclear Medicine and Biology 2021, 92, 149-55; Zhang, M. et. Al. Bioconjugate Chemistry 2019, 30, 1745-53.

[00290] In some embodiments, the composition comprising a cleavable linker comprises an amino acid sequence comprising any one of SEQ ID NOs: 7-11, 17-23, 26, or 27. In some embodiments, the composition comprising a cleavable linker comprises a compound selected from any of C15, C17-C22, C26-C28, C34-C42, C55-66, C68, C70, or C72-C77..

[00291] In some embodiments, the cleavable linker is connected to M at the Na-carboxyl of lysine. In some embodiments, the cleavable linker comprises hydrogen, (PEG4)1-4, 4-aminomethyl-phenylacetic acid (AmPA), aminomethylbenzoyl (AmBz), (succinic acid-(PEG4)1-4, norleucine, ileucine, glutamine, methoxinine, phenylalanine, tyrosine, beta alanine, MWK or MVK, glycine, citrulline (Cit), or sarcosine (Sar). In some embodiments, the linker is cleaved by cathepsin B in a lysosome or a neutral endopeptidase, metalloprotease, or dipeptidyl peptidase in a kidney brush border membrane.

[00292] Described in Example 10 and Example 11 are the level of kidney retention measured in mice treated with radioactively-labeled peptides and imaged via SPECT/CT,. In some embodiments, the cleavable linker reduces the uptake of M in the kidney, as shown in FIG. 5 and FIG. 13.

[00293] In some embodiments, the cleavable linker is a disulfide bond or protease sensitive. In a further embodiment, the groups adjacent to the disulfide bond are modified to control the hindrance of the disulfide bond, and by this the rate of cleavage. Published work established the potential for modifying the susceptibility of the disulfide bond to reduction by introducing steric hindrance on either side of the disulfide bond (Kellogg et al (2011) Bioconjugate

Chemistry, 22, 717). A greater degree of steric hindrance reduces the rate of reduction by intracellular glutathione and also extracellular (systemic) reducing agents, consequentially reducing the ease by which the rest of the conjugate is released, both inside and outside the cell. Thus, selection of the optimum in disulfide stability in the circulation (which minimizes undesirable side effects of the radionuclide) versus efficient release in the intracellular milieu (which maximizes the therapeutic effect) can be achieved by careful selection of the degree of hindrance on either side of the disulfide bond. The hindrance on either side of the disulfide bond is modulated through introducing one or more methyl groups on either the miniprotein or radionuclide side of the molecular construct.

Co-Administration

[00294] In alternative aspects to reduce kidney clearance, in some embodiments, a composition comprises M-L-C-R and one or more additional proteins, e.g., a decoy peptide. In some embodiments, co-administration of the composition with a decoy peptide results in reduced kidney uptake due to competitive inhibition of the proximal tubule cell receptors. In some embodiments, co-administration with a functional inhibitor results in reduced kidney uptake. See Xiong, C. et. al. Mol. Pharmaceutics 2019, 16, 808-15; Melis, M. et. al. Eur J Nucl Med Imaging, 2009, 36, 1968-76.

[00295] In some embodiments, the decoy peptide has an amino acid sequence comprising any one of SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NOs: 16-52, or SEQ ID NOs: 68. In some embodiments, the decoy peptide is a compound selected from any of C7, C31, C100-C108, or C131-C139. In some embodiments, the decoy peptide is present at a concentration of 10X, 20X, 30X, 40X, 50X, 60X, 70X, 80X, 90X or 100X the concentration of M. Example 10 and Example 11 describe, the level of kidney retention measured in mice treated with radioactively-labeled peptides and imaged via SPECT/CT, using the approach as provided herein. In some embodiments, the decoy peptide reduces the uptake of M in the kidney, as shown in, e.g., FIG. 2, FIG. 4, and FIG. 12.

[00296] In some embodiments, the decoy peptide reduces the uptake of M in the kidney by competitive inhibition. In some embodiments, the decoy peptide can be of a desirable length. In some aspects, amino acid residues added to the N-terminal end or the C-terminal end of the decoy peptides disclosed herein may prevent ubiquitination, improve stability, help maintain the three dimensional structure of the peptide, or a combination thereof.

[00297] In some aspects, the decoy peptides disclosed herein can further comprise a peptide or polypeptide having one or more amino acid residues with a modified side chain. In some aspects, one or more amino acids of any of the decoy peptides disclosed here can have a

modified side chain. Examples of side chain modifications include but are not limited to modifications of amino acid groups, such as reductive alkylation; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino acid with 2,4,6-trinitrobenzene sulfonic acid (TNBS); alkylation of amino groups with succinic anhydride; and pyridoxylation with pridoxal-5-phosphate followed by reduction with NaBH₄.

[00298] In some aspects, the decoy peptides described herein can be further modified to improve stability. In some aspects, any of the amino acid residues of the decoy peptides described herein can be modified to improve stability. In some aspects, decoy peptide can have at least one amino acid residue that has an acetyl group, a fluorenylmethoxy carbonyl group, a formyl group, a palmitoyl group, a myristyl group, a stearyl group, or polyethylene glycol. In some aspects, an acetyl protective group can be bound to the decoy peptide described herein.

[00299] As used herein, the term “decoy peptide” can also be used to include functional equivalents of the decoy peptides described herein. As used herein, the term “functional equivalents” can refer to amino acid sequence variants having an amino acid substitution, addition, or deletion in some of the amino acid sequence of the decoy peptide while simultaneously having similar or improved biological activity, compared with the decoy peptide as described herein. In some aspects, the amino acid substitution can be a conservative substitution. Examples of the naturally occurring amino acid conservative substitution include, for example, aliphatic amino acids (Gly, Ala, and Pro), hydrophobic amino acids (Ile, Leu, and Val), aromatic amino acids (Phe, Tyr, and Trp), acidic amino acids (Asp and Glu), basic amino acids (His, Lys, Arg, Gln, and Asn), and sulfur-containing amino acids (Cys and Met). In some aspects, the amino acid deletion can be located in a region that is not directly involved in the activity of the decoy peptide disclosed herein.

[00300] In some aspects, the amino acid sequence of the decoy peptides described herein can include a peptide sequence that has substantial identity to any of the sequences of the decoy peptides disclosed herein. As used herein, the term “substantial identity” means that two amino acid sequences, when optimally aligned and then analyzed by an algorithm normally used in the art, such as BLAST, GAP, or BESTFIT, or by visual inspection, share at least about 60%, 70%, 80%, 85%, 90%, or 95% sequence identity. Methods of alignment for sequence comparison are known in the art.

[00301] In some aspects, the amino acid sequence of the decoy peptides described herein can include a peptide sequence that has some degree of identity or homology to any of

sequences of the decoy peptides disclosed herein. The degree of identity can vary and be determined by methods known to one of ordinary skill in the art. The terms “homology” and “identity” each refer to sequence similarity between two polypeptide sequences. Homology and identity can each be determined by comparing a position in each sequence which can be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same amino acid residue, then the polypeptides can be referred to as identical at that position; when the equivalent site is occupied by the same amino acid (e.g., identical) or a similar amino acid (e.g., similar in steric and/or electronic nature), then the molecules can be referred to as homologous at that position. A percentage of homology or identity between sequences is a function of the number of matching or homologous positions shared by the sequences. The decoy peptides described herein can have at least or about 25%, 50%, 65%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity or homology to the decoy peptide.

Host Cell Transformants

[00302] In some embodiments, the present disclosure provides host cells transformed with polynucleotides, polypeptides, and/or vectors of the present disclosure, and any combinations as well as any descendants thereof. In some embodiments host cells comprise and carry nucleic acid sequences of the present disclosure on vectors. In some embodiments, a host cell is a cell line. In some embodiments, a host cell is a primary cell, such as an immune cell. In some embodiments, such a primary cell is derived from or made compatible with a subject. In some embodiments, a subject is a mammal. In some embodiments, a mammal is a human. In some embodiments, a human is at risk of having or has been diagnosed as having cancer.

[00303] In some such embodiments, such vectors may but need not be freely replicating vectors. In some embodiments, nucleic acid sequences or polynucleotides provided by the present disclosure have been integrated into a genome of a host cell.

[00304] In some embodiments, host cells of the present disclosure can be mutated by recombination with a disruption, deletion or mutation of the isolated nucleic acid of the present disclosure so that the activity of one or more functional activities in the host cell is reduced or eliminated compared to a host cell lacking the mutation.

[00305] Without limitation, and as will be appreciated by those of skill in the art, a wide variety of host cells is contemplated in various embodiments in order to express binders of the present disclosure (via use of, e.g., nucleic acid sequences, amino acid sequences, and/or additional components as provided here).

Pharmaceutical Compositions

[00306] The present disclosure provides, among other things, pharmaceutical compositions comprise a polypeptide, polynucleotide, vector and/or host cell encoding a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) as provided herein. It is to be understood that a pharmaceutical composition comprising a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) is interpreted as a pharmaceutical composition comprising a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) per se and/or one or more components encoding a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) (*e.g.*, a vector, *e.g.*, a host cell). In some embodiments, a pharmaceutical composition comprises a linker and a chelator. In some embodiments, a pharmaceutical composition comprises a linker, chelator, and radionuclide. In some embodiments, a composition comprises a miniprotein, optional linker, and chelator. In some embodiments, a composition comprises a miniprotein, optional linker, chelator, and radionuclide. In some embodiments, a pharmaceutical composition provided by the present disclosure comprises a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) that selectively binds to any one of the target proteins in Table 10. In some embodiments, the target protein selected from Table 10 is a human target protein selected from Table 10.

[00307] In certain embodiments, a pharmaceutical composition comprises a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) comprising one or more cysteine-rich domains. In some embodiments, the pharmaceutical composition comprises a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) having one or more disulfide bonds. In some embodiments, the pharmaceutical composition comprises a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) represented or encoded by an amino acid sequence having <100AAs, <90AAs, <80AAs, <85AAs, <75AAs, <70AAs, <65AAs, <60AAs, <55AAs, <50AAs, <45AAs, <40AAs, <35AAs, <30AAs, <25AAs, <20AAs, <15AAs, <10AAs, or <5AAs.

[00308] In some embodiments, a pharmaceutical composition comprising a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) as provided herein is characterized as having a molecule weight equal to or less than 12 kDa.

[00309] In some embodiments, a pharmaceutical composition comprising a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) does not elicit an undesirable immune response or elicits a tolerable immune response. In some embodiments, a pharmaceutical composition of the present disclosure comprises a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) having high tissue penetrating properties.

[00310] In some embodiments, a pharmaceutical composition comprising a miniprotein comprises acceptable half-life and/or stability. In some such embodiments, acceptable stability is between about 30 minutes to 48 hours in serum and 1-4 days or more in a tumor or tumor microenvironment. By way of non-limiting example, for instance, in some embodiments, a miniprotein of the present disclosure has stability of about 2.5 hours in serum. In some embodiments, stability of a miniprotein is about 30 minutes, 60 minutes, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 30, 36, 40, or 48 hours in serum. In some embodiments, stability in a tumor or tumor microenvironment is 24, 36, 48, 60, 72, 84, 96 hours or more.

[00311] In some embodiments, the pharmaceutical composition is characterized as stable *in vivo*. In some embodiments, a pharmaceutical composition provided herein is not taken up in kidney or liver.

[00312] In some embodiments, a pharmaceutical composition of the present disclosure exhibits solubility of >0.05 mg/mL, >0.1 mg/mL, >0.2 mg/mL, >0.3 mg/mL, >0.4 mg/mL, >0.5 mg/mL, >0.6 mg/mL, >0.7 mg/mL, >0.8 mg/mL, >0.9 mg/mL, >1 mg/mL, >2 mg/mL, >3 mg/mL, >4 mg/mL, >5 mg/mL, >6 mg/mL, >7 mg/mL, >8 mg/mL, >9 mg/mL, or >10 mg/mL.

[00313] In some embodiments, a pharmaceutical composition provided by the present disclosure exhibits stability of >80%, >81%, >82%, >83%, >84%, 85%, >86%, >87%, >88%, >89%, >90%, >91%, >92%, >93%, >94%, 95%, >96%, >97%, >98%, or >99%.

[00314] In some embodiments, a pharmaceutical composition of the present disclosure is characterized as comprising a certain purity, represented as a percentage of parent molecule still intact. In some embodiments, a pharmaceutical composition of the present disclosure

comprises about 85% purity or greater at 5 days at room temperature. In some embodiments, a pharmaceutical composition of the present disclosure is characterized as having about 90% purity or greater at 40°C for 4 hr. In some embodiments, a pharmaceutical composition of the present disclosure comprises cyclic or acyclic sequence.

[00315] In some embodiments, a pharmaceutical composition in accordance with the present disclosure comprises a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) and one or more additional components. For example, in some embodiments, one or more additional components may be a linker and/or a conjugate such as a cytotoxic payload or detectable moiety for use in diagnosis and/or imaging. In some such embodiments, a pharmaceutical composition comprises a linker, chelator, and/or radionuclide as provided herein.

[00316] In some embodiments, pharmaceutical compositions modulating, binding, or inhibiting a human isoform of any one of the target proteins in Table 10 (or any related activity thereto) are provided. In some embodiments, a pharmaceutical composition is or comprises a therapeutic. In some embodiments, a pharmaceutical composition is or comprises a detectable moiety (*e.g.*, as used for imaging such as MRI, CT, PET, *etc.*).

[00317] In preferred embodiments, one or more characteristics of the pharmaceutical compositions are identified for optimized administration parameters including but not limited to dose, effective dose, dose rate, tumor penetration profile, intracellular localization profile, binding specificity, *etc.* (See Sofou S. Radionuclide carriers for targeting of cancer. *Int J Nanomedicine*. 2008;3(2):181-199. doi:10.2147/ijn.s2736).

[00318] In some embodiments, a pharmaceutical composition of the present disclosure does not present toxicity or presents less toxicity than a composition comprising one or more different components such as a larger targeting peptide, or a different radionuclide (*e.g.*, beta emitter, *etc.*).

[00319] In some embodiments, a pharmaceutical composition of the present disclosure does not accumulate in the liver, spleen, and the pancreas and is cleared rapidly. For instance, the biodistribution and t_{1/2} in the kidney is >10% of injected dose (ID; initial dose injected) in tumors at 24 hrs and tumors is >3% ID at 24 hrs.

Theranostic Compositions

[00320] In some embodiments, theranostic compositions are provided. In some embodiments, the present disclosure provides a diagnostic or a screening to detect the

presence or absence, and/or the level of any one of the target proteins in Table 10 in a subject or sample. In some embodiments, the subject is a mammal human subject and the the target protein selected from Table 10 is a human isoform. In some embodiments, presence of any one of the target proteins in Table 10 in a subject is related to an risk of developing a disease, disorder, or condition. In some embodiments, presence of a particular level of any one of the target proteins in Table 10 indicates increased risk of developing or diagnosis of a disease, disorder, or condition. In some embodiments, a reduction in a level of any one of the target proteins in Table 10 (*e.g.*, as compared to a prior measurement) is associated with treatment of a diagnosed disease.

[00321] In certain aspects, theranostic compositions are provided. In some embodiments, the present disclosure provides a diagnostic or a screening to detect the presence or absence, and/or the level of human any one of the target proteins in Table 10 in a subject or sample.

[00322] In certain aspects, the present disclosure provides methods for defining the structure activity relationship of a pharmaceutical composition comprising:

- (i) a miniprotein specific to any one of the target proteins in Table 10;
- (ii) an optional linker;
- (iii) a chelator; and
- (iv) a radioactive molecule, wherein the modified polypeptide sequence modulates activity of the human isoform of any one of the target proteins in Table 10.

Methods of Screening and Development

[00323] In some embodiments, directed evolution and computational folding algorithms can be combined for de novo creation of miniproteins (*e.g.*, CDPs, knottins, binders, affibodies, engineered Kunitz domains, monobodies, anticalins, designed ankyrin repeat domains (DARPs), avimers). For example, in some embodiments, hundreds of miniprotein backbones with various secondary structure elements, orientations, and loop lengths can be matched with hotspot binding motifs on a protein target or antigen of interest (*e.g.*, any one of the target proteins in Table 10). In some such embodiments, if the binding motifs of the miniprotein do not clash with the backbone of the target, the monomer and interaction energies are optimized with Rosetta combinatorial sequence optimization.

[00324] In some embodiments, oligonucleotide pools encoding design sequences selected through the computational approach can be synthesized, amplified, and co-transformed into yeast. The resulting yeast libraries displaying the design sequences can be incubated with fluorescently labeled target protein or antigen. Cells that display the designs that bind the target can be retrieved by fluorescence-activated cell sorting (FACS) and deep sequenced.

Once miniproteins (*e.g.*, CDPs, knottins, binders, affibodies, engineered Kunitz domains, monobodies, anticalins, designed ankyrin repeat domains (DARPin), avimers) are identified either through affinity-maturation or original designs, they can be chemically synthesized or expressed, *e.g.*, in *Escherichia coli*, and purified, and characterized in solution.

[00325] In some embodiments, libraries of stable miniproteins (*e.g.*, CDPs, knottins, binders, affibodies, engineered Kunitz domains, monobodies, anticalins, designed ankyrin repeat domains (DARPin), avimers) may be developed to allow for screening against specific chosen targets. Such a library designs a hydrophobic core to the miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) to enable folding in addition to cysteine crosslinking, improving the number of folded structures in a library.

[00326] In some embodiments, once miniproteins (*e.g.*, CDPs, knottins, binders, affibodies, engineered Kunitz domains, monobodies, anticalins, designed ankyrin repeat domains (DARPin), avimers) are identified or engineered, they may be produced via chemical synthesis or recombinant expression. In some embodiments, a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) may be produced by solid phase peptide synthesis followed by *in vitro* folding. Standard 9-fluorenylmethyloxycarbonyl (Fmoc)-based solid phase peptide chemistry may be employed. In some such embodiments, the linear peptide may then be folded under conditions that promote oxidation of cysteine side chain thiols to form disulfide bonds, followed by purification, *e.g.*, by reversed-phase high-performance liquid chromatography (RP-HPLC). An approach using recombinant DNA may also be employed to produce a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) as provided herein.

[00327] Iterations between data-driven model improvement and experimental testing with miniproteins (*e.g.*, CDPs, knottins, binders, affibodies, engineered Kunitz domains, monobodies, anticalins, designed ankyrin repeat domains (DARPin), avimers) is likely to optimize the folding and binding abilities of miniproteins (*e.g.*, CDPs, knottins, binders, affibodies, engineered Kunitz domains, monobodies, anticalins, designed ankyrin repeat domains (DARPin), avimers), to develop pharmaceutically superior specific molecules.

Characterization, Analysis & Synthesis

[00328] In some embodiments, a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin),

avimer) of the present disclosure is characterized. For example, in some embodiments, binding specificity, binding affinity, binding localization, *etc.* are performed using methods known to those of skill in the art. For instance, in some embodiments, binding localization is performed using one or more techniques such as immunohistochemistry/immunocytochemistry (*e.g.*, using cell lines or tissue biopsy samples). In some embodiments, binding affinity is performed using surface plasmon resonance measurements.

[00329] In some such embodiments, binding affinity (*e.g.*, dissociation constant expressed as K_D) is measured in one or more assays (*e.g.*, a yeast-based assay where the target is recombinantly expressed in yeast and exposed to a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) provided by the present disclosure).

[00330] In some embodiments, synthesis and analysis techniques including, without limitation, HPLC, LCMS, CD, quantitative thin layer chromatography and others known to those of skill in the art are used to efficiently synthesize via solid phase peptide synthesis methods, characterize miniproteins (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) and fully optimized clinical pharmaceutical composition candidates.

Methods of Screening and Development of Miniproteins

[00331] Directed evolution and computational folding algorithms can be combined for de novo creation of miniproteins as provided herein. In some embodiments, hundreds of miniprotein backbones with various secondary structure elements, orientations, and loop lengths can be matched with hotspot binding motifs on a target of interest (*e.g.*, any one of the target proteins in Table 10). In some such embodiments, if binding motifs of the miniprotein do not clash with the backbone of the target, the monomer and interaction energies are optimized with Rosetta combinatorial sequence optimization.

[00332] In some embodiments, oligo pools encoding the design sequences selected through the computational approach can be synthesized, amplified, and co-transformed into yeast. In some embodiments, resulting yeast libraries displaying the design sequences can be incubated with fluorescently labeled target. In some embodiments, cells that display the designs that bind the target can be retrieved by fluorescence-activated cell sorting (FACS) and deep sequencing. In some embodiments, once miniproteins are identified either through affinity-maturation or original designs, miniproteins can be chemically synthesized or expressed in *Escherichia coli*, purified, and characterized in solution.

[00333] In some embodiments, libraries of stable CDPs or knottin peptides may be developed to allow for screening against specific chosen targets. The library designs a hydrophobic core to the miniproteins to enable folding in addition to cystine crosslinking, improving the number of folded structures in a library.

[00334] In some embodiments, once miniproteins are identified or engineered, they may be produced via chemical synthesis or recombinant expression. In some embodiments, a miniprotein peptide may be produced by solid phase peptide synthesis followed by in vitro folding. Standard 9-fluorenylmethyloxycarbonyl (Fmoc)-based solid phase peptide chemistry may be employed. In some such embodiments, a linear peptide may then be folded under conditions that promote oxidation of cysteine side chain thiols to form disulfide bonds, followed by purification, *e.g.*, by reversed-phase high-performance liquid chromatography (RP-HPLC). In some embodiments, an approach using recombinant DNA may also be employed to produce a desired miniprotein.

[00335] In some embodiments, iterations between data-driven model improvement and experimental testing with miniproteins is likely to optimize the folding and binding abilities of the miniproteins, in order to develop pharmaceutically superior specific molecules.

[00336] In some embodiments, the miniprotein or a portion thereof is engineered at the DNA level (*e.g.*, degenerate codons can be introduced by oligonucleotide assembly using overlap extension PCR; or the genetic material can be amplified using flanking primers with sufficient overlap with the yeast display vector for homologous recombination).

Modifications to Miniproteins

[00337] In some embodiments, the present disclosure further provides one or more modifying components. In some embodiments, a modifying component comprises or consists of an inducible or repressible promoter that is operably linked to the coding sequence of a miniprotein as provided herein. In some embodiments, expression profile of a miniprotein or its underlying amino acid sequence can be altered via the promoter. In some aspects, the expression profile of the miniprotein can be temporally altered or controlled by temporally altering or controlling promoter function. In some embodiments, a promoter may be spatially and/or environmentally controlled. In some embodiments, a modifying component comprises or consists of an enhancer. In some such embodiment, an enhancer is used to modify expression profile of a binder but not necessarily operably linked to the coding sequence of the binder; rather, in some embodiments, an enhancer is located upstream or downstream of a coding sequence of a binder of the present disclosure. In some

embodiments, an enhancer may be temporally controlled. In some embodiments, an enhancer may be spatially and/or environmentally controlled.

[00338] In some embodiments, an expression profile of a binder and/or a sequence encoding it (*e.g.*, a nucleic acid sequence, *e.g.*, an amino acid sequence such as, *e.g.*, a gene or portion thereof) of the present disclosure can be altered via one or more modifications. In some such embodiments, the one or more modifications comprise one or more mutations in a sequence (*e.g.*, nucleic acid sequence, *e.g.*, amino acid sequence) provided by the present disclosure. In some aspects, a sequence of the present disclosure comprises a deletion relative to a parental sequence or portion thereof.

Binding Assays

[00339] In some embodiments, binding assays are used to determine binding affinities and/or binding/dissociation constants or a composition or one or more components thereof (*e.g.*, of a miniprotein with or without one or more additional components as provided herein). For example, in some embodiments, an equilibrium dissociation constant (K_d) is determined using fluorescent labeling and detection methods. In some embodiments, a cell population (*e.g.*, yeast cells) engineered to express a library of miniproteins (*e.g.*, CDPs, knottins, binders, affibodies, engineered Kunitz domains, monobodies, anticalins, designed ankyrin repeat domains (DARPs), avimers) (*e.g.*, binders that bind to a target) is produced. In some embodiments, the cells express a target. Depending on whether a set of cells expresses targets or miniproteins (*e.g.*, CDPs, knottins, binders, affibodies, engineered Kunitz domains, monobodies, anticalins, designed ankyrin repeat domains (DARPs), avimers), in some embodiments, a cell library is incubated with a target (*e.g.*, any one of the target proteins in Table 10) or with a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) or set of miniproteins (*e.g.*, CDPs, knottins, binders, affibodies, engineered Kunitz domains, monobodies, anticalins, designed ankyrin repeat domains (DARPs), avimers) as provided herein. In some such embodiments, cells and miniproteins (*e.g.*, CDPs, knottins, binders, affibodies, engineered Kunitz domains, monobodies, anticalins, designed ankyrin repeat domains (DARPs), avimers) are assessed using flow cytometry and/or FACS analysis to determine binding affinity using methods known to those of skill in the art. (*See, e.g.*, “Chapter Nine - Engineering CDPs as Novel Binding Agents.” *Methods in Enzymology*, edited by K. Dane Wittrup and Gregory L. Verdine, vol. 503, Academic Press, 2012, pp. 223–51. ScienceDirect, doi:10.1016/B978-0-12-396962-0.00009-4.).

[00340] In some embodiments, affinity measurements can be measured on live cells using methods known to those of skill in the art, such as DELFIA[®] time-resolved fluorescence (TRF) intensity technology. As is known to those in the art, a DELFIA assay can be performed using a Europium-labeled ligand (such as a miniprotein and/or conjugate capable of binding to a target, *e.g.*, any one of the target proteins in Table 10). In some such embodiments, at equilibrium, a binding constant (K_D) can be estimated following the addition of increasing amounts of a europium-labeled composition capable of binding to mammalian cells expressing any one of the target proteins in Table 10 (*e.g.*, a europium-DOTA chelated peptide). To measure affinities with this approach, following addition up to saturating concentrations, unbound fluorescent peptide can be separated from bound reagent through a series of washing and aspiration steps. Detection of remaining fluorescence, which is also considered a reflection of bound peptide, occurs following dissociation of europium (*e.g.*, from the DOTA-peptide) at low pH, and fluorescence can be measured on a fluorescent plate reader following the addition of a signal enhancing reagent. Concentration-dependent increases in fluorescence can be plotted as a function of peptide concentration and a modelled curve fit can be used to estimate the equilibrium binding constant using GraphPad Prism software.

[00341] Another approach to estimate binding affinity is to use a peptide inhibition constant (K_i) to estimate peptide binding affinity following the co-addition of increasing concentrations of an unlabeled peptide with a single sub-saturating amount of a labeled binding agent (*e.g.*, a europium-DOTA chelated target protein selected from Table 10 binding peptide). In some embodiments, it is contemplated that unlabeled peptide competes for the same binding site on the target (*e.g.*, any one of the target proteins in Table 10) to displace the labeled binding agent (*e.g.*, europium-DOTA chelated binding peptide of any one of the target proteins in Table 10). In some such embodiments, using similar fluorescent enhancement and detection steps as in DELFIA europium dissociation measurements, a binding affinity for the unlabeled peptide can be estimated following a modelled curve fit of the data upon loss of fluorescent signal.

Affinity Maturation

[00342] In some embodiments, a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) of the present disclosure comprises or consists of a sequence that exhibits certain desired affinity ranges for a target. In some embodiments, affinity maturation is performed on a sequence as provided by the present disclosure wherein the affinity matured sequence

displays the same or better selectivity and/or affinity for any one of the target proteins in Table 10 as compared to the starting sequence or another sequence with “less” affinity as compared to the affinity matured sequence. In some embodiments, affinity maturation is performed using an antigen of any one of the target proteins in Table 10 and a sequence that binds to the target protein of a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) as provided herein.

[00343] In some embodiments, affinity maturation is performed on a sequence that selectively binds to any one of the target proteins in Table 10. In some embodiments, the target protein selected from Table 10 is a human isoform

[00344] In preferred aspects of the present disclosure, the modified polypeptide sequence of the pharmaceutical composition comprises nM or sub-nM binding affinity to a target on a cell line expressing a human isoform of any one of the target proteins in Table 10 binding potency on protein target or in a cell-based assay.

[00345] In certain embodiments, the modified polypeptide sequence comprises a binding affinity of 900, 800, 700, 600, 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 nM binding affinity to the human isoform of any one of the target proteins in Table 10.

[00346] In one embodiment, the modified polypeptide sequence comprises a binding affinity of 500 nM binding affinity to the human isoform of any one of the target proteins in Table 10.

[00347] In more preferred embodiments, the modified polypeptide sequence comprises a picomolar binding affinity.

[00348] In other preferred embodiments, the modified polypeptide sequence exhibits selectivity with which the sequence binds to only the target protein selected from Table 10. In some embodiments, affinity maturation is performed using magnetic-based assays. In some embodiments, affinity maturation is performed using flow cytometry/FACS-based assays in accordance with procedures known to those of skill in the art.

Methods of Miniprotein Manufacturing

[00349] In some embodiments, synthesis of a miniprotein comprises solid phase synthesis. In some embodiments, miniproteins are synthesized using standard solid phase peptide synthesis as is known to those of skill in the art. (*See, e.g.*, Johannes Meienhofer, *Hormonal Proteins and Peptides*, Volume II, 1973, Pages 45-267). In some embodiments, SPS comprises synthesis using methods known to those of skill in the art including, for example,

Fmoc or Boc amino protecting groups. In some embodiments, synthesis comprises protection from reaction with incoming N-protected amino acids. In some embodiments, synthesized polypeptides are analyzed to determine sequence, structure, and related properties using HPLC/LC-MS.

[00350] In some embodiments, a miniprotein is manufactured using recombinant production methods as are known to those of skill in the art including, for example, yeast-based approaches and chemical synthesis.

Methods of Conjugation

[00351] In some embodiments, conjugates of the present disclosure comprise a linker and a chelator. In some embodiments, the chelator has a bound radionuclide. In some embodiments, a linker and chelator, or a linker, chelator, and radionuclide are conjugated to a miniprotein. In some embodiments, a chelator and/or radionuclide are conjugated to a miniprotein. In some embodiments, a miniprotein is conjugated to a chelator either directly or through a linker (*e.g.*, a linker described herein). Any known conjugation chemistry can be utilized to conjugate a miniprotein directly to a chelator or to conjugate a linker to the miniprotein and to the chelator.

[00352] In some embodiments, a miniprotein comprises a surface exposed functional group to allow for site specific conjugation. In some embodiments, a miniprotein comprises a surface exposed lysine or cysteine residue that can serve for site specific conjugation. In some embodiments, a miniprotein conjugate comprises one or more non-naturally occurring amino acids that can serve for site specific conjugation.

[00353] A person of ordinary skill in the art will recognize that numerous chemical conjugation strategies provide ready access to present technology, whereby exposed amino acid residues on a protein undergo well-known reactions with reactive moieties on a chelator.

[00354] A person of ordinary skill in the art will recognize that cysteine coupling reactions may be employed to conjugate chelators with thiol-reactive termini to protein surfaces through exposed thiol side chains on cysteine residues on the protein surface. (See generally Tsuchikama & An, *supra*, at 36-37; see also, *e.g.*, Pierre Adumeau et al., Thiol-Reactive Bifunctional Chelators for the Creation of Site-Selectively Modified Radioimmunoconjugates with Improved Stability, 29 Bioconjugate Chem. 1364 (2018)). In some embodiments, because cysteine residues readily form disulfide linkages with nearby cysteine residues under physiological conditions, rather than existing as free thiols, some cysteine coupling strategies may rely upon selective reduction of disulfides to generate a higher number of reactive free thiols. Cysteine coupling techniques known in the art include, but are

not limited to, cys alkylation reactions, cysteine rebridging reactions, and cys-aryl coupling using organometallic palladium reagents. (See, *e.g.*, C.R. Behrens et al., Antibody-Drug Conjugates (ADCs) Derived from Interchain Cysteine Cross-Linking Demonstrates Improved Homogeneity and Other Pharmacological Properties Over Conventional Heterogeneous ADCs, 12 Mol. Pharm. 3986 (2015); Vinogradova et al., Organometallic Palladium Reagents for Cysteine Bioconjugation, 526 Nature 687 (2015); see also Tsuchikama, *supra*, at 37).

[00355] Protein conjugation strategies using non-natural amino acid side chains are also well known in the art. For example, in some embodiments, “click chemistries” provide access to conjugated proteins, by rapid and selective chemical transformations under a diverse range of reaction conditions. In some embodiments, click chemistries are known to yield peptide conjugates with limited by-product formation, despite the presence of unprotected functional groups, in aqueous conditions. For instance, in some embodiments, a click reaction in the formation of conjugated peptides is the copper(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition reaction (CuAAC). (See Liyuan Liang & Didier Astruc, The Copper(I)-Catalysed Alkyne-Azide Cycloaddition (CuAAC) “Click” Reaction and Its Applications: An Overview, 255 COORD. CHEM. Rev 2933 (2011); see also, *e.g.*, Herman S. Gill & Jan Marik, Preparation of 18F-labeled Peptides using the Copper(I)-Catalyzed Azide-Alkyne 1,3-Dipolar Cycloaddition, 6 Nature Protocols 1718 (2011)). In some embodiments, a CuAAC click reaction may be carried out in the presence of ligands to enhance reaction rates. In some such embodiments, such ligands may include, for example, polydentate nitrogen donors, including amines (*e.g.*, tris(triazolyl)methyl amines) and pyridines. (See Liang & Astruc, *supra*, at 2934 (collecting examples); P.L. Goias et al., 39 Macromolecules 6451 (2006)). In some embodiments, other widely-utilized click reactions include, but are not limited to, thiol-ene, oxime, Diels-Alder, Michael addition, and pyridyl sulfide reactions.

[00356] In some embodiments, copper-free (Cu-free) click methods are also known in the art for delivery of therapeutic and/or diagnostic agents, such as radionuclides (*e.g.*, 18F), chemotherapeutic agents, dyes, contrast agents, fluorescent labels, chemiluminescent labels, or other labels, to protein surfaces. In some embodiments, Cu-free click methods may permit stable covalent linkage between target molecules and prosthetic groups. In some embodiments, Cu-free click chemistry may include reacting an antibody, antigen binding fragment, or target protein binding fragment, which has been modified with a non-natural amino acid side chain that includes an activating moiety such as a cyclooctyne (*e.g.*, dibenzocyclooctyne (DBCO)), a nitron or an azide group, with a prosthetic group that presents a corresponding or complementary reactive moiety, such as an azide, nitron or

cyclooctyne (*e.g.*, DBCO). (See, *e.g.*, David. J. Donnelly et al., Synthesis and Biologic Evaluation of a Novel 18F-Labeled Adnectin as a PET Radio ligand for Imaging PD-L1 Expression, 59 J. NUCL. MED. 529 (2018)). For instance, in some embodiments, where a targeting molecule comprises a cyclooctyne, the prosthetic group may include an azide, nitron, or similar reactive moiety. In some embodiments, where a targeting molecule includes an azide or nitron, the prosthetic group may present a complementary cyclooctyne, alkyne, or similar reactive moiety. In some embodiments, Cu-free click reactions may be carried out at room temperature, in aqueous solution, in the presence of phosphate-buffered saline (PBS). In some such embodiments, the prosthetic group may be radiolabeled (*e.g.*, with 18F) or may be conjugated to any alternative diagnostic and/or therapeutic agent (*e.g.*, a chelator). (See *id.* at 531.)

[00357] In some embodiments, conjugation chemistries such as the Huisgen cyclo-addition (“click” reaction) are available for synthesis of chelates and peptides. In some embodiments, an efficient, high-yielding three-step synthesis of a versatile monofluoro-substituted cyclooctyne (MFCO) has been shown to facilitate a variety of bioconjugation processes (M. Martin et al., 2013). In some embodiments, MFCO can be utilized to prepare a DOTA derivative for copper-free click chemical addition at an internal azide-modified lysine residue of the CDP or knottin peptide.

[00358] In some embodiments, a miniprotein conjugate provided herein has a lysine at a specific position (*e.g.*, in a cysteine knot, or cysteine-dense region) and can be replaced with an azide derivative for “click” chemistry with DOTA-MFCO.

[00359] In some embodiments, a DOTA-MFCO-CDP conjugate can be prepared by first coupling an amine-modified DOTA to MFCO, then conjugating the DOTA-MFCO to the azide on the desired lysine of the miniprotein.

[00360] In some embodiments, the chelator and miniprotein are joined together by a cycloaddition reaction in the presence of a transition metal catalyst. In some embodiments, a metal catalyst is based on Cu or Rh.

[00361] In some embodiments, utilizing solution phase conjugation, a chelator (DOTA) and a miniprotein are joined with 1-ethyl-3-[3-(dimethylamino)propyl] (EDC) and N-hydroxysulfonosuccinimide (SNHS) in water (pH 5.5) for 40 min at room temperature using a 1:1:1 molar ratio of DOTA:EDC:SNHS. In some such embodiments, peptides are dissolved in sodium phosphate buffer and added to the above sulfosuccinimidyl ester of DOTA (DOTA-OSSu). In some such embodiments, a molar excess of DOTA-OSSu is used to drive the conjugation on the N-termini of the peptide (See, *e.g.*, Kimura, Richard H et al.

“Engineered knottin peptides: a new class of agents for imaging integrin expression in living subjects.” *Cancer research* vol. 69,6 (2009): 2435-42. doi:10.1158/0008-5472.CAN-08-2495).

[00362] In some embodiments, a new DOTA derivative, α -amino-DOTA is prepared with the objective of attaching DOTA to the C-terminus of a peptide, since in some scenarios, the peptide function might be compromised because of DOTA conjugation to the N-terminus or to lysine side chains.

[00363] In some embodiments, a miniprotein is generated by solid-phase peptide synthesis (SPPS). The tris-tert-butyl ester of DOTA, a bifunctional ligand (in the salt free, zwitterionic form), is readily soluble in most organic solvents and the tert-butyl ester protection is fully compatible with standard SPPS techniques. The most convenient way of conjugation comprises the addition of DOTA to the N-terminus of the protected peptide chain as the last amino acid in an automated peptide synthesizer followed by cleavage from the resin and removal of the acid-labile protecting groups. It can also be attached to Lys side chains. The preformed activated NHS ester of DOTA-tris(tert-butyl ester) has also been synthesized, and this reagent does not require a coupling agent to couple DOTA to free amino groups. The DOTA unit is linked to peptides through one of the acetate sidearms, and the conjugate has four amino, three carboxylates, and one amide group available for metal binding (See, *e.g.*, De León-Rodríguez LM, Kovacs Z. The synthesis and chelation chemistry of DOTA-peptide conjugates. *Bioconjug Chem.* 2008 Feb;19(2):391-402. doi: 10.1021/bc700328s. Epub 2007 Dec 12. PMID: 18072717).

[00364] In some embodiments, a more general method involves the use of preformed DOTA-amino acid derivatives which allows the introduction of a DOTA unit into any desired position in the peptide sequence without the need of orthogonal protection. Protected DOTA-Lys and DOTA-Phe derivatives that are fully compatible with standard SPPS conditions (N-R-Fmoc protection, free carboxyl for the coupling, and acid-labile tert-butyl protection of the remaining acetate sidearms of the DOTA unit) have been synthesized. These DOTA-amino acids can be used in SPPS to build peptides that incorporate the DOTA moiety in any desired position (See, *e.g.*, De León-Rodríguez LM, Kovacs Z. The synthesis and chelation chemistry of DOTA-peptide conjugates. *Bioconjug Chem.* 2008 Feb;19(2):391-402. doi: 10.1021/bc700328s. Epub 2007 Dec 12. PMID: 18072717).

[00365] General methods for coupling DOTA-type macrocycles to targeting groups through a linker (*e.g.* by activation of one of the carboxylates of the DOTA to form an active ester,

which is then reacted with an amino group on the linker to form a stable amide bond), are known to those skilled in the art. (See *e.g.* Tweedle et al. U.S. Pat. No. 4,885,363).

[00366] A linker may be incorporated between the chelator and the targeting vector to influence the pharmacokinetic properties of the conjugate. Hydrocarbon, PEG, or polypeptide linkers can alter the pharmacokinetics and biodistribution by changing the overall charge and hydrophilicity of the radiopharmaceutical (See, *e.g.*, De León-Rodríguez LM, Kovacs Z. The synthesis and chelation chemistry of DOTA-peptide conjugates. *Bioconjug Chem.* 2008 Feb;19(2):391-402. doi: 10.1021/bc700328s. Epub 2007 Dec 12. PMID: 18072717).

Miniprotein Conjugate Orientation

[00367] In some embodiments, a conjugate has the following orientation: linker-chelator, linker-chelator-radionuclide, linker-radionuclide, chelator-radionuclide. In some such embodiments a conjugate has the following orientation: miniprotein-linker-Radionuclide, miniprotein-Chelator, Chelator-miniprotein, miniprotein-linker-Chelator, Chelator-linker-miniprotein, miniprotein-Chelator-Radionuclide, Radionuclide-Chelator-miniprotein, miniprotein-linker-Chelator-Radionuclide, or Radionuclide-Chelator-linker-miniprotein.

[00368] In some embodiments, a conjugate provided by the present disclosure comprises a miniprotein. In some such embodiments, the miniprotein functions as a targeting moiety, *e.g.*, specifically binding to a target, *e.g.*, a protein expressed on the surface of a target tumor cell. Accordingly, in some embodiments, the miniprotein in the conjugates of the present disclosure may vary depending on the target of interest.

Exemplary Miniprotein Conjugates

[00369] The following provides exemplary embodiments of miniprotein conjugates as provided herein. In some such embodiments, such miniproteins specifically bind to any one of the target proteins in Table 10 expressed on the surface of a cancer cell (*e.g.*, a solid tumor cell). In some embodiments, a conjugate comprises a linker, a chelator, and/or a radionuclide.

[00370] In some embodiments, a conjugate comprises a miniprotein, optional linker, chelator, and radionuclide. In some embodiments, a miniprotein comprises or consists of a binder, CDP, knottin, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), or avimer. In some embodiments, a chelator comprises or consists of DOTA, Crown, NOPO, or Macropa. In some embodiments, a radionuclide comprises or consists of an alpha emitter. In some embodiments, a radionuclide comprises or

consists of a beta emitter. In some embodiments, a radionuclide comprises or consists of Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211.

[00371] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a DOTA chelator, and Ac-225.

[00372] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a DOTA chelator, and Lu-177.

[00373] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a DOTA chelator, and Ga-68.

[00374] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a DOTA chelator, and La-132.

[00375] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a DOTA chelator, and La-135.

[00376] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a DOTA chelator, and Cu-64.

[00377] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a DOTA chelator, and In-111.

[00378] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a DOTA chelator, and Cu-67.

[00379] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a DOTA chelator, and Ce-134.

[00380] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a Crown chelator, and Ac-225.

[00381] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a Crown chelator, and Lu-177.

[00382] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a Crown chelator, and Ga-68.

[00383] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a Crown chelator, and La-132.

[00384] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a Crown chelator, and La-135.

[00385] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a Crown chelator, and Cu-64.

[00386] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a Crown chelator, and In-111.

[00387] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a Crown chelator, and Cu-67.

[00388] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a Crown chelator, and Ce-134.

[00389] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a NOPO chelator, and Ga-68.

[00390] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a NOPO chelator, and La-132.

[00391] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a NOPO chelator, and La-135.

[00392] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a NOPO chelator, and Cu-64.

[00393] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a NOPO chelator, and In-111.

[00394] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a NOPO chelator, and Cu-67.

[00395] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a NOPO chelator, and Ce-134.

[00396] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a Macropa chelator, and Ac-225.

[00397] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a Macropa chelator, and Lu-177.

[00398] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a Macropa chelator, and Ga-68.

[00399] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a Macropa chelator, and La-132.

[00400] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a Macropa chelator, and La-135.

[00401] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a Macropa chelator, and Cu-64.

[00402] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a Macropa chelator, and In-111.

[00403] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a Macropa chelator, and Cu-67.

[00404] In some embodiments, a conjugate comprises or consists of a miniprotein that specifically binds to any one of the target proteins in Table 10, an optional PEG linker, a Macropa chelator, and Ce-134.

[00405] In some embodiments, an exemplary imaging/diagnostic miniprotein conjugate described herein comprises a miniprotein that specifically binds to any one of the target proteins in Table 10 or a fragment or portion thereof (*e.g.*, expressed on the surface of a solid tumor cell), a PEG linker, a DOTA chelator, and Gallium-68.

[00406] In some embodiments, an exemplary imaging/diagnostic miniprotein conjugate described herein comprises a miniprotein that specifically binds to any one of the target proteins in Table 10 or a fragment or portion thereof (*e.g.*, expressed on the surface of a solid tumor cell), a PEG linker, a DOTA chelator, and Copper-64.

[00407] In some embodiments, an exemplary imaging/diagnostic miniprotein conjugate described herein comprises a miniprotein that specifically binds to any one of the target proteins in Table 10 or a fragment or portion thereof (*e.g.*, expressed on the surface of a solid tumor cell), a PEG linker, a DOTA chelator, and Indium-111.

[00408] In some embodiments, an exemplary imaging/diagnostic miniprotein conjugate described herein comprises a miniprotein that specifically binds to any one of the target proteins in Table 10 or a fragment or portion thereof (*e.g.*, expressed on the surface of a solid tumor cell), a PEG linker, a DOTA chelator, and Lutetium-177.

[00409] In some embodiments, exemplary imaging/diagnostic CDP conjugate described herein comprises a miniprotein that specifically binds to any one of the target proteins in Table 10 or a fragment or portion thereof (*e.g.*, expressed on the surface of a solid tumor cell), a PEG linker, a DOTA chelator, and Lead-212.

[00410] In some embodiments, exemplary imaging/diagnostic CDP conjugate described herein comprises a miniprotein that specifically binds to any one of the target proteins in Table 10 or a fragment or portion thereof (*e.g.*, expressed on the surface of a solid tumor cell), a PEG linker, a DOTA chelator, and Cerium-134.

[00411] In some embodiments, a pharmaceutical composition comprising the radionuclide is employed in imaging scans to detect or diagnosis one or more diseases. Further embodiments include use as companion diagnostics.

[00412] In some embodiments, one or more different linkers and different chelators for Ga-68 (*e.g.*, NOPO) and Ac-225 (*e.g.*, Crown or DOTA) for both are operably linked to the same miniprotein.

Methods of Use

[00413] In some embodiments, the present disclosure provides methods of treating or preventing disease or disorder in a subject, the method comprising administering to the subject the pharmaceutical composition in an amount effective that modulates, binds or inhibits a human isoform of any one of the target proteins in Table 10 to treat or prevent disease or disorder in the subject. In preferred embodiments, the disease or disorder associated with any one of the target proteins in Table 10 is treated (*e.g.*, prevented, progression is slowed, symptoms are relieved, tumor size reduced to improve overall survival, *etc.*).

[00414] In some embodiments, subjects, *e.g.*, patients or patients inclusion criteria include, without limitation, candidates positive for any one of the target proteins in Table 10 shown via imaging (*e.g.*, DOTA PET/CT), candidates with progressive disease, advanced or metastatic disease, candidates who are not candidates for surgery, refractory or relapsed candidates.

[00415] In some embodiments, possible certain side effects including nausea, suppression of blood cell counts are managed through one or more medications. In some embodiments, side effects may include renal toxicity, myelodysplastic syndrome, however, it is contemplated that the pharmaceutical compositions are manageable, and treatment is generally well-tolerated. The present disclosure is directed to address, ameliorate or preempt such renal toxicity before, during or after radionuclide therapy while maintaining and/or improving therapeutic effectiveness through the administration of the composition.

[00416] In some embodiments, the present disclosure comprises a method for treatment, comprising administering a pharmaceutical composition as provided herein in the absence of administering targeted conditioning or pre-conditioning regimens where conditioning is necessary prior to administration of therapies, *e.g.*, adoptive cell therapies and gene therapies to ablate certain cells.

[00417] In some embodiments, methods and compositions of the present disclosure include multistep or pre-targeting approaches. For instance, in some embodiments, a radionuclide can be decoupled to a provided composition and may be subsequently administered after an initial step of administering a miniprotein or an antibody (*e.g.*, a first ligand binding moiety). In such embodiments, the first ligand binding moiety is not conjugated to a radionuclide and

has the desired affinity and specificity for the tumor cells. The first ligand binding moiety is then targeted by a second moiety carrying the radionuclide. For instance, the first ligand binding moiety may comprise an antibody to the target protein selected from Table 10 and the second moiety may be the pharmaceutical composition comprising the miniprotein, linker, chelator and the radionuclide wherein the miniprotein may exhibit a desired avidity to the first ligand binding moiety.

[00418] In some embodiments, the present disclosure provides methods of use (*e.g.*, treatment, manufacture, *etc.*) of miniproteins (*e.g.*, CDPs, knottins, binders, affibodies, engineered Kunitz domains, monobodies, anticalins, designed ankyrin repeat domains (DARPs), avimers) as provided herein.

[00419] In some embodiments, anti-target protein selected from Table 10 compositions and pharmaceutical compositions are produced, *e.g.*, using miniproteins (*e.g.*, CDPs, knottins, binders, affibodies, engineered Kunitz domains, monobodies, anticalins, designed ankyrin repeat domains (DARPs), avimers), as provided herein.

[00420] In some embodiments, miniproteins of any one of the target proteins in Table 10 (*e.g.*, CDPs, knottins, binders, affibodies, engineered Kunitz domains, monobodies, anticalins, designed ankyrin repeat domains (DARPs), avimers) provided by the present disclosure are formatted to generate peptides, antibodies, antibody and antibody fragments, ADCs, BiTEs, CAR-Ts, and TRuCs, Fc-domain components, portions, or modifications, bispecific antibodies *etc.* In some such embodiments, such compositions and pharmaceutical compositions are used in treatment of a disease, disorder, or condition wherein expression of any one of the target proteins in Table 10 is suspected or detected. In some such embodiments, the disease, disorder, or condition is related to overexpression and/or aberrant expression of any one of the target proteins in Table 10. In some embodiments, the disease, disorder and/or condition is cancer. Accordingly, the present disclosure provides various anti-target protein selected from Table 10 compositions and pharmaceutical compositions for the treatment of disease related to any one of the target proteins in Table 10.

[00421] Among other things, the present disclosure provides methods of treating a subject in need thereof by administering a composition as provided herein. In some such embodiments, a composition is or comprises a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer). In some embodiments, the composition is a miniprotein conjugate comprising a miniprotein and one or more of a chelator and radionuclide, as well as, optionally, a linker (*e.g.*, linking the chelator to the miniprotein).

[00422] In some embodiments, a subject treated herein is at risk of having or has been diagnosed as having a cancer. In some embodiments, the subject is a mammal. In some embodiments, the mammal is a human. In some embodiments, the human is a fetus, infant, child, adolescent, adult, or elderly adult. In some embodiments, a human subject having a cancer is treated by administering a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) as described herein.

[00423] In some embodiments, the cancer expresses a target protein (*e.g.*, any one of the target proteins in Table 10) specifically bound by a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) of the present disclosure. In some embodiments, the target protein selected from Table 10 or portion thereof is expressed on the surface of a cancer cell of the subject. In some such embodiments, the target protein selected from Table 10 is expressed on the cancer cell and has lower or non-detectable expression on cells of normal tissues, and/or is expressed at much higher density on cancer cells versus normal cells.

[00424] In some embodiments, the cancer is a solid tumor. In some embodiments, the cancer is metastatic. In some embodiments, the cancer is recurrent. In some embodiments, the cancer is remitting. In some embodiments, the cancer is selected from the group consisting of bladder, breast, pancreas, ovary, stomach, gastrointestinal tract, liver, lung, prostate, skin, colon, rectum, colon and rectum, skin.

[00425] In some embodiments, miniproteins (*e.g.*, CDPs, knottins, binders, affibodies, engineered Kunitz domains, monobodies, anticalins, designed ankyrin repeat domains (DARPs), avimers) provided herein can be used in conjunction with one or more additional components. For example, in some embodiments, a miniprotein (*e.g.*, CDP, knottin, binder affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) may be combined with one or more other components for use in imaging, diagnosis, prognosis/monitoring and/or treating a disease, disorder or condition. In some embodiments, the disease is cancer. In some embodiments, a miniprotein (*e.g.*, CDP, knottin, binder affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) of the present disclosure may be used in a wide variety of cancers, including, but not limited to, breast cancer, ovarian cancer, melanoma, pancreatic cancer, peripheral neuroma, glioblastoma, adrenocortical carcinoma, AIDS-related lymphoma, anal cancer, bladder cancer, meningioma, glioma, astrocytoma, cervical cancer, chronic myeloproliferative disorders, colon cancer, endometrial cancer, ependymoma,

esophageal cancer, Ewing's sarcoma, extracranial germ cell tumors, extrahepatic bile duct cancer, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumors, gestational trophoblastic tumors, hairy cell leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, hypopharyngeal cancer, islet cell carcinoma, Kaposi sarcoma, laryngeal cancer, leukemia, lip cancer, oral cavity cancer, liver cancer, male breast cancer, malignant mesothelioma, medulloblastoma, Merkel cell carcinoma, metastatic squamous neck cell carcinoma, multiple myeloma and other plasma cell neoplasms, mycosis fungoides and the Sezary syndrome, myelodysplastic syndromes, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, small cell lung cancer, head and neck cancer, skin cancer, oropharyngeal cancer, bone cancers, including osteosarcoma and malignant fibrous histiocytoma of bone, paranasal sinus cancer, parathyroid cancer, penile cancer, pheochromocytoma, pituitary tumors, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, small intestine cancer, soft tissue sarcoma, supratentorial primitive neuroectodermal tumors, pineoblastoma, testicular cancer, thymoma, thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis and ureter, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Wilm's tumor and other childhood kidney tumors.

[00426] In some embodiments, treatment (*e.g.*, including with a miniprotein (*e.g.*, CDP, knottin, binder affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) of the present disclosure), diagnosis, prognosis/monitoring, or imaging is in a subject who does not exhibit signs or symptoms of a disease, disorder, and/or condition. In some embodiments, treatment is in a subject who exhibits one or more signs or symptoms of a disease, disorder, or condition even if, for example, such signs or symptoms are not objectively observable without further testing such as laboratory diagnostics. In some embodiments, a subject is susceptible to having or at risk of developing a disease, disorder, or condition (*e.g.*, cancer), based on one or more factors (*e.g.*, level of any one of the target proteins in Table 10, *etc.*) that are related to increased risk of developing of the disease, disorder or condition. In some embodiments, a subject has been diagnosed as having a disease, disorder, or condition (*e.g.*, cancer).

[00427] In some embodiments, present disclosure provides methods of treating or preventing disease or disorder in a subject, the method comprising administering to the subject a pharmaceutical composition comprising a miniprotein (*e.g.*, CDP, knottin, binder affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) of the present disclosure in an amount effective that modulates, binds or inhibits human isoform of any one of the target proteins in Table 10 to treat or prevent

disease or disorder in the subject. In some embodiments, the disease or disorder is cancer. In some embodiments, the cancer is treated (*e.g.*, resolved, prevented, progression is slowed, symptoms are relieved, tumor size reduced to improve overall survival, *etc.*).

Formulation and administration

[00428] In various aspects formulations of the pharmaceutical compositions of the present disclosure include parenteral *e.g.*, subcutaneous, intravenous, intraarterial, intramuscular, intradermal, intraperitoneal, interperitoneal, and intrathecal administration. See for instance, Remington: The Science and Practice of Pharmacy, Lippincott Williams & Wilkins; 22nd ed. (2013) described in more detail.

[00429] In some embodiments, a pharmaceutical composition comprising a miniprotein (*e.g.*, CDP, knottin, binder affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) of the present disclosure is administered to a subject in need thereof. In some embodiments, the subject has or is at risk of having cancer. By way of non-limiting example, in some embodiments, the cancer is selected from breast cancer, ovarian cancer, melanoma, pancreatic cancer, peripheral neuroma, glioblastoma, adrenocortical carcinoma, AIDS-related lymphoma, anal cancer, bladder cancer, meningioma, glioma, astrocytoma, cervical cancer, chronic myeloproliferative disorders, colon cancer, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extracranial germ cell tumors, extrahepatic bile duct cancer, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumors, gestational trophoblastic tumors, hairy cell leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, hypopharyngeal cancer, islet cell carcinoma, Kaposi sarcoma, laryngeal cancer, leukemia, lip cancer, oral cavity cancer, liver cancer, male breast cancer, malignant mesothelioma, medulloblastoma, Merkel cell carcinoma, metastatic squamous neck cell carcinoma, multiple myeloma and other plasma cell neoplasms, mycosis fungoides and the Sezary syndrome, myelodysplastic syndromes, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, small cell lung cancer, head and neck cancer, skin cancer, oropharyngeal cancer, bone cancers, including osteosarcoma and malignant fibrous histiocytoma of bone, paranasal sinus cancer, parathyroid cancer, penile cancer, pheochromocytoma, pituitary tumors, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, small intestine cancer, soft tissue sarcoma, supratentorial primitive neuroectodermal tumors, pineoblastoma, testicular cancer, thymoma, thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis and ureter, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Wilm's tumor and other childhood kidney tumors.

[00430] In some embodiments, determination of an appropriate dose and regimen of a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) of the present disclosure can be made by the clinician, *e.g.*, using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment. Actual dosage levels of the active ingredients (*e.g.*, miniproteins (*e.g.*, CDPs, knottins, binders, affibodies, engineered Kunitz domains, monobodies, anticalins, designed ankyrin repeat domains (DARPs), avimers) as provided by compositions of the present disclosure may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. In some embodiments, the selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present disclosure employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors known in the medical arts.

[00431] In some embodiments, administration is by one or more routes including, but not limited to bronchial, buccal, enteral, interdermal, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, within a specific organ and/or tissue, mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (including by intratracheal instillation), transdermal, vaginal and vitreal.

[00432] In some embodiments, administration may comprise or consist of continuous dosing (*e.g.*, intravenous administration) for a period of time.

[00433] In some embodiments, administration may comprise or consist of intermittent dosing.

[00434] In some embodiments, administration may comprise or consist of dosing separated by a selected period of time and with one or more doses, based on clinical response and/or activity following one or more doses.

[00435] In some embodiments, administration is to a subject is suffering from a relevant disease, disorder or condition. In some embodiments, administration is to a subject susceptible to or at risk of developing a disease, disorder, or condition. In some such embodiments, a subject displays one or more symptoms or characteristics of a disease,

disorder or condition. In some embodiments, a subject does not display any symptom or characteristic of a disease, disorder, or condition. In some such embodiments, a subject is someone with one or more features characteristic of susceptibility to or at risk of developing a disease, disorder, or condition. In some embodiments, a subject has received a diagnosis of a disease, disorder, or condition.

[00436] In some embodiments, the present disclosure provides a method for modulating biological activity of any one of the target proteins in Table 10 in a subject. In some such embodiments, the method comprises administering a pharmaceutical composition provided by the disclosure to the subject in an amount effective to modulate the biological activity of any one of the target proteins in Table 10 in the subject.

[00437] In some embodiments, the present disclosure provides a method for treating or preventing cancer in a subject. In some embodiments, the method comprises administering to the subject a pharmaceutical composition provided by the present disclosure, wherein the miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) of the pharmaceutical composition selectively binds to any one of the target proteins in Table 10 in an amount effective to treat or prevent the cancer in the subject.

[00438] In some embodiments, the present disclosure provides methods and compositions that bind target (*e.g.*, any one of the target proteins in Table 10) and are capable of activating or inhibiting immune cell response. In some embodiments, compositions are administered for the treatment of non-small-cell lung cancer (NSCLC), cutaneous squamous cell carcinoma, pancreatic cancer, primary hepatocellular carcinoma, colorectal carcinoma, clear cell renal carcinoma, breast cancer and prostate cancer. (Yang, S et al., Int J of Bio Sci 2020 Mar 25 (16): 11; 1767-1773).

[00439] In some embodiments, the present disclosure provides a method for detecting the presence or extent of a cancer in a subject. In some such embodiments, the method comprises measuring a level of any one of the target proteins in Table 10 in a sample comprising one or more cells from the subject; wherein detection of the level of the target protein selected from Table 10 in the subject relative to the levels of the target protein selected from Table 10 in one or more control subjects is indicative of the presence or extent of the cancer.

[00440] In some embodiments, a composition provided by the present disclosure is used to downregulate an inhibitory immune response in a subject. For example, in some embodiments, a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain,

monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) of the present disclosure specifically binds such that it may be used to deliver a cytotoxic payload and promote cellular cytotoxicity of T cells for specific - cells expressing any one of the target proteins in Table 10 (*e.g.*, tumor). (See Goodman A, Patel SP, Kurzrock R Nat Rev Clin Oncol. 2017 Apr; 14(4):203-220.) In some such embodiments, the miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) modulates IFN- γ IL-2, IL-10, and IL-13 production during T-cell activation.

Treatment, Imaging, and Diagnostic/Prognostic Methods of Use

[00441] In some embodiments, the present disclosure provides methods of treating cancer in a human subject by administering a miniprotein conjugate described herein. In some embodiments, the cancer expresses the target (*e.g.*, any one of the target proteins in Table 10) specifically bound by the miniprotein of the conjugate. In some embodiments, the target protein is expressed on the surface of malignant cells with limited expression on cells of normal tissues, and/or expressed at much higher density on malignant versus normal cells.

[00442] In some embodiments, the cancer is a solid tumor. In some embodiments, the cancer is metastatic. In some embodiments, the cancer is recurrent. In some embodiments, the cancer is remitting. In some embodiments, the cancer is selected from the group consisting of bladder, breast, pancreas, ovary, stomach, gastrointestinal tract, liver, lung, prostate, skin, colon, rectum, colon and rectum, skin.

[00443] In some embodiment, miniprotein conjugates provided herein can be used for imaging and treating a wide variety of cancers, including, but not limited to, breast cancer, ovarian cancer, melanoma, pancreatic cancer, peripheral neuroma, glioblastoma, adrenocortical carcinoma, AIDS-related lymphoma, anal cancer, bladder cancer, meningioma, glioma, astrocytoma, cervical cancer, chronic myeloproliferative disorders, colon cancer, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extracranial germ cell tumors, extrahepatic bile duct cancer, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumors, gestational trophoblastic tumors, hairy cell leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, hypopharyngeal cancer, islet cell carcinoma, Kaposi sarcoma, laryngeal cancer, leukemia, lip cancer, oral cavity cancer, liver cancer, male breast cancer, malignant mesothelioma, medulloblastoma, Merkel cell carcinoma, metastatic squamous neck cell carcinoma, multiple myeloma and other plasma cell neoplasms, mycosis fungoides and the Sezary syndrome, myelodysplastic syndromes,

nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, small cell lung cancer, head and neck cancer, skin cancer, oropharyngeal cancer, bone cancers, including osteosarcoma and malignant fibrous histiocytoma of bone, paranasal sinus cancer, parathyroid cancer, penile cancer, pheochromocytoma, pituitary tumors, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, small intestine cancer, soft tissue sarcoma, supratentorial primitive neuroectodermal tumors, pineoblastoma, testicular cancer, thymoma, thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis and ureter, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Wilm's tumor and other childhood kidney tumors.

[00444] As will be known to those of skill in the art, determination of the appropriate dose and regimen of a composition provided by the present disclosure can be made by the clinician, *e.g.*, using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment. In some embodiments, actual dosage levels of the active ingredients in compositions provided by the present disclosure may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. In some embodiments, the selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present disclosure employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors known in the medical arts.

[00445] In some embodiments, the present disclosure provides methods of imaging, diagnosing and/or monitoring (including determining prognosis) of presence of a target in a subject. In some embodiments, a conjugate (*e.g.*, a miniprotein conjugate comprising, *e.g.*, a chelator and/or radionuclide) of the present disclosure is useful for PET, SPECT, or MRI imaging.

[00446] In some embodiments, conjugates (*e.g.*, miniprotein conjugates comprising a chelator and/or radionuclide) of the present disclosure can be used in image-guided surgery. For example, in some embodiments, tissue of interest suspected of containing cancerous cells or a tumor can be contacted with a miniprotein targeted to any one of the target proteins in Table 10 (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody,

anticalin, designed ankyrin repeat domain (DARPin), avimer), such that the miniprotein targeted to any one of the target proteins in Table 10 (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) or component(s) thereof (chelator, radionuclide) accumulates in metastatic cancerous cells. Imaging of tissues labeled with the miniprotein conjugate of any one of the target proteins in Table 10 wherein the conjugate comprises more additional detectable components (*e.g.*, chelator, *e.g.*, radionuclide, *e.g.*, other detectable imaging moiety) can be used, for example, for detection of metastatic cells, tumor margin delineation, evaluation of the completeness of resection, and evaluation of the efficacy of treatment.

[00447] In some embodiments, the present disclosure provides methods of imaging a cancer in a subject. In some embodiments, miniprotein conjugates of the present disclosure are useful for PET, SPECT, or MRI imaging. In some embodiments, a detectably effective amount of a miniprotein conjugate is administered to a subject; that is, an amount that is sufficient to yield an acceptable image using the imaging equipment that is available for clinical use. In some embodiments, a detectably effective amount of a miniprotein conjugate may be administered in more than one injection if needed. In some such embodiments, a detectably effective amount of miniprotein conjugate needed for an individual may vary according to factors such as the degree of uptake of miniprotein conjugates into cancerous tissue, the age, sex, and weight of the individual, and the particular medical imaging method used. Optimization of such factors is within the level of skill in the art.

[00448] In some embodiments, imaging with miniprotein conjugates can be used in assessing efficacy of therapeutic drugs in treating cancer. For example, images can be acquired after treatment with an anti-cancer therapy to determine if the individual is responding to treatment. In some embodiments, in a subject with cancer, imaging with miniprotein conjugate can be used to evaluate whether a tumor is shrinking or growing. Further, the extent of cancerous disease (how far and where the cancer has spread) can be determined to aid in determining prognosis and evaluating optimal strategies for treatment (*e.g.*, surgery, radiation, or chemotherapy).

[00449] In some embodiments, miniprotein conjugates can be used in image-guided surgery. Tissue of interest suspected of containing cancerous cells or a tumor can be contacted with a miniprotein conjugate, such that the miniproteins or components thereof (*e.g.*, chelator, *e.g.*, radionuclide) accumulate in metastatic cancerous cells. In some embodiments, imaging of tissues labeled with miniprotein conjugate in this way can be used,

for example, for detection of metastatic cells, tumor margin delineation, evaluation of the completeness of resection, and evaluation of the efficacy of treatment.

Kits

[00450] In one aspect, provided herein are kits comprising a pharmaceutical composition described herein for therapeutic, imaging, or diagnostic uses. In some embodiments, kits typically include a label indicating the intended use of the contents of the kit and instructions for use. The term label includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit. Accordingly, this disclosure provides a kit for treating a subject afflicted with a cancer, the kit comprising: (a) a dosage of pharmaceutical composition described herein and (b) instructions for using the in methods of therapy methods disclosed herein. In certain embodiments for treating human patients, the kit comprises a pharmaceutical composition described herein comprising a miniprotein conjugate described herein.

[00451] In some embodiments, a kit comprises a cold miniprotein conjugate as provided herein (*i.e.*, a miniprotein conjugate that does not contain a radionuclide), and instructions for chelation of the miniprotein conjugate to the radionuclide. In some embodiments, the kit comprises a hot miniprotein conjugate as provided herein (*i.e.*, a miniprotein conjugate described herein comprising the radionuclide), with instructions for administration to a subject.

[00452] In some embodiments, the present disclosure provides kits comprising a miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) as provided herein. In some embodiments, the kit comprises compositions for detecting the miniprotein of any one of the target proteins in Table 10 (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) (*e.g.*, conjugation to one or more detectable moieties).

[00453] In some embodiments, a kit comprises a label indicating the intended use of the contents of the kit and instructions for use. The term label includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit.

[00454] In some embodiments, the present disclosure provides a kit for treating, monitoring, or diagnosing a subject having or suspected of having cells overexpressing any one of the target proteins in Table 10, the kit comprising: (a) a unit of a pharmaceutical composition described herein and (b) instructions for using the in methods of administration disclosed herein. In certain embodiments for treating human patients, the kit comprises a

pharmaceutical composition described herein comprising miniprotein (*e.g.*, CDP, knottin, binder, affibody, engineered Kunitz domain, monobody, anticalin, designed ankyrin repeat domain (DARPin), avimer) as provided herein.

Certain Exemplary Embodiments

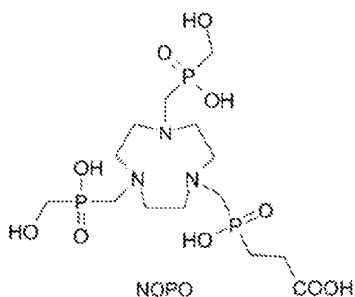
[00455] In some embodiments, the present disclosure provides compositions comprising a miniprotein (M), an optional linker (L), and one or both of a chelator (C) and a radionuclide (R), represented a formula selected from M-L-C-R, M-L-C, M-C-R, M-L-R, M-C, M-L, and M-R. In some embodiments, the composition comprises a folded polypeptide held together by disulfide bonds, covalent bonds, or non-covalent interactions, comprising cysteine-dense peptides, knittin peptides, and affibodies. In some embodiments, the miniprotein comprises or consists of a linear polypeptide, a folded polypeptide (*e.g.*, covalently linked polypeptide, non-covalently linked polypeptide, or polypeptide include a di-sulfide linkage), cysteine-dense peptide, a knottin peptide, a binder, an affibody, an engineered Kunitz domain, a monobody, an anticalin, a designed ankyrin repeat domain (DARPin), or an avimer. In some embodiments, the binder comprises or consists of a linear polypeptide, a folded polypeptide, and/or a non-disulfide sequence. In some embodiments, M is characterized in that it comprises 10-100 amino acids, and no more than 100 amino acids. In some preferred embodiments, the miniprotein is characterized in that it comprises (i) no more than 100 amino acids and/or 12 kDa; (ii) at least one secondary structure elements; (iii) a sequestered hydrophobic core; and/or displays cooperative folding. In some embodiments, the miniprotein comprises no more than about 100 amino acids or less, 90 amino acids, 85 amino acids, 80 amino acids, 75 amino acids, 70 amino acids, 65 amino acids, 60 amino acids, 55 amino acids, 50 amino acids, 45 amino acids, 40 amino acids, 35 amino acids, 30 amino acids, 25 amino acids, 20 amino acids, 15 amino acids, or 10 amino acids. In some embodiments, the miniprotein comprises at least one disulfide bridge.

[00456] In some embodiments, the present disclosure provides compositions represented by the formula L-C, wherein L comprises or consists of a linker, C comprises or consists of a chelator, and wherein the linker is designed to be conjugated to a polypeptide.

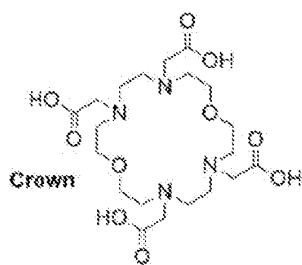
In some embodiments, the present disclosure provides compositions represented by the formula L-C-R, wherein L comprises or consists of a linker, C comprises or consists of a chelator, and R comprises or consists of a radionuclide, and wherein the composition is capable of being conjugated to a miniprotein. In some embodiments, L comprises or consists of a polyethylene glycol (PEG) linker of PEG4, PEG6, PEG8, PEG12, PEG24, an ester linker, an amide linker, a maleimide linker, a succinimidyl-4-(N-maleimidomethyl)

cyclohexane-1-carboxylate (SMCC) linker, , a propanoic acid linker, a caproic acid linker, or (Gly) n -(γ Glu) n - (SEQ ID NO: 79) or (PEG) n , wherein n is from 1 to 10, (Gly) $_{1-10}$ (SEQ ID NO: 80), or any fragment or combination via covalent bond thereof. In some embodiments, C comprises or consists of NOPO, Crown, Macropa or tetrazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) as set forth as follows:

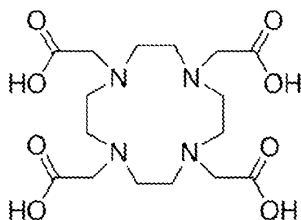
i) NOPO



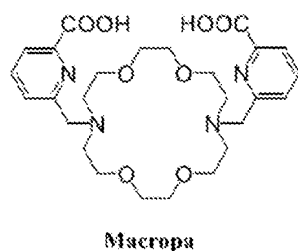
ii) Crown



iii) DOTA



or iv) Macropa



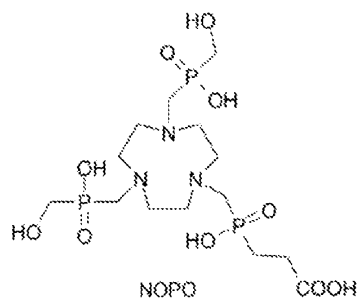
[00457] In some embodiments, C comprises or consists NOPO, Crown, Macropa, or tetrazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) or a derivative thereof.

[00458] In some embodiments, the present disclosure provides isolated constructs or pharmaceutically acceptable salts thereof comprising a miniprotein, optional linker, and at least one of a chelator or radionuclide. In some embodiments, R comprises or consists of Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211. In some embodiments, the composition comprises at least one additional component. In some embodiments, the composition can penetrate tumor tissue. In some embodiments, the miniprotein specifically binds to a target. In some embodiments, the composition displays μM or nM binding affinity to the target in an in vitro assay. In some embodiments, the composition binds to the target with an affinity of 1 pM to 100 nM as measured by an in vitro binding assay. In some embodiments, the miniprotein binding to the target modulates biological function. In some embodiments, the miniprotein binding to the target does not elicit an immune response. In some embodiments, the immune response includes a systemic immune response or a local immune response. In some embodiments, the target is located in, on, or near a cell. In some embodiments, the target is a protein expressed on the surface of the cell. In some embodiments, the cell is a tumor cell. In some embodiments, the tumor cell is a solid tumor cell. In some embodiments, the cell is a human cell. In some embodiments, the target is any one of the target proteins in Table 10. In some embodiments, the miniprotein selectively binds to any one of the target proteins in Table 10 or a portion thereof.

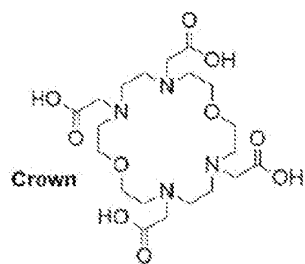
[00459] In some embodiments, the present disclosure provides pharmaceutical compositions comprising a miniprotein, (M) an optional linker (L) and one or both of a chelator (C) and a nuclide. In some embodiments, when L is present, L comprises or consists of a polyethylene glycol (PEG) linker, an ester linker, an amide linker, a maleimide linker, a valine-citrulline linker, a hydrazone linker, a N-succinimidyl-4-(2-pyridyldithio)butyrate (SPDB) linker, a succinimidyl-4-(N-maleimidomethyl)cyclohexane-

1-carboxylate (SMCC) linker, a vinylsulfone-based linker, a propanoic acid linker, a caproic acid linker, or any fragment or combination thereof. In some embodiments, when C is present, C comprises or consists of NOPO, Crown, Macropa, or tetrazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) as set forth as follows:

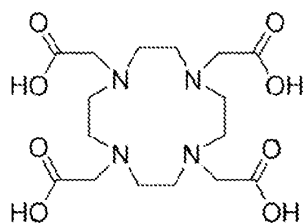
i) NOPO



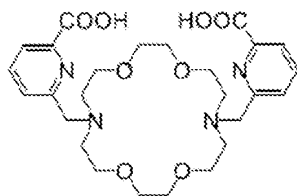
ii) Crown



iii) DOTA



or iv) Macropa

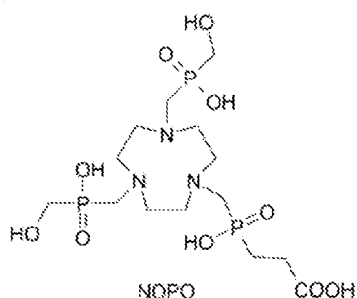
**Macropa**

[00460] In some embodiments, the chelator covalently attaches to the miniprotein. In some embodiments, the chelation efficiency is > 90%. In some embodiments, when R is present, the radionuclide is an alpha-emitter. In some embodiments, the radionuclide is Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211. In some embodiments, the miniprotein specifically binds to a target. In some embodiments, the target is any one of the target proteins in Table 10. In some embodiments, the target protein selected from Table 10 is expressed on a cell. In some embodiments, the cell is a human cell. In some embodiments, the human cell is a tumor cell. In some embodiments, the tumor cell is a solid tumor cell. In some embodiments, the miniprotein comprises or consists of a linear polypeptide, a folded polypeptide (e.g., covalently linked polypeptide, non-covalently linked polypeptide, or polypeptide include a di-sulfide linkage), cysteine-dense peptide, a knottin peptide, a binder, an affibody, an engineered Kunitz domain, a monobody, an anticalin, a designed ankyrin repeat domain (DARPin), or an avimer. In some embodiments, the miniprotein comprises one or more disulfide bonds. In some embodiments, the miniprotein is characterized in that it has nM or sub-nM binding affinity on the target in vivo or in a cell-based assay. In some embodiments, the miniprotein a binding affinity of 1 pM to 100 nM to any one of the target proteins in Table 10 on a cell line expressing human isoform of any one of the target proteins in Table 10. In some embodiments, the miniprotein has an amino acid sequence no more than about 100 amino acids or less, 90 amino acids, 85 amino acids, 80 amino acids, 75 amino acids, 70 amino acids, 65 amino acids, 60 amino acids, 55 amino acids, 50 amino acids, 45 amino acids, 40 amino acids, 35 amino acids, 30 amino acids, 25 amino acids, 20 amino acids, 15 amino acids, or 10 amino acids. In some embodiments, the miniprotein does not elicit an immune response or wherein the immune response elicited is tolerable. In some embodiments, the pharmaceutical composition comprises high tumor tissue penetration. In some embodiments, the pharmaceutical composition is not taken up and/or

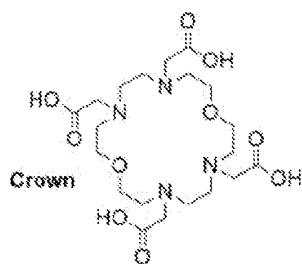
retained in the kidney or liver. In some embodiments, the pharmaceutical composition is internalized in a cell expressing human isoform of any one of the target proteins in Table 10.

[00461] In some embodiments, the present disclosure provides methods of treating a subject in need thereof comprising administering a composition comprising a miniprotein (M), an optional linker (L), and one or both of a chelator (C) and a radionuclide (R). In some embodiments, when L is present, L comprises or consists of a polyethylene glycol (PEG) linker, an ester linker, an amide linker, a maleimide linker, a valine-citrulline linker, a hydrazone linker, a N-succinimidyl-4-(2-pyridyldithio)butyrate (SPDB) linker, a succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) linker, a vinylsulfone-based linker, a propanoic acid linker, a caproic acid linker, or any fragment or combination thereof. In some embodiments, when C is present, C comprises or consists of NOPO, Crown, Macropa or tetrazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) as set forth as follows:

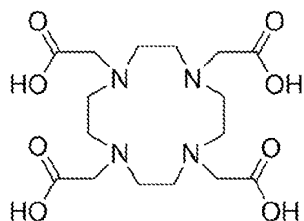
i) NOPO



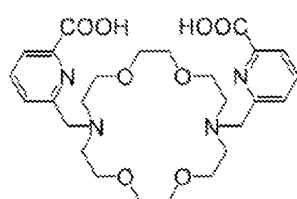
ii) Crown



iii) DOTA



iv) Macropa

**Macropa**

[00462] In some embodiments, when R is present, R comprises or consists of Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211. In some embodiments, M comprises or consists of a cysteine-dense peptide, a knottin peptide, a binder, an affibody, an engineered Kunitz domain, a monobody, an anticalin, a designed ankyrin repeat domain (DARPin), or an avimer. In some embodiments, M is characterized in that it comprises 10-100 amino acids, and no more than 100 amino acids. In some preferred embodiments, M is characterized in that it comprises (i) no more than 100 amino acids and/or 12 kDa; (ii) at least two secondary structure elements; (iii) a sequestered hydrophobic core; and/or displays cooperative folding. In some embodiments, a composition comprising M, optional L, and one or both of C and R, for use in a method of the present disclosure, comprises at least one additional component. In some embodiments, the composition can penetrate tumor tissue. In some embodiments, the miniprotein comprises no more than about 100 amino acids or less, 90 amino acids, 85 amino acids, 80 amino acids, 75 amino acids, 70 amino acids, 65 amino acids, 60 amino acids, 55 amino acids, 50 amino acids, 45 amino acids, 40 amino acids, 35 amino acids, 30 amino acids, 25 amino acids, 20 amino acids, 15 amino acids, or 10 amino acids. In some embodiment, the miniprotein comprises at least one disulfide bridge. In some embodiments, the miniprotein specifically binds to a target. In some embodiments, the composition displays mM or nM binding affinity to the target in an in vitro

assay. In some embodiments, the composition binds to the target with an affinity of 1 pM to 100 nM as measured by an *in vitro* binding assay. In some embodiments, the composition is characterized in that it has high tissue penetrating properties relative to a composition comprising a full-size protein that binds to the same target. In some embodiments, the miniprotein binding to the target modulates biological function. In some embodiments, administration of the composition does not elicit an immune response. In some embodiments, the immune response includes a systemic immune response or a local immune response. In some embodiments, the target is located in, on, or near a cell. In some embodiments, the target is a protein expressed on the surface of the cell. In some embodiments, the cell is a tumor cell. In some embodiments, the tumor cell is a solid tumor cell. In some embodiments, the cell is a human cell. In some embodiments, the target is any one of the target proteins in Table 10. In some embodiments, the subject has or is at risk of having cancer. In some embodiments, the cancer is selected from breast cancer, ovarian cancer, melanoma, pancreatic cancer, peripheral neuroma, glioblastoma, adrenocortical carcinoma, AIDS-related lymphoma, anal cancer, bladder cancer, meningioma, glioma, astrocytoma, cervical cancer, chronic myeloproliferative disorders, colon cancer, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extracranial germ cell tumors, extrahepatic bile duct cancer, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumors, gestational trophoblastic tumors, hairy cell leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, hypopharyngeal cancer, islet cell carcinoma, Kaposi sarcoma, laryngeal cancer, leukemia, lip cancer, oral cavity cancer, liver cancer, male breast cancer, malignant mesothelioma, medulloblastoma, Merkel cell carcinoma, metastatic squamous neck cell carcinoma, multiple myeloma and other plasma cell neoplasms, mycosis fungoides and the Sezary syndrome, myelodysplastic syndromes, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, small cell lung cancer, head and neck cancer, skin cancer, oropharyngeal cancer, bone cancers, including osteosarcoma and malignant fibrous histiocytoma of bone, paranasal sinus cancer, parathyroid cancer, penile cancer, pheochromocytoma, pituitary tumors, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, small intestine cancer, soft tissue sarcoma, supratentorial primitive neuroectodermal tumors, pineoblastoma, testicular cancer, thymoma, thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis and ureter, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Wilm's tumor and other childhood kidney tumors. In some embodiments, after administration of the composition, the cancer is treated. In some embodiments, the composition is administered intravenously or subcutaneously.

[00463] In some embodiments, the present disclosure provides methods of characterizing miniprotein conjugates comprising contacting a population of cells expressing any one of the target proteins in Table 10 with a miniprotein conjugate and measuring one or more of: (i) binding affinity; (ii) internalization; (iii) binding specificity; (iv) immune response as characterize by secretion or expression of one or more cytokines.

[00464] In some embodiments, the present disclosure provides methods of detecting cancer comprising administering to a subject a composition comprising a miniprotein specific to any one of the target proteins in Table 10, further comprising a detectable moiety, and detecting the presence and/or quantity of the composition in the subject, wherein detection of the miniprotein is associated with risk of developing or having cancer. In some embodiments, the miniprotein of the composition is designed for conjugation to one or more additional components. In some embodiments, the composition penetrates tumor tissue. In some embodiments, the miniprotein of the composition comprises less than 12 kDa. In some embodiments, the miniprotein of the composition comprises no more than about 100 amino acids or less, 90 amino acids, 85 amino acids, 80 amino acids, 75 amino acids, 70 amino acids, 65 amino acids, 60 amino acids, 55 amino acids, 50 amino acids, 45 amino acids, 40 amino acids, 35 amino acids, 30 amino acids, 25 amino acids, 20 amino acids, 15 amino acids, or 10 amino acids. In some embodiments, the miniprotein of the composition comprises at least one disulfide bond. In some embodiments, the miniprotein that does not comprise multiple cysteine residues such as, for example, a miniprotein comprising a single cysteine residue.

[00465] In some embodiments, the method identifies a subject as having a cancer. In some embodiments, the cancer is selected from breast cancer, ovarian cancer, melanoma, pancreatic cancer, peripheral neuroma, glioblastoma, adrenocortical carcinoma, AIDS-related lymphoma, anal cancer, bladder cancer, meningioma, glioma, astrocytoma, cervical cancer, chronic myeloproliferative disorders, colon cancer, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extracranial germ cell tumors, extrahepatic bile duct cancer, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumors, gestational trophoblastic tumors, hairy cell leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, hypopharyngeal cancer, islet cell carcinoma, Kaposi sarcoma, laryngeal cancer, leukemia, lip cancer, oral cavity cancer, liver cancer, male breast cancer, malignant mesothelioma, medulloblastoma, Merkel cell carcinoma, metastatic squamous neck cell carcinoma, multiple myeloma and other plasma cell neoplasms, mycosis fungoides and the Sezary syndrome, myelodysplastic syndromes, nasopharyngeal cancer, neuroblastoma, non-small cell lung

cancer, small cell lung cancer, head and neck cancer, skin cancer, oropharyngeal cancer, bone cancers, including osteosarcoma and malignant fibrous histiocytoma of bone, paranasal sinus cancer, parathyroid cancer, penile cancer, pheochromocytoma, pituitary tumors, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, small intestine cancer, soft tissue sarcoma, supratentorial primitive neuroectodermal tumors, pineoblastoma, testicular cancer, thymoma, thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis and ureter, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Wilm's tumor and other childhood kidney tumors. In some embodiments, a cancer cell from the subject expresses any one of the target proteins in Table 10, or a portion thereof. In some embodiments, the expression of the target is higher in the cancer cell than in a non-cancer cell.

[00466] In some embodiments, the present disclosure provides methods of targeting a population of cancer cells expressing any one of the target proteins in Table 10, the method comprising: (i) determining a level of expression of a target in a population of cancer cells; (ii) administering to a subject in need thereof a composition according to the present disclosure or a pharmaceutical composition according to the present disclosure, wherein the composition specifically binds any one of the target proteins in Table 10; and (iii) wherein the composition targets the cells expressing the target protein selected from Table 10 and is internalized into the cells expressing the target proteins in Table 10; (iv) wherein the patient is treated after the administering as compared to prior to the administering and (v) wherein the treatment does not damage cells not expressing the target protein selected from Table 10.

[00467] A miniprotein conjugate comprising: (i) miniprotein (M) that specifically binds to any one of the target proteins in Table 10; (ii) a chelator (C) conjugated to (M) through an optional linker (L), wherein (C) comprises DOTA, and (L), when present, comprises PEG; and (ii) a radionuclide (R) chelated to (C), wherein (R) is Actinium-225.

[00468] A miniprotein conjugate comprising: (i) miniprotein (M) that specifically binds to any one of the target proteins in Table 10; (ii) a chelator (C) conjugated to (M) through an optional linker (L), wherein (C) comprises DOTA, and (L), when present, comprises PEG; and (ii) a radionuclide (R) chelated to (C), wherein (R) is Lead-212.

[00469] A miniprotein conjugate comprising: (i) miniprotein (M) that specifically binds to any one of the target proteins in Table 10; (ii) a chelator (C) conjugated to (M) through an optional linker (L), wherein (C) comprises DOTA, and (L), when present, comprises PEG; and (ii) a radionuclide (R) chelated to (C), wherein (R) is Lutetium-177.

[00470] A miniprotein conjugate comprising: (i) miniprotein (M) that specifically binds to any one of the target proteins in Table 10; (ii) a chelator (C) conjugated to (M) through an optional linker (L), wherein (C) comprises DOTA, and (L), when present, comprises PEG; and (ii) a radionuclide (R) chelated to (C), wherein (R) is Gallium-68.

[00471] In some embodiments, the PEG linker is PEG (4-24).

[00472] In some embodiments, the present disclosure provides pharmaceutical compositions comprising a conjugate in accordance with the present disclosure and a pharmaceutically acceptable excipient. In some embodiments, the composition is formulated for parenteral or oral administration.

[00473] In some embodiments, the present disclosure provides methods of imaging a cell or population of cells in a subject having or suspected of having cancer comprising administering a pharmaceutical composition in accordance with the present disclosure and detecting a presence of the pharmaceutical composition in the subject.

[00474] In some embodiments, the present disclosure provides methods of treating cancer in a subject in need thereof comprising administering a pharmaceutical composition in accordance with the present disclosure to the subject, wherein the subject is treated and non-cancer cells of the subject are not killed.

[00475] The present disclosure is further illustrated by the following examples which should not be construed as further limiting.

[00476] The contents of all references cited throughout this application are expressly incorporated herein by reference.

EXAMPLES

EXAMPLE 1: SYNTHESIS OF NECTIN-4 MINIPROTEIN-PEG-DOTA CONJUGATES

Synthesis of Polypeptide

[00477] Polypeptides are synthesized on a Prelude peptide synthesizer (Protein Technologies Inc., Tucson, AZ)) by solid-phase methods using Fmoc strategy with N-[(dimethylamino)-1H-1,2,3-triazolo-[4,5-b]pyridin-1-ylmethylene]-N-methylmethanaminium hexafluorophosphate N-oxide (HATU) or 2-(6-chloro-1-H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) activation (5-fold molar excess to amino acid) in N,N-dimethylformamide (DMF), and N,N-diisopropylethylamine (DIEA) is used as a base. A 20% piperidine/DMF solution is used for Fmoc deprotection. The resin is Rink Amide MBHA LL (Novabiochem) with loading of (0.30 - 0.40) mmol/g on a (20-400) pmol

scale. Final deprotection and cleavage of the peptide from the solid support is performed by treatment of the resin with (92.5% TFA, 2.5% phenol, 2.5% water and 2.5% triisopropylsilane) for 2-3 hours.

[00478] The cleaved peptide is precipitated using cold diethyl ether. The diethyl ether is decanted, and the solids are triturated with cold diethyl ether and pelleted by centrifugation. The crude solids are dissolved in a 1:1 solution of ACN/water, 0.01% TFA. Disulfide bridge formation is accomplished via orthogonally protected cys residues and after selective deprotection and/or natural folding cyclization, the crude product solution is lyophilized in preparation for final purification. The lyophilized solid is re-dissolved in a 1:1 solution of acetonitrile/water, with 0.1% TFA (10-15 mL), purified via reverse phase HPLC on a Waters XBridge™ BEH 130, CIS, 10 pm, 130 Å, 30 X 250 mm ID column, using a 30 gradient within the ranges of 5 -75% acetonitrile/water with 0.1% TFA over 30 - 45 minutes at a flow rate of 30 mL/min, λ -215 nm.

Conjugation of Chelator to Polypeptide

[00479] Metal-chelating motifs are covalently attached to polypeptide chains at the end of the linear SPPS. First, orthogonally protected linkers (e.g., amino-discrete polyethylene glycols, amino-hexanoic acids, and amino acids; QuantaBioscience, Plain City, OH, US) are coupled to either the N-terminal amine, or alternatively the ϵ -amino side chain of a lysine. After an appropriate deprotection step (20% piperidine in dimethylformamide for Fmoc), metal chelators are coupled to the linker-polypeptide chain with conventional HATU activation in DMF containing 2% N,N-diisopropylethylamine (DIEA). Chelators such as tri-t-butyl-DOTA, bis-t-butyl-NOTA, tetra-t-butyl-DTPA, (Macrocyclics, Plano, TX, US) or CROWN are coupled to the linker also using HATU activation in DMF containing 2% N,N-diisopropylethylamine (DIEA). Finally, polypeptides are cleaved from solid support and acid-labile protecting groups removed with a cocktail of TFA.

Formation of Metal Complex with Polypeptide

[00480] Peptides with covalently attached metal chelators are complexed with either their cold-metal surrogates (e.g., Lanthanum, 69/71-gallium, or europium) or radionuclides (e.g., 225-Ac or 68-Ga). These metalation reactions are carried out in slightly alkaline aqueous buffers. Peptides are dissolved in buffers comprised of 100 mM ammonium acetate, pH 7-8 with 3 molar equivalents of metal. Additional weakly coordinating formulants are also used to avoid oxidation of some metals. Metalation reactions are monitored by RP-HPLC under neutral mobile phases such as 10 mM triethylammonium acetate as a modifier. Ultra-violet signals are used to identify analytes and their shifting retention times are indicative of

metalated peptides. Elevated temperatures of 60-90 °C are used, as appropriate, for efficient complexation. After the completion of the metal complexation, excess metal is separated from metalated peptide by semi-prep HPLC under neutral conditions. Mass spectroscopy is used to confirm the metalated peptide species. Radioactive species are characterized for radiochemical yield and purity with either radio-HPLC or radio-TLC.

EXAMPLE 2: RADIOLABELING OF POLYPEPTIDE BINDER-PEG-DOTA COMPOUNDS

[00481] *Labeling of polypeptide binder-PEG-DOTA with ^{114}In or ^{113}In :* 1 mg of peptide (DOTA-A4) was dissolved in 100 μl of pH 5 sodium acetate solution (0.1M) and 2 eq of $^{111}\text{InCl}_3$ or $^{114}\text{InCl}_3$ was added. Reaction mixture was heated at 40°C for 15 min and mass spec analysis was shown indium chelation to DOTA was completed. Product of ^{114}In 14 was purified using semi-prep system (column: C18, 10 mm x 250 mm, 5 μm , gradient: 5-50-15 min-4.6 ml/min, mobile phase: A: pH 6.5 100 mM TEAA, B: 10% pH 6.5 100 mM TEAA in acetonitrile). Analytical HPLC was performed using XBridge Protein BEH C4 column, 4.6 X 100 mm, 3.5 μm , 300 Å and Mobile phase gradient of 5-40% to 10 min, hold at 40% to 15 min at 60°C, 1 ml/min. Mobile phase: A: pH 6.5 100 mM TEAA, B: 10% pH 6.5 100 mM TEAA in acetonitrile.

[00482] Polypeptides (including peptides and conjugates thereof) along with SEQ ID NOs and molecular weight are reported in Tables 4A and 4B.

[00483] *Labeling of DOTA-compounds with ^{225}Ac , ^{111}In , or ^{177}Lu :* DOTA-compound was dissolved in 0.1^{-3} M sodium acetate buffer pH 5.5 followed by the addition of radiometal (^{225}Ac , ^{111}In , or ^{177}Lu) in 0.05 M HCl. The reaction was vortexed and heated at 70 °C for 30 minutes. Radionuclide incorporation was assessed by thin-layer chromatography developed in 0.1 M ammonium acetate solution with 0.1 M EDTA at pH 6. Radioactivity was measured by gamma counting.

[00484] *Labeling of polypeptide binder-PEG-DOTA with natural abundance In (^{115}In):* 1 mg of peptide (DOTA-A4) was dissolved in 100 μl of pH 6 ammonium acetate solution (0.1 M) (or pH 5 sodium acetate solution (0.1 M)) and 2 molar equivalents of InCl_3 in separated reaction buffer solution were added. Reaction mixture was heated at 70 °C (or 60 °C) for 30 - 40 minutes and mass spec analysis was shown indium chelation to DOTA was completed. Metalated product was purified using semi-prep system (column: C4 or C18, 10 mm x 250 mm, 5 μm , gradient: 5-50%-10 min and hold at 50 % for 5 min-4.0 ml/min (or 5-60%-10 min and hold at 60 % for 5 min-4.0 ml/min depending on the peptide hydrophobicity), column

temperature: RT or 60 °C (depending on the peptide stickiness), mobile phase: A: pH 6.5 100 mM TEAA, B: 10% pH 6.5 100 mM TEAA in acetonitrile).

[00485] Results for radionuclide incorporation for exemplary test articles are reported in Table 1.

EXAMPLE 3: RADIOLABELING OF NECTIN-4-PEG-DOTA with Ac²²⁵

[00486] For radiolabeling reaction, miniprotein-PEG-DOTA conjugates are incubated with 1 to 1 molar ratio of Ac225 (in the form of a nitrate salt) in 0.1 N sodium acetate (pH 5) for 5 hrs at 70 °C. The reaction is terminated with the addition of EDTA. Ac225-CDP-PEG-DOTA was purified using a PD-10 column and eluted with PBS (pH 7.4), and passed through a 0.22 µm filter. The radiochemical purity is determined as the ratio of the main product peak to other peaks. The radiochemical yield is determined as the ratio of final activity of the product over the starting activity used for the reaction adjusted for the radioactive decay.

EXAMPLE 4: ESTIMATING PEPTIDE BINDING AFFINITY ON LIVE CELLS: DELFIA BINDING ASSAY

[00487] A whole cell binding assay was used to estimate the equilibrium binding affinity (K_D) or binding inhibition constant (K_i) of miniprotein ligands to cells expressing a target of interest. Cells were dispensed into a 384-well plate and incubated at 37 °C in 5% CO₂ overnight. The next day cells were gently washed once in assay buffer prior to addition of europium chelated ligand. For K_D determination, test agent conjugated with europium chelate (DOTA or DTPA) in the presence or absence of 100-fold excess un-conjugated ligand was incubated with cells over 8 different concentrations ranging from 100 times below and above expected K_D values. For K_i determination, a single concentration (K_D equivalent concentration) of europium-chelate miniprotein was added to each well followed immediately by the addition of unconjugated competing ligand over 8 different concentrations ranging from 100 times below and above expected K_i values as before. The plate was incubated 1.5-hours at room temperature. Cells were then washed three times at room temperature with 1X PBS followed by the addition of 2 M HCl and incubated for two-hours at 37 °C. Following this step, 2 M NaOH and fluorescent inducer solution were added to each well and incubated for 30 minutes at room temperature according to the manufacturer's protocol (Perkin-ELMER DELFIA assay). Plates were read on an Envision plate reader and a curve-fitting model was applied to estimate K_D and K_i values using GraphPad Prism software. DELFIA saturation and competitive inhibition binding curves of exemplar Compound ID NO: C34 and

Compound ID NO: 40 are reported in FIG. 6 and FIG. 7 respectively. Mean DELFIA K_i values for exemplary test articles are recorded in Table 5.

EXAMPLE 5: ESTIMATING PEPTIDE BINDING AFFINITY BY SURFACE PLASMON RESONANCE

[00488] Surface plasmon resonance (SPR) analyses were performed using Biacore T200 (GE Healthcare). Target protein (“ligand”) was immobilized to a CM5 Series S sensor chip (Cytiva) using amine-coupling chemistry. Running buffer was 0.05% Surfactant P20 in HEPES buffered saline (HBS-P), pH 7.4 (Cytiva). Immobilization was performed at 25 °C. Carboxyl groups in each flow cell were activated using a 1:1 mixture of 0.4 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide in water (EDC) and 0.1 M N-hydroxysuccinimide in water (NHS) for 7 minutes. The ligand was prepared in 10 mM Sodium acetate pH 5 (Cytiva). Pulsatile injections were made to target a specific immobilization level. Any remaining amine-reactive esters on the chip surface were blocked with a 7-minute injection of 1 M ethanolamine, pH 8.5. Three 10-second pulses of 50 mM sodium hydroxide were then made to remove any unbound ligand. Reference cells were modified using amine coupling chemistry, omitting ligand injection. A second method with a nickel (Ni^{2+})-nitriloacetic acid (NTA) surface was used to capture the target protein (“ligand”) by polyhistidine tag.

[00489] Test peptides (“analyte”) were injected to the prepared ligand surface and blank reference surface in increasing concentrations to cover 0.1 – 10x the expected K_D range at 25 °C. Increase in signal (RU) is proportional to an increase in binding events between the analyte and ligand surface. The injection was then stopped, and dissociation was measured. The resulting sensorgram is reference surface and blank injection subtracted prior to curve fitting. Measured association (k_a , $\text{M}^{-1}\text{s}^{-1}$) and dissociation (k_d , s^{-1}) were fit to a 1:1 binding model to calculate an equilibrium dissociation constant, K_D (M) for each analyte. Equilibrium binding constants (K_D) and binding inhibition constants (K_i) estimated from surface plasmon resonance (SPR) or DELFIA® binding assays, respectively, for exemplar miniprotein scaffolds are recorded in Tables 4A and 4B and binding kinetics to immobile ligand of Compound ID NO: C11 is recorded in FIG. 8. K_D values for exemplary test articles are recorded in Table 5.

EXAMPLE 6: RADIOLIGAND ALBUMIN SHIFT COMPETITION ASSAY

[00490] Albumin shift competition assay was performed to determine albumin binding of compounds. DOTA-compounds were radiolabeled under standard conditions with lutetium-

177. Live cancer cells were seeded in 96-wells 24-48 hours prior to assay at 20,000 to 40,000 cells per well. On the day of assay, the culture medium was removed and radioligand was added to cells at a single concentration (selected from the range of 1-10 nM), followed by either unlabeled competitor in the presence or absence of 4% human serum albumin at concentrations of competitor ranged from 10 pM to 10 μ M. In some assays, 1% bovine serum albumin was used. The assay was incubated at 4 °C for 1 hour. At the end of incubation, cells were washed with assay buffer and bound radioactivity was quantified by liquid scintillation counting. Data was fitted to a non-linear regression function to determine the competitive inhibition constant (K_i) for each assay condition. The K_i shift of competitor in the presence of albumin was used to determine the percent free fraction of competitor as a measure of albumin binding. K_i constants and percent free fraction measurements for exemplar miniprotein scaffolds are recorded in Table 2 for human serum albumin and bovine serum albumin, respectively.

EXAMPLE 7: EVALUATING PEPTIDE UPTAKE IN OPOSSUM (OK) KIDNEY CELLS

[00491] Fluorophore conjugated test agent-based assay: Opossum kidney proximal tubule cells were plated in 24-well transwell plates containing 200 μ L of growth media in the upper chamber and 600 μ L growth media in the lower chamber and incubated overnight at 37 °C in 5% CO₂. The following day, the media was changed, and cells were incubated an additional 72 hours with orbital shaking at 146 rpm, changing media daily. Next, cells were washed and treated with Alexafluor-647-labelled (AF647) test agent at several concentrations for 1-hour at 37 °C and 5% CO₂ with continuous orbital shaking. Following incubation, cells were washed three times with 1x PBS prior to adding 4% paraformaldehyde and incubated for 15 minutes. Following paraformaldehyde aspiration, cells were washed three times with 1x PBS, and membranes were excised and mounted on glass slides and a coverslip using ProGold antifade mounting solution containing DAPI. The following day 2-channel fluorescence images were captured using an Echo Revolution fluorescent microscope, and fluorescence quantitated from each channel using ImageJ software. Raw fluorescence values from test agent were normalized to cell number using the DAPI channel for each treatment and results were expressed as a normalized fluorescence value. Quantitative uptake in the OK-PTC model is shown in FIGS. 9A, 9B, and 9C.

[00492] Biotin conjugated test agent-based assay: Opossum kidney proximal tubule cells were plated in the upper chamber of 24-well transwell plates containing 600 μ L of growth

media in the lower chamber and were incubated overnight at 37°C in 5% CO₂. The following day, the media were changed, and cells were incubated an additional 24 hours with orbital shaking at 146 RPM. Next, cells were washed and treated in duplicates with biotinylated test agents pre-complexed with streptavidin-AF647 for 1-hour at 37°C and 5% CO₂ with continuous orbital shaking. Following incubation, the cells were washed three times with 1X PBS and lysed with RIPA buffer containing protease and phosphatase inhibitor cocktail and Pierce universal nuclease for 30 minutes. Uptake was measured on 100 µL of lysates using a plate-based fluorimeter. Quantitative uptake shown in FIGS. 10 and 11.

EXAMPLE 8: EVALUATING *IN VITRO* METABOLIC STABILITY OF TEST ARTICLES

[00493] Miniprotein test articles (TAs) were spiked into biological matrices at initial concentration between 1-2 µM. Tested matrices were varied by species (mouse, rat, human) and type (plasma, serum, and kidney brush border membrane preparations tested each from 0.625-100 µg/mL protein content). Tested conditions were incubated with TAs at 37 °C for 4 hours. At collection points of 0, 15, 30, 60, 120, 240 minutes, 50 µL of each condition were quenched by organic solvent (methanol or acetonitrile) or the addition of 4% phosphoric acid solution. Fractions of each quenched timepoint were subsequently assayed for parent TA concentration by high resolution LC-MS. Percent-parent-remaining-time plots were constructed from the ratio of each given time collection to the time-zero parent TA. In the case of cleavable linkers, cleaved products were also identified by high resolution LC-MS and in some cases, doubling time of cleavage products was calculated. Percent-parent-remaining and cleavage product doubling time in mouse and human sera and plasmas for exemplar miniproteins are recorded in Tables 7 and 8.

EXAMPLE 9: EVALUATING PHARMACOKINETICS OF TEST ARTICLES IN RAT PLASMA

[00494] Double jugular vein-cannulated Sprague Dawley rats were dosed with bolus intravenous injections of miniprotein test articles (TAs) (0.03-0.3 mg/kg) and FITC-sinistrin. Nine blood collection timepoints were taken from 2-24 hours, processed to plasma with K₂EDTA, and frozen for subsequent bioanalysis. Plasma TA concentrations were measured by LC-MS/MS. Plasma FITC-sinistrin was measured fluorometrically. All unknown sample measurements were interpolated against known authentic standards spiked into normal rat plasma to calculate concentration. Finally, non-compartmental analysis was performed to estimate plasma pharmacokinetic parameters. Plasma terminal half-life measurements for exemplar TAs are recorded in Table 9.

EXAMPLE 10: ^{111}In LABELING OF TEST ARTICLES FOR BIODISTRIBUTION

[00495] *In-111 labeling of miniproteins with MES buffer:* In-111 was neutralized with 0.5 M MES buffer pH 5.5. This mixture was added to miniproteins test articles prepared at 2 mg/mL in water with an equivalent amount of 0.5 M MES buffer pH 5.5, in a 1.5 mL Eppendorf vial and heated at 75°C for 30 minutes. After the reaction, an HPLC sample was taken and an equivalent amount of 10 mM DTPA in 0.1 M ammonium acetate pH 5 was added and incubated for at least 15 minutes. The sample was then used for HPLC analysis.

[00496] *In-111 labeling of miniproteins with Sodium Acetate buffer:* In-111 was neutralized with 0.1 M sodium acetate buffer pH 5. This mixture was added to miniprotein prepared at 5 mg/mL in sodium acetate buffer, with various amounts of 0.1 M sodium acetate buffer pH 5, in a 1.5 mL Eppendorf vial and heated at 75°C for 30 minutes. After the reaction, an HPLC sample was taken and an equivalent amount of 10 mM DTPA in 0.1 M ammonium acetate pH 5 was added and incubated for at least 15 minutes. The sample was then used for HPLC analysis.

[00497] *Purification of ^{111}In -labeled miniproteins:* Purification was done on two of the crude reaction mixtures mixed with 10 mM DTPA in 0.1 M ammonium acetate pH 5 and incubated for 15 minutes. A 3 kDa 0.5 mL amicon filter was used for purification and the filter was spun at 15,000 RCF for 9 minutes. Saline was used as a formulation buffer.

EXAMPLE 11: IN VIVO BIODISTRIBUTION INVOLVING ^{111}In -LABELED TEST ARTICLES

[00498] *Animals:* Female athymic nude mice (6-8 weeks of age) were purchased from Charles River Laboratories and housed according to IACUC guidelines with ad libitum feeding.

[00499] *Animal grouping and Treatment:* Animals were monitored for body weight bi-weekly and at the time of experimentation, grouped into groups of n=3. Animals were administered test-agents (TAs) that were prepared as described above. Animals received 350 μCi of activity (^{111}In) at approximately 3-5 μg of total peptide per mouse.

[00500] *Animal imaging:* Animals were sedated using isoflurane gas and imaged in a 3 bed hotel using a NanoScan SPECT/CT scanner (Mediso). Animals were imaged at 4 and 24 h post-dosing via SPECT scan followed CT scans.

[00501] *Humane endpoints:* All animals were euthanized following the final imaging time point and carcasses were discarded according to IACUC protocols.

EXAMPLE 12: IMAGE ANALYSIS METHODS

[00502] *Image Processing:* Images were generated as SPECT/CT pair with the SPECT reported in units of activity. Namely, the values assigned to the voxels (volume elements) comprising the SPECT images were in units of μCi . SPECT images were co-registered to the CT scan for anatomical reference, resampled to 0.2 mm^3 voxels, masked to remove the CT background, and cropped to a uniform size prior to analysis.

[00503] *Estimating tissue uptake:* Regions of interest (ROIs) were defined using VivoQuant software. The kidneys and bladder were segmented as fixed volume phantoms and registered using the CT for anatomical reference. Two fixed volume spheres were used to create the liver ROI. Spheres were placed at appropriate anatomical locations based on CT and SPECT signal. Group and individual master spreadsheets were generated which included the volume, activity, and concentration (Activity/Volume) at each time point for each ROI generated. Additionally, plots of activity were generated using Matplotlib based python tools to highlight trends in the data. Outputs of each region were plotted and reported in percent injected dose per gram (%ID/g) and regions which were fully segmented were additionally presented in percent injected dose (%ID). Plots of body weights and tumor volumes measured manually in the lab were also created in the same manner.

[00504] *Uptake unit definitions:* Results were presented in units of percent injected dose (%ID) and percent injected dose per gram (%ID/g). The definition of these units can be found in the equations below: The %ID for each analyzed region from the in vivo imaging data can be defined as stated in Equation 1:

$$\%ID = \frac{Uptake}{Injected Dose} * 100$$

where, Uptake = Radioactivity (μCi) in a particular ROI, decay-corrected to the time of injection, and Injected dose = Radioactivity (μCi) injected into the subject.

[00505] The %ID/g for each analyzed region from the in vivo imaging data can be defined as stated in Equation 2:

$$\frac{ID}{g} = \frac{\frac{Uptake}{Injected Dose} * 100}{ROI weight}$$

where, Uptake = Radioactivity (μCi) in a particular ROI, decay-corrected to the time of injection. Injected dose= Radioactivity (μCi) injected into the subject. Weight = For in vivo, this is the volume of the particular ROI in mL.

[00506] *Image generation:* After the preprocessing described in A, individual maximum intensity projections (MIPs) were created with VivoQuant software for each subject at each time point and scaled in percent injected dose per gram (%ID/g). The CT for each image was windowed from -500 to 4500 Hounsfield Units (HU). The SPECT was windowed at various ranges to highlight different regions of uptake. Images were then stitched together using Image Magick based python tools to create montages of subjects over time and time points over groups.

[00507] ROIs for quantitative analysis were generated from CT scans in order to quantify the injected dose per gram (%ID/g) of kidney tissue in mice as indicated of the different miniprotein scaffolds are illustrated in FIGs. 1-5 and FIGs. 12-14. All ROIs were generated based on data at both 4 and 24 hours post-administration. Regions of Interest (ROIs) were determined in mice treated with exemplary Nectin-4 charge variant conjugate (Compound ID NOs: C14, C45, C48, and C52) at 4 and 24 hours post-administration. Reduced levels of kidney retention in mouse biodistribution were observed (FIG. 1).

[00508] In mice co-administered an exemplary decoy peptide (Compound ID NO: C15) in combination with an exemplary Nectin-4 conjugate (Compound ID NO: C14), as compared to those administered an exemplary Nectin-4 targeting conjugate (Compound ID NO: C14) alone, kidney uptake was reduced at both 4 and 24 hours post-administration (FIG. 2).

[00509] . Regions of Interest (ROIs) were determined in mice treated with exemplary B7H3 charge variant conjugate (Compound ID NOs: C1, C3, C5, C9, C43) at both 4 and 24 hours post-administration. Reduced levels of kidney retention in mouse biodistribution were observed (FIG. 3).

[00510] In mice co-administered an exemplary decoy peptide (Compound ID NO: C7) in combination with an exemplary B7H3 conjugate (Compound ID NO: C9), as compared to those administered an exemplary B7H3 conjugate (Compound ID NO: C9) alone, kidney uptake was reduced at both 4 and 24 hours post-administration (FIG. 4).

[00511] Regions of Interest (ROIs) were determined in mice treated with exemplary Nectin-4 conjugate with an addition of a Version 1 cleavable linker (Compound ID NO: C64), as compared to those administered an exemplary Nectin-4 conjugate (Compound ID

NO: C14) at both 4 and 24 hours post-administration. Levels of kidney uptake and retention in mouse biodistribution showed minimal alterations between treatment groups (FIG. 5).

[00512] Regions of Interest (ROIs) were determined in mice treated with exemplary Nectin-4 conjugate (Compound ID NO: C79), as compared to those administered an exemplary Nectin-4 conjugate (Compound ID NO: C67 at both 4 and 24 hours post-administration. Levels of kidney uptake and retention in mouse biodistribution showed minimal alterations between treatment groups (FIG. 12).

[00513] Regions of Interest (ROIs) were determined in mice treated with exemplary Nectin-4 conjugate with an addition of a Version 2 cleavable linker (Compound ID NO: C68), as compared to those administered an exemplary Nectin-4 conjugate (Compound ID NO: C67 at both 4 and 24 hours post-administration. Reduced levels of kidney retention in mouse biodistribution were observed (FIG. 13)

[00514] Regions of Interest (ROIs) were determined in mice treated with exemplary Nectin-4 conjugate with an addition of an albumin-binding motif (Compound ID NO: C69), as compared to those administered an exemplary Nectin-4 conjugate (Compound ID NO: C67) at both 4 and 24 hours post-administration. Reduced levels of kidney uptake and retention in mouse biodistribution were observed (FIG. 14).

EXAMPLE 13: MOUSE EFFICACY STUDIES INVOLVING ²²⁵Ac-LABELED TEST ARTICLES

[00515] *Animals:* Female athymic nude mice (6-8 weeks of age) were purchased from Charles River Laboratories and housed according to IACUC guidelines with ad libitum feeding. In vivo efficacy study experiments were performed in tumor bearing athymic nude mice. Tumor xenograft models were generated by inoculating mice subcutaneously with 3x10⁶ HT1376 cells, in 200 μ L (50:50 PBS/Matrigel) in the right shoulder or right flank. Tumors were monitored for a minimum of 14 days prior to group stratification and study initiation dates. Mice with tumor volumes between 150mm³ and 250mm³ were selected for study inclusion and randomized to treatment arms. An excess of 60% of required study mice were inoculated with tumor cells to ensure enough mice with appropriate tumor ranges were generated.

[00516] *Animal grouping and Treatment:* One day prior to treatment, ²²⁵Ac labelled test articles were prepared as described above at a specific activity of approx. 1 μ Ci/ μ g, with activity measurements made at secular equilibrium on a dose calibrator. On the day of treatment, dose measurements for a sample injected dose were measured and confirmed on a

gamma counter and corrected for decay. Indicated doses of vehicle or radiolabeled test article were prepared corresponding to the indicated administered dose levels per group. Doses were administered via tail vein injection while restrained and awake. Syringes with prepared doses were weighed pre and post injection and weights recorded.

[00517] *Animal monitoring:* Tumor volume (caliper measurement) and body weight measurements for enrolled mice were performed twice a week for an initial planned monitoring period of 8 weeks. More frequent gross observations of mice were performed.

[00518] *Humane endpoints:* Mice remained on study until they reached the end of the 8-week monitoring period or a number of pre-defined humane endpoints, including:

An increase in tumor volume size that exceeds >1500 mm³, or 20 mm in one dimension, or tumor becomes ulcerated or necrotic.

A decrease in body weight $\geq 20\%$ from maximum recorded weight.

Any signs of pain or distress (i.e., consistent hunched posture, rough coat, squinted eyes, slowed gait).

Tumors that compromise mobility or ability to eat or drink.

BCS score ≤ 2 .

[00519] Measurement of tumor volumes and body weight were compared in mice treated with exemplary test article (Compound ID NO: C116) at two doses (X nCi and 2X nCi) to mice treated with vehicle for a period of 8 weeks. Reduction in tumor volume in mouse efficacy studies were observed (FIG. 15). Body weight in mouse efficacy studies showed no changes between treatment groups (FIG. 16).

TABLES

Table 1. Radionuclide Incorporation For Exemplary Compounds

Compound	% radionuclide incorporation		
	Actinium-225	Indium-111	Lutetium-177
C9	>90	>90	>90
C14		94.5	>90
C48		98	
C52		97	>90
C45		99	
C43	>90	>90	>90
C1		95	
C3		98	
C5		95	
C67	>90	>90	>90
C64		94.7	
C68		94.1	
C69		98	

Table 2. Albumin Shift Assay For Exemplary Compounds Using [¹⁷⁷Lu]Lu- Radioligand and Bovine Serum Albumin

Compound	(-) BSA 1%		(+) BSA 1%		Ki ₁ /Ki ₂	% Free Fraction
	Ki ₁	(CI)	Ki ₂	(CI)		
C14	3.16	(2.506 to 3.978)	7.378	(5.864 to 9.265)	2.3	42.80%
C24	9.414	(7.761 to 11.41)	36.05	(29.59 to 43.85)	3.8	26.10%
C23	42.53	(35.65 to 50.75)	307.8	(252.3 to 378.0)	7.2	13.80%

Table 3. Exemplary Miniprotein Sequences and Structures

Compound # ¹	Target	N-terminal	Linker	Sequence ²	SEQ ID NO (referring to sequences in the "Sequence" column)	C-terminal
C1	B7H3	DOTA-PEG4		AEAK (Ac) YAK (Ac) EK (Ac) IAALSEIIWLPNLTHGQI (homo-Leucine) AFIAALNDPSSQSELLSEAK (Ac) K (Ac) LND SQAPK (Ac)	1	NH2

¹ Each compound is identified via a compound # (e.g., "C1", "C2", "C3", etc.) and refers to the combination of the N-terminal, Linker (if present), Sequence, and C-terminal.

² "Kme3" refers to trimethyllysine; "K(Ac)" and "Lys(Ac)" refer to acetylated lysine; "Cit" refers to citrulline

Compound # ¹	Target	N-terminal	Linker	Sequence ²	SEQ ID NO (referring to sequences in the "Sequence" column)	C-terminal
C2	B7H3	In:DOTA- PEG4		AEAK (Ac) YAK (Ac) EK (Ac) IAALSEIIWLPNLTHGQI (homo-Leucine) AFIAALNDDP SQSSELLSEAK (Ac) K (Ac) LND SQAPK (Ac)	1	NH2
C3	B7H3	DOTA- PEG4		AEAK (Ac) YAK (Ac) EK (Ac) IAALSEIIWLPNLTHGQI (homo-Leucine) AFIAALNDDP SQSSELLSEAKKLNDSQAPK (Ac)	2	NH2
C4	B7H3	In:DOTA- PEG4		AEAK (Ac) YAK (Ac) EK (Ac) IAALSEIIWLPNLTHGQI (homo-Leucine) AFIAALNDDP SQSSELLSEAKKLNDSQAPK (Ac)	2	NH2
C5	B7H3	DOTA- PEG4		AEAK (Ac) YAKEKIAALS EIIWLPNLTHGQI (homo-Leucine) AFIAALNDDP SQSSELLSEAKKLNDSQAPK (Ac)	3	NH2
C6	B7H3	In:DOTA- PEG4		AEAK (Ac) YAKEKIAALS EIIWLPNLTHGQI (homo-Leucine) AFIAALNDDP SQSSELLSEAKKLNDSQAPK (Ac)	3	NH2
C7	B7H3	Acetyl		AEAKYAKEKIAALSEII (3-(1-naphthyl)-L-alanine) LPNLTHGQIM AFIAALNDDP SQSSELLS EAKKLNDSQAPK	4	OH
C8	B7H3	Acetyl		AEAKYAKEKIAALSEIIW LPNLTHGQI (homo-Leucine) AFIAALNDDP SQSSELLSEAKKLNDSQAPK	5	OH
C9	B7H3, affibody	DOTA- PEG4		AEAKYAKEKIAALSEIIW LPNLTHGQI (norleucine) AFIAALNDDP SQSSELLSEAKKLNDSQAPK	6	OH
C10	B7H3	Acetyl		AEAKYAKEKIAALSEIIW LPNLTHGQI (norleucine) AFIAALNDDP SQSSELLSEAKKLNDSQAPK	6	OH
C11	B7H3	In:DOTA- PEG4		AEAKYAKEKIAALSEIIW LPNLTHGQI (norleucine) AFIAALNDDP SQSSELLSEAKKLNDSQAPK	6	OH
C12	Nectin-4	Ac-		CEYDEEFFTALKRLRGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	OH

Compound # ¹	Target	N-terminal	Linker	Sequence ²	SEQ ID NO (referring to sequences in the "Sequence" column)	C-terminal
C13		DOTA-PEG4		CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	OH
C14	Nectin-4	DOTA-PEG4-		CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C15	Nectin-4	Ac-		CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C16		In:DOTA-PEG4		CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C17	Nectin-4	DOTA-PEG4	rGF-Beta-Ala-PEG4 (SEQ ID NO: 82)	CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	OH
C18	Nectin-4	In-DOTA-PEG4	rGF-Beta-Ala-PEG4 (SEQ ID NO: 82)	CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	OH
C19	Nectin-4	DOTA-PEG4	D-Tyr-GF-β-Ala-PEG4 (SEQ ID NO: 83)	CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	OH
C20	Nectin-4	DOTA-PEG4	rGF-Lys(succinimide) (SEQ ID NO: 83)	CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	OH
C21	Nectin-4	DOTA-PEG4	rGF-Lys(succinimide)-PEG4 (SEQ ID NO: 83)	CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	OH
C22	Nectin-4	DOTA-PEG4	rGF-β-Ala (SEQ ID NO: 82)	CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	OH
C23	Nectin-4	C16-(γGlu)(dPEG2)(Lys(DOTA))PEG4		CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C24	Nectin-4	(C12alkyl)(gGlu)(dPEG2)(dPEG2)(Lys(DOTA))(PEG4)		CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C25	Nectin-4	In:DOTA-C12		CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C26	Nectin-4	DOTA-PEG4	γGF-Lys(succinimide)-PEG4 (SEQ ID NO: 85)	CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	OH

Compound # ¹	Target	N-terminal	Linker	Sequence ²	SEQ ID NO (referring to sequences in the "Sequence" column)	C-terminal
C27	Nectin-4	DOTA-PEG4	yGF-Lys(succinimide)-PEG12 (SEQ ID NO: 85)	CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	OH
C28	Nectin-4	DOTA-PEG4	yGF-β-Ala-PEG4-Gly10 (SEQ ID NOs: 83 and 86) ³	CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	OH
C29	Nectin-4	DOTA-C16-diacid		CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C30	Nectin-4	In:DOTA-C16-diacid		CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C31	Nectin-4	Acetyl		CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C32	Nectin-4	DOTA-(C18 diacid)lys-PEG4		CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C33	Nectin-4	In:DOTA-(C18 diacid)lys-PEG4		CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C34	B7H3	Eu:DOTA-PEG4		CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	OH
C35	Nectin-4	Eu:DOTA-PEG4		CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C36	B7H3	DOTA-PEG4	EV(Cit)-PEG4	AEAKYAKEKIAALSEIIW LPNLTHGQI (norleucine) AFIAALNDDPSQSSE LLSEAKKLND SQAPK	6	NH2
C37	B7H3	In:DOTA-PEG4	EV(Cit)-PEG4	AEAKYAKEKIAALSEIIW LPNLTHGQI (norleucine) AFIAALNDDPSQSSE LLSEAKKLND SQAPK	6	NH2
C38	B7H3	DOTA-PEG4	GG(Sar)-PEG4	AEAKYAKEKIAALSEIIW LPNLTHGQI (norleucine) AFIAALNDDPSQSSE LLSEAKKLND SQAPK	6	NH2
C39	B7H3	DOTA-PEG4	MVK-PEG4	AEAKYAKEKIAALSEIIW LPNLTHGQI (norleucine) AFIAALNDDPSQSSE LLSEAKKLND SQAPK	6	NH2
C40	B7H3	In:DOTA-PEG4	MVK-PEG4	AEAKYAKEKIAALSEIIW LPNLTHGQI (norleucine) AFIAALNDDPSQSSE LLSEAKKLND SQAPK	6	NH2

³ SEQ ID NO: 83 refers to "yGF-β-Ala" and SEQ ID NO: 86 refers to "Gly10"

Compound # ¹	Target	N-terminal	Linker	Sequence ²	SEQ ID NO (referring to sequences in the "Sequence" column)	C-terminal
C41	B7H3	DOTA-PEG4	MWK-PEG4	AEAKYAKEKIAALSEIIW LPNLTHGQI (norleucine) AFIAALNDDPSQSSE LLSEAKKLNDSSQAPK	6	NH2
C42	B7H3	In:DOTA-PEG4	MWK-PEG4	AEAKYAKEKIAALSEIIW LPNLTHGQI (norleucine) AFIAALNDDPSQSSE LLSEAKKLNDSSQAPK	6	NH2
C43	B7H3	DOTA-PEG4		YAKEKIAALSEIIWLPNL THGQI (homo-Leucine) AFIAALNDDPSQSSELLSEAKKLNDSSQAP	8	NH2
C44	B7H3	In:DOTA-PEG4		YAKEKIAALSEIIWLPNL THGQI (homo-Leucine) AFIAALNDDPSQSSELLSEAKKLNDSSQAP	8	NH2
C45	Nectin-4	DOTA-PEG4		CEYDEEFFTALKRLRGD ICYIYK(Ac)K(Ac)K(Ac) FK(Ac)K(Ac)VPK(Ac) LCIEEILDKLGC	9	NH2
C46	Nectin-4	FITC-PEG4		CEYDEEFFTALKRLRGD ICYIYK(Ac)K(Ac)K(Ac) FK(Ac)K(Ac)VPK(Ac) LCIEEILDKLGC	9	NH2
C47	Nectin-4	In:DOTA-PEG4		CEYDEEFFTALKRLRGD ICYIYK(Ac)K(Ac)K(Ac) FK(Ac)K(Ac)VPK(Ac) LCIEEILDKLGC	9	NH2
C48	Nectin-4	DOTA-PEG4		CEYDEEFFTALK(Ac)(Citrulline) L(Citrulline)GGDICYYIKKKFK KVPKLCIEEILDK(Ac)LC	10	NH2
C49	Nectin-4	FITC-PEG4		CEYDEEFFTALK(Ac)(Citrulline) L(Citrulline)GGDICYYIKKKFK KVPKLCIEEILDK(Ac)LC	10	NH2
C50	Nectin-4	In:DOTA-PEG4		CEYDEEFFTALK(Ac)(Citrulline) L(Citrulline)GGDICYYIKKKFK KVPKLCIEEILDK(Ac)LC	10	NH2
C51	Nectin-4	Acetyl		CEYDEEFFTALK(Ac)(Citrulline) L(Citrulline)GGDICYYIKKKFK KVPKLCIEEILDK(Ac)LC	10	NH2
C52	Nectin-4	DOTA-PEG4		CEYDEEFFTALK(Ac)(Citrulline) L(Citrulline)GGDICYYIK(Ac) K(Ac)K(Ac)FK(Ac)K(Ac)	11	NH2

Compound # ¹	Target	N-terminal	Linker	Sequence ²	SEQ ID NO (referring to sequences in the "Sequence" column)	C-terminal
				Ac) VPK (Ac) LCIEEILD K (Ac) LGC		
C53	Nectin-4	FITC-PEG4		CEYDEEFFTALK (Ac) (C itrulline) L (Citrul line) GGDICYIK (Ac) K (Ac) K (Ac) FK (Ac) K (Ac) VPK (Ac) LCIEEILD K (Ac) LGC	11	NH2
C54	Nectin-4	In:DOTA-PEG4		CEYDEEFFTALK (Ac) (C itrulline) L (Citrul line) GGDICYIK (Ac) K (Ac) K (Ac) FK (Ac) K (Ac) VPK (Ac) LCIEEILD K (Ac) LGC	11	NH2
C55	Nectin-4	DOTA-PEG4	EV(Cit)-PEG4	CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C56	Nectin-4	In:DOTA-PEG4	EV(Cit)-PEG4	CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C57	Nectin-4	In:DOTA-PEG4	GG(Sar)-PEG4	AEAKYAKEKIAALSEIIW LPNLTHGQI (norleuci ne) AFIAALNDPSQSSE LLSEAKKLNDSQAPK	6	NH2
C58	Nectin-4	DOTA-PEG4	GG(Sar)-PEG4	CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C59	Nectin-4	In:DOTA-PEG4	GG(Sar)-PEG4	CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C60	Nectin-4	DOTA-PEG4	MVK-PEG4	CEYDEEFFTALKRLRGGD ICYYIKK KFKKVPKLCIEEILDKLG C	12	NH2
C61	Nectin-4	In:DOTA-PEG4	MVK-PEG4	CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C62	Nectin-4	In:DOTA-PEG	MWK(succinic acid-PEG4)	CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C63	Nectin-4	DOTA-PEG	MWK(succinic acid-PEG4)	CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C64	Nectin-4	DOTA-PEG4	MWK-PEG4	CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C65	Nectin-4	In:DOTA-PEG4	MWK-PEG4	CEYDEEFFTALKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	7	NH2
C66	Nectin-4	Ac-		CEYDEEFFTALKRLRGGD ICYYIQASFQYLPGLCIE EILDNLGCS	13	OH
C67	Nectin-4	DOTA-PEG4-		CEYDEEFFTALKRLRGGD ICYYIQASFQYLPGLCIE EILDNLGCS	13	OH

Compound # ¹	Target	N-terminal	Linker	Sequence ²	SEQ ID NO (referring to sequences in the "Sequence" column)	C-terminal
C68	Nectin-4	DOTA-PEG4	yGF-β-Ala-PEG4-Gly10 (SEQ ID NOs: 83 and 86)	CEYDEEFFTALKRLRGGD ICYYIQASFQYLPGLCIE EILDNLGCS	13	OH
C69	Nectin-4	DOTA-(MPB)lys-PEG4-		CEYDEEFFTALKRLRGGD ICYYIQASFQYLPGLCIE EILDNLGCS	13	OH
C70	Nectin-4	In:DOTA-PEG4	yGF-β-Ala-PEG4-Gly10 (SEQ ID NOs: 83 and 86)	CEYDEEFFTALKRLRGGD ICYYIQASFQYLPGLCIE EILDNLGCS	13	OH
C71	Nectin-4	In:DOTA-(MPB)lys-PEG4		CEYDEEFFTALKRLRGGD ICYYIQASFQYLPGLCIE EILDNLGCS	13	OH
C72	Nectin-4	Ac-		CEYDEEFFTALKRLRGGD ICYYIKKKFAKVPGLCIE EILDKLGC	14	OH
C73	Nectin-4	DOTA-PEG4	yGF-Beta-Ala-PEG4-Gly10 (SEQ ID NOs: 83 and 86)	CEYDEEFFTALKRLRGGD ICYYIKKKFAKVPGLCIE EILDKLGC	14	NH2
C74	Nectin-4	In:DOTA-PEG4	yGF-Beta-Ala-PEG4-Gly10 (SEQ ID NOs: 83 and 86)	CEYDEEFFTALKRLRGGD ICYYIKKKFAKVPGLCIE EILDKLGC	14	OH
C75	Nectin-4	Ac-		CEYDEEFFTALKRLRGGD ICYYIKKKFDYLPKLCIE EILDNLGC	15	OH
C76	Nectin-4	DOTA-PEG4	yGF-Beta-Ala-PEG4-Gly10 (SEQ ID NOs: 83 and 86)	CEYDEEFFTALKRLRGGD ICYYIKKKFDYLPKLCIE EILDNLGC	15	NH2
C77	Nectin-4	In:DOTA-PEG4	yGF-Beta-Ala-PEG4-Gly10 (SEQ ID NOs: 83 and 86)	CEYDEEFFTALKRLRGGD ICYYIKKKFDYLPKLCIE EILDNLGC	15	OH
C78	Nectin-4	Acetyl		CEYDEEFFTELERLKGGD ICYYIKKKFDKVPDLCK EIRDKLGC	16	NH2
C79	Nectin-4	Acetyl		CEYDEEFFTELERLKGGD ICYYIKKKFDKVPRLCK EIRDKLGC	17	NH2
C80	Nectin-4	Acetyl		CEYDEEFFTELERLNGGD ICYYIKKKFDKVPRLCIN EILDKLGC	18	NH2
C81	Nectin-4	Acetyl		CEYDEEFFTELKRLRGGD ICYYIKKKFKKVPDLCK RIRDKLGC	19	NH2

Compound # ¹	Target	N-terminal	Linker	Sequence ²	SEQ ID NO (referring to sequences in the "Sequence" column)	C-terminal
C82	Nectin-4	Acetyl		CEYDEEFFTELKRLRGGK ICYYIKKKFKKVPKLCIE EILDKLGC	20	NH2
C83	Nectin-4	Acetyl		CEYDEEFFTGLERLAGGD ICYYIKKKFKKVPKLCIE EILDKLGC	21	NH2
C84	Nectin-4	Acetyl		CEYDEEFFTGLERLNGGD ICYYIKKKFKKVPKLCIE EILDKLGC	22	NH2
C85	Nectin-4	Acetyl		CEYDEEFFTKLRLRGGK ICKYIQASFQYLPDLCIE EILDNLGCK	23	NH2
C86	Nectin-4	Acetyl		CEYDEEFFTKLRLRGGK ICKYIQASFQYLPGLCIE EILDNLGCK	24	OH
C87	Nectin-4	Acetyl		CEYDEEFFTKLRLRGGK ICKYIQASFQYLPGLCIE EILDNLGCS	25	OH
C88	Nectin-4	Acetyl		CEYDEEFFTKLRLRGGK ICYYIKKKFKKVPKLCIE EILDKLGC	26	NH2
C89	Nectin-4	Acetyl		CEYDEEFFTKLRLRGGK ICYYIQASFQYLPGLCIE EILDNLGCS	27	OH
C90	Nectin-4	Acetyl		CEYDEEFFTKLRLYGGK ICKYIQAKFQYLPDLCIE EILDNLGCK	28	NH2
C91	Nectin-4	Acetyl		CEYKEEFFTELKRLRGGD ICYYIKKKFKKVPDLCIE EILDKLGC	29	NH2
C92	Nectin-4	Acetyl		CEYKEEFFTELKRLRGGD ICYYIKKKFKKVPDLCIE EIRDKLGC	30	NH2
C93	Nectin-4	Acetyl		CEYKEEFFTELKRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	31	NH2
C94	Nectin-4	Acetyl		CEYKEEFFTELKRLRGGK ICYYIKKKFKKVPDLCIE EILDKLGC	32	NH2
C95	Nectin-4	Acetyl		CEYKEEFFTELKRLRGGK ICYYIKKKFKKVPKLCIE EILDKLGC	33	NH2
C96	Nectin-4	Acetyl		CEYKEEFFTELKRLYGGD ICYYIKKKFKKVPDLCIE EILDKLGC	34	NH2
C97	Nectin-4	Acetyl		CEYKEEFFTGLKRLRGGD ICYYIKKKFKKVPDLCIE EILDKLGC	35	NH2
C98	Nectin-4	Acetyl		CEYKEEFFTKLRLRGGD ICYYIKKKFKKVPDLCIE EILDKLGC	36	NH2
C99	Nectin-4	Acetyl		CEYKEEFFTKLRLRGGD ICYYIKKKFKKVPDLCIE EIRDKLGC	37	NH2

Compound # ¹	Target	N-terminal	Linker	Sequence ²	SEQ ID NO (referring to sequences in the "Sequence" column)	C-terminal
C100	Nectin-4	Acetyl		CEYKEEFFTKLRLRGGD ICYYIKKKFKKVPKLCIE EILDKLGC	38	NH2
C101	Nectin-4	Acetyl		CEYKEEFFTKLRLRGGD ICYYIQASFQYLPGLCIE EILDNLGCS	39	OH
C102	Nectin-4	Acetyl		CEYKEEFFTKLRLRGGK ICYYIKKKFKKVPKLCIE EIRDKLGC	40	NH2
C103	Nectin-4	Acetyl		CEYKEEFFTKLRLRGGK ICYYIKKKFKKVLDCIK RIRDKLGC	41	NH2
C104	Nectin-4	Acetyl		CEYKEEFFTKLRLRGGK ICYYIKKKFKKVPDLCK RIRDKLGC	42	NH2
C105	Nectin-4	Acetyl		CEYKEEFFTKLRLRGGK ICYYIKKKFKKVPKLCIE EILDKLGC	43	NH2
C106	Nectin-4	Acetyl		CEYKEEFFTKLRLRGGK ICYYIQASFQYLPGLCIE EILDNLGCS	44	OH
C107	Nectin-4	Acetyl		CEYKEEFFTKLRLRLGK ICYYIKKKFKKVPDLCK RIRDKLGC	45	NH2
C108	Nectin-4	Acetyl		CEYKEEFFTKLRLYGGD ICYYIKKKFKKVPDLCK EILDKLGC	46	NH2
C109	Nectin-4	DOTA-PEG4		CEYDEEFFTALARLRGDD ICSYIQAKFQYLPGLCIE EILDNLGCS	47	Acid
C110	Nectin-4	DOTA-PEG4		CEYDEQFFTALKRLRGAD ICSYIQASFQYLPGLCIE EILDNLGCS	48	Acid
C111	Nectin-4	DOTA-PEG4		CDYDEQFFTALKRLRGGD ICEYIQASFQYLPGLCIE EILDNLGCS	49	Acid
C112	Nectin-4	DOTA-PEG4		CEYDEEFFTALARLRGGD ICQYIQAKFQYLPQCIE EILDNLGCS	50	Acid
C113	Nectin-4	DOTA-PEG4		CEYDEQFFTALKRLRGDD ICDYIQASFQYLPGLCIE EILDNLGCS	51	Acid
C114	Nectin-4	DOTA-PEG4		CDYDEQFFTALKRLRGAD ICEYIQASFQYLPGECK EILDNLGCS	52	Acid
C115	Nectin-4	DOTA-PEG4		CDYDEQFFTALARLRGAD ICEYIQASFQYLPGECK EILDNLGCS	53	Acid
C116	Nectin-4	DOTA-PEG4		CEYDEEFFTALARLRGGD ICQYIQA (Kme3) FQYLP ALCIEEILDNLGCS	54	Acid
C117	Nectin-4	DOTA-PEG4		CDYDEQFFTALA (Cit) L RGGDICEYIQA (Kme3) F QYLPGLCIEEILDNLGCS	55	Acid

Compound # ¹	Target	N-terminal	Linker	Sequence ²	SEQ ID NO (referring to sequences in the "Sequence" column)	C-terminal
C118	Nectin-4	DOTA-PEG4		CEYDEEFFTALKRLRGGD ICYYIKKKFDYLPKLCIE EILDNLGC	15	NH2
C119	Nectin-4	Acetyl		CEYDEEFFTALKRLRGGD ICYYIKKKFQYLPKLCIE EILDNLGC	56	OH
C120	Nectin-4	Acetyl		CEYDEQFFTALKRLRGGD ICYYISAQFNTLPDLCIE EILENLGC	57	OH
C121	Nectin-4	Acetyl		CEYDEEFFTALKRLRGGD ICYYIQAFNYLPGICIE EILDNLGC	58	OH
C122	Nectin-4	DOTA-PEG4		CEYDEEFFTALKRLRGGD ICYYIQAKFQYLPKLCIE EILDNLGCS	59	OH
C123	Nectin-4	DOTA-PEG4		CEYDEQFFTALKRLRGGD ICYYIQASFNYLPGICIE EILDNLGCS	60	OH
C124	Nectin-4	DOTA-PEG4		CEYDEEFFTALKRLRGGD ICYYIQAKFQYLPGLCIE EILDNLGCS	61	OH
C125	Nectin-4	DOTA-PEG4		CEYDEQFFTALARLRGGD ICYYIQEQFATVPGLCIE EILDQLGCS	62	OH
C126	Nectin-4	DOTA-PEG4		CEYDEEFFTALSRLRGGD ICYYIQAFQYLPGLCIE EILDNLGCS	63	OH
C127	Nectin-4	DOTA-PEG4		CEYDEQFFTALSSLRGGD ICYYIQEQFANVPGLCIE EILDNLGCS	64	OH
C128		Biotin-PEG4		CEYDEEFFTAL(Lys(Ac)) (Citruilline)L(Citruilline) GGDICYYIKKKFKKVPKLCIE EILD(Lys(Ac))LGC	65	NH2
C129		Biotin-PEG4		CEYDEEFFTALLys(Ac) CitruillineLCitruilline GGDICYYILys(Ac) Lys(Ac)Lys(Ac)FLys(Ac) Lys(Ac)VPLys(Ac)LCIE EILD(Lys(Ac))LGC	66	NH2
C130		Biotin-PEG4		CEYDEEFFTALKRLRGGD ICYYI(Lys(Ac))(Lys(Ac)) (Lys(Ac))F(Lys(Ac)) (Lys(Ac))VP(Lys(Ac)) LCIEEILDKLGC	67	NH2
C131		Acetyl		CEYDEEFFTALARLRGGD ICYYIQAKFQYLPGLCIE EILDNLGCS	68	OH
C132		Biotin-PEG4		CEYDEEFFTALARLRGGD ICYYIQAKFQYLPGLCIE EILDNLGCS	68	OH

Compound # ¹	Target	N-terminal	Linker	Sequence ²	SEQ ID NO (referring to sequences in the "Sequence" column)	C-terminal
C133		In:DOTA- PEG4		CEYDEEFFTALARLRGGD ICYYIQAKFQYLPGLCIE EILDNLGCS	68	OH
C134		Biotin- PEG4		CEYDEEFFTALARLRGDD ICSYIQAKFQYLPGLCIE EILDNLGCS	47	OH
C135		Biotin- PEG4		CEYDEEFFTALARLRGGD ICQYIQAKFQYLPQCIE EILDNLGCS	50	OH
C136		Biotin- PEG4		CEYDEQFFTALKRLRGAD ICSYIQASFQYLPGLCIE EILDNLGCS	48	OH
C137		Biotin- PEG4		CDYDEQFFTALKRLRGDD ICEYIQASFQYLPGLCIE EILDNLGCS	49	OH
C138		Biotin- PEG4		CEYDEQFFTALKRLRGDD ICDYIQASFQYLPGLCIE EILDNLGCS	51	OH
C139		Biotin- PEG4		CDYDEQFFTALKRLRGAD ICEYIQASFQYLPQCIE EILDNLGCS	52	OH

Table 4A. Exemplary B7H3 Mini-protein Sequences and Structures

Compound ID NO ⁴	DELFI A Ki (M)	DELFI A KD (M)	SPR KD (M)	N-Term.	Linker	Sequence	SEQ ID NO (referring to sequences in the "Sequence" column)	C-Ter m.	Calc. Mass ⁵	Obs. Mass ⁶
C7	766E-09±94.4E-09		1.06E-05	Acetyl		AEAKYAKEKIAALSEII (3-(1-naphthyl)-L-alanine) LPNLTHGQIMAFIAALNDDPSQ SELLSEAKKLNDSQAPK	4	OH	1591.82	1593.1
C10	1.65E-08		5.84E-09	Acetyl		AEAKYAKEKIAALSEIIWLPNL THGQI (norleucine)AFIAA LNDDPSQSS ELLSEAKKLNDSQAPK	6	OH	1584.55	1585.7
C9				DOTA-PEG4		AEAKYAKEKIAALSEIIWLPNL THGQI (norleucine)AFIAA LNDD PSQSS ELLSEAKKLNDSQAPK	6	OH	1732.4	1733.2
C34		3.27E-09		Eu:DOTA-PEG4		CEYDEEFFTALKRLRGDICY IKKKFKKVPKLCIEEILDKLGC	7	OH	1507.72	1508.7

⁴ Each compound is identified via a compound # (e.g., "C1", "C2", "C3", etc.) and refers to the combination of the N-terminal, Linker (if present), Sequence, and C-terminal.
⁵ Calculated Mass: (M+4/4) of parent MW unless noted as (M+5/5) in the cell
⁶ Observed Mass: (M+4/4) of parent MW unless noted as (M+5/5) in the cell

Compound ID NO ⁴	DELFI A Ki (M)	DELFI A KD (M)	SPR KD (M)	N-Term.	Linker	Sequence	SEQ ID NO (referring to sequences in the "Sequence" column)	C-Ter m.	Calc. Mass ⁵	Obs. Mass ⁶
C11			5.28E-09	In:DOTA-PEG4		AEAKYAKETIAALSEIWLPLNL THGQI (norleucine)AFIAA LNDD PSQ SSELLSEAKKLND SQAPK	6	OH	1760.4 1	1761.3
C8				Acetyl		AEAKYAKETIAALSEIWLPLNL THGQI (homo-Leucine) AFIAALNDDPSQSSELLSEAKK LND SQAPK	5	OH	1588.0 5	1589.1
C43			4.28E-09	DOTA-PEG4		YAKEKTAALSEIWLPLNLTHGQ I (homo-Leucine)AFIAALN DDPSQSSELLSEAKKLND SQAP	8	NH2	1603.8 2	1605.4
C1			>5.00E-07	DOTA-PEG4		AEAK (Ac) YAK (Ac) EK (Ac) I AALSEIWLPLNLTHGQI (homo - Leucine)AFIAALNDDPSQSS ELLSEAK (Ac) K (Ac) LND SQA PK (Ac)	1	NH2	1798.7 8	1799.7

Compound ID NO ⁴	DELFI A Ki (M)	DELFI A KD (M)	SPR KD (M)	N-Term.	Linker	Sequence	SEQ ID NO (referring to sequences in the "Sequence" column)	C-Ter m.	Calc. Mass ⁵	Obs. Mass ⁶
C5			>1.00E-07	DOTA-PEG4		AEAK (Ac) YAKEKIALALSEIIWLPNLTHGQI (homo-Leucine) AFTAAINDDPSQSSELLSEAKKLNDSQAPK (Ac)	3	NH2	1756.7	1757.8
C3			1.10E-08	DOTA-PEG4		AEAK (Ac) YAK (Ac) EK (Ac) I AALSEIIWLPLNLTHGQI (homo-Leucine) AFTAAALNDDPSQSSELLSEAKKLNDSQAPK (Ac)	2	NH2	1777.76	1779.8
C44				In:DOTA-PEG4		YAKEKIALALSEIIWLPLNLTHGQI (homo-Leucine) AFTAAINDDPSQSSELLSEAKKLNDSQAP	8	NH2	1631.76	1633.1
C2				In:DOTA-PEG4		AEAK (Ac) YAK (Ac) EK (Ac) I AALSEIIWLPLNLTHGQI (homo-Leucine) AFTAAALNDDPSQSSELLSEAK (Ac) K (Ac) LNDSSQA PK (Ac)	1	NH2	1826.7	1828.2

Compound ID NO ⁴	DELFI A Ki (M)	DELFI A KD (M)	SPR KD (M)	N-Term.	Linker	Sequence	SEQ ID NO (referring to sequences in the "Sequence" column)	C-Ter m.	Calc. Mass ⁵	Obs. Mass ⁶
C6				In:DOTA-PEG4		AEAK (Ac) YAKEKIAALSEIIWLPNLTHGQI (homo-Leucine) AFTAAALNDDPSQSS ELLSEAKKLNDSQAPK (Ac)	3	NH2	1784.69	1786
C4				In:DOTA-PEG4		AEAK (Ac) YAK (Ac) EK (Ac) I AALSEIIWLPNLTHGQI (homo-Leucine) AFTAAALNDDPSQSS ELLSEAKKLNDSQAPK (Ac)	2	NH2	1805.71	1807.1
C41				DOTA-PEG4	MWK-PEG4	AEAKYAKEKIAALSEIIWLPNLTH GQI (norleucine) AFTAAALNDDPSQSS ELLSEAKKLNDSQAPK	6	NH2	1524.74 (M+5/5)	1526.2 (M+5/5)
C39				DOTA-PEG4	MVK-PEG4	AEAKYAKEKIAALSEIIWLPNLTHGQI (norleucine) AFTAAALNDDPSQSS ELLSEAKKLNDSQAPK	6	NH2	1883.9	1885.8
C36				DOTA-PEG4	EV (Cit) -PEG4	AEAKYAKEKIAALSEIIWLPNLTHGQI (norleucine) AFTAAALNDDPSQSS ELLSEAKKLNDSQAPK	6	NH2	1890.64	1891.4

Compound ID NO ⁴	DELFI A Ki (M)	DELFI A KD (M)	SPR KD (M)	N-Term.	Linker	Sequence	SEQ ID NO (referring to sequences in the "Sequence" column)	C-Ter m.	Calc. Mass ⁵	Obs. Mass ⁶
C38				DOTA-PEG4	GG (Sar) -PEG4	AEAKYAKEKIAALSEIIWLPNL THGQI (norleucine)AFIAA LNDDPSQSSELLSEAKKLNDSQ APK	6	NH2	1840.5	1841.8
C42	13.6 E-09		5.57E-09	In:DOTA-PEG4	MWK-PEG4	AEAKYAKEKIAALSEIIWLPNL THGQI (norleucine)A FIAALNDDPSQSSELLSEAKKL NDSQAPK	6	NH2	1933.6 3	1935.1
C40	13.7 E-09		8.56E-09	In:DOTA-PEG4	MVK-PEG4	AEAKYAKEKIAALSEIIWLPNL THGQI (norleucine)A FIAALNDDPSQSSELLSEAKKL NDSQAPK	6	NH2	1911.8 6	1913.6
C37	13.1 E-09		2.74E-09	In:DOTA-PEG4	EV (Cit) -PEG4	AEAKYAKEKIAALSEIIWLPNL THGQ I (norleucine)A FIAALNDDPSQSSELLSEAKKL NDSQAPK	6	NH2	1918.5 9	1919.8

Table 4B. Exemplary Nectin-4 Miniprotein Sequences and Structures

Compound ID	DELFI A Ki (M)	DELFI IA KD (M)	SPR KD (M)	N-Term.	Linker	Sequence	SEQ ID NO (referring to sequences in the "Sequence" column)	C-Term.	Calc. Mass ⁸	Obs. Mass ⁹
C14				DOTA-PEG4		CEYDEEFFTALKRLRGGDICYI IKKKFKKVPKLCIEEILDKLGC	7	NH2	1470.24	1471.6
C15	11.8E-09±6.65E-09		6.13E-10	Acetyl		CEYDEEFFTALKRLRGGDICYI IKKKFKKVPKLCIEEILDKLGC	7	NH2	1322.32	1323.4
C25	21.8E-09 ± 0.84E-09			In:DOTA-C12		CEYDEEFFTALKRLRGGDICYI IKKKFKKVPKLCIEEILDKLGC	7	NH2		
C30	38.4E-09			In:DOTA-C16-diacid		CEYDEEFFTALKRLRGGDICYI IKKKFKKVPKLCIEEILDKLGC	7	NH2		

⁷ Each compound is identified via a compound # (e.g., "C1", "C2", "C3", etc.) and refers to the combination of the N-terminal, Linker (if present), Sequence, and C-terminal.
⁸ Calculated Mass: (M+4/4) of parent MW
⁹ Observed Mass: (M+4/4) of parent MW

Compound ID NO ⁷	DELFI A Ki (M)	DELFI A Ki (M)	SPR KD (M)	N-Term.	Linker	Sequence	SEQ ID NO (referring to sequences in the "Sequence" column)	C-Term.	Calc. Mass ⁸	Obs. Mass ⁹
C33	20.6E-09			In(DOTA-(C18 diacid)lys-PEG4		CEYDEEFFTALKRLRGGDICYY IKKKFKKVPKLCIEEILDKLGC	7	NH2		
C35		5.22E-09		Eut(DOTA-PEG4		CEYDEEFFTALKRLRGGDICYY IKKKFKKVPKLCIEEILDKLGC	7	NH2	1507.4 7	1508.7
C48				DOTA-PEG4		CEYDEEFFTAL(Lys(Ac)) (CitruLLine) L (CitruLLine) GGDI CY Y I K K K F K KVPKLCIEEILD Lys (Ac) LGC	10	NH2	1491.7 5	1492.3
C52				DOTA-PEG4		CEYDEEFFTAL Lys (Ac) (CitruLLine) L (CitruLLine) G G D I C Y Y I L y s (A c) L y s (A c) L y s (A c) F L y s (A c) L y s (A c) V P L y s (A c) L C I E E I L D L y s (A c) L G C	11	NH2	1554.8	1556.5
C45				DOTA-PEG4		CEYDEEFFTALKRLRGGDICYY I L y s (A c) L y s (A c) L y s (A c) F L y s (A c)	9	NH2	1533.2 9	1534.2

Compound ID NO ⁷	DELFI A Ki (M)	DELFI A Ki (M)	SPR KD (M)	N-Term.	Linker	Sequence	SEQ ID NO (referring to sequences in the "Sequence" column)	C-Term.	Calc. Mass ⁸	Obs. Mass ⁹
						Lys (Ac) VPLys (Ac) LCIEEI LDKLG				
C50			1.65E-08	In:DOTA-PEG4		CEYDEEFFTALys (Ac) (Cit rulline) L (Citruiline) G GDICYI KKK FKK VPKLCIEEIL D Lys (Ac) LGC	10	NH2	1519.6 9	1520.4
C54			> 1.00E-06	In:DOTA-PEG4		CEYDEEFFTALys (Ac) (Cit rulline) L (Citruiline) G GDICYI Lys (Ac) Lys (Ac) Lys (Ac) FLys (Ac) Lys (Ac) VPLys (Ac) LCIEEI LDLys (Ac) LGC	11	NH2	1582.7	1584
C47			8.07E-09	In:DOTA-PEG4		CEYDEEFFTALKRLRGDCYY I Lys (Ac) Lys (Ac) Lys (Ac) F Lys (Ac) Lys (Ac) VPLys (Ac) LCIEEI LDKLG	9	NH2	1561.2 4	1562.3

Compound ID NO ⁷	DELFI A Ki (M)	DELFI IA KD (M)	SPR KD (M)	N-Term.	Linker	Sequence	SEQ ID NO (referring to sequences in the "Sequence" column)	C- Term.	Calc. Mass ⁸	Obs. Mass ⁹
C64				DOTA-PEG4	MWK- PEG4	CEYDEEFFTALK PLRGDICYIKKKKVPKL CIEEILDKLGC	7	NH2	1643.4 5	1645
C60				DOTA-PEG4	MWK- PEG4	CEYDEEFFTALKRLR GGDICYIKK KKKVPKL CIEEILDKLGC	12	NH2	1621.6 8	1623
C55				DOTA-PEG4	EV (Cit) -PEG4	CEYDEEFFTALKRLRG GDICYIKKKKVPKL CIEEILDKLGC	7	NH2	1628.4	1629.7
C58				DOTA-PEG4	GG (Sar) -PEG4	CEYDEEFFTALKRLRGDI CYYIKKKKVPK LCIEEILDKLGC	7	NH2	1578.3 5	1579.7
C65	1.22E- 08		7.08E- 10	In:DOTA-PEG4	MWK- PEG4	CEYDEEFFTALKRLRGDICYI IKKKKKVPK LCIEEILDKLGC	7	NH2	1671.6 5	1672.9

Compound ID NO ⁷	DELFI A Ki (M)	DELFI IA KD (M)	SPR KD (M)	N-Term.	Linker	Sequence	SEQ ID NO (referring to sequences in the "Sequence" column)	C-Term.	Calc. Mass ⁸	Obs. Mass ⁹
C61	1.82E-08		7.00E-10	In:DOTA-PEG4	MVK-PEG4	CEYDEEFFTALKRLRGGDICYI IKKFKKKVPK LCIEEILDKLGC	7	NH2	1649.8 8	1650.5
C56	1.38E-08		9.44E-10	In:DOTA-PEG4	EV (Cit) -PEG4	CEYDEEFFTALKRLRGGDICYI IKKFKKKVPK LCIEEILDKLGC	7	NH2	1656.6 1	1657.4
C59	1.19E-08		6.33E-10	In:DOTA-PEG4	GG (Sar) -PEG4	CEYDEEFFTALKRLRGGDICYI IKKFKKKVPK LCIEEILDKLGC	7	NH2	1603.0 4	1607.5
C57	10.0 E -09		2.69E-09	In:DOTA-PEG4	GG (Sar) -PEG4	AEAKYAKEKIAALSEIILWLPNL THGQ I (norleucine) AFTALNDD PSQSSELLSEAKKLNDSPAK	6	NH2	1868.5 3	1869.8
C63				DOTA-PEG	MWK (suc cinic acid) -PEG4	CEYDEEFFTALKRLRGGDICYI IKK KFKKVPKLCIEEILDKLGC	7	NH2	1668.4 7	1669.2

Compound ID	DELFI A Ki (M)	DELFI IA KD (M)	SPR KD (M)	N-Term.	Linker	Sequence	SEQ ID NO (referring to sequences in the "Sequence" column)	C-Term.	Calc. Mass ⁸	Obs. Mass ⁹
C62				In:DOTA-PEG	MWK(suc cinic acid)-PEG4	CEYDEEFFTALKRLRGGDICYI IKKK FKKVPKLCIEEILDKLG	7	NH2	1697.18	1696.09
C12	17.5E-09		2.86E-10	Ac-		CEYDEEFFTALKRLRGGDICYI IKKKFKKVPKLCIEEILDKLG	7	OH		
C72	4.62E-09		7.06E-10	Ac-		CEYDEEFFTALKRLRGGDICYI IKKKFAKVPGLCIEEILDKLG	14	OH		
C75	2.48E-09 +/- 0.54E-09		3.32E-10	Ac-		CEYDEEFFTALKRLRGGDICYI IKKKFDYLPKLCIEEILDNLG	15	OH		
C47			7.14E-09	In:DOTA-PEG4		CEYDEEFFTALKRLRGGDICYI IK(Ac)K(Ac)K(Ac)FK(Ac) K(Ac)VPK(Ac)LCIEEILDKL GC	9	NH2		

Compound ID	DELFI A Ki (M)	DELFI IA KD (M)	SPR KD (M)	N-Term.	Linker	Sequence	SEQ ID NO (referring to sequences in the "Sequence" column)	C-Term.	Calc. Mass ⁸	Obs. Mass ⁹
C97	3.11E-06 +/- 0.37E-06			Acetyl		CEYKEEFFFTGLKRLRGGDICYY IKKKFKKVPDLCIEEILDKLG	35	NH2		
C80	>100E-06			Acetyl		CEYDEEFFTELERLNGGDICYY IKKKFDKVPRLCINEILDKLG	18	NH2		
C79	>100E-06			Acetyl		CEYDEEFFTELERLKGGDICYY IKKKFDKVPRLCIKEIRDKLG	17	NH2		
C91	13.3E-06			Acetyl		CEYKEEFFFTGLKRLRGGDICYY IKKKFKKVPDLCIEEILDKLG	29	NH2		
C96	>100E-06			Acetyl		CEYKEEFFFTGLKRLRGGDICYY IKKKFKKVPDLCIEEILDKLG	34	NH2		

Compound ID NO ⁷	DELFI A Ki (M)	DELFI IA KD (M)	SPR KD (M)	N-Term.	Linker	Sequence	SEQ ID NO (referring to sequences in the "Sequence" column)	C-Term.	Calc. Mass ⁸	Obs. Mass ⁹
C78	>100E-06			Acetyl		CEYDEEFFTELERLKGGDICYY IKKKFDKVPDLICIKEIRDKLGC	16	NH2		
C70	2.94E-09			In:DOTA-PEG4	YGF-β-Ala-PEG4-Gly10 (SEQ ID NOS: 83 and 86)	CEYDEEFFTALKRLRGGDICYY IQASFQYLPGLCIEEILDNLGC	13	OH		
C66	16.3E-09 ± 10.9E-09			Ac-		CEYDEEFFTALKRLRGGDICYY IQASFQYLPGLCIEEILDNLGC	13	OH		
C128	2.14E-06			Biotin-PEG4		CEYDEEFFTAL(Lys(Ac))(Citru itrulline)L(Citrulline) GGDICYYIKKKFKKVPKLCIE EILD(Lys(Ac))LGC	65	NH2	1451. 17	1453. 2

Compound ID NO ⁷	DELFI A Ki (M)	DELFI A KD (M)	SPR KD (M)	N-Term.	Linker	Sequence	SEQ ID NO (referring to sequences in the "Sequence" column)	C-Term.	Calc. Mass ⁸	Obs. Mass ⁹
C129	>10E-06			Biotin-PEG4		CEYDEEFFTALys(Ac)Citru ullineLCitrullineGGDIC YYILys(Ac)Lys(Ac)Lys(A c)FLys(Ac)Lys(Ac)VPLys (Ac)LCIEEILDlys(Ac)LGC	66	NH2	1514. 7	1516
C130	48.9E-09			Biotin-PEG4		CEYDEEFFTALKRLRGGDICYY I(Lys(Ac))(Lys(Ac))(Ly s(Ac))F(Lys(Ac))(Lys(A c))VP(Lys(Ac))LCIEEILD KLGC	67	NH2	1494. 3	1493. 27

Table 5. Albumin Binding Affinity Measurements for Exemplary Mini-proteins

Compound ID NO	SPR K_D (M)
C25	3.21E-05
C30	3.69E-05
C15	3.75E-05

Table 6: Exemplary Target Protein Amino Acid Sequences

Target Protein (Uniprot Acc. No.)	Amino Acid Sequence	SEQ ID NO.
Human B7H3 (Q5ZPR3)	MLRRRGS PGMGVHVGAAALGALWFCLTGALEVQVPEDPVVALVGT DATLCCSFSPGPGFSLAQLNLIWQLTDTKQLVHSFAEGDQGS YANRTALFPDLLAQGNASRLRQVRVADEGSFTCFVSIIRDFGSA AVSLQVAAPYSKPSMTLEPNKDLRPGDTVTITCSSYQGYPEAEV FWQDGGQVPLTGNVTTSMANEQGLFDVHSILRVVLGANGTYSC LVRNPVLQQDAHSVTITPQRSPTGAVEVQVPEDPVVALVGTDA TLRCSFSPGPGFSLAQLNLIWQLTDTKQLVHSFTEGRDQGSAYA NRTALFPDLLAQGNASRLRQVRVADEGSFTCFVSIIRDFGSAAV SLQVAAPYSKPSMTLEPNKDLRPGDTVTITCSSYRGYPEAEVFW QDGGQVPLTGNVTTSMANEQGLFDVHSILRVVLGANGTYSC LVRNPVLQQDAHGSVTITGQPMTPFPPEALWVTVGLSVCLIALV ALAFVCWRKIKQSCEENAGAEDQDGESEKSKTALQPLKHSKSD KEDDGQEI	69
Murine B7H3 (Q8VE98)	MLRGWGGPSVGVVVRTALGVLCCLTGALEVQVSEDPVVALVDT DATLRCSFSPGPGFSLAQLNLIWQLTDTKQLVHSFTEGRDQGS YANRTALFPDLLVQGNASRLRQVRVTDEGSYTCFVSIQDFDSA AVSLQVAAPYSKPSMTLEPNKDLRPGNMVTITCSSYQGYPEAEV FWKDGQGVPLTGNVTTSMANERGLFDVHSILRVVLGANGTYSC LVRNPVLQQDAHGSVTITGQPLTFPPEALWVTVGLSVCLVLLV ALAFVCWRKIKQSCEENAGAEDQDGDGEGSKTALRPLKPSNK EDDGQEI	70
Human TROP2 (P09758)	MARGPGLAPPPLRLPLLLLVLAAVTGHTAAQDNCTCPTNKMTVC SPDGPGGRCQCRAIGSGMAVDCSTLTSKCLLLKARMSAPKNART LVRPSEHALVDNDGLYDPCDPEGFRKARQCNQTSVCWCVNSVG VRRTDKGLSLRCDELVRTHHILIDLHRPTAGAFNHSDLDLAE RLFRERYRLHPKFVAHVHVEPTQIELRQNTSQKAAGDVIDG AAYYFERDIKGESLFQGRGGLDLVRGEPLOVERTLIYYLDEIP PKFSMKRLTAGLIAVIVVVVALVAGMAVLVITNRRKSGKYKKV EIKELGELRKEPSL	73
Murine TROP2 (Q8BGV3)	MARGLDLAPLLLLLAMATRFCTAQSNCCTCPTNKMTVCNTNGPG GVCQCRAMGSQVLVDCSTLTSKCLLLKARMSARKSGRSLVMPSE HAILDNDGLYDPECDDKGRFKARQCNQTSVCWCVNSVGVRRTDK GDQSLRCDEVVRTHHILIELRHRPTDRAFNHSDLDSELRRLFQE RYKLHPSFLSAVHYEPTIQLIELRQNASQKGLRDVDIADAAAYF ERDIKGESLFMGRRLDVQVRGEP LHVERTLIYYLDEKPPQFSM KRLTAGVIAVIAVSVAVVAGVVVLTNRKSGKYKKVELKEL GEMRSEPSL	74

Target Protein (Uniprot Acc. No.)	Amino Acid Sequence	SEQ ID NO.
Human FAP (Q12884)	MKTWVKIVFGVATSAVLALLVMCIVLRPSRVHNSEENTMRALTL KDI LN GTFSYKTF FPNWISGQEYLHQ SADNNIVLYNIETGQSYT ILSNRTMKS VNASNYGLSPDRQFVYLES DY SKLWRYSYTATYYI YDL SNGEFVRGNE LRP IQYLCWSPVGS KLAYVYQNNIYLKQRP GDPPFQITFNGRENKIFNGIPDWVYEEEMLATKYALWWS PNGKF LAYAEFNDTDI PVIAYSYYGDEQYPTINIPYPKAGAKNPVVR I FIIDTTYPAYVGPQEV PVPAMIASSDYF SWLTWVTD ERVCLQW LKR VQNVSVLSICDFREDWQTWDCPKTQEHIEESRTGWAGGFFV STPVFSYDAISYYKIFSDKDG YKHIHYIKDTVENAIQITSGKWE AINIFRV TQDSL FYSSNEFEEY PGR RN IYRISIGSYPPSKKCVT CHLRKERCQYYTASFSDYAKYYALVCYGP GIP ISTLHDGR TDQE IKI LEENKELE NALKNIQLPKEEIKKLEVDEITLWYKMILPPQF DRSKKYPLLIQVYGGPCSQSVRSVFAVNWISY LASKEGMVIALV DGRGTAFQGD KLLYAVYRKLGVYEVEDQITAVRKFIEMGFIDEK RIAIWGSYGGYVSSLALASGTGLFKCGIAPVSSWEY YASVY TEREMGLPTKDDNLEHYKNSTVMARAEYFRNV DYLIIHG TADDN VHFQNSAQIAKALVNAQVDFQAMWYSDQNHGLSGLSTNHLYTHM THFLKQCFSLSD	75
Murine FAP (P97321)	MKTWLKTVFGVTTLAALALVVICIVLRPSRVYKPEGNTKRALTL KDI LN GTFSYKTY FPNWISEQEYLHQ SEDDNIVFYNIETRESYI ILSNSTMKS VNATDYGLSPDRQFVYLES DY SKLWRYSYTATYYI YDLQNGEFVRGYELRP IQYLCWSPVGS KLAYVYQNNIYLKQRP GDPPFQITYTGRENRI FNGIPDWVYEEEMLATKYALWWS PDGKF LAYVEFNDS DIPIIAYSYYGDGQYPTINIPYPKAGAKNPVVRV FIVDTTYPHHVGPMEVPVPEMIASSDYF SWLTWVSSERVCLQW LKR VQNVSVLSICDFREDWHAWEC PKNQEHVEESRTGWAGGFFV STPAFSQDATSYKYIFSDKDG YKHIHYIKDTVENAIQITSGKWE AIYIFRV TQDSL FYSSNEFEGY PGR RN IYRISIGNSPPSKKCVT CHLRKERCQYYTASF SYKAKYYALVCYGP GLPISTLHDGR TDQE IQVLEENKELE NSLRNIQLPKVEIKKLDGGLTFWYKMILPPQF DRSKKYPLLIQVYGGPCSQSVKSVFAVNWITYLASKEGIVIALV DGRGTAFQGD KFLHAVYRKLGVYEVEDQLTAVRKFIEMGFIDEE RIAIWGSYGGYVSSLALASGTGLFKCGIAPVSSWEY YASIY SEREMGLPTKDDNLEHYKNSTVMARAEYFRNV DYLIIHG TADDN VHFQNSAQIAKALVNAQVDFQAMWYSDQNHGISSGRSQNHLYTH MTHFLKQCFSLSD	76
Human Nectin-4 (Q96NY8)	MPLSLGAEMWGPEAWLLLLLLLASFTGRCPAGELETSDVVTVVL GQDAKLPCFYRGDSGEQVGQVAWARVDAGEGAQELALLHSKYGL HVSPAYEGRVEQPPPPRNPLDGSVLLRNAVQADEGEYECRVSTF PAGSFQARLRLRVLPPLPSLNP GPAL EEGQGLTLAASCTAEGS PAPSVTWDTEVKGTSSRSFKHSRSAAVTSEFHLVPSRSMNGQP LTCVVSHPGLLQDQRITHILHVSFLAEASVRGLEDQNLWHIGRE GAMLKCLSEGQPPPSYNWTRLDGPLESGVRVDGDTLGFPPLTTE HSGIYVCHVSNEFSSRDSQVTVDVLD PQEDSGKQVDLV SASVVV VGVI AALLFCLLVVVVVLMSRYHRRKAQQMTQKYEEELTLTREN SIRRLHSHHTDPRSQPEESVGLRAEGHPDSLKD NSSCSVMSEEP EGRSYSTLT TVREIETQTELLSPGSGRAEEEEEDQDEGIKQAMNH FVQENGTLRAKPTNGIYINGRGLV	71

Target Protein (Uniprot Acc. No.)	Amino Acid Sequence	SEQ ID NO.
Murine Nectin-4 (Q8R007)	MPLSLGAEMWGPEAWLLLLLLASFTGRCPAGELETSDVVTVVV GQDAKLPCFYRGDSGEQVGVAVARVDAGEGAQELALLHSKYGL HVS PAYEGRVEQPPPPRNPLDGSVLLRNAVQADEGEYECRVSTF PAGSFQARLRRLRVLPPLPSLNP GPAL EEGQGLT LAASCTAEGS PAPSVTWDETVKGTTSRSRKFHRSAAVTSEFHLVPSRSMNGQP LTCVVSHPGLLQDQRITHILHVSFLAEASVRGLEDQNLWHIGRE GAMLKCLSEGQPPPSYNWTRLDG?LP SGVRVDGDTLGFPPTTE HSGIYVCHVSNEFSSRDSQVTVVDLPQEDSGKQVDLVASVVV VGVIALLFCLLVVVVMSRYHRRKAQQTQKYEEELTILTREN SIRRLHSHHTDPRSQPEESVGLRAEGHPDSLKDNSSCSVMSEEP EGRSYSTLTTVREIETQTELLSPGSGRAEEEDQDEGIKQAMNH FVQENGTLRAKPTNGIYINGRHLV	72
Human Mesothelin (Q13421)	MALPTARPLLGSCTPALGSLFLFLFSLGWVQPSRTL AGETGQE AAPLDGVLANPPNISSLSRQLLGFP CAEVSGLSTERVRELA LAQKNVKLSTEQRLCLAHRLSEP EDLDALPLDLLFLNPD GPOACTRFFSRITKANVDLLPRGAPERQRLPAALACWGVRS LSEADVRLGGGLACDLPGRFVAESA EVLLPRLVSCPGPLDQDQ EAAARALQGGGPPYGPSTWSVSTMDALRGLLPVLGQPIIRSI QGI VAAWRQSSRDPSWRQPERTILRPFRREVEKTACPSGKKA REIDESLI FYKKWELEACVDAALLATQMDRVNAI PFTYEQLDVL KHKLD ELYPQGYPESVIQHLGYLFLKMSPEDIRKWNVTSL ETLK ALLEVNKGHEMSPQAPRRPLPQVATLIDRFVKGRQLDKD TLDLTAFYPGYLCSLSP EELSSVPPSSIWAVRPQDLDTCDPRQ LDVLYPKARLAFQNMNGSEYFVKIQSFLGGAPTEDLKALSQQNV SMDLATFMKLRTDAVLP LTVAEVQKLLGPHVEGLKAEERHRP VRDWILRQRQDDLTGLGLQGGIPNGYLVLDLSMQEALSGTPC LLGPGP VLTVLALLLASTLA	77
Murine Mesothelin (Q61468)	MALPTARPLLGSCTPICSRSFLLLLLSLGWI PRIQTQTTKTSQ EATLLHAVNGAADFASLPTGLFLGLTCEEVSDLSMEQAKGLAMA VRQKNITLRGHQLRCLARLRPHLTDEELNALPLDLLFLNPMAM FPGQACAHFFSLISKANVDVLP RRSLEQRLLMEALKCQGVYG FQVSEADVRLGGIACDLP GK FVARSEVLLPWLACQGP LDQS QEKAVREVLRSGRTOYGPSPKWSVSTLDALQSLVAVLDESIVQS IPKDVKAEWLQHSRDP SRLGSKLTVIHPFRFRDAEQKACPPGK EPYKVDEDLIF YQNWELEACVDGTM LARQMDLVNEI PFTYEQLS IFKHKLDKTYPQGYPESLIQQLGHFFRYVSPEDIHQWNVTS PDT VKTLLKVS KGQKMNAQAI ALVACYLRGGQLDEDMVKALGDI PLSYLCDFSPQDLHSVPSSVMWLVG?QDLDKCSQRHLGLLYQKACS AFQNVSGLEYFEKIKTFLGGASVKDLRALSQHNVSMDIATFKRL QVDSLVLGLSVAEVQKLLGPNIVDLKT EEDKSPVRDWLFRQHQKD LDRLGLGLQGGIPNGYLVLD FNVREAFSSRASLLGPGFVLIWIP ALLPALRLS	78

Table 7. Exemplary In Vitro Stability and Pharmacokinetics Analyses

Compound	mkBBM (t1/2 hr)	mSerum (t1/2 hr)	mPlasm a (t1/2 hr)	hkBBM (t1/2 hr)	hPlasma (t1/2 hr)	Plasma terminal t1/2 (min)
C2			>12			26
C4			>12			32.5
C7	>12		>12			50.2
C15	>12	1.5	>12	>12		

Compound	mkBBM (t _{1/2} hr)	mSerum (t _{1/2} hr)	mPlasma (t _{1/2} hr)	hkBBM (t _{1/2} hr)	hPlasma (t _{1/2} hr)	Plasma terminal t _{1/2} (min)
C17	>12					
C18	>12	1.8				
C19	>12	1.9				
C20	>12	8.3				
C21	>12	>12				
C22	>12	4.7				
C25						21.8
C26	>12	>12				
C27	>12	>12				
C28	7.2	4.5				
C30						107
C33	>12		>12			332
C44			>12			26.4
C45			>12			
C47						29.8
C48			>12			
C50						32
C52			>12			
C63	>12		>12			
C66			>12			
C68	>12			>12		
C70						31.9
C71	>12		>12			81.2
C73	>12			>12		
C76	>12			>12		
C77						26.1
C78	>12		>12			27.6
C79	>12		>12			26.3
C80						23.5
C81	>12		>12			
C83						25
C91	>12		>12	>12	>12	32.3
C96	>12		>12			25.8
C98	6.6		>12	6.8	>12	
C102	2.85		>12			

Table 8. Exemplary In Vitro Cleavage Doubling Time Analysis

Compound	Mouse kBBM (min)	Human kBBM (min)	Human Plasma (min) ¹⁰
C28	90	90	ND
C68	79	84	ND
C76	117	115	ND

Table 9. Exemplary In Vivo Kidney Biodistribution Analysis – Cleavage Product Doubling Times Reported

Compound ID NO	4h (%ID/g)		24h (%ID/g)	
	Average	StDev	Average	StDev
C14	144.57	14.35	104.05	21.11
C120	111.82	2.20	61.79	7.29
C121	69.63	6.39	40.56	9.58
C67	77.43	17.72	36.88	5.48
C122	149.73	13.22	93.33	9.39
C123	87.90	3.86	42.66	5.00
C124	100.39	15.82	60.42	12.74
C125	43.45	24.17	19.94	11.05
C126	43.83	0.15	20.94	2.26
C127	78.82	9.08	42.86	5.65
C109	55.78	6.10	32.60	2.06
C110	67.48	20.71	34.85	8.14
C111	60.07	8.96	38.28	17.31
C112	27.72	13.57	15.88	7.45
C113	65.14	6.38	42.08	5.73
C114	59.10	5.34	34.34	0.72
C115	61.02	18.99	33.54	9.39
C116	16.58	4.00	10.19	2.63
C117	12.82	3.36	7.36	1.43

Table 10. Candidate Proteins Targeted By Each Candidate

Target
5T4
ADAM9
AG-7
AGS-16/ENPP3
ALPV
ASCT2
AXL

¹⁰ “ND” refers to “not detected”.

Target
B7H3/CD276
B7H4
BCMA
C4,4a
CA6
CA9
CAIX
CCR2
CCR7
CD123
CD138
CD142
CD166
CD19
CD20
CD205
CD22
CD228
CD25
CD30
CD33
CD352
CD37
CD38
CD44v6
CD45
CD46
CD47
CD48
CD5
CD51
CD56
CD7
CD70
CD71
CD74
CD79B
CDH6
CEACAM5
Cholecystokinin 2 receptor
cKIT
CLDN18
CLDN18.2
CLDN6

Target
CLDN6+CLDN9
CLL-1
cMET
cMET+EGFR
Cripto
CXCR4
DLL3
DPEP3
EFNA4
EGFR
EGFR+HER3
EGFR+MUC1
EGFRvIII
ENPP3
EPHA2
ETBR
FAP
FAPI
FCRH5
FGFR2
FGFR3
FLT3
FR α
GCC
GD2
GD3
Globo H
GPC3
GPCR5D
gpNMB
GPR20
HER2
HER2+HER3
HER3
IGF1R
IL13Ra
IL-4R
Integrin β -6
KAAG1
L1CAM
LAMP1
Lewis Y Ag
LHRH receptor
LIV1
LIV1A

Target
LRRC15
LY6E
Ly75/CD205
MC1R
Melanocyte stimulating hormone
MELTF
Mesothelin
MSLN
MT1-MMP
MUC1
MUC16/CA-125
NaPi-2b
Nectin-4
Neurokinin 1 receptor
NKG2D
Norepinephrine transporter
NOTCH3
NTSR1
P-Cadherin
PDL1
PRLR
PSMA
PTK7
RNF43
ROR1
ROR2
SEZ6
SLAMF7
SLC44A4
SLITRK6
SS2R
STEAP1
TF
TIM1
TNFSF9
Trop-2

CLAIMS

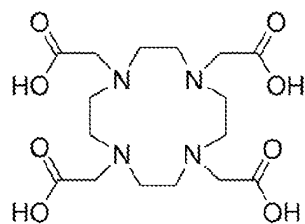
What is claimed is:

1. A composition represented by the formula selected from one or more of M-L-C-R, M-L-C, M-C-R, M-L-R, M-C, M-L, and M-R, wherein M comprises a miniprotein (M), L comprises a linear, branched, or enzymatically cleavable linker (L), C comprises a chelator (C), and R comprises a radionuclide (R), wherein the composition is characterized to exhibit reduced kidney uptake.
2. The composition of claim 1, wherein M comprises a linear polypeptide, a folded polypeptide (e.g., covalently linked polypeptide, non-covalently linked polypeptide, or polypeptide include a di-sulfide linkage), cysteine-dense peptide, a knottin peptide, a binder, an affibody, an engineered Kunitz domain, a monobody, an anticalin, a designed ankyrin repeat domain (DARPin), or an avimer.
3. The composition of claim 1, wherein M comprises an amino acid sequence that shares at least 90% identity to any one of SEQ ID NOs: 1-68.
4. The composition of claim 3, wherein M comprises an amino acid sequence that shares 100% identity to any one of SEQ ID NOs: 1-68.
5. The composition of claim 4, wherein the composition comprises any one of C1-C139.
6. The composition of claim 4, wherein M comprises an amino acid sequence that shares 100% identity to any one of SEQ ID NOs: 1-3, 5-15, and 47-67.
7. The composition of claim 6, wherein the composition comprises any one of C1-C6, C8-C77, or C118.
8. The composition of claim 4, wherein M comprises an amino acid sequence that shares 100% identity to SEQ ID NO: 16.
9. The composition of claim 8, wherein the composition comprises C78.
10. The composition of any one of claims 1-9, wherein M comprises an amino acid sequence comprising a percentage of charged amino acids between 1-5% of the total amino acid sequence, wherein the charged amino acids are selected from Lys, Arg, His, or non-canonical amino acids such as trimethyllysine.
11. The composition of any one of claims 1-9, wherein M comprises an amino acid sequence comprising a percentage of charged amino acids between 5-10% of the total amino acid sequence, wherein the charged amino acids are selected from Lys, Arg, His, or non-canonical amino acids such as trimethyllysine.

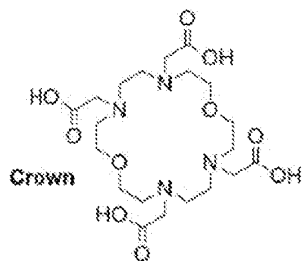
12. The composition of any one of claims 1-9, wherein M comprises an amino acid sequence comprising a percentage of charged amino acids between 10-15% of the total amino acid sequence, wherein the charged amino acids are selected from Lys, Arg, His, or non-canonical amino acids such as trimethyllysine..
13. The composition of any one of claims 1-9, wherein M comprises an amino acid sequence comprising a percentage of charged amino acids between 1-5% of the total amino acid sequence, wherein the charged amino acids are selected from Asp or Glu.
14. The composition of any one of claims 1-9, wherein M comprises an amino acid sequence comprising a percentage of charged amino acids between 5-10% of the total amino acid sequence, wherein the charged amino acids are selected from Asp or Glu.
15. The composition of any one of claims 1-9, wherein M comprises an amino acid sequence comprising a percentage of charged amino acids between 10-15% of the total amino acid sequence, wherein the charged amino acids are selected from Asp or Glu.
16. The composition of any one of the preceding claims, wherein the composition is characterized to exhibit increased circulating half-life.
17. The composition of any one of claims 10-12, wherein the composition is characterized to exhibit 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, or greater than 10% ID/g in a tumor 24 hours post administration.
18. The composition of any one of claims 10-12, wherein the composition is characterized to exhibit less than 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or less than 20% ID/g in a kidney 4 hours post administration.
19. The composition of any one of claims 10-12, wherein the composition is characterized to exhibit an adherence in a tumor and normal, non-ablatable tissues at a ratio of greater than 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1 or 10:1 24 hours post administration.
20. The composition of claim 13, wherein the composition is characterized to exhibit 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, or greater than 10% ID/g in a tumor 24 hours post administration.
21. The composition of claim 13, wherein the composition is characterized to exhibit less than less than 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, or less than 20% ID/g in a kidney 4 hours post administration.

22. The composition of claim 13, wherein the composition is characterized to exhibit an adherence in a tumor and normal, non-ablatable tissues at a ratio of greater than 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1 or 10:1 24 hours post administration.
23. The composition of any one of claims 1-22, wherein, when the chelator (C) is present, C comprises or consists of:

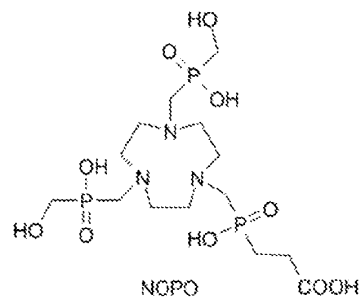
i) DOTA



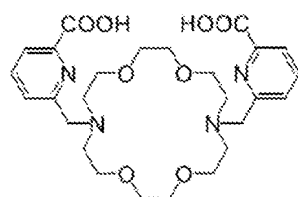
ii) Crown



iii) NOPO, or



iv) Macropa



Macropa

24. The composition of any one of claims 1-23, wherein when R is present, R comprises or consists of Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211.
25. The composition of any one of claims 1-24, wherein M comprises no more than 100 amino acids or less, 90 amino acids, 85 amino acids, 80 amino acids, 75 amino acids, 70 amino acids, 65 amino acids, 60 amino acids, 55 amino acids, 50 amino acids, 45 amino acids, 40 amino acids, 35 amino acids, 30 amino acids, 25 amino acids, 20 amino acids, 15 amino acids, 10 amino acids, or 5 amino acids.
26. The composition of any one of claims 1-25, wherein the miniprotein comprises at least one disulfide bridge.
27. The composition of any one of claims 1-26, wherein the composition is characterized to exhibit reduced kidney uptake using a biotin conjugated test agent-based assay.
28. The composition of claim 27, wherein the biotin conjugated test agent-based assay is performed by:

providing a plurality of kidney cells;

contacting the plurality of kidney cells with a composition represented by the formula selected from one or more of M-L-C-R, M-L-C, M-C-R, M-L-R, M-C, M-L, and M-R, wherein M comprises a Mini protein (M), L comprises a linear, branched, or enzymatically cleavable linker (L), C comprises a chelator (C), and R comprises a radionuclide (R), wherein the composition further comprises a biotin group complexed with a fluorescently labeled streptavidin; and

lysing the plurality of kidney cells and measuring kidney uptake of the composition by detecting fluorescence of the fluorescently labeled streptavidin.
29. A composition represented by the formula selected from one or more of M-L-C-R, M-L-C, M-C-R, M-L-R, M-C, M-L, and M-R, wherein M comprises a miniprotein (M),

L comprises a linear, branched, or enzymatically cleavable linker (L), C comprises a chelator (C), and R comprises a radionuclide (R), wherein the composition is characterized to have charge reduced miniprotein, or modifications of the miniprotein associated with increased kidney resorption, wherein the composition exhibits reduced kidney uptake.

30. A composition of a miniprotein (M) and a linker (L), wherein L is linked to the C-terminus or N-terminus of M at the N α -Carboxyl of cysteine, and L comprises an enzymatically cleavable linker of the formula L= R1-X1-X2-Lys, wherein:

R1= H, PEG(4-36), 4-aminomethyl-phenylacetic acid (AmPA),
Aminomethylbenzoyl (AmBz), or (succinic acid-PEG(4-36)); and
X1 or X2, = Met, Leu, norleu (Nle), Ile, Glu, Methoxinine, Phe, Tyr, beta
Ala, MWK or MVK, yGF, rGF, Gly(1-10), Citrulline, or Sar.
31. A composition of a miniprotein (M) and a linker (L), wherein L is linked via a succinic acid derivative to the N ϵ -of Lysine of M and L comprises an enzymatically cleavable linker of the formula L= R1-X1-X2-Lys, wherein:

R1= H, PEG(4-36), 4-aminomethyl-phenylacetic acid (AmPA),
Aminomethylbenzoyl (AmBz), or (succinic acid-PEG(4-36)); and
X1, or X2, = Met, Leu, norleu (Nle), Ile, Glu, Methoxinine, Phe, Tyr, beta
Ala, MWK or MVK, yGF, rGF, Gly(1-10), Citrulline, or Sar.
32. The composition of any one of the preceding claims, wherein L is cleaved in the kidney.
33. The composition of any one of the preceding claims, wherein L is cleaved by cathepsin B in a lysosome or a neutral endopeptidase, metalloprotease, or dipeptidyl peptidase in a kidney brush border membrane.
34. A method of treating cancer in a patient, the method comprising administering a composition of any one of claims 1-33 to the patient.
35. The method of claim 34, wherein the cancer is selected from breast cancer, ovarian cancer, melanoma, pancreatic cancer, peripheral neuroma, glioblastoma, adrenocortical carcinoma, AIDS-related lymphoma, anal cancer, bladder cancer, meningioma, glioma, astrocytoma, cervical cancer, chronic myeloproliferative disorders, colon cancer, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extracranial germ cell tumors, extrahepatic bile duct cancer, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumors, gestational

trophoblastic tumors, hairy cell leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, hypopharyngeal cancer, islet cell carcinoma, Kaposi sarcoma, laryngeal cancer, leukemia, lip cancer, oral cavity cancer, liver cancer, male breast cancer, malignant mesothelioma, medulloblastoma, Merkel cell carcinoma, metastatic squamous neck cell carcinoma, multiple myeloma and other plasma cell neoplasms, mycosis fungoides and the Sezary syndrome, myelodysplastic syndromes, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, small cell lung cancer, head and neck cancer, skin cancer, oropharyngeal cancer, bone cancers, including osteosarcoma and malignant fibrous histiocytoma of bone, paranasal sinus cancer, parathyroid cancer, penile cancer, pheochromocytoma, pituitary tumors, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, small intestine cancer, soft tissue sarcoma, supratentorial primitive neuroectodermal tumors, pineoblastoma, testicular cancer, thymoma, thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis and ureter, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Wilm's tumor and other childhood kidney tumors.

36. A method of co-administering the composition of any one of claims 1-35 and a peptide comprising an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NOs: 16-52, or SEQ ID NO: 68.
37. The method of claim 36, wherein the composition comprises any one of C7, C12-C35, C55, C56, C58, C59, C61-C65, or C131-133.
38. The method of claim 36 or 37, wherein the method reduces uptake of M, L, C, and R, and combinations thereof in a kidney.
39. The method of claim 38, wherein co-administering the composition of any one of claims 1-27 and a peptide comprising an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NOs: 16-52, or SEQ ID NO: 68 results in competitive inhibition of M.
40. The method of claim 39, wherein the composition comprises any one of C7, C12-C35, C55, C56, C58, C59, C61-C65, or C131-133.
41. The method of any one of claims 36-40, wherein the composition is administered intravenously or subcutaneously.
42. The method of any one of claims 36-41, wherein the composition of any one of claims 1-27 and the peptide comprising an amino acid sequence selected from SEQ

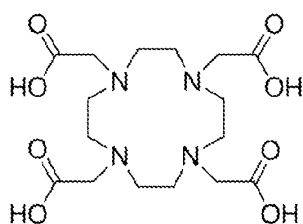
- ID NO: 4, SEQ ID NO: 7, SEQ ID NOs: 16-52, or SEQ ID NO: 68 are co-formulated with a pharmaceutically acceptable buffer.
43. The method of claim 42, wherein the composition comprises any one of C7, C12-C35, C55, C56, C58, C59, C61-C65, or C131-133.
44. The method of any one of claims 36-43, wherein the method produces a treatment characterized to exhibit reduced uptake of M in a kidney and/or exhibit increased circulating half-life.
45. A composition comprising a compound represented by the formula selected from one or more of M-L-C-R, M-L-C, M-C-R, M-L-R, M-C, and M-L, wherein M comprises a miniprotein (M), L comprises a linear, branched, or enzymatically cleavable linker (L), C comprises a chelator (C), and R comprises a radionuclide (R), and a peptide, wherein the peptide is characterized as competitively inhibiting the target for uptake in a kidney.
46. The composition of claim 45, wherein the peptide comprises an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NOs: 16-52, or SEQ ID NO: 68, and wherein the peptide is present at a concentration of 10-1000X relative to the concentration of M.
47. The composition of claim 46, wherein the composition comprises any one of C7, C12-C35, C55, C56, C58, C59, C61-C65, or C131-133.
48. The composition of claim 47, wherein the peptide comprises an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NOs: 16-52, or SEQ ID NO: 68, and wherein the peptide is present at a concentration of 100X or greater than 100X relative to the concentration of M.
49. The composition of claim 48, wherein the composition comprises any one of C7, C12-C35, C55, C56, C58, C59, C61-C65, or C131-133.
50. A composition comprising a compound represented by the formula selected from one or more of M-L-C, M-C, and M-L, wherein M comprises a miniprotein (M) comprising an amino acid selected from SEQ ID NO: 4 or SEQ ID NO: 7, L comprises a linear, branched, or enzymatically cleavable linker (L), and C comprises a chelator (C), and a peptide comprising an amino acid sequence selected from SEQ ID NO: 4, SEQ ID NO: 7, SEQ ID NOs: 16-52, or SEQ ID NO: 68, wherein the peptide is characterized as competitively inhibiting the target for uptake in a kidney.
51. The composition of claim 50, wherein the composition comprises any one of C7, C12-C35, C55, C56, C58, C59, C61-C65, or C131-133.

52. The composition of claim 50, wherein the peptide is present at a concentration of 10-1000X relative to the concentration of M.
53. The peptide of claim 50, wherein the peptide is present at a concentration of 100X or greater than 100X relative to the concentration of M.
54. The composition of any one of claims 45-53, wherein the peptide reduces the uptake of M-L-C-R, M-L-C, or R in a kidney.
55. The composition of any one of claims 45-54, wherein when C is present, C comprises or consists of DOTA, NOPO, Crown, or Macropa.
56. A composition according to any one of claims 45-55, wherein when R is present, R comprises or consists of Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211.
57. A pharmaceutical composition comprising (M)-L-(C)-R and at least one drug moiety.
58. The composition of any one of claims 45-57, wherein the composition binds to a target expressed on tumor cell with an affinity of 1 pM to 100 nM as measured by an in vitro binding assay.
59. The composition of any one of claims 43-56, wherein the composition is characterized by peptide solubility of 1-100 mg/mL in an assay formulation and peptide stability of 80-95% at 75°C for at least 1 hour.
60. The composition of any one of claims 45-59, wherein the composition is cleared from a kidney at or near the glomerular filtration rate of a subject.
61. The composition of any one of claims 45-60, wherein the composition displays passage through a kidney, a liver, a bone marrow, or a spleen.
62. The composition of any one of claims 45-61, wherein expression of the target is higher in a cancer cell than in a non-cancer cell.
63. The composition of any one of claims 45-62, wherein the target is 5T4, ADAM9, AG-7, AGS-16/ENPP3, ALPV, ASCT2, AXL, B7H3/CD276, B7H4, BCMA, C4.4a, CA6, CA9, CAIX, CCR2, CCR7, CD123, CD138, CD142, CD166, CD19, CD20, CD205, CD22, CD228, CD25, CD30, CD33, CD352, CD37, CD38, CD44v6, CD45, CD46, CD47, CD48, CD5, CD51, CD56, CD7, CD70, CD71, CD74, CD79B, CDH6, CEACAM5, Cholecystokinin 2 receptor, cKIT, CLDN18, CLDN18.2, CLDN6, CLDN6+CLDN9, CLL-1, cMET, cMET+EGFR, Cripto, CXCR4, DLL3, DPEP3, EFNA4, EGFR, EGFR+HER3, EGFR+MUC1, EGFRvIII, EPHA2, ETBR, FAP, FAPI, FCRH5, FGFR2, FGFR3, FLT3, FR α , GCC, GD2, GD3, Globo H, GPC3, GPCR5D, gpNMB, GPR20, HER2, HER2+HER3, IGF1R, IL13Ra, IL-4R, Integrin

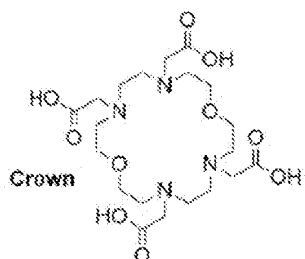
β -6, KAAG1, L1CAM, LAMP1, Lewis Y Ag, LHRH receptor, LIV1, LIV1A, LRRC15, LY6E, Ly75/CD205, MC1R, MELTF, Mesothelin, MSLN, MT1-MMP, MUC1, MUC16/CA-125, NaPi-2b, Nectin-4, Neurokinin 1 receptor, NKG2D, Norepinephrine transporter, NOTCH3, NTSR1, P-Cadherin, PDL1, PRLR, PSMA, PTK7, RNF43, ROR1, ROR2, SEZ6, SLAMF7, SLC44A4, SLITRK6, SS2R, STEAP1, TF, TIM1, TNFSF9, Trop-2.

64. The composition of any one of claims 45-63, wherein, when the chelator (C) is present, C comprises or consists of:

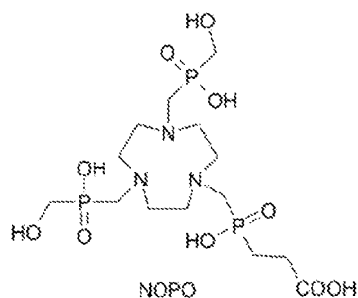
i) DOTA



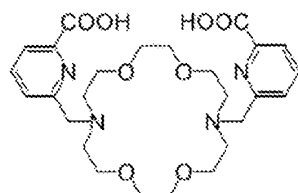
ii) Crown



iii) NOPO, or



iv) Macropa

**Macropa**

65. The composition of any one of claims 45-64, wherein when R is present, R comprises or consists of Ac-225, Ga-68, Pb-212, Lu-177, Cu-67, Cu-64, La-132, La-135, In-111, Ce-134, F-18, or At-211.
66. The composition of any one of claims 45-65, wherein M comprises no more than 100 amino acids or less, 90 amino acids, 85 amino acids, 80 amino acids, 75 amino acids, 70 amino acids, 65 amino acids, 60 amino acids, 55 amino acids, 50 amino acids, 45 amino acids, 40 amino acids, 35 amino acids, 30 amino acids, 25 amino acids, 20 amino acids, 15 amino acids, 10 amino acids, or 5 amino acids.
67. The composition of any one of claims 45-66, wherein the miniprotein comprises at least one disulfide bridge.
68. A method of treating cancer in a patient, the method comprising administering a composition of any one of claims 45-67 to the patient.
69. The method of claim 68, wherein the cancer is selected from breast cancer, ovarian cancer, melanoma, pancreatic cancer, peripheral neuroma, glioblastoma, adrenocortical carcinoma, AIDS-related lymphoma, anal cancer, bladder cancer, meningioma, glioma, astrocytoma, cervical cancer, chronic myeloproliferative disorders, colon cancer, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma, extracranial germ cell tumors, extrahepatic bile duct cancer, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumors, gestational trophoblastic tumors, hairy cell leukemia, Hodgkin lymphoma, non-Hodgkin lymphoma, hypopharyngeal cancer, islet cell carcinoma, Kaposi sarcoma, laryngeal cancer, leukemia, lip cancer, oral cavity cancer, liver cancer, male breast cancer, malignant mesothelioma, medulloblastoma, Merkel cell carcinoma, metastatic squamous neck cell carcinoma, multiple myeloma and other plasma cell neoplasms,

mycosis fungoides and the Sezary syndrome, myelodysplastic syndromes, nasopharyngeal cancer, neuroblastoma, non-small cell lung cancer, small cell lung cancer, head and neck cancer, skin cancer, oropharyngeal cancer, bone cancers, including osteosarcoma and malignant fibrous histiocytoma of bone, paranasal sinus cancer, parathyroid cancer, penile cancer, pheochromocytoma, pituitary tumors, prostate cancer, rectal cancer, renal cell cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, small intestine cancer, soft tissue sarcoma, supratentorial primitive neuroectodermal tumors, pineoblastoma, testicular cancer, thymoma, thymic carcinoma, thyroid cancer, transitional cell cancer of the renal pelvis and ureter, urethral cancer, uterine sarcoma, vaginal cancer, vulvar cancer, and Wilm's tumor and other childhood kidney tumors.

70. Use of a composition of any one of claims 1-33 or 45-47 to treat cancer in a subject.
71. An isolated polynucleotide comprising one or more nucleic acid sequences encoding a polypeptide selected from SEQ ID NO: 1-68; or a nucleic acid sequence encoding a polypeptide comprising at least 90%, 95%, 96%, 97%, 98%, 99% or greater identity to SEQ ID NO: 1-68.
72. A vector comprising the isolated polynucleotide of claim 71.
73. A host cell transformed with the isolated polynucleotide of claim 71 or the vector of claim 72.
74. A method for characterizing kidney uptake of a composition, the method comprising: providing a plurality of kidney cells; contacting the plurality of kidney cells with a composition represented by the formula selected from one or more of M-L-C-R, M-L-C, M-C-R, M-L-R, M-C, M-L, and M-R, wherein M comprises a miniprotein (M), L comprises a linear, branched, or enzymatically cleavable linker (L), C comprises a chelator (C), and R comprises a radionuclide (R), wherein the composition further comprises a biotin group complexed with a fluorescently labeled streptavidin; and lysing the plurality of kidney cells and measuring kidney uptake of the composition by detecting fluorescence of the fluorescently labeled streptavidin.
75. The method of claim 74, wherein the plurality of kidney cells are provided in a well.
76. The method of claim 74 or 75, wherein M comprises an amino acid sequence that shares at least 90% identity to any one of SEQ ID NOs: 1-68.
77. The method of claim 76, wherein M comprises an amino acid sequence that shares 100% identity to any one of SEQ ID NOs: 1-68.