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(71) Applicant: **THE BOARD OF REGENTS OF THE UNIVERSITY OF OKLAHOMA** [US/US]; 660 Parrington Oval, Room 119, Norman, OK 73019 (US).

(72) Inventors: **MAO, Chuanbin**; 910 East Lindsey Street, #3, Norman, OK 73071 (US). **QU, Xuewei**; 207 Wadsack Drive, Apt. G, Norman, OK 73072 (US).

(74) Agent: **SMITH, Michael, H.**; Hall Estill Hardwick Gable Golden & Nelson, P.C., 100 North Broadway, Suite 2900, Oklahoma, OK 73102 (US).

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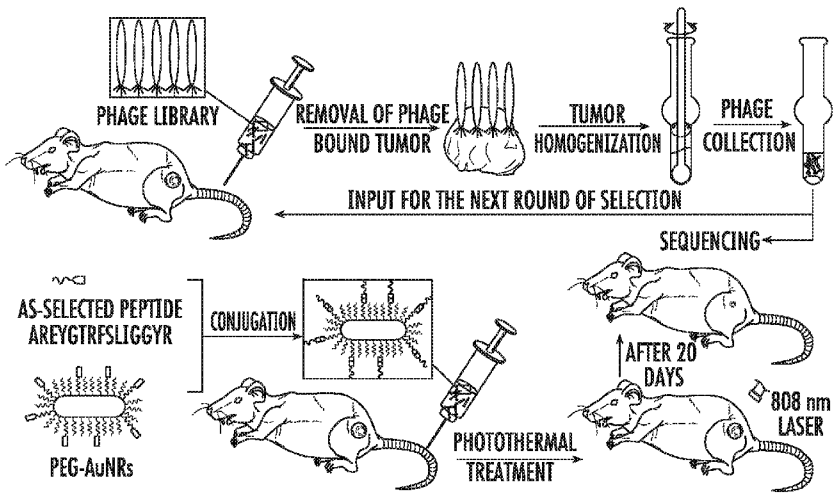

FIG. 1

(57) Abstract: Peptides conjugated to therapeutic, imaging, or diagnostic moieties to form peptide conjugates for use in treating, imaging, and/or diagnosing a cancer, such as breast cancer, in a subject in need of such treatment are disclosed.

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[Continued on next page]

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PEPTIDES AND METHODS FOR TARGETED DELIVERY TO TUMORS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Application Serial No. 62/478,805, filed March 30, 2017, which claims the benefit under 35 U.S.C. 119(e), the disclosure of which is hereby expressly incorporated herein by reference.

GOVERNMENT SUPPORT

[0002] This invention was made with government support under Contract Numbers CA200504 and EB021339 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

[0003] Recently, targeted drug delivery has been studied intensively in cancer treatment. It involves the use of tumor-recognizing molecules in the drug formulation to enhance drug accumulation in the cancerous tissue. This strategy can result in significantly enhanced cancer treatment efficacy, decreased side effects as well as reduced drug use. Up to date, different types of molecules with tumor affinity, such as antibodies, folic acid/folate, and hyaluronic acid, have been reported and employed for targeted drug delivery. These molecules interact preferentially with certain types of proteins that are overexpressed or specifically expressed at the tumor sites. Namely, in this type of tumor-targeting approach, it requires unambiguous pre-knowledge of the corresponding tumor receptors that are paired with these targeting molecules. However, such strategy neglects the patient-specific tumor difference for the same type of cancer. Ideally, a molecule targeting patient-specific tumors should be identified and then integrated with the cancer therapeutics to achieve customized cancer therapy. This is the goal of precision medicine in the context of cancer therapy. Precision medicine is a new emerging research area that aims to provide personalized drug formulations or treatment plans for individual patients. It is expected that by using the patient-specific solution, cancer treatment can be significantly more effective. Since each type of cancer differs in many ways on different patients, especially on the molecular level, the tumor receptor-based targeted delivery strategy may not work quite well for precision medicine, as the expression level of receptors may vary dramatically among different patients. This is also one of the reasons why the current cancer therapeutics result in low survival rate. In addition, for unknown types of tumor or new tumor variants on individual patients, it is practically difficult to explore the specific ligand-receptor

pairs for them when the patient-specific receptors have not been identified. New treatment strategies for receptor-based targeted delivery to tumors continue to be needed.

BRIEF DESCRIPTION OF THE DRAWINGS

[0004] Several embodiments of the present disclosure are hereby illustrated in the appended drawings. It is to be noted however, that the appended drawings only illustrate several typical embodiments and are therefore not intended to be considered limiting of the scope of the inventive concepts disclosed herein. The figures are not necessarily to scale and certain features and certain views of the figures may be shown as exaggerated in scale or in schematic in the interest of clarity and conciseness. The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0005] FIG. 1 is a schematic description of a phage display-based breast cancer treatment strategy disclosed herein. In phase (a), patient-specific breast cancer-targeting peptides were identified through in vivo phage display. A phage library was first injected through tail vein into tumor-bearing mice and was allowed to circulate for 24 h. Following this period, tumors with preferentially bound phages were removed from mice and homogenized. Tumor-binding phages were then collected by centrifugation, amplified and used as input for the next round of in vivo selection. In phase (b) selected peptides were coupled with an anticancer nanomedicine (e.g., gold nanorods (AuNRs)) to enhance their accumulation inside tumors so as to achieve (in this example) highly efficient cancer treatment by photothermal therapy.

[0006] FIG. 2 shows a transmission electron microscopy (TEM) image (left) and UV-Visible light absorbance spectrum (right) of the as-synthesized AuNRs used for the photothermal treatment.

[0007] FIG. 3 shows in vivo imaging for the detection of selective accumulation of tumor-homing peptides at the MCF-7 tumors. Peptides 1-5 (SEQ ID NOS:1-5), and a control peptide (SEQ ID NO:6) were first labelled with biotin at the C-terminal end, and then conjugated to Cy-5 tagged streptavidin. Images were taken 24 h after the intravenous injection of 5 nmol of peptides (100 μl in PBS). All of the images were taken under the same parameters, thus the intensity difference represents the actual difference of peptide accumulation at the tumor sites.

[0008] FIG. 4 shows Dynamic Light Scattering (DLS) measurement of AuNRs with different surface ligands. The effective hydrodynamic radii are (a) 40.8 ± 2.1 nm for the as-synthesized CTAB-AuNRs, (b) 44.3 ± 2.5 nm for the PEG functionalized AuNRs, and (c) 47.0 ± 2.4 nm for the peptide functionalized AuNRs.

[0009] FIG. 5 shows blood circulation of AuNRs. 1.0 mg of tumor targeting peptide conjugated AuNRs or control peptide conjugated AuNRs were injected intravenously. To track the gold concentration in peripheral blood (represented as the mass of gold per ml of blood), 20 μl of blood was collected from suborbital space of the mouse each time at 3 min, 20 min, 1 h , 4 h, 7 h, 12 h, 24 h and 48 h after injection, and immediately added into 200 μl of freshly prepared aqua regia. The concentration of AuNRs was quantified by ICP-AES.

[0010] FIG. 6 shows organ distribution of AuNRs. Mice were sacrificed 1 day, 2 days, 3 days, 4 days and 5 days after the intravenous injection of 1 mg of AuNRs. Target group was the group injected with peptide 1 conjugated AuNRs whereas control group was the one injected with control peptide conjugated AuNRs. Gold concentration was calculated as mass permillage of Au in 1 gram of the corresponding tissue. The inset chart shows the percentage increase (in tumor, represented as positive values) or decrease (in liver, represented as negative values) of gold content in target group when compared to the control group on different days. The data indicate that conjugation of our selected peptide 1 onto AuNRs can effectively increase their uptake by tumors but decrease their uptake by livers.

[0011] FIG. 7 shows results after photothermal treatment of MCF-7 tumors. (a) Photographs of tumors taken out from the mice 20 days after the photothermal therapy (for the target-AuNRs group, only two animals had visible tumors). (b) Average tumor volume before and 20 days after the treatment. Only tumors treated with peptide 1-AuNR conjugate showed a decrease in size after 20 days. (c) Images of HE stained sections of tumors obtained 3 h after 808 nm laser treatment. Target-AuNRs: AuNRs modified with peptide 1; PEG-AuNRs: PEG modified AuNRs; Control-AuNRs: AuNRs modified with a control peptide; Saline: saline only and without AuNRs. Comparing to the normal nucleus of cancer cells in the control tumor, contiguous cell necrosis is determined by the extensive pale eosinophilic cytoplasm (typically highlighted by dashed ovals) as well as karyolysis and nuclear swelling (typically highlighted by solid ovals). The cells apoptosis with the presence of nuclear pyknosis, hepereosinophilic cytoplasm (typically highlighted by arrows) and apoptotic bodies (typically highlighted by arrow heads) are marked. The normal tumor tissue area in each AuNRs treated group is also circled by angled dash frames.

[0012] FIG. 8 is a schematic showing the phage display based solution for breast cancer precision medicine as developed herein. Patient-specific tumor targeting peptide is first selected through in vivo biopanning. The selected peptide is then coupled with an anti-cancer drug forming a peptide-drug conjugate, which is then administered to the tumor-bearing

subject. Enhanced peptide-drug conjugate accumulation in the tumor is achieved, resulting in improved tumor killing efficacy.

DETAILED DESCRIPTION

[0013] In the present disclosure, a set of tumor-targeting peptides are identified via *in vivo* biopanning of a tumor, then the peptides were tested for effectiveness in tumor homing. In certain embodiments, a specific tumor-targeting peptide is conjugated to a therapeutic drug to form a peptide conjugate for killing the tumor in vivo, or to a fluorescent or radiographic moiety for imaging the tumor, or for use in diagnosis. Non-limiting examples of several peptides of the present disclosure are shown in Table 1. The targeting peptides disclosed herein can optionally comprise an amino acid linker sequence such as described in further non-limiting detail below. In at least one embodiment, the disclosed method uses a phage display technique for identifying peptides that target specific receptors on cancer tumor for use in a customized, patient-specific anti-cancer therapy. In this embodiment the method doesn't require the identification of receptors which are specific or unique to a tumor desired to be treated. Instead the method identifies one or more tumor-specific targeting peptides independently of the identification of the tumor receptors. This method can performed in a fast and cost-effective manner, enabling the discovery of patient-specific tumor-homing peptides for use in producing receptor-specific therapeutic conjugates.

[0014] Before further describing various embodiments of the compositions and methods of the present disclosure in more detail by way of exemplary description, examples, and results, it is to be understood that the embodiments of the present disclosure are not limited in application to the details of methods and compositions as set forth in the following description. The embodiments of the compositions and methods of the present disclosure are capable of being practiced or carried out in various ways not explicitly described herein. As such, the language used herein is intended to be given the broadest possible scope and meaning; and the embodiments are meant to be exemplary, not exhaustive. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting unless otherwise indicated as so. Moreover, in the following detailed description, numerous specific details are set forth in order to provide a more thorough understanding of the disclosure. However, it will be apparent to a person having ordinary skill in the art that the embodiments of the present disclosure may be practiced without these specific details. In other instances, features which are well known to persons of ordinary skill in the art have not been described in detail to avoid unnecessary complication of the description. All of

the compositions and methods of production and application and use thereof disclosed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of the present disclosure have been described in terms of particular embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit, and scope of the inventive concepts as described herein. All such similar substitutes and modifications apparent to those having ordinary skill in the art are deemed to be within the spirit and scope of the inventive concepts as disclosed herein.

[0015] All patents, published patent applications, and non-patent publications referenced or mentioned in any portion of the present specification are indicative of the level of skill of those skilled in the art to which the present disclosure pertains, and are hereby expressly incorporated by reference in their entirety to the same extent as if the contents of each individual patent or publication was specifically and individually incorporated herein, including U.S. Provisional Application Serial No. 62/478,805, filed March 30, 2017.

[0016] 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 having ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0017] As utilized in accordance with the methods and compositions of the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[0018] The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one." The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or when the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." The use of the term "at least one" will be understood to include one as well as any quantity more than one, including but not limited to, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 100, or any integer inclusive therein. The term "at least one" may extend up to 100 or 1000 or more, depending on the term to which it is attached; in addition, the quantities of 100/1000 are not to be considered limiting, as higher limits may also produce satisfactory results. In addition, the use of the term "at least one of X,

Y and Z" will be understood to include X alone, Y alone, and Z alone, as well as any combination of X, Y and Z.

[0019] As used in this specification and claims, the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[0020] The term "or combinations thereof" as used herein refers to all permutations and combinations of the listed items preceding the term. For example, "A, B, C, or combinations thereof" is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, CBA, BCA, ACB, BAC, or CAB. Continuing with this example, expressly included are combinations that contain repeats of one or more item or term, such as BB, AAA, AAB, BBC, AAABCCCC, CBBAAA, CABABB, and so forth. The skilled artisan will understand that typically there is no limit on the number of items or terms in any combination, unless otherwise apparent from the context.

[0021] Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the composition, the method used to administer the composition, or the variation that exists among the objects, or study subjects. As used herein the qualifiers "about" or "approximately" are intended to include not only the exact value, amount, degree, orientation, or other qualified characteristic or value, but are intended to include some slight variations due to measuring error, manufacturing tolerances, stress exerted on various parts or components, observer error, wear and tear, and combinations thereof, for example. The term "about" or "approximately", where used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass, for example, variations of ± 20% or ± 10%, or ± 5%, or ± 1%, or ± 0.1% from the specified value, as such variations are appropriate to perform the disclosed methods and as understood by persons having ordinary skill in the art. As used herein, the term "substantially" means that the subsequently described event or circumstance completely occurs or that the subsequently described event or circumstance occurs to a great extent or degree. For example, the term "substantially" means that the subsequently described event or circumstance occurs at least 90% of the time, or at least 95% of the time, or at least 98% of the time.

[0022] As used herein any reference to "one embodiment" or "an embodiment" means that a particular element, feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment. The appearances of the phrase "in one

embodiment" in various places in the specification are not necessarily all referring to the same embodiment.

[0023] As used herein, all numerical values or ranges include fractions of the values and integers within such ranges and fractions of the integers within such ranges unless the context clearly indicates otherwise. Thus, to illustrate, reference to a numerical range, such as 1-10 includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, as well as 1.1, 1.2, 1.3, 1.4, 1.5, etc., and so forth. Reference to a range of 1-50 therefore includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etc., up to and including 50, as well as 1.1, 1.2, 1.3, 1.4, 1.5, etc., 2.1, 2.2, 2.3, 2.4, 2.5, etc., and so forth. Reference to a series of ranges includes ranges which combine the values of the boundaries of different ranges within the series. Thus, to illustrate reference to a series of ranges, for example, a range of 1-1,000 includes, for example, 1-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-75, 75-100, 100-150, 150-200, 200-250, 250-300, 300-400, 400-500, 500-750, 750-1,000, and includes ranges of 1-20, 10-50, 50-100, 100-500, and 500-1,000. Any two values within the above ranges, e.g., 88 and 444 therefore can be used to set the lower and upper boundaries of a range (e.g., 88 - 444) in accordance with the embodiments of the present disclosure.

[0024] The term “pharmaceutically acceptable” refers to compounds and compositions which are suitable for administration to humans and/or animals without undue adverse side effects such as toxicity, irritation and/or allergic response commensurate with a reasonable benefit/risk ratio.

[0025] By “biologically active” is meant the ability to modify the physiological system of an organism without reference to how the active agent has its physiological effects.

[0026] As used herein, “pure, “substantially pure,” or “isolated” means an object species is the predominant species present (i.e., on a molar basis it is more abundant than any other object species in the composition thereof), and particularly a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80% of all macromolecular species present in the composition, more particularly more than about 85%, more than about 90%, more than about 95%, or more than about 99%. The term “pure” or “substantially pure” also refers to preparations where the object species (e.g., the peptide compound) is at least 60% (w/w) pure, or at least 70% (w/w) pure, or at least 75% (w/w) pure, or at least 80% (w/w) pure, or at least 85% (w/w) pure, or at least 90% (w/w) pure, or at least 92% (w/w) pure, or at least 95% (w/w) pure, or at least 96% (w/w) pure, or at least 97% (w/w) pure, or at least 98% (w/w) pure, or at least 99% (w/w) pure, or 100%

(w/w) pure. Where used herein the term "high specificity" refers to a specificity of at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%. Where used herein the term "high sensitivity" refers to a sensitivity of at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%.

[0027] The terms "subject" and "patient" are used interchangeably herein and will be understood to refer to a warm blooded animal, particularly a mammal or bird. Non-limiting examples of animals within the scope and meaning of this term include dogs, cats, rats, mice, guinea pigs, horses, goats, cattle, sheep, zoo animals, Old and New World monkeys, non-human primates, and humans.

[0028] "Treatment" refers to therapeutic treatments. "Prevention" refers to prophylactic treatment measures to stop a condition from occurring. The term "treating" refers to administering the composition to a patient for therapeutic purposes, and may result in an amelioration of the condition or disease.

[0029] The terms "therapeutic composition" and "pharmaceutical composition" refer to an active agent-containing composition that may be administered to a subject by any method known in the art or otherwise contemplated herein, wherein administration of the composition brings about a therapeutic effect as described elsewhere herein. In addition, the compositions of the present disclosure may be designed to provide delayed, controlled, extended, and/or sustained release using formulation techniques which are well known in the art.

[0030] The term "effective amount" refers to an amount of an active agent which is sufficient to exhibit a detectable biochemical and/or therapeutic effect, for example without excessive adverse side effects (such as toxicity, irritation and allergic response) commensurate with a reasonable benefit/risk ratio when used in the manner of the inventive concepts. The effective amount for a patient will depend upon the type of patient, the patient's size and health, the nature and severity of the condition to be treated, the method of administration, the duration of treatment, the nature of concurrent therapy (if any), the specific formulations employed, and the like. Thus, it is not possible to specify an exact effective amount in advance. However, the effective amount for a given situation can be determined by one of ordinary skill in the art using routine experimentation based on the information provided herein.

[0031] The term "ameliorate" means a detectable or measurable improvement in a subject's condition or or symptom thereof. A detectable or measurable improvement includes a subjective or objective decrease, reduction, inhibition, suppression, limit or control in the

occurrence, frequency, severity, progression, or duration of the condition, or an improvement in a symptom or an underlying cause or a consequence of the condition, or a reversal of the condition. A successful treatment outcome can lead to a "therapeutic effect," or "benefit" of ameliorating, decreasing, reducing, inhibiting, suppressing, limiting, controlling or preventing the occurrence, frequency, severity, progression, or duration of a condition, or consequences of the condition in a subject.

[0032] A decrease or reduction in worsening, such as stabilizing the condition, is also a successful treatment outcome. A therapeutic benefit therefore need not be complete ablation or reversal of the condition, or any one, most or all adverse symptoms, complications, consequences or underlying causes associated with the condition. Thus, a satisfactory endpoint may be achieved when there is an incremental improvement such as a partial decrease, reduction, inhibition, suppression, limit, control or prevention in the occurrence, frequency, severity, progression, or duration, or inhibition or reversal of the condition (*e.g.*, stabilizing), over a short or long duration of time (e.g., seconds, minutes, hours).

[0033] The following abbreviations may be used herein for amino acids: alanine:ala:A; arginine:arg:R; asparagine:asn:N; aspartic acid:asp:D; cysteine:cys:C; glutamic acid:glu:E; glutamine:gln:Q; glycine:gly:G; histidine:his:H; isoleucine:ile:I; leucine:leu:L; lysine:lys:K; methionine:met:M; phenylalanine:phe:F; proline:pro:P; serine:ser:S; threonine:thr:T; tryptophan:trp:W; tyrosine:tyr:Y; and valine:val:V.

[0034] The term "peptide" is used herein to designate a series of amino acid residues, connected one to the other typically by peptide bonds between the alpha-amino and carbonyl groups of the adjacent amino acids to form an amino acid sequence. In certain embodiments, the peptides can range in length from 2 to 10 to 15 to 25 to 40 to 60 to 75 to 100 amino acids, for example, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 amino acids. The term "polypeptide" or "protein" is used herein to designate a series of amino acid residues, connected one to the other typically by peptide bonds between the alpha-amino and carbonyl groups of the adjacent amino acids, wherein the length is longer than a single peptide. A peptide conjugate refers, in a non-limiting embodiment, to a compound comprising a peptide of the present disclosure which is conjugated (e.g., covalently linked, directly or indirectly via a linker sequence) to another molecule, such as a carrier molecule such as a protein or other polymeric molecule, e.g., a serum albumin molecule or antibody, or

other theraputic compound such as a drug, or an imaging or diagnostic moiety and wherein the peptide retains its activity (e.g., binding, targeting, imaging, or inhibitory) even when conjugated to the molecule. A “fusion protein” or “fusion polypeptide” refers to proteins or polypeptides (and may be used interchangeably) which have been created by recombinant or synthetic methods to combine peptides in a serial configuration. The peptides of the present disclosure may be produced using any nucleotide sequence which encodes the desired amino acid sequence. Any of the peptides described herein or active variants thereof may be used to make the peptide conjugates of the present disclosure.

[0035] Peptides of the present disclosure and the nucleic acids which encode them include peptide and nucleic acid variants which comprise substitutions (conservative or non-conservative) of the native amino acids or bases. For example, the peptide variants include, but are not limited to, variants that are not exactly the same as the sequences disclosed herein, but which have, in addition to the substitutions explicitly described for various sequences listed herein, additional substitutions of amino acid residues (conservative or non-conservative) which substantially do not impair the activity or properties of the variants described herein. Examples of such conservative amino acid substitutions may include, but are not limited to, ala to gly, ser, or thr; arg to gln, his, or lys; asn to asp, gln, his, lys, ser, or thr; asp to asn or glu; cys to ser; gln to arg, asn, glu, his, lys, or met; glu to asp, gln, or lys; gly to pro or ala; his to arg, asn, gln, or tyr; ile to leu, met, or val; leu to ile, met, phe, or val; lys to arg, asn, gln, or glu; met to gln, ile, leu, or val; phe to leu, met, trp, or tyr; ser to ala, asn, met, or thr; thr to ala, asn, ser, or met; trp to phe or tyr; tyr to his, phe or trp; and val to ile, leu, or met.

[0036] One of ordinary skill in the art would readily know how to make, identify, select or test such variants for receptor targeting activity against the same receptors targeted by the native peptides. Particular examples of conservative amino acid substitutions include, but are not limited to, gly:ala substitutions; val:ile:leu substitutions; asn:glu:his substitutions; asp:glu substitutions; ser:thr:met substitutions; lys:arg:his substitutions; and phe:tyr:trp substitutions. Other types of substitutions, variations, additions, deletions and derivatives that result in functional variant peptides are also encompassed by the present disclosure, and one of skill in the art would readily know how to make, identify, or select such variants or derivatives, and how to test for receptor binding activity of those variants.

[0037] The term "homologous" or “% identity” as used herein means a nucleic acid (or fragment thereof) or a peptide having a degree of homology to the corresponding natural reference nucleic acid or peptide that may be in excess of 60%, or in excess of 65%, or in excess of 70%, or in excess of 75%, or in excess of 80%, or in excess of 85%, or in excess of

90%, or in excess of 91%, or in excess of 92%, or in excess of 93%, or in excess of 94%, or in excess of 95%, or in excess of 96%, or in excess of 97%, or in excess of 98%, or in excess of 99%, or other specific percentages described herein. For example, in regard to peptides, the percentage of homology or identity as described herein is typically calculated as the percentage of amino acid residues found in the smaller of the two sequences which align with identical amino acid residues in the sequence being compared, when four gaps per 100 amino acids may be introduced to assist in that alignment (as set forth by Dayhoff, in Atlas of Protein Sequence and Structure, Vol. 5, p. 124, National Biochemical Research Foundation, Washington, D.C. (1972)). In one embodiment, the percentage homology as described above is calculated as the percentage of the components found in the smaller of the two sequences that may also be found in the larger of the two sequences (with the introduction of gaps), with a component being defined as a sequence of four, contiguous amino acids. Also included as substantially homologous is any protein product which may be isolated by virtue of cross-reactivity with antibodies to the native protein product. Sequence identity or homology can be determined by comparing the sequences when aligned so as to maximize overlap and identity while minimizing sequence gaps. In particular, sequence identity may be determined using any of a number of mathematical algorithms. A non-limiting example of a mathematical algorithm used for comparison of two sequences is the algorithm of Karlin & Altschul, Proc. Natl. Acad. Sci. USA 1990, 87, 2264-2268, modified as in Karlin & Altschul, Proc. Natl. Acad. Sci. USA 1993, 90, 5873-5877.

[0038] In at least one embodiment "% identity" represents the number of amino acids or nucleotides which are identical at corresponding positions in two sequences of a peptide or nucleic acids encoding similar peptides. For example, two amino acid sequences each having 15 residues will have at least 60% identity when at least 9 of the amino acids at corresponding positions are the same, at least 66% identity when at least 10 of the amino acids at corresponding positions are the same, at least 73% identity when at least 11 of the amino acids at corresponding positions are the same, at least 80% identity when at least 12 of the amino acids at corresponding positions are the same, at least 86% identity when at least 13 of the amino acids at corresponding positions are the same, and at least 93% identity when at least 14 of the amino acids at corresponding positions are the same. In another example, two amino acid sequences each having 19 residues will have at least 73% identity when at least 14 of the amino acids at corresponding positions are the same, at least 78% identity when at least 15 of the amino acids at corresponding positions are the same, at least 84% identity when at least 16 of the amino acids at corresponding positions are the same, at least 89% identity when at least

17 of the amino acids at corresponding positions are the same, and at least 94% identity when at least 18 of the amino acids at corresponding positions are the same.

[0039] Similarly, two amino acid sequences each having 20 residues will have at least 95% identity when 19 of the amino acids at corresponding positions are the same, or at least 90% identity when at least 18 of the amino acids at corresponding positions are the same, or at least 85% identity when at least 17 of the amino acids at corresponding positions are the same, or at least 80% identity when at least 16 of the amino acids at corresponding positions are the same. In other non-limiting examples, two amino acid sequences each having 100 residues will have 95% identity when 95 of the amino acids at corresponding positions are the same. Two amino acid sequences each having 100 residues will have at least 90% identity when at least 90 of the amino acids at corresponding positions are the same. Further, where a sequence is described herein as having "at least X% identity to" a reference sequence, this is intended to include, unless indicated otherwise, all percentages greater than X%, such as for example, (X+1)%, (X+2)%, (X+3)%, (X+4)%, and so on, up to 100%.

[0040] The terms "polynucleotide sequence" or "nucleic acid," as used herein, include any polynucleotide sequence which encodes a peptide product including polynucleotides in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced by chemical synthetic techniques or by a combination thereof. The DNA may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand. The peptide may be expressed using polynucleotide sequence(s) which differ in codon usage due to the degeneracies of the genetic code or allelic variations.

[0041] The terms "infection," "transduction," and "transfection" are used interchangeably herein and mean introduction of a gene, nucleic acid, or polynucleotide sequence into cells such that the encoded peptide or protein is expressed. The polynucleotides which encode peptides or proteins of the present disclosure may comprise additional sequences, such as additional coding sequences within the same transcription unit, controlling elements such as promoters, ribosome binding sites, transcription terminators, polyadenylation sites, additional transcription units under control of the same or different promoters, sequences that permit cloning, expression, homologous recombination, and transformation of a host cell, and any such construct as may be desirable to provide embodiments of the present disclosure.

[0042] In certain embodiments, the present disclosure includes expression vectors capable of expressing one or more peptide molecules described herein. Expression

vectors for different cell types are well known in the art and can be selected without undue experimentation. Generally, the DNA encoding the fusion polypeptide is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression. If necessary, the DNA may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences recognized by the desired host, although such controls are generally available in the expression vector. The vector is then introduced into the host through standard techniques. Guidance can be found e.g., in Sambrook et al. Molecular Cloning: A Laboratory Manual, 3d ed., Cold Spring Harbor Laboratory Press, NY 2001)).

[0043] In at least certain embodiments, the peptide conjugates of the present disclosure, whether wholly or partially synthetically or recombinantly produced, may be used as a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may contain, in addition to the peptide conjugate and carrier, diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. Suitable carriers, vehicles and other components of the formulation are described, for example, in Remington: The Science and Practice of Pharmacy, 22nd ed. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the peptide conjugate. The characteristics of the carrier will depend on the route of administration.

[0044] The pharmaceutical compositions of the present disclosure may be in the form of liposomes in which the peptide conjugate is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent Nos. 4,235,871; 4,501,728; 4,837,028; and 4,737,323, all of which are incorporated herein by reference.

[0045] An effective amount of the peptide conjugate used herein for treatment of a particular condition can be determined by the attending diagnostician, as one skilled in the art, by the use of conventional techniques and by observing results obtained under analogous circumstances. In determining the therapeutically effective dose, a number of factors may be considered by the attending diagnostician, including, but not limited to: the species of the subject; its size, age, and general health; the response of the individual subject; the mode of

administration; the bioavailability characteristic of the preparation administered; the dose regimen selected; the use of concomitant medication; and other relevant circumstances. An effective amount of a peptide conjugate of the present disclosure also refers to an amount of the peptide conjugate which is effective in controlling or reducing the particular condition.

[0046] An effective amount of a composition of the present disclosure will generally contain sufficient active ingredient (i.e., the peptide conjugate) to deliver from about 0.1 μg/kg to about 100 mg/kg (weight of active ingredient/body weight of patient). Particularly, the composition will deliver at least 0.5 μg/kg to 50 mg/kg, and more particularly at least 1 μg/kg to 10 mg/kg. Without wishing to be held to a specific dosage, it is contemplated that the various pharmaceutical compositions used to practice the method of the present disclosure may contain, but are not limited to, about 0.01 mg to about 25 mg of the peptide conjugate per kg body weight per dose.

[0047] Practice of the method of the present disclosure may include administering to a subject an effective amount of the peptide conjugate in any suitable systemic or local formulation, in an amount effective to deliver the dosages listed above. In one embodiment, an effective, particular therapeutic dosage of the peptide conjugate is 1 μg/kg to 10 mg/kg. The dosage can be administered on a one-time basis, or (for example) from one to five times per day or once or twice per week, or continuously via a venous drip, depending on the desired therapeutic effect. In one therapeutic method of the present disclosure, the peptide conjugate is provided in an IV infusion in the range of from 1 mg/kg-10 mg/kg of body weight once a day. The duration of an intravenous therapy using the pharmaceutical composition of the present disclosure will vary, depending on the condition being treated and the condition and potential idiosyncratic response of each individual patient. In at least one embodiment, it is contemplated that the duration of each application of the peptide conjugate may be in the range of 1 to 4 hours and given once every 12 or 24 hours by continuous intravenous administration. Other therapeutic drugs, intravenous fluids, cardiovascular and respiratory support could also be provided if requested by the attending physician in a manner known to one of ordinary skill in the art.

[0048] In practicing the method of treatment or use of the peptide conjugates of the present disclosure, an effective amount of the peptide conjugate is administered to a mammal having a condition to be treated, such as a breast cancer tumor or any other cancer to which the peptide conjugate binds with high specificity. The peptide conjugate may be administered in accordance with the method of the present disclosure either alone or in combination with other therapies.

[0049] Administration of the peptide conjugate used in the pharmaceutical composition or to practice the method of the present disclosure can be carried out in a variety of conventional ways, such as, but not limited to, orally, by inhalation, rectally, or by cutaneous, subcutaneous, intraperitoneal, vaginal, or intravenous injection. In certain embodiments, oral formulations may be formulated such that the peptide conjugate passes through a portion of the digestive system before being released, for example it may not be released until reaching the small intestine, or the colon.

[0050] When an effective amount of the peptide conjugate is administered orally, the compound may be in the form of a tablet, capsule, powder, solution or elixir. The pharmaceutical composition may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder particularly contains from about .05 to 95% of the peptide compound by dry weight. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, 35 propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition particularly contains from about 0.005 to 95% by weight of peptide. For example, a dose of 10-1000 mg once to twice a day could be administered orally.

[0051] For oral administration, the peptide conjugates can be formulated into solid or liquid preparations such as capsules, pills, tablets, lozenges, melts, powders, suspensions, or emulsions. Solid unit dosage forms can be capsules of the ordinary gelatin type containing, for example, surfactants, lubricants and inert fillers such as lactose, sucrose, and cornstarch or they can be sustained release preparations.

[0052] In another embodiment, the peptide conjugates of the present disclosure can be tableted with conventional tablet bases such as lactose, sucrose, and cornstarch in combination with binders, such as acacia, cornstarch, or gelatin, disintegrating agents such as potato starch or alginic acid, and a lubricant such as stearic acid or magnesium stearate. Liquid preparations are prepared by dissolving the peptide conjugate in an aqueous or non-aqueous pharmaceutically acceptable solvent which may also contain suspending agents, sweetening agents, flavoring agents, and preservative agents as are known in the art.

[0053] For parenteral administration, for example, the peptide conjugates may be dissolved in a physiologically acceptable pharmaceutical carrier and administered as either a solution or a suspension. Illustrative of suitable pharmaceutical carriers are water, saline, dextrose

solutions, fructose solutions, ethanol, or oils of animal, vegetative, or synthetic origin. The pharmaceutical carrier may also contain preservatives, and buffers as are known in the art.

[0054] When an effective amount of the peptide conjugate is administered by intravenous, cutaneous or subcutaneous injection, the peptide conjugate may be in the form of a pyrogen-free, parenterally acceptable aqueous solution or suspension. The preparation of such parenterally acceptable peptide solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A particular pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection may contain, in addition to the peptide conjugate, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical compositions of the present disclosure may also contain stabilizers, preservatives, buffers, antioxidants, or other additive known to those of skill in the art.

[0055] As noted above, the compositions can also include an appropriate carrier. For topical use, any of the conventional excipients may be added to formulate the peptide compound into a lotion, ointment, powder, cream, spray, or aerosol. For surgical implantation, the peptide conjugate may be combined with any of the well-known biodegradable and bioerodible carriers, such as polylactic acid and collagen formulations. Such materials may be in the form of solid implants, sutures, sponges, wound dressings, and the like. In any event, for local use of the materials, the peptide conjugate is usually present in the carrier or excipient in a weight ratio of from about 1:1000 to 1:20,000, but is not limited to ratios within this range. Preparation of compositions for local use is detailed in Remington: The Science and Practice of Pharmacy, 22nd ed.

[0056] As noted, particular amounts and modes of administration are able to be determined by one skilled in the art. One skilled in the art of preparing formulations can readily select the proper form and mode of administration depending upon the particular characteristics of the peptide conjugate selected, the condition to be treated, and other relevant circumstances using formulation technology known in the art, described, for example, in Remington: The Science and Practice of Pharmacy, 22nd ed. The pharmaceutical compositions of the present disclosure can be manufactured utilizing techniques known in the art. As noted above, typically the effective amount of the peptide conjugate will be admixed with a pharmaceutically acceptable carrier.

[0057] Additional pharmaceutical methods may be employed to control the duration of action of the peptide conjugate. Increased half-life and controlled release preparations may be

achieved through the use of polymers to conjugate, complex with, or absorb the peptide conjugates described herein. The controlled delivery and/or increased half-life may be achieved by selecting appropriate macromolecules (for example, polysaccharides, polyesters, polyamino acids, homopolymers polyvinyl pyrrolidone, ethylenevinylacetate, methylcellulose, or carboxymethylcellulose, and acrylamides such as N-(2-hydroxypropyl) methacrylamide, proteins (e.g., bovine serum albumin or human serum albumin) and the appropriate concentration of macromolecules as well as the methods of incorporation, in order to control release.

[0058] Another possible method useful in controlling the duration of action by controlled release preparations and half-life is incorporation of the peptide conjugate into particles of a polymeric material such as polyesters, polyamides, polyamino acids, hydrogels, poly(lactic acid), ethylene vinylacetate copolymers, copolymer micelles of, for example, PEG and poly(l-aspartamide).

[0059] It is also possible to entrap the peptide conjugates in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatine-microcapsules and poly-(methylmethacylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules), or in macroemulsions. Such techniques are well known to persons having ordinary skill in the art.

[0060] When the peptide composition is to be used as an injectable material, it can be formulated into a conventional injectable carrier. Suitable carriers include biocompatible and pharmaceutically acceptable phosphate buffered saline solutions, which are particularly isotonic.

[0061] For reconstitution of a lyophilized product in accordance with the present disclosure, one may employ a sterile diluent, which may contain materials generally recognized for approximating physiological conditions and/or as required by governmental regulation. In this respect, the sterile diluent may contain a buffering agent to obtain a physiologically acceptable pH, such as sodium chloride, saline, phosphate-buffered saline, and/or other substances which are physiologically acceptable and/or safe for use. In general, the material for intravenous injection in humans should conform to regulations established by the Food and Drug Administration, which are available to those in the field. The pharmaceutical composition may also be in the form of an aqueous solution containing many of the same substances as described above for the reconstitution of a lyophilized product.

[0062] In certain embodiments the peptide conjugates of the present disclosure can also be administered as a pharmaceutically acceptable acid- or base-addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines.

[0063] As mentioned above, the peptide conjugates of the present disclosure may be incorporated into pharmaceutical preparations which may be used for therapeutic purposes. However, the term "pharmaceutical preparation" is intended in a broader sense herein to include preparations containing a peptide composition in accordance with present disclosure, used not only for therapeutic purposes but also for reagent, imaging, or diagnostic purposes as known in the art. The pharmaceutical preparation intended for therapeutic use should contain a "pharmaceutically acceptable" or "effective amount" of the peptide compound, i.e., that amount necessary for a therapeutic response in a patient or subject in need of such treatment. If the pharmaceutical preparation is to be employed as a reagent, imaging, or diagnostic, then it should contain reagent, imaging, or diagnostic amounts of the peptide conjugates.

[0064] Peptides of the present disclosure include, but are not limited to, amino acid sequences which include the 15-amino acid sequence shown in Table 1, or variants of the peptides of Table 1 as discussed in further detail elsewhere herein. The peptide may optionally further comprise an amino acid linker sequence of, for example, 1 to 25 additional amino acids.

Table 1. Selected binding peptides.

Peptide No.	Amino Acid Sequence	SEQ ID NO:
1	AREYGTRFSLIGGYR	1
2	PKAFQYGGRAVGGLW	2
3	PVRYGFSGPRLAELW	3
4	RNVPPIFKEVYWIAQ	4
5	RTLIRMGTGAHAFAV	5

[0065] Examples of drugs, reagents, imaging, and/or diagnostic agents that may be conjugated, directly or indirectly, to the peptides disclosed herein include, but are not limited to:

(a) chemotherapeutic drugs including those having suitable sites for linking directly to or via a linker to the peptide, wherein the drug has activity while still conjugated to the peptide,

or becomes active upon release from the peptide at the tumor site; for example the linker can be an acid-labile linker, an ester linker or a carbamate linker, and the drug can be, for example, doxorubicin or taxol, or any other drug which can be linked to the peptide.

(b) diagnostic reagents, such as radioisotopes for PET imaging, quantum dots, and near-infrared red excited dye for fluorescence imaging, and magnetic nanoparticles such as iron oxide for MRI.

(c) oligonucleotides (DNA or RNA) or oligopeptides can be used as detectable reagent for diagnosis or anti-cancer reagent to interfere cancer cell behavior.

(d) drug carriers including nano-carriers (nano-cages, nano- dendrites and etc.), liposomes, polymeric drug scaffolds and polymeric micelles those are able to load, transport and release drugs, and

(e) functional particles such as gold-nanorods and gold-nanoparticles, or photosensitizer reagents such as aminolevulinic acid (ALA), and Silicon Phthalocyanine, for example, which are sensitive to either wide spectrum or specific wavelength laser that can be used for photothermal therapy and Photodynamic therapy (PDT).

[0066] In at least one embodiment of the present disclosure, the peptide comprises an amino acid sequence which includes the 15-amino acid sequence AREYGTRFSLIGGYR (SEQ ID NO:1), or variants thereof. The peptide may optionally comprise an amino acid linker sequence of, for example, 1 to 25 additional amino acids such that the peptide comprises or consists of 16-40 total amino acids (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 amino acids), or more particularly 16-25 amino acids (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25), including SEQ ID NO: 1 or variants thereof. In the peptides which include SEQ ID NO:1 or variants thereof, and a linker sequence, the linker sequence may extend from the N-terminal or C-terminal end of the amino acid sequence SEQ ID NO:1, or variant thereof. The amino acids of the linker sequence, or the substituted amino acids of the SEQ ID NO:1 variant may be selected from, but are not limited to, the group consisting of gly, L-ala, L-arg, L-asn, L-asp, L-cys, L-glu, L-gln, L-his, L-ile, L-leu, L-lys, L-met, L-phe, L-pro, L-ser, L-thr, L-trp, L-tyr, L-val, D-ala, D-arg, D-asn, D-asp, D-cys, D-glu, D-gln, D-his, D-ile, D-leu, D-lys, D-met, D-phe, D-pro, D-ser, D-thr, D-trp, D-tyr, and D-val. In certain embodiments, the peptides of the present disclosure include variants of SEQ ID NO:1 which may include substitutions, such as conservative substitutions, or any amino acid in a D or L configuration (such as listed above), wherein the variant peptide has the activity of the non-variant version of the peptide. Examples of substitutions include but are not limited to those

described elsewhere herein. Examples of substitutions include but are not limited to those described elsewhere herein. In at least certain embodiments, the variant peptides of SEQ ID NO:1 comprises at least at least 86% or greater sequence identity (as defined elsewhere herein) with SEQ ID NO:1, i.e., it is identical except for one or two amino acid substitutions.

[0067] In at least one embodiment of the present disclosure, the peptide comprises an amino acid sequence which includes the 15-amino acid sequence PKAFQYGGRAVGGLW (SEQ ID NO:2), or variants thereof. The peptide may optionally comprise an amino acid linker sequence of, for example, 1 to 25 additional amino acids such that the peptide comprises or consists of 16-40 total amino acids (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 amino acids), or more particularly 16-25 amino acids (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25), including SEQ ID NO: 2 or variants thereof. In the peptides which include SEQ ID NO:2 or variants thereof, and a linker sequence, the linker sequence may extend from the N-terminal or C-terminal end of the amino acid sequence SEQ ID NO:2, or variant thereof. The amino acids of the linker sequence, or the substituted amino acids of the SEQ ID NO:2 variant may be selected from, but are not limited to, the group consisting of gly, L-ala, L-arg, L-asn, L-asp, L-cys, L-glu, L-gln, L-his, L-ile, L-leu, L-lys, L-met, L-phe, L-pro, L-ser, L-thr, L-trp, L-tyr, L-val, D-ala, D-arg, D-asn, D-asp, D-cys, D-glu, D-gln, D-his, D-ile, D-leu, D-lys, D-met, D-phe, D-pro, D-ser, D-thr, D-trp, D-tyr, and D-val. In certain embodiments, the peptides of the present disclosure include variants of SEQ ID NO:2 which may include substitutions, such as conservative substitutions, or any amino acid in a D or L configuration (such as listed above), wherein the variant peptide has the activity of the non-variant version of the peptide. Examples of substitutions include but are not limited to those described elsewhere herein. In at least certain embodiments, the variant peptides of SEQ ID NO:2 comprises at least at least 86% or greater sequence identity (as defined elsewhere herein) with SEQ ID NO:2, i.e., it is identical except for one or two amino acid substitutions.

[0068] In at least one embodiment of the present disclosure, the peptide comprises an amino acid sequence which includes the 15-amino acid sequence PVRYGFSGPRLAELW (SEQ ID NO:3), or variants thereof. The peptide may optionally comprise an amino acid linker sequence of, for example, 1 to 25 additional amino acids such that the peptide comprises or consists of 16-40 total amino acids (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 amino acids), or more particularly 16-25 amino acids (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25), including SEQ ID NO: 3 or variants thereof. In the peptides which include SEQ ID NO:3 or variants thereof, and a linker sequence, the linker sequence may extend from the N-terminal or C-terminal end of the amino acid sequence SEQ ID NO:3,

or variant thereof. The amino acids of the linker sequence, or the substituted amino acids of the SEQ ID NO:3 variant may be selected from, but are not limited to, the group consisting of gly, L-ala, L-arg, L-asn, L-asp, L-cys, L-glu, L-gln, L-his, L-ile, L-leu, L-lys, L-met, L-phe, L-pro, L-ser, L-thr, L-trp, L-tyr, L-val, D-ala, D-arg, D-asn, D-asp, D-cys, D-glu, D-gln, D-his, D-ile, D-leu, D-lys, D-met, D-phe, D-pro, D-ser, D-thr, D-trp, D-tyr, and D-val. In certain embodiments, the peptides of the present disclosure include variants of SEQ ID NO:3 which may include substitutions, such as conservative substitutions, or any amino acid in a D or L configuration (such as listed above), wherein the variant peptide has the activity of the non-variant version of the peptide. Examples of substitutions include but are not limited to those described elsewhere herein. In at least certain embodiments, the variant peptides of SEQ ID NO:3 comprises at least at least 86% or greater sequence identity (as defined elsewhere herein) with SEQ ID NO:3, i.e., it is identical except for one or two amino acid substitutions.

[0069] In at least one embodiment of the present disclosure, the peptide comprises an amino acid sequence which includes the 15-amino acid sequence RNVPPIFKEVYWIAQ (SEQ ID NO:4), or variants thereof. The peptide may optionally comprise an amino acid linker sequence of, for example, 1 to 25 additional amino acids such that the peptide comprises or consists of 16-40 total amino acids (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 amino acids), or more particularly 16-25 amino acids (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25), including SEQ ID NO: 4 or variants thereof. In the peptides which include SEQ ID NO:4 or variants thereof, and a linker sequence, the linker sequence may extend from the N-terminal or C-terminal end of the amino acid sequence SEQ ID NO:4, or variant thereof. The amino acids of the linker sequence, or the substituted amino acids of the SEQ ID NO:4 variant may be selected from, but are not limited to, the group consisting of gly, L-ala, L-arg, L-asn, L-asp, L-cys, L-glu, L-gln, L-his, L-ile, L-leu, L-lys, L-met, L-phe, L-pro, L-ser, L-thr, L-trp, L-tyr, L-val, D-ala, D-arg, D-asn, D-asp, D-cys, D-glu, D-gln, D-his, D-ile, D-leu, D-lys, D-met, D-phe, D-pro, D-ser, D-thr, D-trp, D-tyr, and D-val. In certain embodiments, the peptides of the present disclosure include variants of SEQ ID NO:4 which may include substitutions, such as conservative substitutions, or any amino acid in a D or L configuration (such as listed above), wherein the variant peptide has the activity of the non-variant version of the peptide. Examples of substitutions include but are not limited to those described elsewhere herein. In at least certain embodiments, the variant peptides of SEQ ID NO:4 comprises at least at least 86% or greater sequence identity (as defined elsewhere herein) with SEQ ID NO:4, i.e., it is identical except for one or two amino acid substitutions.

[0070] In at least one embodiment of the present disclosure, the peptide comprises an amino acid sequence which includes the 15-amino acid sequence RTLIRMGTGAHAFAV (SEQ ID NO:5), or variants thereof. The peptide may optionally comprise an amino acid linker sequence of, for example, 1 to 25 additional amino acids such that the peptide comprises or consists of 16-40 total amino acids (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 amino acids), or more particularly 16-25 amino acids (e.g., 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25), including SEQ ID NO: 5 or variants thereof. In the peptides which include SEQ ID NO:5 or variants thereof, and a linker sequence, the linker sequence may extend from the N-terminal or C-terminal end of the amino acid sequence SEQ ID NO:5, or variant thereof. The amino acids of the linker sequence, or the substituted amino acids of the SEQ ID NO:5 variant may be selected from, but are not limited to, the group consisting of gly, L-ala, L-arg, L-asn, L-asp, L-cys, L-glu, L-gln, L-his, L-ile, L-leu, L-lys, L-met, L-phe, L-pro, L-ser, L-thr, L-trp, L-tyr, L-val, D-ala, D-arg, D-asn, D-asp, D-cys, D-glu, D-gln, D-his, D-ile, D-leu, D-lys, D-met, D-phe, D-pro, D-ser, D-thr, D-trp, D-tyr, and D-val. In certain embodiments, the peptides of the present disclosure include variants of SEQ ID NO:5 which may include substitutions, such as conservative substitutions, or any amino acid in a D or L configuration (such as listed above), wherein the variant peptide has the activity of the non-variant version of the peptide. Examples of substitutions include but are not limited to those described elsewhere herein. In at least certain embodiments, the variant peptides of SEQ ID NO:5 comprises at least at least 86% or greater sequence identity (as defined elsewhere herein) with SEQ ID NO:5, i.e., it is identical except for one or two amino acid substitutions.

[0071] Various embodiments of the present disclosure will be more readily understood by reference to the following examples and description, which as noted above are included merely for purposes of illustration of certain aspects and embodiments of the present disclosure, and are not intended to be limiting. The following detailed examples and methods describe how to make and use various peptides, peptide conjugates, and other peptide compositions of the present disclosure and are to be construed, as noted above, only as illustrative, and not limitations of the disclosure in any way whatsoever. Those skilled in the art will promptly recognize appropriate variations from the materials and procedures described herein.

[0072] EXPERIMENTAL

[0073] Methods

[0074] Cell line and MCF-7 tumor xenograft

[0075] Human breast cancer cells MCF-7 (ATCC) were cultured in Eagle's Minimal Essential Medium (EMEM, ATCC) with 10% fetal bovine serum (GIBco Inc). To generate tumors on the mice, 2.5- 3 × 106 cells in 0.1 ml of saline were hypodermically injected on the right flank for each mouse (Athymic Nude-Foxn1nu, 3-5 weeks, female, Harlan Lab). All the tests in the *in vivo* phage display and photothermal therapy were started when the tumor reached 0.5-0.8 cm in diameter.

[0076] *In vivo* phage display

[0077] The f3-15mer phage library (aka fUSE/15mer; GenBank Accession AF246445) displaying 15-mer peptide on all five copies of pIII coat proteins at N-terminal on fd phages were provided as a gift from Dr. George Smith at the University of Missouri. At least 1 × 1012 transducing units (TU) of f3-15mer phage library were intravenously injected into each tumor-bearing mouse through the tail vein and allowed to circulate for 1 hour before the euthanasia by CO_2 asphyxiation. Then 20 ml of PBS buffer was used to wash the unassociated phages by heart perfusion. The tumor-recognized phages were purified from the tumor homogenate and then amplified to be used as new input phages for the next round of selection. The tumor-homing peptide sequences in every round of selection were identified by phage genome sequencing from the third to fifth round.

[0078] *In vivo* fluorescence imaging.

[0079] All the peptides with biotin labeled at C-terminal for conjugation with streptavidin were commercially purchased from United BioSystems. A linker of GGG was added to the C-terminal during the peptide synthesis for each peptide. The streptavidin proteins, tagged with a fluorescence dye (Cy5) at a dye/protein ratio of 6.5 were purchased from USBiological. To conduct conjugation, 16 μl of 1 mg/ml streptavidin was added to 800 μl of 6.0 μM peptide saline solution, and the mixture was then incubated at room temperature for 3 h. Free unconjugated peptide was then removed by using Slide-A-Lyzer Dialysis Cassettes (10,000 MWCO, Thermo Inc.) according to the protocols provided with the kit. The Cy5-peptide conjugates were stored at 4 °C and used within 24 h after preparation. For in vivo imaging, 5 nmol of the as-prepared Cy5-peptides conjugates dissolved in 100 μl saline was injected through tail vein of each tumor model. All of the mice were given anesthesia of 4% isoflurane

/oxygen mixture and scanned under the In Vivo Xtreme Imaging System (CarestreamInc.) 24 h after the injection.

[0080] Synthesis of gold nanorods

[0081] Gold nanorods were synthesized through a seed mediated process. Seed solution was prepared by adding 600 μl of $NaBH_4$ (100 mM) into 10 ml mixture solution of 0.25 mM $HAuCl_4$ and 0.1 M CTAB under vigorous stirring. A total of 30 ml gold nanorod (AuNRs) solution was prepared each time. Briefly, to a 30 ml growth solution, which contained 0.5 mM $HAuCl_4$ and 0.1 M CTAB, varying amounts of 4 mM $AgNO_3$ solution and 210 μl of 78.8 mM ascorbic acid solution were added in sequence. After gentle shaking, 36 μl of the as-prepared gold seed solution was added, and the growth of AuNRs took place under room temperature for overnight. The resultant nanoparticles solution was considered as the as-synthesized AuNRs.

[0082] Preparation of peptide conjugated tumor-homing AuNRs

[0083] The peptides used for AuNRs conjugation were synthesized with an amino acid linker comprising four lysine amino acids (KKKK) at the C-terminal. It is expected that the majority of the conjugation reaction takes place through the amine groups at the C-terminal, leaving the $-NH_2$ at the N-end free. The AuNRs with extinction band maximized at around 808 nm were synthesized according to the well-developed seed-mediated method in cetyltrimethylammonium bromide (CTAB). Conjugation of peptides onto AuNRs can be finished, for example, through a three-step surface modification. To begin with, 100 ml of the as-prepared AuNRs were centrifuged twice at 10,000 rpm for 10 min, and then redispersed into 50 ml of 0.1 mM HS-PEG aqueous solution and placed overnight to allow the CTAB molecules on the AuNRs surface to be replaced by HS-PEG molecules. The AuNRs were then centrifuged once and mixed with 50 ml of 0.1 mM HS-PEG-COOH for a few hours to achieve partial ligand exchange of HS-PEG by HS-PEG-COOH. Afterwards, the nanoparticles were subjected to another round of centrifugation and redispersed into 10 ml of pH 6.0 MES buffer. The peptide conjugation was then initiated by activation of –COOH with EDC (4 mg) and NHS (6 mg) at room temperature for 15 min, followed by centrifugation at 4 °C for 10 min. Thereafter, the nanoparticle pellet was immediately resuspended into 2 ml of 1 mM peptide/ PBS solution and left on a rocker shaker for 2 h under room temperature. The resultant peptide-conjugated tumor-homing AuNRs were about 5 mg/ml in concentration and 200 μl of the solution containing about 1 mg of AuNRs were used for each mouse for intravenous injection.

[0084] Quantification of AuNRs tissue distribution through ICP-MS

[0085] Peptide-AuNRs or PEG-AuNRs (200 μl, 5 mg/ml) were intravenously injected into each tumor-bearing mouse through the tail vein and circulated for 1 day, 3 days and 5 days before euthanasia. The tumors and other organs including liver, kidney, intestine, heart, lung, brain and thigh muscle, were excised after heart perfusion and completely dried in a freeze drying system. Some peripheral blood was collected before heart perfusion and dried as well. Afterwards, all these tissues or dried blood were weighed and then digested completely by freshly prepared aqua regia. Finally, the solution was diluted with large amount of water, filtered and used for ICP-MS quantification.

[0086] To track the gold concentration in peripheral blood, 20 μl of blood was collected from suborbital space of 5 anesthesia mice each time at 3 min, 20 min, 1 h, 4 h, 7 h, 12 h, 24 h and 48 h after injecting either peptide conjugated AuNRs or PEG modified AuNRs. The gold was then digested and quantified following the same procedures as above.

[0087] Photothermal treatment of MCF-7 tumor

[0088] Twenty tumor-bearing mice were randomly separated into four groups. Mice in each group were injected intravenously with target peptide-AuNRs, control peptide-AuNRs, PEG-AuNRs and saline respectively. The amount of AuNRs was 1 mg per mouse. Twenty four hours after injection and under general anesthesia, all the mice were irradiated at the tumor site by 808 nm laser for 5 min at a power density of 2.25 W/cm^2. The laser spot size is about 1 cm in diameter. After laser irradiation, the mice were housed for another 20 days, after which the tumors were measured in dimensions and were then taken out of the mice and weighed. The tumor volume was calculated by the following equation: Volume= length × $width^2/2$.

[0089] HE staining

[0090] For HE staining, tumors were excised from mice 3 h after laser treatment and then placed immediately into 10% fresh formalin solution and soaked for 24 h. The fixed tumors were embedded in paraffin block following standard procedures. Thereafter, they were cut into 5 μm thick sections and mounted onto glass slides. Before imaging, the slices were deparaffinated and stained subsequently with hematoxylin and eosin staining solutions. The imaging was carried out on Eclipse Ti microscope system (Nikon Instruments Inc.).

[0091] Results

[0092] Briefly, as illustrated in FIG. 1a, *in vivo* phage display was carried out by several rounds of affinity test against MCF-7 tumors *in vivo*. In the first round, a phage library, which

contains billions of phage clones with each displaying a unique 15-mer peptide at the five copies of minor coat protein (pIII) of the phage, was injected intravenously into tumor-bearing mice. After an hour, the mice were sacrificed and the tumors were excised and homogenized. Tumor-associated phages (output phages) were extracted from tumor homogenate and used as an input for the next round of selection. Similarly, in the rest rounds of selections, output from the previous round was employed as an input for the next round and injected intravenously into a new tumor-bearing mouse. In this work, a total of 5 rounds of selection were conducted. It is expected that phages appear in the output of subsequent rounds should have higher tumor affinity. Hence, we randomly isolated some output phage clones in round 3, 4 and 5. Since the DNA inside the phage genetically encodes the peptides displayed on the surface of the phage, the DNA of the tumor-associated phage clones was sequenced to identify the sequences of 15-mer peptides (FIG. 1b and Tables 2-5). A total of 246 colonies were identified from 3-5 rounds of *in vivo* selection and the top 12 identified peptides with the number of repeats no less than 2 after the 5th round are listed in Table 2. The present work was mainly focused on the top 5 peptides with the highest occurrence frequency shown in Table 1 as they appeared to have higher affinity to MCF-7 tumors than the other peptides identified. Among these peptides, peptide 1 (SEQ ID NO:1) has the highest occurrence frequency, indicating that peptide 1 has the highest tumor affinity of the set.

Table 2. Summary of peptide sequences and frequencies in the 3rd to 5th round of in vivo phage display against MCF-7 tumors *

SEQ ID NO:	Peptide Sequences	Count/Frequency (%)			
		3rd Rnd (30 cln)	4th Rnd (54 cln)	5th Rnd (162 cln)	Sum (246 cln)
1	AREYGTRFSLIGGYR	1 / 3.33	3 / 5.56	7 / 4.32	11 / 4.30
2	PKAFQYGGRAVGGLW	0	3 / 5.56	7 / 4.32	10 / 4.07
3	PVRYGFSGPRLAELW	0	0	3 / 1.85	3 / 1.22
4	RNVPPIFKEVYWIAQ	0	0	3 / 1.85	3 / 1.22
5	RTLIRMGTGAHAFAV	0	0	3 / 1.85	3 / 1.22
22	DRYLPINGVSMFGVP	0	0	2 / 1.23	2 / 0.81
75	IPVQFSTIDFVAASY	0	0	2 / 1.23	2 / 0.81
108	PGHSLGKLSVLHSFF	0	0	2 / 1.23	2 / 0.81
125	QADGPNSVVRPFTLT	0	0	2 / 1.23	2 / 0.81
134	RSFAYAAAPTSFPWV	0	0	2 / 1.23	2 / 0.81
158	SVGCPVVGTVGYLRCG	0	0	2 / 1.23	2 / 0.81
191	VRMFDYGVPRRAVY	0	0	2 / 1.23	2 / 0.81

* Only peptides having total colonies no less than 2 are included in this table. Rd = round of biopanning; cln = total phage colonies.

Table 3. Thirty sequences randomly picked and identified from the 3rd round of *in vivo* biopanning.

SEQ. ID NO:	Sequence	Count (30 seq. total)	SEQ ID NO:	Sequence	Count (30 seq. total)
1	AREYGTRFSLIGGYR	1	121	PSVPVFRGRFFQVDY	1
7	AVVSSGGALYXRIVR	1	122	PVIAIPPSFANMFLF	1
29	FAFSPCPLESNVIGC	1	123	PWSSRPWYLQFLGAA	1
38	FSLGVSSVIFSPVSA	1	148	RLVCWRLGCVSPMGS	1
36	FVFPRPNAY*	1	149	RPPVVDAAHFGASRW	1
61	GHSPRCSSSFVRCEA	1	150	RQQDGRLIYTTASVR	1
62	GQDVRIRNEVNQYGL	1	175	SEELLVESSAIRSRE	1
63	GVQVPFSGGSSLLGM	1	173	SNPGLFVSGYWRLFP	1
64	GVSEDASVQHYYRSP	1	180	TRLECFSAGWRLSAC	1
95	LRMGLCSDQIRLSCA	1	184	TRVYWAPVSEGDVSP	1
96	MFRTFVGRSSNAVVG	1	195	VSSQHGXGREVNSAV	1
97	MQSVSGWFPWESVAY	1	196	VYASPASIPWSFAGL	1
103	NAVRVAFWSVPLYPF	1	201	YFTTPATLLPFGVGT	1
104	NAVRVAFWSVPLYXF	1	202	YRVREPQLFCCEGPD	1
105	PGGGANFLLSPFSGG	1			

The * marker indicates the incomplete peptides displayed on particular phages, which might be the result of either the failure of sequencing or the insertion of a stop codon into the fusion DNA sequence.

Table 4. Fifty-four sequences randomly picked and identified from the 4th round of *in vivo* biopanning.

SEQ ID NO:	Sequence	Count (54 seq. total)	SEQ ID NO:	Sequence	Count (54 seq. total)
8	ACAIGCXQANGLALV	1	116	PFPVSSRVVPRFTAV	1
9	AGGGGPTVGDDRVRR	1	117	PIGVFWDDLLIRH	1
10	ALGRGRVVGVRLVSL	1	2	PKAFQYGGRAVGGLW	3
1	AREYGTRFSLIGGYR	3	118	PKAXQYGGRAVGGLW	1
11	AVYPLLVICLLGRFW	1	119	PVQSLYAIGGVSLDT	1
25	DGLRVGXWDAVSX	1	120	PWSSILVGRSSSLLS	1
26	DKCVRRXILFMLVLM	1	124	QDFFAFCGGLAAVCG	1
27	DVLYRFGHSSVVFPG	1	144	RCSSFAMCGSIVPGS	1
39	FPYTRVPHFGNVHSS	1	145	RFPSASWSFSGHAAT	1
55	GASILGGXXGXXP*	1	146	RGAPFFISVSERAFR	1
56	GLAPVGSHSATPRPW	1	147	RSSGAVXNGVGVSALG	1
57	GLDLLHTTWRCCAPP	1	170	SGTGLXYPSWTAGSS	1
58	GPVVLPWPLFDGHLS	1	171	SGVHSYLAXRPVRL	1
59	GRXRLGVVSVLSXDG	1	172	SIGEMPSGLVTLSST	1
60	GTLKVGMMCSLGACLG	1	173	SNPGLFVSGYWRLFP	1
73	HRWMPHVFAVRQGAS	1	174	SRHVRSLGNLGDVSVG	1

SEQ ID NO:	Sequence	Count (54 seq. total)	SEQ ID NO:	Sequence	Count (54 seq. total)
77	KTWPXLVPGSASGRA	1	181	TSASTSVFIXRASST	1
89	LDPRRASFHIGRAAP	1	182	TTCLDFFNRPEVFWG	1
90	LFTPFFSCHEFRCWD	1	183	TYHSVVWSEPVVWS	1
91	LGRAGQSYPSFARGL	1	194	VRCEGVLINGDRCGL	1
92	LGRARDRLSIQFPHF	1	195	VSSQHGXGREVNSAV	1
93	LRFISVAVAGNLSWA	1	199	WSNRMPPLFTXWYX	1
84	LRPPVAGMSVARVYG	1	203	YFTTPATLLPFGV*	1
94	LVRALPLWPLVGPV	1	98	MCAVRDPAFSRS*	1
99	MPLRFFXPSXGPLAP	1	107	PFARAPVEHHDVVGL	1

The * marker indicates the incomplete peptides displayed on particular phages, which might be the result of either the failure of sequencing or the insertion of a stop codon into the fusion DNA sequence.

Table 5. 162 sequences were randomly picked and identified from the 5th round of *in vivo* biopanning.

SEQ ID NO:	Sequence	Count (162 seq. total)	SEQ ID NO:	Sequence	Count (162 seq. total)
12	AHPPLASVWHVSVPL	1	2	PKAFQYGGRAVGGLW	7
13	ALESSGSVPSRDLPP	1	109	PLRYGPSRSRLDEVW	1
1	AREYGTRFSLIGGYR	7	110	PMLGPHLCVSEFCGR	1
14	ARIGFASGSRPYVSS	1	111	PNRNYLPNTSMDGSR	1
15	ASSFSVSEVSVVRRV	1	112	PPDSFSRGYRVRDTF	1
16	ASSVPYVDANWSYNR	1	113	PPQTISGKARYLPSV	1
17	AVFDGSLCVPGFYSC	1	114	PRAASIFRAGHVSML	1
18	AVTPQNPYGIQRDRR	1	115	PSALRVKGFTVVSRS	1
19	AYGLVVPDLPAMPSL	1	3	PVRYGFSGPRLAELW	3
20	CAGFQSRQVAL*	1	125	QADGPNSVVRPFTLT	2
21	CSGCAPGFRSERAIR	1	126	QPVSAVPLCRLHCRS	1
22	DRYLPINGVSMFGVP	2	127	QPWHPGVYGVASSVA	1
23	DSALRVSRWRLSHSV	1	128	RAPRSAPGIFVFRSF	1
24	DSSSLGNSSGSRGWR	1	129	RERIHSPGSTQILFL	1
28	EGLFSFPRGASE*	1	130	RFGFSATWDQSNLLL	1
29	FAFSPCPLESNVIGC	1	131	RGFLSDLHALASRDR	1
30	FGHIIPSRFDRLSLG	1	4	RNVPPIFKEVYWIAQ	3
31	FGRIPSPLAYTYSFR	1	132	RRVP*	1
32	FLLTGLQHATSSGFR	1	133	RSDHLGVGPASASRYG	1
33	FSDSFVTGVWAPSRP	1	134	RSFAYAAAPTSFPWV	2
34	FSSGGTSYRLRHIPF	1	135	RSLSHGGRWGPGYAI	1
35	FSVSFPSLPAPPDRS	1	136	RSPWSDFYASASRGP	1
36	FVFPRPNAY*	1	5	RTLIRMGTGAHAFAV	3
37	FVRHYLLGGQGSLPR	1	137	RVLFLVAALAIGSLA	1
40	GAKPGLGPRAHLGGV	1	138	RVLGRDGSVFYELAA	1
41	GALHQTRPVGPAFAW	1	139	RVNFLFKPSVIFNAP	1

SEQ ID NO:	Sequence	Count (162 seq. total)	SEQ ID NO:	Sequence	Count (162 seq. total)
42	GAPGAFFGSVRDVVPRR	1	140	RVPPRYHAKISPMVK	1
43	GAPLSANFNNPAFWR	1	141	RVQSTILSGLRGFSS	1
44	GAVDFVSLYAAATVA	1	142	RVTHHAFLGAHRTVG	1
45	GAYPSLDFPRAPSFG	1	143	RYRPVFPGFEETLPR	1
46	GDAFGFFPPFRTMG	1	161	SDHSPTQSQRASHDA	1
47	GFTDVHLHLPGNSHR	1	162	SDWFPTACFDCRSVR	1
48	GGAVFPEYPLARAFL	1	163	SGASRNSAFWAVSVA	1
49	GGPLVSPLSEDARPW	1	164	SGAYSSFRPSHHTTR	1
50	GKGEVSLYSLGSPGM	1	165	SGLCRYESPSGRPSC	1
51	GLHGATPAHRLFHTG	1	166	SGNSAIWPRVRLLHG	1
52	GSGAVSPPLWAARGR	1	167	SGYMTSALIRPSRFP	1
53	GSGTAYMMRPSLGPD	1	168	SKKPQSSNEGYRIAD	1
54	GTRAVLVVGLDALSA	1	169	SKTFQYGGRAIEGPW	1
65	GVGHVRAGHLRSVGI	1	151	SLPHLAPFGTTFFGP	1
66	GWSHFYRSPNFLRIQ	1	152	SPPLAPYGGTRVGLT	1
67	HAALSLPWYRINSVY	1	153	SPPLAPYGGTRVGLTG	1
68	HGSLGLGWPGHTSVR	1	154	SSGFRDAFRGWDGSA	1
69	HKAIQHGGRAVGGAW	1	155	SSVA*	1
70	HPTTSYSTSVFSWGW	1	156	STFFGFVGNSIVRPW	1
71	HVSGYVSRFDRSVGA	1	157	STSGVLSSILHVVSV	1
72	HVTCVHSVSSLRSIV	1	158	SVGCPVVGTVGYLRCG	2
74	IPLLVNLPHLPRAAL	1	159	SVLHPALDFTSLA	1
75	IPVQFSTIDFVAASY	2	160	SVVXLRTRHFSTDSA	1
76	KVGVALVGP*	1	176	TGSVLSRFASGRLAP	1
78	LAGVQMSLRSLDTRR	1	177	TGTVVQVADPFAGGH	1
79	LFLNSHDDDRSFLSS	1	178	TNVPPISNDA*	1
80	LGAHVVLSGSSDFFP	1	179	TPFVFRAGRFFLHAG	1
81	LGGAGPFFGLGLVES	1	180	TRLECFSAGWRLSAC	1
82	LLGELPPTPRSPRLW	1	185	VCSPVFSPFCKMSVA	1
83	LPSIGPWEPXPDALS	1	186	VFGATSVVRDLYSLR	1
84	LRPPVAGMSVARVYG	1	187	VFRRVDTAVYKPSYP	1
85	LRSLVSYSGGHNYSG	1	188	VGAASFYLERGSRXS	1
86	LTLSHPHWVLNHFVS	1	189	VLERSRRVLGGAAKV	1
87	LVGTLLGAHGFVAIP	1	190	VRINNCIGFDSNCTS	1
88	LVSRLTPCDLSFYAC	1	191	VRMFDYGVPRRAVYG	2
100	MAAWLPHSYSITRLL	1	192	VRVTPSFFREPSGFV	1
101	MHFGPGFGH*	5	193	VTATAAQPGDAFIGV	1
102	NAAGYNSRVVTLPLF	1	197	WFPTHPFWTYSSWTG	1
106	PAQSNFVTWGYNVAV	1	198	WRYRLVYALLAMLTI	1
107	PFARAPVEHHDVVGL	1	200	YSRVLSSSSYRFFDR	1
108	PGHSLGKLSVLHSFF	2			

The * marker indicates the incomplete peptides displayed on particular phages, which might be the result of either the failure of sequencing or the insertion of a stop codon into the fusion DNA sequence.

[0093] We then conducted in vivo experiments to test the targeting activity of the selected peptides in tumor targeting. First, we employed the in vivo imaging system to verify peptide accumulation at tumor sites. Second, we conjugated the peptides to gold nanorods (AuNRs, FIG. 2), a well-known photothermal reagent, to confirm the effectiveness of the selected peptides in enhancing the targeted delivery of a payload (e.g., drug) to cancerous tissues.

[0094] For *in vivo* imaging, the 5 peptides (SEQ ID NOS:1-5) were conjugated to streptavidin with a Cy5 fluorescent tag, and were injected into mice through tail vein. In vivo imaging was carried out 24 h later. For comparison, a random peptide, KGYGVGLRFPAWQGA (SEQ ID NO:6), was used as a control peptide. The amount of peptides accumulated at the MCF-7 tumors is judged by the fluorescence intensity, and the stronger fluorescence indicates more peptide accumulation. As shown in FIG. 3, all of the 5 selected peptides exhibited significantly higher tumor accumulation in comparison to the control peptide. Among them, peptide 1 showed the strongest fluorescence intensity, suggesting that it has the best tumor-homing capability. In addition, peptide 2 presented slightly lower fluorescence intensity compared to peptide 1, which is consistent with the fact that peptide 1 has slightly higher occurrence frequency than peptide 2 (Table 1). Thus, peptide 1 was chosen for further targeted drug delivery testing.

[0095] The peptide-assisted *in vivo* drug delivery was tested by quantification of tissue distribution of the nanoparticles as well as the effectiveness of photothermal treatment on MCF-7 tumor-bearing mice. To conduct the test, peptide 1 was first conjugated with AuNRs at the C-terminal through a well-developed method, in which the as-synthesized CTAB coated AuNRs were first replaced by HS-PEG-COOH, followed by peptide conjugation through the EDC/NHS chemistry (see experimental section). The resultant nanoparticles are termed Peptide-AuNRs. The hydrodynamic radius of the CTAB coated AuNRs, PEG coated AuNRs and peptide conjugated AuNRs is 40.8 ± 2.1 nm, 44.3 ± 2.5 nm and 47.0 ± 2.4 nm, respectively (FIG. 4), suggesting the successful conjugation of peptides onto the AuNRs. For quantitative studies, 1 mg of Peptide-AuNRs (200 μl, 5 mg/ml) per mouse was used for intravenous injection. We first monitored for 48 h the in vivo blood circulation of AuNRs. We discovered that the half-life ($t_{1/2}$) of the Target-AuNRs in the blood is about 16 h (FIG. 5), and the value for the control peptide conjugated AuNRs (Control-AuNRs) is about 18 h; both are comparable to the previously reported $t_{1/2}$ of PEG coated AuNRs.

[0096] For *in vivo* tissue distribution study, the nanoparticles were allowed to circulate for five different periods of time (1 day, 2 days, 3 days, 4 days and 5 days). At the end of each time point, mice were sacrificed and the organs were then freeze dried, weighed, and digested.

Finally, the concentration of gold in each organ was quantified by the Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). The Control-AuNRs were used as a control. In the presence of the tumor-homing peptide, the tumor uptake of AuNRs after 1 day circulation is 63% higher than that of the case with control peptide and it remained to be 61%-83% higher during the whole period of five-day monitoring (FIG. 6). This result confirms that peptide 1 selected from the *in vivo* phage display can result in significantly enhanced drug delivery to the MCF-7 tumors. The distribution of peptide-AuNRs in other healthy organs is either less than or comparable to that of the Control-AuNRs. It is worth noting that the gold content in liver in the target group is 56% lower than that in the control group on day 1, and remained to be 22-44% lower in the following 4 days, suggesting that modification of AuNRs with the tumor targeting peptides can effectively reduce the non-specific AuNRs uptake by livers.

[0097] The photothermal treatment was conducted 24 h after injection because the concentration of peptide-AuNRs in tumors was maximized at this time point according to the ICP-MS based biodistribution study. During the treatment, MCF-7 tumors were irradiated directly by a 808 nm laser at a power density of 2.25 W/cm2 for 5 min. FIG. 7a and FIG. 7b show photographs of tumors and the corresponding average tumor volumes in each group 20 days after the photothermal therapy. The size and volume of the tumors in the targeting group (AuNRs functionalized with peptide 1) is significantly smaller than those in the other groups. In fact, two of the tumors in this group were destructed so severely that it was hard to collect any tumor tissue even after 20 days following the treatment. The development of tumors treated by non-targeting AuNRs, modified with PEG and control peptide, has also been retarded, although not as significant as that in the targeting group. This is because even without the assistance of targeting peptide, AuNRs can still be delivered to tumors through the passive targeting mechanism.

[0098] Hematoxylin and Eosin (HE) staining study further illustrates the death of tumor cells as a result of photothermal treatment (FIG. 7c). Tumors used for this study were collected from mice 3 h after the laser treatment. For control tumors without AuNRs (Saline group), both apoptosis and necrosis, the two typical cell death mechanisms, are barely found in the tumor cells, suggesting that the laser power density used for the photothermal treatment was safe to normal tissues. For tumors in the target group (Target-AuNRs), however, the classic necrosis characters, including karyolysis, nuclear swelling and extensive pale eosinophilic cytoplasm are all observed, indicating contiguous cell necrosis upon acute injury caused by photothermal treatment. In addition, the apoptosis characters such as nuclear pyknosis, hypereosinophilic

cytoplasm and apoptotic bodies were also seen, which are indications of programmable cell death following the laser treatment. In contrast, such damages on the other two AuNRs groups (PEG-AuNRs and Control-AuNRs) were not as significant. Overall, our photothermal investigation is in accordance with the study of organ distribution of AuNRs by ICP-AES, namely, the in vivo selected targeting peptides, when conjugated onto AuNRs, can indeed result in enhanced delivery and accumulation of AuNRs at the tumor sites, which further leads to the remarkably improved efficiency in the photothermal cancer therapy.

[0099] Phage display can be carried out both *in vitro* and *in vivo*. Phage library is allowed to interact cancer cells in the culture media in an *in vitro* phage display whereas it is intravenously injected into animals and circulated in blood stream to enable the discovery of phages homing to tumors in an *in vivo* phage display. In principle, *in vivo* selection against tumors should be more target-specific and more effective for clinic applications, as the peptides were selected under conditions similar to the physiological environment. So far, to our knowledge, no one has studied the discovery and identification of tumor-targeting peptides using *in vivo* phage display, and then tested the use of such peptides in *in vivo* homing and destruction of tumors, all using the same animal models and in the same lab. Most of the reported studies were based on peptides reported previously by others, rather than selected through exactly the same tumor model in the same work, thus these peptides cannot be considered as patient-specific. Hence, the results in those studies did not meet the requirement of precision medicine. In particular, for MCF-7 breast cancer, such studies were exclusively based on peptides selected through *in vitro* phage display. In addition, the results shown herein can be extended beyond photothermal treatment to be readily applied to other cancer therapeutics, such as chemo-, gene and photodynamic therapies.

[0100] Phage library screening methods of the present disclosure can be applied to design personalized therapy for individual cancer patient either *in vitro* or *in vivo*. For example, for *in vitro* phage library screening, a section of excised tumor or the isolation of patient's cancerous cells could be used as the target (e.g., see Krag, D.N., et al., Selection of tumor-binding ligands in cancer patients with phage display libraries. Cancer Res, 2006. 66(15): p. 7724-33; and Galili, N., E. Devemy, and A. Raza, Isolation of specific and biologically active peptides that bind cells from patients with acute myeloid leukemia (AML). J Hematol Oncol, 2008. 1: p. 8). The approach is very similar as the common *in vitro* phage biopanning so far.

[0101] For *in vivo* biopanning on a cancer patient, in one non-limiting embodiment, 1.0×10^{11}TU/kg of phage library, for example, diluted in 100 ml of warm saline, is i.v injected into the patient at 15 min before the tumor is surgically excised. Then a piece of the fresh tumor

is used to recovery the binding phages, and the rest of the same tumor is frozen (e.g., at -80°C). Once the tumor binding phages are recovered and isolated to individual clones, a portion of the frozen tumor is used to evaluate the affinity between the selected phage clone and the tumor (e.g., see Shukla, G.S., et al., Intravenous infusion of phage-displayed antibody library in human cancer patients: enrichment and cancer-specificity of tumor-homing phage-antibodies. Cancer Immunol Immunother, 2013. 62(8): p. 1397-410; and Sun, Y., et al., Biopanning Phage-Display Libraries on Small Tissue Sections Captured by Laser Capture Microdissection. J Biotech Res, 2009. 1: p. 55-63).

[0102] In summary, we have demonstrated a new strategy of breast cancer precision medicine by using the MCF-7 breast cancer mice model. In this strategy, as represented in FIG. 8, a patient specific tumor-targeting peptide was first identified through *in vivo* phage display. Our *in vivo* imaging study showed that these peptides can actively target MCF-7 tumors. By conjugating the as-selected peptides with the model drug, e.g., AuNRs, enhanced accumulation of the nanomedicine to the tumors was observed by the ICP-MS measurement, which further leads to improved cancer killing efficiency.

[0103] In at least one embodiment, the present disclosure is directed to a peptide conjugate, comprising a peptide conjugated to a therapeutic, imaging, or diagnostic moiety, wherein the peptide comprises an amino acid sequence selected from the group consisting of AREYGTRFSLIGGYR (SEQ ID NO:1), PKAFQYGGRAVGGLW (SEQ ID NO:2), PVRYGFSGPRLAELW (SEQ ID NO:3), RNVPPIFKEVYWIAQ (SEQ ID NO:4), and RTLIRMGTGAHAFAV (SEQ ID NO:5), and variant sequences thereof, and wherein the variant sequence has at least 86% identity with one of SEQ ID NO:1-5. The peptide may be directly linked by a covalent bond to the therapeutic, imaging, or diagnostic moiety, or indirectly via a linker sequence. The linker sequence may comprise 1 to 25 amino acids. The peptide conjugate may be disposed in a pharmaceutically-acceptable carrier or vehicle to form a pharmaceutical composition. In at least one embodiment, the present disclosure is directed to a method of treating a cancer in a subject in need of such treatment, comprising administering to the subject an effective amount of said peptide conjugate.

[0104] While the present disclosure has been described herein in connection with certain embodiments so that aspects thereof may be more fully understood and appreciated, it is not intended that the present disclosure be limited to these particular embodiments. On the contrary, it is intended that all alternatives, modifications and equivalents are included within the scope of the present disclosure as defined herein. Thus the examples described above, which include particular embodiments, will serve to illustrate the practice of the inventive

concepts of the present disclosure, it being understood that the particulars shown are by way of example and for purposes of illustrative discussion of particular embodiments only and are presented in the cause of providing what is believed to be the most useful and readily understood description of procedures as well as of the principles and conceptual aspects of the present disclosure. Changes may be made in the formulation of the various compositions described herein, the methods described herein or in the steps or the sequence of steps of the methods described herein without departing from the spirit and scope of the present disclosure. Further, while various embodiments of the present disclosure have been described in claims herein below, it is not intended that the present disclosure be limited to these particular claims. Applicants reserve the right to amend, add to, or replace the claims indicated herein below in subsequent patent applications.

What is claimed is:

1. A peptide conjugate, comprising:

a peptide conjugated to a therapeutic, imaging, or diagnostic moiety, the peptide comprising an amino acid sequence selected from the group consisting of AREYGTRFSLIGGYR (SEQ ID NO:1), PKAFQYGGRAVGGLW (SEQ ID NO:2), PVRYGFSGPRLAELW (SEQ ID NO:3), RNVPPIFKEVYWIAQ (SEQ ID NO:4), and RTLIRMGTGAHAFAV (SEQ ID NO:5), and variant sequences thereof, and wherein the variant sequence has at least 86% identity with one of SEQ ID NO:1-5.

2. The peptide conjugate of claim 1, wherein the variant sequence has at least 93% identity to one of SEQ ID NO:1-5.

3. The peptide conjugate of claim 1, wherein the peptide is directly linked by a covalent bond to the therapeutic, imaging, or diagnostic moiety.

4. The peptide conjugate of claim 1, wherein the peptide is linked to the therapeutic, imaging, or diagnostic moiety via a linker sequence.

5. The peptide conjugate of claim 4, wherein the linker sequence comprises 1 to 25 amino acids.

6. The peptide conjugate of claim 4, wherein the linker sequence comprises 1 to 10 amino acids.

7. A pharmaceutical composition comprising the peptide conjugate of claim 1, disposed in a pharmaceutically-acceptable carrier, vehicle, or diluent.

8. A method of treating a cancer in a subject in need of such treatment, comprising, administering to the subject an effective amount of a peptide conjugate comprising a peptide conjugated to a therapeutic, imaging, or diagnostic moiety, the peptide comprising an amino acid sequence selected from the group consisting of AREYGTRFSLIGGYR (SEQ ID NO:1), PKAFQYGGRAVGGLW (SEQ ID NO:2), PVRYGFSGPRLAELW (SEQ ID NO:3), RNVPPIFKEVYWIAQ (SEQ ID NO:4), and RTLIRMGTGAHAFAV (SEQ ID NO:5), and variant sequences thereof, and wherein the variant sequence has at least 86% identity with one of SEQ ID NO:1-5.

9. The method of claim 8, wherein the variant sequence has at least 93% identity to one of SEQ ID NO:1-5.

10. The method of claim 8, wherein the peptide of the peptide conjugate is directly linked by a covalent bond to the therapeutic, imaging, or diagnostic moiety.

11. The method of claim 8, wherein the peptide of the peptide conjugate is linked to the therapeutic, imaging, or diagnostic moiety via a linker sequence.

12. The method of claim 11, wherein the linker sequence of the peptide conjugate comprises 1 to 25 amino acids.

13. The method of claim 11, wherein the linker sequence of the peptide conjugate comprises 1 to 10 amino acids.

14. The method of claim 8, wherein the peptide conjugate is disposed in a pharmaceutically-acceptable carrier, vehicle, or diluent.

15. The peptide conjugate of either of claims 1 or 2 , wherein the peptide is directly linked by a covalent bond to the therapeutic, imaging, or diagnostic moiety.

16. The peptide conjugate of any one of claims 1, 2, or 15, wherein the peptide is linked to the therapeutic, imaging, or diagnostic moiety via a linker sequence.

17. The peptide conjugate of claim 16, wherein the linker sequence comprises 1 to 25 amino acids.

18. The peptide conjugate of claim 16, wherein the linker sequence comprises 1 to 10 amino acids.

19. A pharmaceutical composition comprising the peptide conjugate of any one of claims 1, 2, or 15-18, disposed in a pharmaceutically-acceptable carrier, vehicle, or diluent.

20. The method of either of claims 8 or 9, wherein the peptide of the peptide conjugate is directly linked by a covalent bond to the therapeutic, imaging, or diagnostic moiety.

21. The method of any one of claims 8, 9, or 20, wherein the peptide of the peptide conjugate is linked to the therapeutic, imaging, or diagnostic moiety via a linker sequence.

22. The method of claim 21, wherein the linker sequence of the peptide conjugate comprises 1 to 25 amino acids.

23. The method of claim 21, wherein the linker sequence of the peptide conjugate comprises 1 to 10 amino acids.

24. The method of any one of claims 8, 9, or 19-23, wherein the peptide conjugate is disposed in a pharmaceutically-acceptable carrier, vehicle, or diluent.

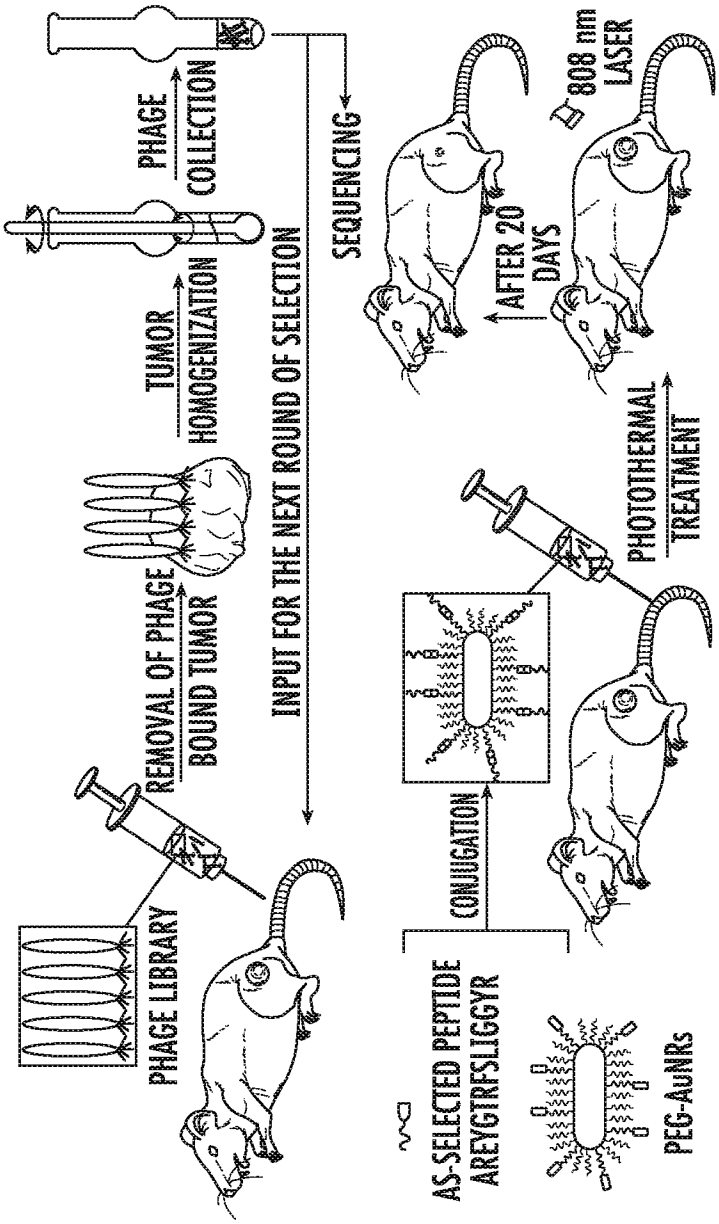
PHAGE LIBRARY
REMOVAL OF PHAGE BOUND TUMOR
TUMOR HOMOGENIZATION
PHAGE COLLECTION
INPUT FOR THE NEXT ROUND OF SELECTION
SEQUENCING
AS-SELECTED PEPTIDE AREYGTRFSLIGGYR
CONJUGATION
PEG-AuNRs
PHOTOTHERMAL TREATMENT
808 nm LASER
AFTER 20 DAYS

FIG. 1

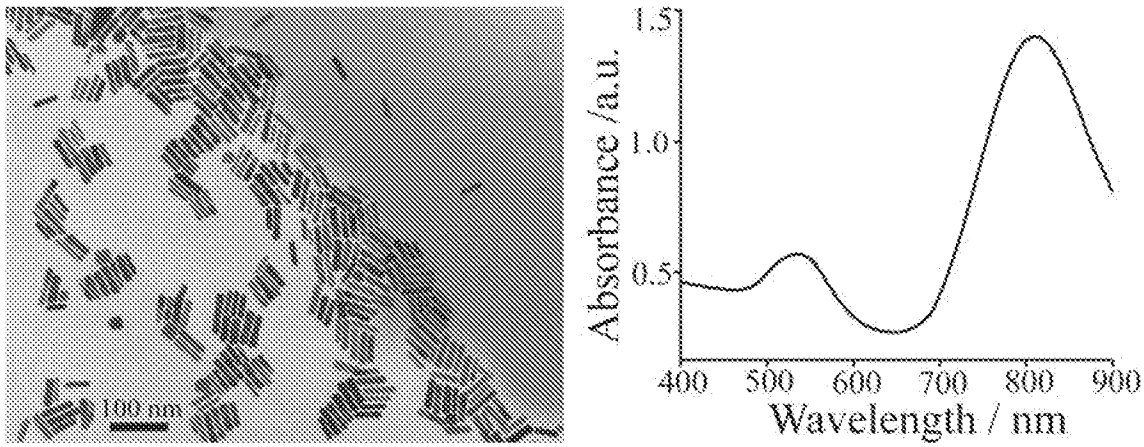
100 nm
Absorbance /a.u.
1.5
1.0
0.5
400
500
600
700
800
900
Wavelength / nm

FIG. 2

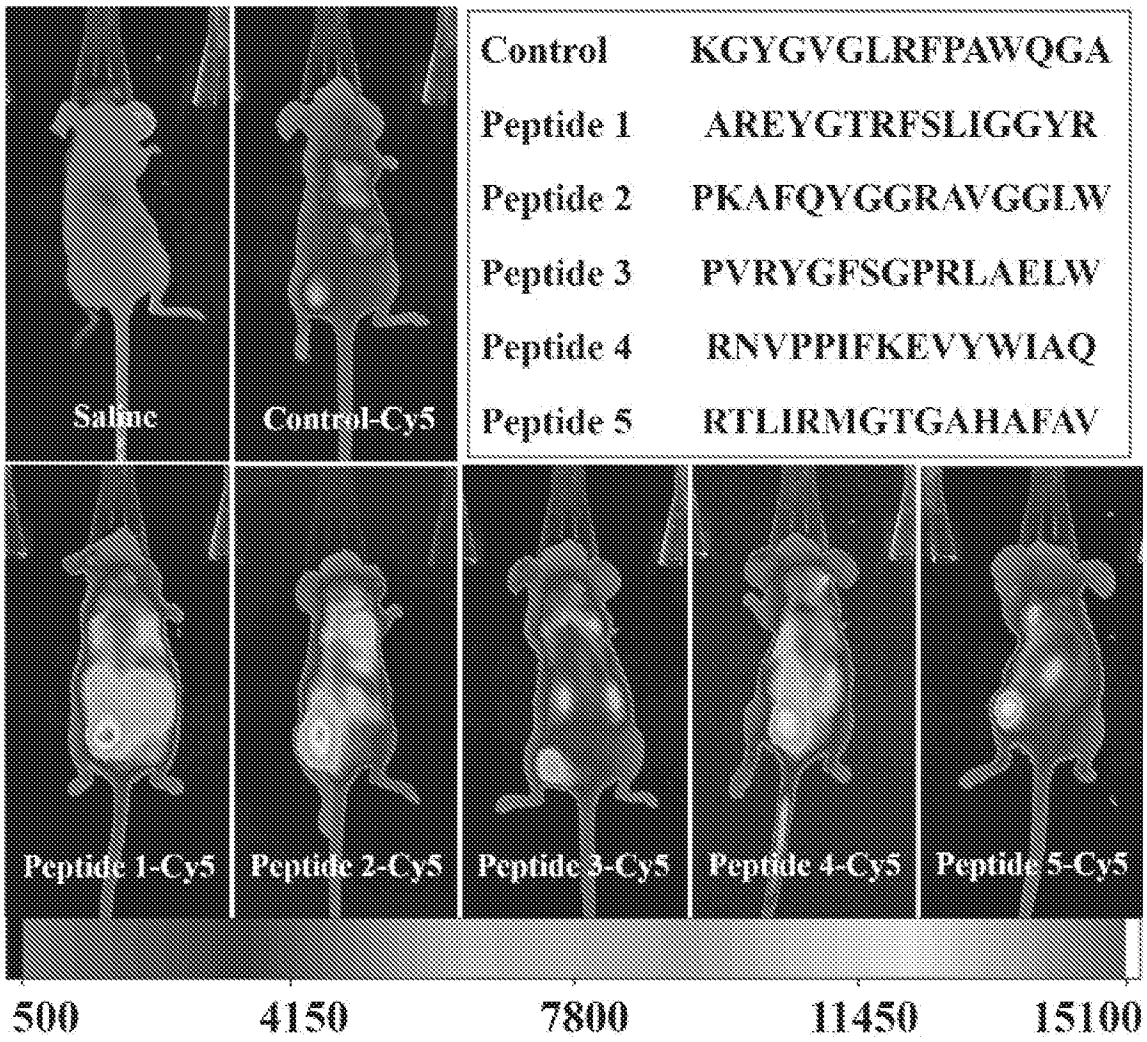
Saline
Control-Cy5
Control KGYGVGLRFPAWQGA
Peptide 1 AREYGTRFSLIGGYR
Peptide 2 PKAFQYGGRAVGGLW
Peptide 3 PVRYGFSGPRLAELW
Peptide 4 RNVPPIFKEVYWIAQ
Peptide 5 RTLIRMGTGAHAFAV
Peptide 1-Cy5
Peptide 2-Cy5
Peptide 3-Cy5
Peptide 4-Cy5
Peptide 5-Cy5
500
4150
7800
11450
15100

FIG. 3

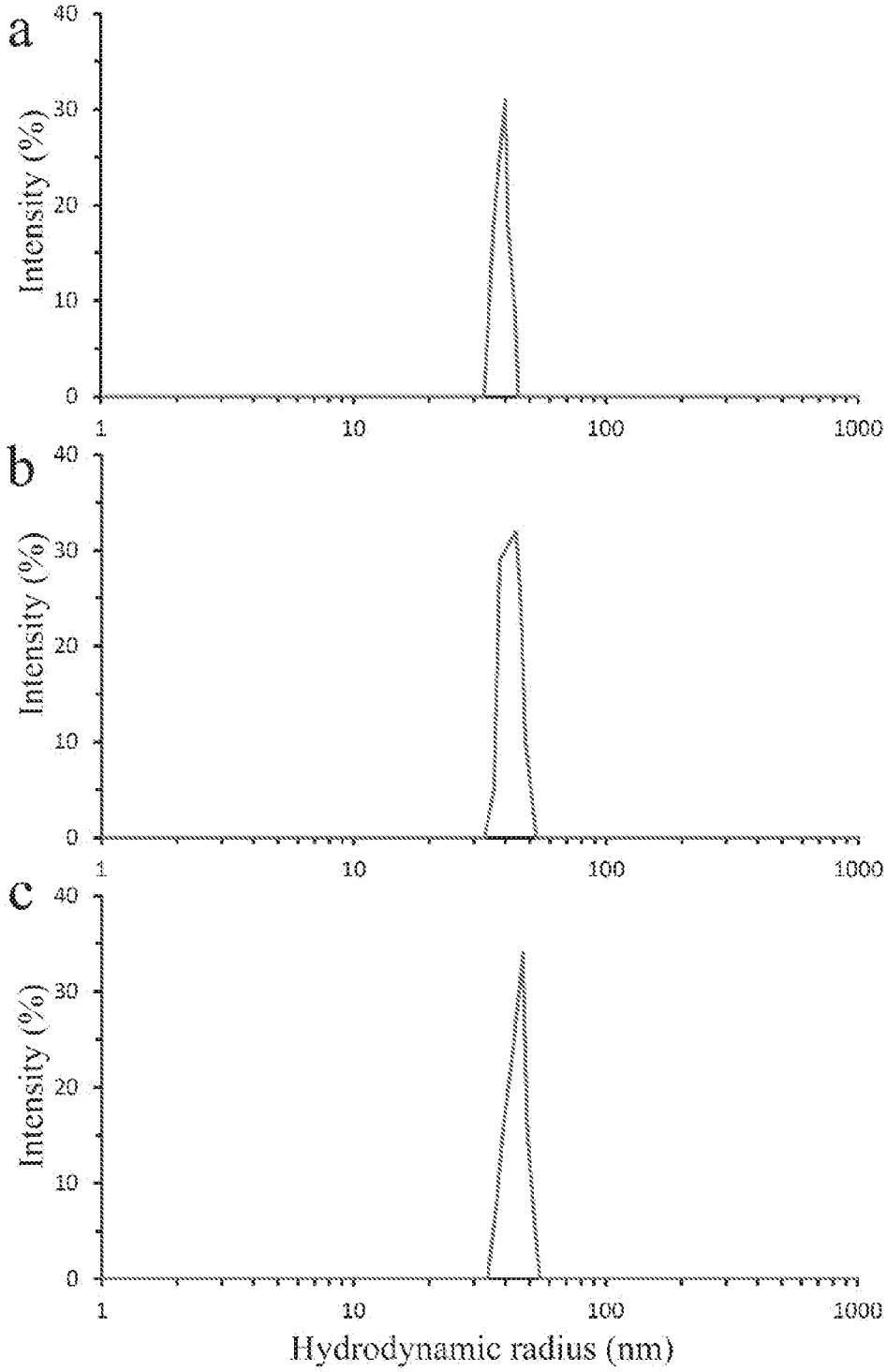
a
b
c
Intensity (%)
0
10
20
30
40
1
10
100
1000
Hydrodynamic radius (nm)

FIG. 4

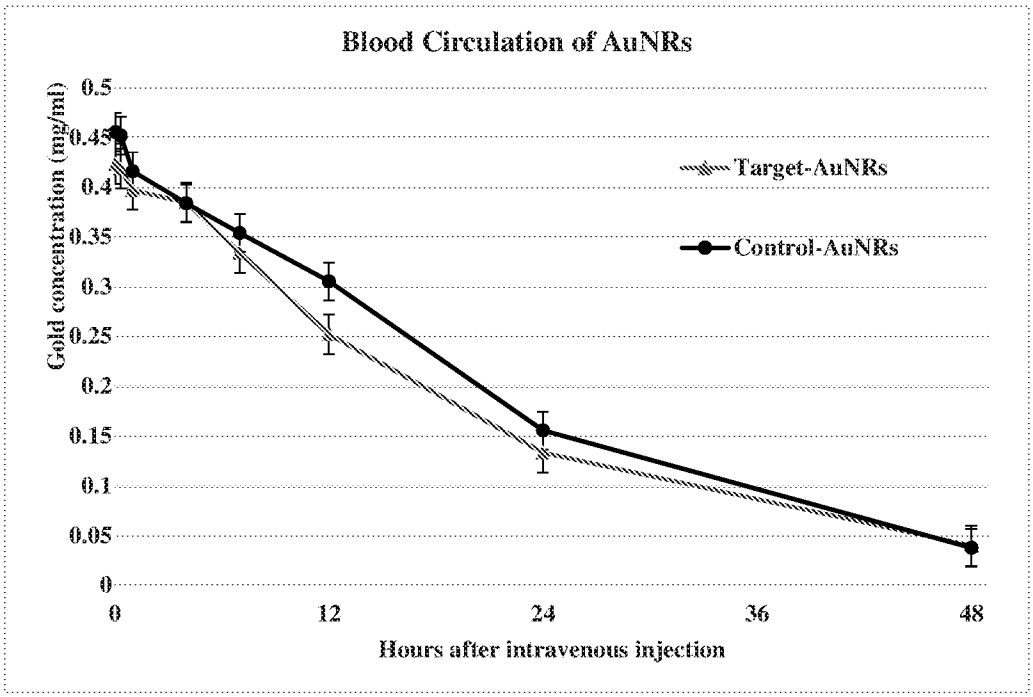
Blood Circulation of AuNRs
Gold concentration (mg/ml)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Target-AuNRs
Control-AuNRs
0
12
24
36
48
Hours after intravenous injection

FIG. 5

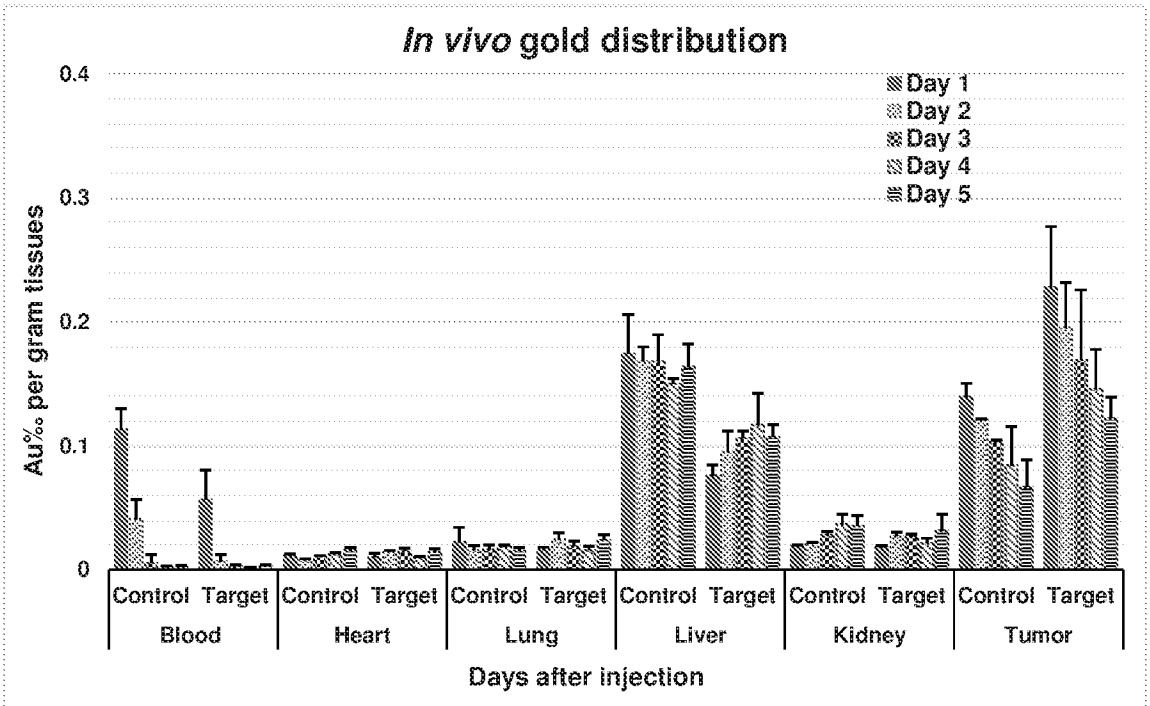
In vivo gold distribution
Au‰ per gram tissues
0.4
0.3
0.2
0.1
0
Day 1
Day 2
Day 3
Day 4
Day 5
Control Target
Blood
Heart
Lung
Liver
Kidney
Tumor
Days after injection

FIG. 6

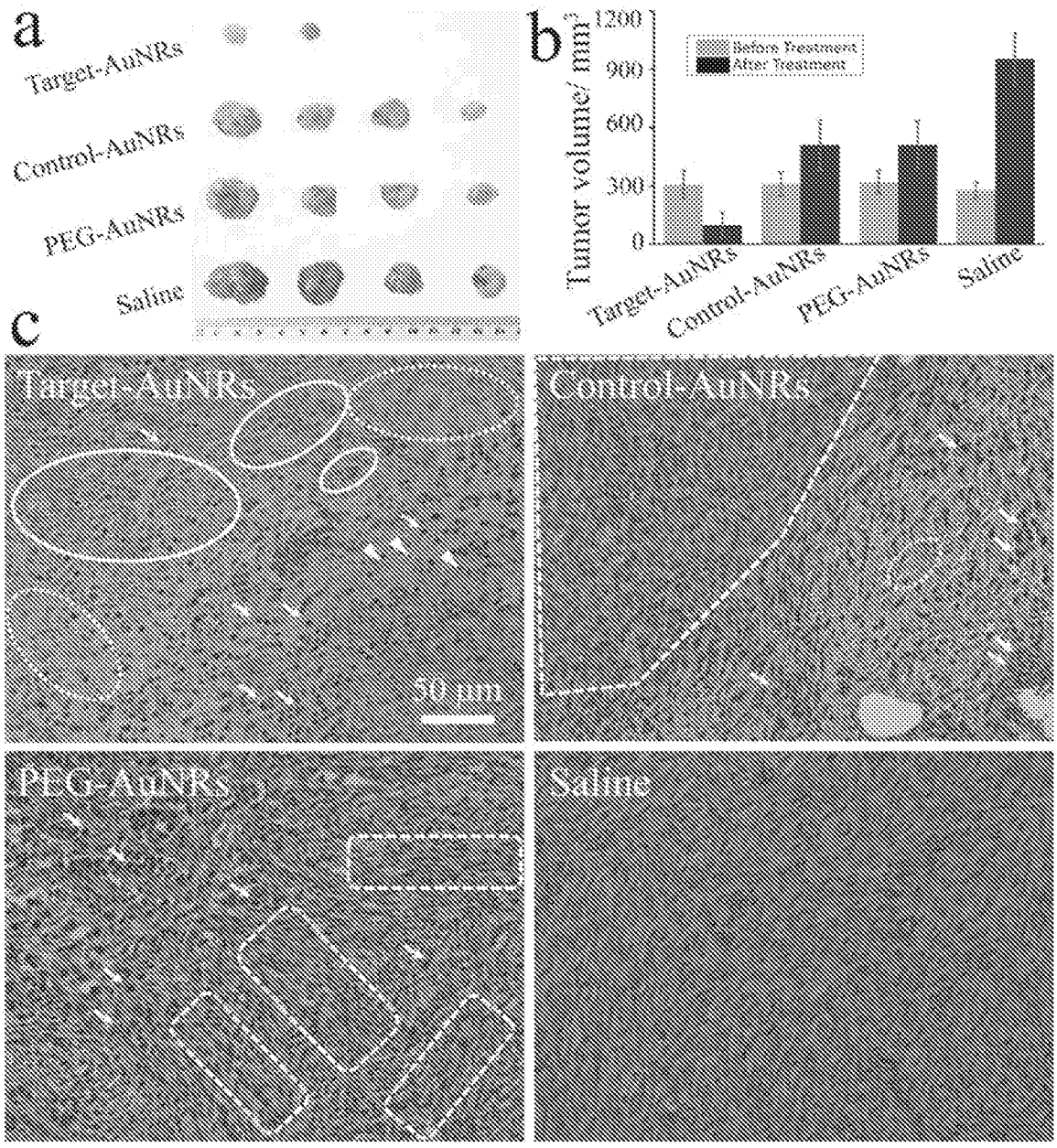
a
Target-AuNRs
Control-AuNRs
PEG-AuNRs
Saline
b
Tumor volume/ mm³
1200
900
600
300
0
Before Treatment
After Treatment
Target-AuNRs
Control-AuNRs
PEG-AuNRs
Saline
c
Target-AuNRs
50 nm
Control-AuNRs
PEG-AuNRs
Saline

FIG. 7

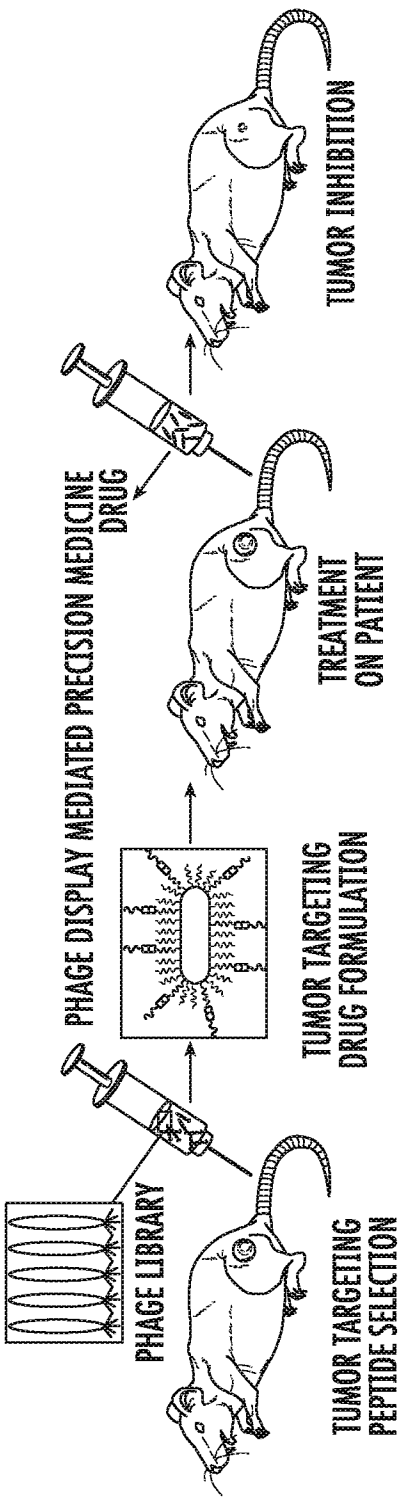
TUMOR TARGETING
PEPTIDE SELECTION
PHAGE LIBRARY
PHAGE DISPLAY MEDIATED PRECISION MEDICINE
DRUG
TUMOR TARGETING
DRUG FORMULATION
TREATMENT
ON PATIENT
TUMOR INHIBITION

FIG. 8