

(43) International Publication Date
10 November 2016 (10.11.2016)

(51) International Patent Classification:

C07K 7/64 (2006.01) A61K 38/12 (2006.01)
C07K 4/00 (2006.01) A61K 38/03 (2006.01)
A61K 38/08 (2006.01)

(21) International Application Number:

PCT/US2016/030893

(22) International Filing Date:

5 May 2016 (05.05.2016)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/157,667 6 May 2015 (06.05.2015) US

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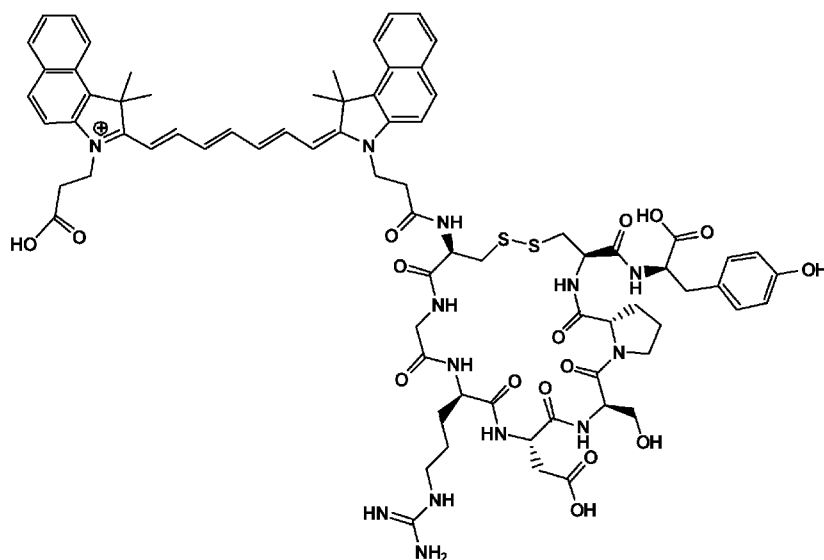
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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: COMPOUNDS HAVING RD TARGETING MOTIFS AND METHODS OF USE THEREOF



(57) Abstract: The present invention provides compounds that have motifs that target the compounds to cells that express integrins. In particular, the compounds have peptides with one or more RD motifs conjugated to an agent selected from an imaging agent and a targeting agent. The compounds may be used to detect, monitor and treat a variety of disorders mediated by integrins.

FIG. 1

**Published:**

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

COMPOUNDS HAVING RD TARGETING MOTIFS AND METHODS OF USE THEREOF

GOVERNMENTAL RIGHTS

[0001] This invention was made with government support under CA171651 awarded by the NIH/NCI. The government has certain rights in the invention.

CROSS REFERENCE TO RELATED APPLICATIONS

[0002] This application claims the benefit of U.S. Provisional Application number 62/157,667, filed May 6, 2015, the disclosure of which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present invention provides compounds that have motifs that target the compounds to cells that express integrins. In particular, the compounds have peptides with one or more RD motifs conjugated to an agent selected from an imaging agent and a targeting agent. The compounds may be used to detect, monitor and treat a variety of disorders mediated by integrins.

BACKGROUND OF THE INVENTION

[0004] Angiogenesis, the formation of new blood vessels, is the cardinal feature of virtually all malignant tumors and because of its commonality, probing tumor-induced angiogenesis and associated proteins is a viable approach to detect and treat a wide range of cancers. Angiogenesis is stimulated by integrins, a large family of transmembrane proteins that mediate dynamic linkages between extracellular adhesion molecules and the intracellular actin skeleton. Integrins are composed of two different subunits, α and β , which are non-covalently bound into $\alpha\beta$ complexes. Particularly, the expression of $\alpha_v\beta_3$ integrin (ABI) in tumor cells undergoing angiogenesis and on the epithelium of tumor-induced neovasculature alters the interaction of cells with the extracellular matrix, thereby increasing tumorigenicity and invasiveness of cancers.

[0005] Numerous studies have shown that ABI and more than 7 other heterodimeric integrins recognize proteins and low molecular weight ligands containing

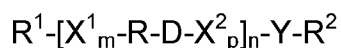
RGD (arginine-glycine-aspartic acid) motifs in proteins and small peptides. Based on structural and bioactivity considerations, cyclic RGD peptide ligands are preferred as delivery vehicles of molecular probes for imaging and treating ABI-positive tumors and proliferating blood vessels. Until recently, most of the *in vivo* imaging studies were performed with radiopharmaceuticals because of the high sensitivity and clinical utility of nuclear imaging methods. Particularly, the use of small monoatomic radioisotopes does not generally interfere with the biodistribution and bioactivity of ligands. Therefore, once a high affinity ligand for a target receptor is identified, the radiolabeled analogue is typically used to monitor the activity, pharmacokinetics and pharmacodynamics of the drug or imaging agent. Despite these advantages, nuclear imaging is currently performed in specialized centers because of regulatory, production and handling issues associated with radiopharmaceuticals. Optical imaging is an alternative, but complementary method to interrogate molecular processes *in vivo* and *in vitro*.

[0006] Optical imaging for biomedical applications typically relies on activating chromophore systems with low energy radiation between 400 and 1500 nm wavelengths and monitoring the propagation of light in deep tissues with a charge-coupled device (CCD) camera or other point source detectors. Molecular optical imaging of diseases with molecular probes is attractive because of the flexibility to alter the detectable spectral properties of the beacons, especially in the fluorescence detection mode. The probes can be designed to target cellular and molecular processes at functional physiological concentrations. For deep tissue imaging, molecular probes that are photoactive in the near infrared (NIR) instead of visible wavelengths are preferred to minimize background tissue autofluorescence and light attenuation caused by absorption by intrinsic chromophores. In contrast to radioisotopes, the NIR antennas are usually large heteroatomic molecules that could impact the biodistribution and activity of conjugated bioactive ligands. However, previous studies have shown that conjugating small peptide carriers with NIR molecular probes successfully delivered the beacons to target proteins *in vivo*, and the nonspecific distribution of the conjugate in non-target tissues can be minimized by adjusting the net lipophilicity and ionic character of the conjugate.

[0007] A need, however, exists for additional compounds that can target and monitor integrin expression. In particular, a need exists for compounds that can target, monitor and/or treat a variety of integrin-mediated disorders.

SUMMARY OF THE INVENTION

[0008] In an aspect, the disclosure provides a compound having the formula:



wherein:

R^1 is a carbocyanine dye or derivative thereof;

R^2 is selected from the group consisting of a treatment agent, hydrogen, hydroxyl, NH_2 , hydrocarbyl, and substituted hydrocarbyl;

$X^1_m-R-D-X^2_p$ together form a linear or cyclic peptide;

X^1 and X^2 are independently selected from any amino acid residue;

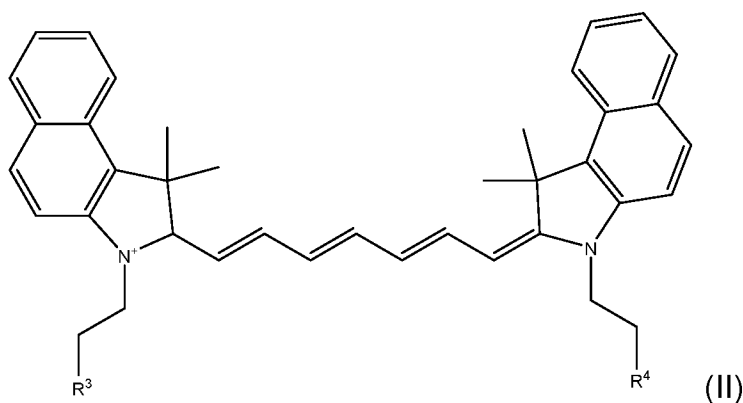
m is an integer from 1 to about 10;

n is an integer from 1 to about 10;

p is an integer from 1 to about 10; and

a dash (-) represent a covalent bond.

[0009] In another aspect, the disclosure provides a compound having formula (II):



wherein:

R^3 is selected from the group consisting of nanoparticles, small organic molecules, peptides, organometallics, metal chelates, proteins, drugs, antibiotics, and carbohydrates; and

R^4 is $[X^1_m-R-D-X^2_p]_n-Y-R^2$, wherein:

R^2 is independently selected from the group consisting of a hydrogen, hydroxyl, NH_2 , hydrocarbyl, and substituted hydrocarbyl;

X^1 and X^2 are independently selected from any amino acid residue;

$X^1_m-R-D-X^2_p$ together form a linear or cyclic peptide;

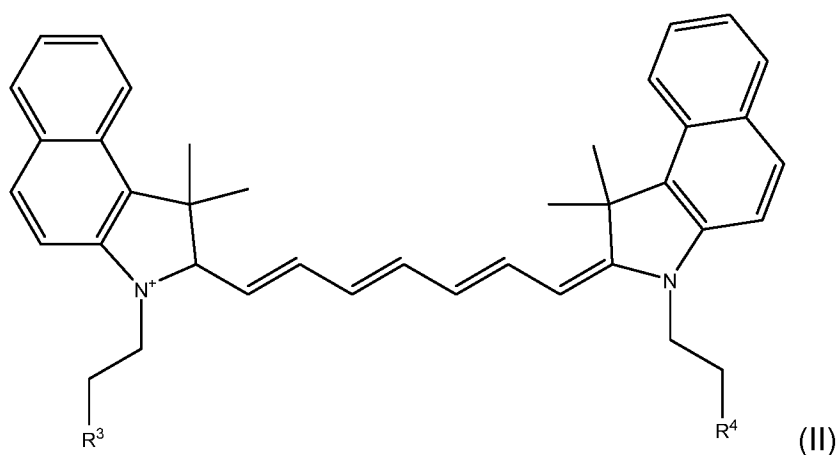
m is an integer from 1 to about 10;

n is an integer from 1 to about 10;

p is an integer from 1 to about 10; and

a dash (-) represent a covalent bond.

[0010] In still another aspect, the disclosure provides a compound having formula (II):



wherein:

R^3 is selected from the group consisting of nanoparticles, small organic molecules, peptides, organometallics, metal chelates, proteins, drugs, antibiotics, and carbohydrates; and

R^4 is selected from the group consisting of:

SEQ ID NO:	R ⁴
8	CONH-[D-Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Lys-Tyr-OH
8	CONH-[D-Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Lys-Tyr-NH ₂
9	CONH-[D-Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Tyr-OH
10	CONH-[D-Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Tyr-Lys-OH
11	CONH-[D-Cys-Gly-Arg-Asp-Ser-Pro-D-Cys]-Tyr-OH
12	CONH-[Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Tyr-OH
13	CONH-[Cys-Arg-Gly-Asp-Ser-Pro-Cys]-Tyr-OH
14	CONH-[D-Cys-Arg-Gly-Asp-Ser-Pro-Cys]-Tyr-OH
15	CONH-[D-Cys-Arg-Gly-Asp-Ser-Pro-D-Cys]-Tyr-OH
17	CONH-Gly-[D-Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Tyr-OH
18	CONH-Gly-[D-Cys-Gly-Arg-Asp-Ser-Pro-Cys]-D-Tyr-OH
19	CONH-Gly-[D-Cys-Gly-Arg-Asp-Ser-Pro-Cys]-D-Tyr-Lys-OH
20	CONH-Gly-[Cys-Gly-Arg-Asp-Ser-Pro-Cys]-D-Tyr-Lys-OH
21	CONH-Gly-[D-Cys-Gly-Arg-Asp-Ser-Pro-D-Cys]-Tyr-OH
22	CONH-Gly-[Cys-Gly-Arg-Asp-Ser-Pro-D-Cys]-Tyr-OH
23	CONH-Gly-[D-Cys-Gly-Arg-Asp-Ser-Pro-D-Cys]-D-Tyr-OH

[0011] In still yet another aspect, the disclosure provides a method for detecting expression of a $\beta 3$ subunit of integrin in a cell, the method comprising: (a) contacting a population of cells with a compound of the disclosure; and detecting the presence of a signal emitted from the carbocyanine dye of the compound of the disclosure in the population of cells, the signal being emitted from a cell expressing a $\beta 3$ subunit of integrin.

[0012] In a different aspect, the disclosure provides a method for detecting cancer, the method comprising: (a) administering a composition comprising an effective amount of a compound of the disclosure to a subject; and (b) detecting the presence of a signal emitted from the carbocyanine dye of the compound of the disclosure in the subject, wherein detection of signal above baseline indicates cancer.

[0013] In other aspects, the disclosure provides a method for detecting pancreatic cancer, the method comprising: (a) administering a composition comprising an effective amount of a compound of the disclosure to a subject; and (b) detecting the presence of a signal emitted from the carbocyanine dye of the compound of the disclosure in the subject, wherein detection of signal above baseline indicates pancreatic cancer.

[0014] In yet other aspects, the disclosure provides a method for detecting early stage pancreatic ductal adenocarcinoma (PDAC) or precursor pancreatic intraepithelial neoplasia (PanIN), the method comprising: (a) administering a composition comprising an effective amount of a compound of the disclosure to a subject; and (b) detecting the presence of a signal emitted from the carbocyanine dye of the compound of the disclosure in the subject, wherein detection of signal above baseline indicates early stage pancreatic ductal adenocarcinoma (PDAC) or precursor pancreatic intraepithelial neoplasia (PanIN).

[0015] In still yet other aspects, the disclosure provides a method for differentiating pancreatic ductal adenocarcinoma (PDAC) and precursor pancreatic intraepithelial neoplasia (PanIN) from pancreatitis, the method comprising: (a) administering a composition comprising an effective amount of a compound of the disclosure to a subject; and (b) detecting the presence of a signal emitted from the carbocyanine dye of the compound of the disclosure in the subject, wherein detection of signal above baseline indicates PDAC or PanIN and detection of a signal at or below baseline indicates pancreatitis.

[0016] In certain aspects, the disclosure provides a method for differentiating pancreatic ductal adenocarcinoma (PDAC) and PanIN-3 from PanIN-1/2 and ductal hyperplasia/metaplasia, the method comprising: (a) administering a composition comprising an effective amount of a compound of the disclosure to a subject; and (b) detecting the presence of a signal emitted from the carbocyanine dye of the compound of the disclosure in the subject, wherein detection of signal above baseline indicates PDAC or PanIN-3 and detection of a signal at or below baseline indicates PanIN-1/2 or ductal hyperplasia/metaplasia.

BRIEF DESCRIPTION OF THE FIGURES

[0017] The application file contains at least one drawing executed in color. Copies of this patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0018] **FIG. 1** depicts the structure of LS838.

[0019] **FIG. 2A, FIG. 2B, FIG. 2C** and **FIG. 2D** depict the structures of NIR dyes with different hydrophobicity. (**FIG. 2A**) LS288; (**FIG. 2B**) LS798; (**FIG. 2C**) LS276; and (**FIG. 2D**) LS843.

[0020] **FIG. 3A, FIG. 3B** and **FIG. 3C** depict the structure of cypates. (**FIG. 3A**) Cypate (cypate 4); (**FIG. 3B**) Cypate 3; and (**FIG. 3C**) Cypate 2.

[0021] **FIG. 4A, FIG. 4B** and **FIG. 4C** depict the absorption and emission spectra of cypates. (**FIG. 4A**) Cypate4; (**FIG. 4B**) Cypate3; (**FIG. 4C**) Cypate2.

[0022] **FIG. 5** depicts images showing LS838 for fluorescence and nuclear (PET and or SPECT) imaging. 4T1luc in Balb/c; 100μl of 50μM LS838.

[0023] **FIG. 6** depicts the distribution of LS838 in tissue at 24 hours.

[0024] **FIG. 7A, FIG. 7B** and **FIG. 7C** depict structures of molecular imaging agents. (**FIG. 7A**) (1) ICG: $R_1 = R_2 = SO_3^-$; (2) Cypate: $R_1 = R_2 = CO_2H$; (3) LS838: $R_1 = CO_2H$; $R_2 = CONH-cyclo-(D-Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Tyr-OH$ (SEQ ID NO:9). (**FIG. 7B**) LS276; (**FIG. 7C**) LS288.

[0025] **FIG. 8A** and **FIG. 8B** depict uptake of LS838 in 4T1luc cells showing internalization of the probe in the cells at 1 h (**FIG. 8A**) and 4 h (**FIG. 8B**) post incubation. Most cells show localization in the lysosomes at early time points, but the punctate fluorescence became more diffuse at 24 h, indicating translocation to the cytosol. Uptake in the mitochondria was negligible. Color scheme: cyan, Mitotracker; green, lysotracker; and red, LS838. (**FIG. 8C**) Internalization of LS838 in cells was inhibited by Filipinin, an inhibitor of albumin endocytosis *via* the gp60 pathway. A similar inhibition was observed when Alexa-680 dye labeled albumin (BSA; blue) was mixed with LS838.

[0026] **FIG. 9A, FIG. 9B, FIG. 9C, FIG. 9D** and **FIG. 9E** depict noninvasive FMT (top), corresponding *ex vivo* (middle), and side-view of the pancreas from planar

fluorescence imaging of pancreatic lesions in mouse models (bottom). **FIG. 9A**, Advanced KPC; **FIG. 9B**, 1 week PDAC; **FIG. 9C**, 2 weeks PDAC; **FIG. 9D**, pancreatitis; and **FIG. 9E**, sham surgery using orthotopic Matrigel implant demonstrated selective uptake of LS838 in PDAC, but not in non-tumor controls. The reconstructed yield is superimposed with the corresponding CT data 10 mm below from the dorsal side. *Dotted red circles*: PDAC; *solid red circles in C*: positive nodules indicated by white arrow.

[0027] **FIG. 10A, FIG. 10B, FIG. 10C, FIG. 10D** and **FIG. 10E** depict H&E staining (*left*) and corresponding immune-fluorescence (*right*) of pancreatic tissues from **FIG. 9** after noninvasive *in vivo* imaging studies. **FIG. 10A**, 1 week after orthotopic PDAC implant; **FIG. 10B**, 2-week orthotopic PDAC implant; **FIG. 10C**, advanced spontaneous KPC model showing high fluorescence uptake in tumor tissue; **FIG. 10D**, chronic pancreatitis showing low fluorescence overall but significant fluorescence in regions suspected to progress toward malignancy; probably PanIN-3; **FIG. 10E**, sham surgery demonstrating minimal LS838 fluorescence.

[0028] **FIG. 11A** depicts representative flow cytometry plots pre-gated for each cell type depicted. Cells are derived from established PDAC tumors treated with LS838. **FIG. 11B**, Tumor-derived mCherry signal is shown in tumor-infiltrating macrophages.

[0029] **FIG. 12A** and **FIG. 12B** depict laparoscopy of the pancreas and pancreatic lesions using different animal models and controls.

[0030] **FIG. 13** depicts further derivatization of compounds for drug delivery. The alkyne group can react with any azido group selectively. We envisage using this molecular as a versatile reagent to incorporate drugs and other biologically active molecules. The method can also be used to introduce stable radio-halogens.

DETAILED DESCRIPTION OF THE INVENTION

[0031] It has been discovered, as detailed in the examples, that a compound comprising a peptide having a RD motif and a tyrosine that is not adjacent to an imaging agent targets to a cell that expresses integrins. Unexpectedly, a peptide comprising a tyrosine non-adjacent to a carbocyanine dye conferred enhanced

brightness relative to the same peptide without a tyrosine. Further, a peptide comprising a tyrosine adjacent to a carbocyanine dye resulted in a compound that was rapidly lost and was not retained in the tumor. Thus, the presence and location of the tyrosine is an essential feature of a compound of the disclosure that unexpectedly improves the properties of the carbocyanine dye comprising the peptide.

[0032] The present invention, accordingly, provides compounds that may be used to detect, monitor, and treat a variety of integrin-mediated biological processes, including the progression of disease states such as diabetes, cardiovascular disease, inflammation and cancer. Specifically a compound of the invention may be used in pancreatic cancer, for example to detect small or microscopic disease as well as differentiate between pancreatic cancer and pancreatitis.

I. COMPOUNDS

[0033] The compounds of the invention typically comprise at least one linear or cyclic peptide conjugated to an imaging agent and, optionally, a treatment agent, wherein the peptide comprises an RD motif and a tyrosine non-adjacent to the imaging agent. The peptide may be conjugated to the imaging agent and/or treatment agent directly by a covalent bond. Alternatively, the peptide may be conjugated to the imaging agent and/or treatment agent by a linker. Suitable linkers are described below. In a specific embodiment, the imaging agent is a carbocyanine dye.

(a) peptide regions of the compound

[0034] Generally, the peptide portion of the compound minimally has a size that includes at least one RD motif and a tyrosine that is non-adjacent to an imaging agent. In an alternative embodiment, the peptide portion of the compound minimally has a size that includes at least one RGD motif and a tyrosine that is non-adjacent to an imaging agent. In some embodiments, the peptide may be linear. In other embodiments, the peptide may be cyclic. Typically, the peptide will have from about 3 to about 50 amino acid residues. In another embodiment, the peptide will have from about 3 to about 30 amino acid residues. In a further embodiment, the peptide will have from about 3 to about 20 amino acid residues. In yet another embodiment, the peptide will

have from about 3 to about 15 amino acid residues. In still another embodiment, the peptide will have from about 4 to about 12 amino acid residues. In a further embodiment, the peptide will have from about 4 to about 8 amino acid residues. In a specific embodiment, the peptide will have from about 8 to about 10 amino acid residues. In another embodiment, the peptide will have 4 amino acid residues. In an additional embodiment, the peptide will have 5 amino acid residues. In still another embodiment, the peptide will have 6 amino acid residues. In an additional embodiment, the peptide will have 7 amino acid residues. In yet another embodiment, the peptide will have 8 amino acid residues. In a different embodiment, the peptide will have 9 amino acid residues. In other embodiments, the peptide will have 10 amino acid residues. In each of the aforementioned embodiments, the peptide, irrespective of its length, has at least one RD motif and a tyrosine that is not adjacent to an imaging agent. It will be appreciated by the skilled artisan that it is possible and, depending upon the embodiment, it may be desirable to have more than one RD motif or RGD motif within a peptide. For example, it is envisioned, depending upon the length of the peptide, that there may be from 2 to about 5 RD motifs or RGD motifs in a given peptide.

[0035] The choice of amino acid residues, in addition to the RD motif or RGD motif, that will comprise the peptide will vary greatly depending upon the particular application for the compound. For example, it may be desirable in certain imaging or treatment applications that the compound be substantially hydrophilic. In other imaging or treatment applications, it may be desirable for the compound to be substantially hydrophobic. Generally, the amino acids may be selected from any amino acid residue including hydrophobic amino acids (e.g., L, A, P, V, M, F, W, and I), polar, uncharged amino acids (e.g., G, S, N, Q, T, Y, and C), acidic amino acids (e.g., D and E) and basic amino acids (e.g., K, H, and R). The amino acid residues may also be modified amino acid residues that are commonly known in the art. For embodiments in which a hydrophobic compound is desired, typically the amino acid residues comprising the peptide will be predominantly selected from hydrophobic amino acids. In embodiments in which a hydrophilic compound is desired, typically the amino acid residues comprising the peptide will be predominantly polar, uncharged or polar, charged amino

residues. An amino acid may be a naturally occurring L-amino acid or a non-natural D-amino acid. In certain embodiments, the D-amino acid is D-cysteine and/or D-tyrosine. In a specific embodiment, the D-cysteine is linked to an imaging agent. In other specific embodiments, the D-cysteine is linked to a carbocyanine dye. The inventors have shown that the unnatural D-cysteine linked to a carbocyanine dye confers high stability on the compound. Without wishing to be bound by theory, it is believed that this is because of the resistance of the compound to degradation by proteases. In an embodiment, a peptide of the invention is a cyclic peptide, wherein the peptide comprises two cysteines which cyclize the peptide by forming a disulfide bridge. In a specific embodiment, a peptide of the invention is a cyclic peptide comprising _DCys-Gly-Arg-Asp-Ser-Pro-Cys (SEQ ID NO:24) or _DCys-Gly-Arg-Asp-Ser-Pro-_DCys (SEQ ID NO:25). Following the cyclic peptide, the peptide further comprises a tyrosine (Tyr). The Tyr residue unexpectedly increases the brightness of the compound such that smaller amounts are needed. Further, the location of the Tyr residue is important, as it was discovered that positioning the Tyr next to the carbocyanine dye led to rapid loss of retention of the compound *in vivo*. In a specific embodiment, a peptide of the invention may comprise Cyclo(_DCys-Gly-Arg-Asp-Ser-Pro-Cys)-Tyr (SEQ ID NO:9).

[0036] Further, the peptide may comprise additional amino acids before and/or after the cyclic peptide and tyrosine. For example, the peptide may comprise 1, 2, 3, 4, or 5 amino acids prior to the cyclic peptide. Additionally, the peptide may comprise 1, 2, 3, 4, or 5 amino acids after the Tyr. Non-limiting examples of peptides for use in a compound of the disclosure include those listed in **Table A**. In a specific embodiment, a peptide for use in a compound may be selected from the peptides of **Table A**.

Table A	
SEQ ID NO:	Peptide
8	_D Cys-Gly-Arg-Asp-Ser-Pro-Cys-Lys-Tyr
9	_D Cys-Gly-Arg-Asp-Ser-Pro-Cys-Tyr
10	_D Cys-Gly-Arg-Asp-Ser-Pro-Cys-Tyr-Lys

11	$\text{D}^{\text{Cys}}\text{-Gly-Arg-Asp-Ser-Pro-D}^{\text{Cys}}\text{-Tyr}$
12	$\text{Cys-Gly-Arg-Asp-Ser-Pro-Cys-Tyr}$
13	$\text{Cys-Arg-Gly-Asp-Ser-Pro-Cys-Tyr}$
14	$\text{D}^{\text{Cys}}\text{-Arg-Gly-Asp-Ser-Pro-Cys-Tyr}$
15	$\text{D}^{\text{Cys}}\text{-Arg-Gly-Asp-Ser-Pro-D}^{\text{Cys}}\text{-Tyr}$
17	$\text{Gly-D}^{\text{Cys}}\text{-Gly-Arg-Asp-Ser-Pro-Cys-Tyr}$
18	$\text{Gly-D}^{\text{Cys}}\text{-Gly-Arg-Asp-Ser-Pro-Cys-D}^{\text{Tyr}}$
19	$\text{Gly-D}^{\text{Cys}}\text{-Gly-Arg-Asp-Ser-Pro-Cys-D}^{\text{Tyr}}\text{-Lys}$
20	$\text{Gly-Cys-Gly-Arg-Asp-Ser-Pro-Cys-D}^{\text{Tyr}}\text{-Lys}$
21	$\text{Gly-D}^{\text{Cys}}\text{-Gly-Arg-Asp-Ser-Pro-D}^{\text{Cys}}\text{-Tyr}$
22	$\text{Gly-Cys-Gly-Arg-Asp-Ser-Pro-D}^{\text{Cys}}\text{-Tyr}$
23	$\text{Gly-D}^{\text{Cys}}\text{-Gly-Arg-Asp-Ser-Pro-D}^{\text{Cys}}\text{-D}^{\text{Tyr}}$

[0037] In an alternative embodiment, the compound may have more than one peptide having a RD motif or RGD motif with the size (i.e., number of amino acid residues) and amino acid composition detailed above. For applications involving more than one peptide, the individual peptides may form a single continuous chain with each individual peptide attached together either directly by a covalent bond or they may be separated by a linker. The single continuous chain of individual peptides may then be conjugated to the agent either directly via a covalent bond or by a linker. Alternatively, individual peptides may each be conjugated directly to the agent by either a covalent bond or by a linker. The number of individual peptides can and will vary. Typically, there may be from about 1 to about 15 peptides having a RD motif or RGD motif. In another embodiment, there may be from about 1 to about 12 peptides. In a further embodiment, there may be from about 1 to about 10 peptides. In yet another embodiment, there may be from about 1 to about 5 peptides. In a further embodiment, there is one peptide. In yet another embodiment, there are two peptides. In an additional embodiment, there are 3 peptides. In an additional embodiment, there are 4 peptides. In still another embodiment, there are 5 peptides.

(b) imaging agents and treatment agents

[0038] The compound of the invention includes at least one imaging agent and, optionally, a treatment agent. In one embodiment, the compound may comprise an imaging agent. In an alternative embodiment, the compound may comprise an imaging agent and a treatment agent. Irrespective of the embodiment, the agent(s) may be either conjugated to the compound by a covalent bond or conjugated via a linker.

[0039] Several imaging agents are suitable for use to the extent that they provide the ability to detect or monitor the localization of the compound(s) of the present invention. In one embodiment, the imaging agent comprises an optical imaging agent. Optical imaging agents suitable for use in the invention can and will vary depending on the embodiment, but include fluorophores, organic fluorescent dyes, luminescent imaging agents, fluorescent lanthanide complexes, and fluorescent semiconductor nanocrystals. Examples of suitable visible (400-700 nm) fluorescent dyes include fluorescein, FITC, rhodamine, Texas Red, CyDyes (e.g., Cy3, Cy5, Cy5.5), Alexa Fluors (e.g., Alexa⁴⁸⁸, Alexa⁵⁵⁵, Alexa⁵⁹⁴, Alexa⁶⁴⁷) and DyDelight Dyes. Examples of suitable near infrared (NIR) (700-900 nm) fluorescent dyes include carbocyanine dyes, such as cypate and its derivatives. Luminescence imaging agents include luminescent lanthanide chelates and bioluminescence compounds (e.g., bacterial Lux, eukaryotic Luc or Ruc systems). In a specific embodiment, an imaging agent is a carbocyanine dye or a derivative thereof. Suitable carbocyanine dyes are known in the art or as described in the Examples. Non-limiting examples of carbocyanine dyes include those depicted in **FIG. 1, FIG. 2A, FIG. 2B, FIG. 2C, FIG. 2D, FIG. 3A, FIG. 3B, FIG. 3C, FIG. 7A, FIG. 7B, FIG. 7C, and FIG. 13**. In a specific embodiment, a compound of the disclosure comprises a carbocyanine dye selected from those depicted in **FIG. 1, FIG. 2A, FIG. 2B, FIG. 2C, FIG. 2D, FIG. 3A, FIG. 3B, FIG. 3C, FIG. 7A, FIG. 7B, FIG. 7C, and FIG. 13**. In another specific embodiment, a compound of the disclosure comprises a carbocyanine dye selected from the group consisting of Cypate (cypate 4), LS288, LS798, LS276, LS843, Cypate 3, and Cypate 2. A derivative of a carbocyanine dye may comprise a nonionic group (i.e. polyethylene glycol) or a positively charged moiety (i.e. ⁺NMe₃) conjugated to a free carboxylic acid group of a cypate. Alternatively, a derivative

of a carbocyanine dye may comprise a functional group for conjugation of a radioisotope, treatment agent or other biologically active molecule. Non-limiting examples of biologically active molecules include nanoparticles, small organic molecules, peptides, proteins, organometallics, drugs, antibiotics, and carbohydrates. In certain embodiments, the biologically active molecule is <500 Da. Exemplary functional groups are depicted in **Table 4** and **FIG. 13**. In a specific embodiment, a functional group is selected from the group consisting of an alkyne, azido (N_3), and a chelating agent. As used herein, a “chelating agent” is a molecule that forms multiple chemical bonds with a single metal atom. Examples of chelating agents include, but are not limited to, iminodicarboxylic and polyaminopolycarboxylic reactive groups, diethylenetriaminepentaacetic acid (DTPA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), tetramethyl heptanedionate (TMHD), 2,4-pentanedione, ethylenediamine-tetraacetic acid disodium salt (EDTA), ethyleneglycol-O,O'-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), N-(2-hydroxyethyl)ethylenediamine-N,N',N'-triacetic acid trisodium salt (HEDTA), nitrilotriacetic acid (NTA), and 1,4,8,11-tetraazacyclotetradecane-N,N',N'',N'''-tetraacetic acid (TETA), deferoxamine (DFO), and derivatives thereof. A radioisotope, treatment agent and biologically active molecule are described below.

[0040] In an alternative embodiment, the imaging agent is a radiological imaging agent. In certain embodiments, a compound of the invention comprises two imaging agents: a carbocyanine dye or derivative thereof and a radioisotope. The radioisotope may be conjugated to the carbocyanine dye or may be conjugated to the Tyr of the peptide. A variety of radioisotopes that are capable of being detected, such as in a PET or SPECT diagnostic imaging procedure, are suitable for use in the present invention. Suitable examples of radiological imaging agents include Antimony-124, Antimony-125, Arsenic-74, Barium-103, Barium-140, Beryllium-7, Bismuth-206, Bismuth-207, Cadmium-109, Cadmium-115, Calcium-45, Cerium-139, Cerium-141, Cerium-144, Cesium-137, Chromium-51, Gadolinium-153, Gold-195, Gold-199, Hafnium-175-181, Indium-111, Iridium-192, Iron-55, Iron-59, Krypton-85, Lead-210, Manganese-54, Mercury-197, Mercury-203, Molybdenum-99, Neodymium-147,

Neptunium-237, Nickel-63, Niobium-95, Osmium-185, Palladium-103, Platinum-195, Praseodymium-143, Promethium-147, Protactinium-233, Radium-226, Rhenium-186, Rubidium-86, Ruthenium-103, Ruthenium-106, Scandium-44, Scandium-46, Selenium-75, Silver-110, Silver-111, Sodium-22, Strontium-85, Strontium-89, Strontium-90, Sulfur-35, Tantalum-182, Technetium-99, Tellurium-125, Tellurium-132, Thallium-204, Thorium-228, Thorium-232, Thallium-170, Tin-113, Titanium-44, Tungsten-185, Vanadium-48, Vanadium-49, Ytterbium-169, Yttrium-88, Yttrium-90, Yttrium-91, Zinc-65, and Zirconium. In a further alternative embodiment, the radiological imaging agent is selected from the group consisting of Technetium-99, Indium-111, Strontium-90, Iodine-125, Thallium-201, fluorine-18, carbon-11, carbon-13, nitrogen-13, Oxygen-15, Copper-64, Lutetium-177, Yttrium-90, and Iodine-123, Iodine-124, Iodine-125, and Iodine-131. In certain embodiments, a radioisotope may be used as an imaging agent and as a treatment agent. It is known in the art which radioisotopes function as both imaging agents and treatment agents. For example, since Iodine-131 has both a beta and gamma decay mode, it can be used for radiotherapy or for imaging.

[0041] A variety of other imaging agents are suitable for use in the invention. For example, other imaging agents include, gadolinium, metalloporphyrins, ferric chloride, ferric ammonium citrate, and ferrioxamine methanesulfonate for magnetic resonance imaging.

[0042] An imaging agent emits a signal that can be detected by a signal transducing machine. In some cases, imaging agent can emit a signal spontaneously, such as when the detectable label is a radionuclide. In other cases the imaging agent emits a signal as a result of being stimulated by an external field such as when the imaging agent is a relaxivity metal. Examples of signals include, without limitation, gamma rays, X-rays, visible light, infrared energy, and radiowaves. Non-limiting examples of modalities of imaging may include magnetic resonance imaging (MRI), ultrasound (US), computed tomography (CT), Positron Emission Tomography (PET), Single Photon Emission Computed Tomography (SPECT), and optical imaging (OI, bioluminescence and fluorescence).

[0043] The compound of the invention optionally includes one or more treatment agents, such as a drug or hormone. In certain embodiments, a compound of the invention comprises an imaging agent and a treatment agent. In other embodiments, a compound of the invention comprises a carbocyanine dye or derivative thereof and a treatment agent. As will be appreciated by the skilled artisan, the choice of a particular treatment agent can and will vary depending upon the indication to be treated and its stage of progression. Because the compounds of the invention are selectively targeted to cells that express integrins, the treatment agents are generally directed toward treatment of an integrin-mediated disorder such as diabetes, inflammation, cardiovascular disease, and cancer. For example, when the indication is diabetes, the treatment agent may be sulfonylureas, biguanides, thiazolidinediones, meglitinides, D-phenylalanine derivatives, amylin synthetic derivatives, and incretin mimetics. In a further embodiment, when the indication is inflammation, the treatment agent may be an NSAID such as aniline derivatives (acetaminophen), indole-3-acetic acid derivatives (indomethacin), specific Cox-2 inhibitors (Celebrex), and aspirin. By way of further example, when the indication is cardiovascular disease, the treatment agent may include sodium-channel blockers (e.g., quinidine), beta-blockers (e.g., propranolol), calcium-channel blockers (e.g., diltiazem), diuretics (e.g., hydrochlorothiazide), ACE inhibitors (e.g., captopril), and thrombolytic agents (e.g., tissue plasminogen activator and streptokinase). In an additional embodiment when the indication is cancer, the treatment agent may include DNA synthesis inhibitors (e.g., daunorubicin, and adriamycin), mitotic inhibitors (e.g., the taxanes, paclitaxel, and docetaxel), the vinca alkaloids (e.g., vinblastine, vincristine, and vinorelbine), antimetabolites (e.g., 5-fluorouracil, capecitabine, 6-mercaptopurine, methotrexate, gemcitabine, cytarabine (ara-C), fludarabine, pemetrexed, cytosine arabinoside, methotrexate, and aminopterin), alkylating agents (e.g., busulfan, cisplatin, carboplatin, chlorambucil, cyclophosphamide, ifosfamide, dacarbazine (DTIC), mechlorethamine (nitrogen mustard), melphalan, and temozolomide), nitrosoureas (e.g., carmustine (BCNU) and lomustine (CCNU) anthracyclines (e.g., daunorubicin, doxorubicin (Adriamycin), epirubicin, idarubicin, and mitoxantrone), topoisomerase inhibitors (e.g.,

topotecan, irinotecan, etoposide (VP-16), and teniposide), cytotoxins (e.g., paclitaxel, vinblastine, and macromycin), anti-cytoskeletals (e.g., taxol and colchicine) and angiogenesis inhibitors (e.g., VEGF inhibitors, anti-VEGF Abs). Summaries of cancer drugs, including information regarding approved indications, may be found via the National Cancer Institute at the National Institutes of Health (www.cancer.gov/cancertopics/druginfo/alphalist), the FDA Approved Drug Product database (www.accessdata.fda.gov/scripts/cder/drugsatfda/) and the National Comprehensive Cancer Network (NCCN) guidelines (www.nccn.org/professionals/physician_gls/f_guidelines.asp). In a specific embodiment, a treatment agent may be a chemotherapeutic. In another specific embodiment, a treatment agent may be a chemotherapeutic for pancreatic cancer. Other suitable treatment agents may include hormones (e.g., steroids), antibodies, antibody fragments, peptides, glycopeptides, peptidomimetic, drug mimic, metal chelating agents, radioactive agents, echogenic agents, various drugs (in addition to the ones specifically delineated), antisense molecules, and small inhibitory RNAs.

(c) linkers

[0044] In certain embodiments, the imaging agent and/or treatment agent is conjugated to the linear peptide via one or more linkers. In other embodiments having more than one linear peptide or one or more cyclic peptides, the individual peptides may optionally be conjugated via one or more linkers.

[0045] A variety of linkers are suitable in the present invention, but typically the linker will impart a degree of flexibility to the compound of the invention. Generally speaking, the chain of atoms defining the linker can and will vary depending upon the embodiment. In certain embodiment, the linker will comprise one or more amino acids. Amino acid residue linkers are usually at least one residue and can be 50 or more residues. In an embodiment, a linker may be about 1 to about 10 amino acids. In another embodiment, a linker may be about 10 to about 20 amino acids. In still another embodiment, a linker may be about 20 to about 30 amino acids. In still yet another embodiment, a linker may be about 30 to about 40 amino acids. In different embodiments, a linker may be about 40 to about 50 amino acids. In other embodiments,

a linker may be more than 50 amino acids. For instance, a linker may be 1, 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 or 50 amino acids. In a specific embodiment, a linker is 1 amino acid.

[0046] Any amino acid residue may be used for the linker. Typical amino acid residues used for linking are glycine, serine, alanine, leucine, lysine, glutamic and aspartic acid, or the like. For example, a linker may be $(AAS)_n$, $(AAAL)_n$, $(G_nS)_n$ or $(G_2S)_n$, wherein A is alanine, S is serine, L is leucine, and G is glycine and wherein n is an integer from 1-20, or 1-10, or 3-10. Accordingly, n may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. Thus, in certain embodiments, a linker includes, but is not limited to, $(AAS)_n$, $(AAAL)_n$, $(G_nS)_n$ or $(G_2S)_n$, wherein A is alanine, S is serine, L is leucine, and G is glycine and wherein n is an integer from 1-20, or 1-10, or 3-10. In a specific embodiment, a linker is one glycine.

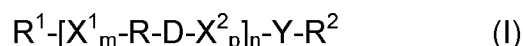
[0047] In a further embodiment, the linker will comprise hydrocarbyl or substituted hydrocarbyl groups. In a typical alternative of this embodiment, the linker is from about 1 to about 50 atoms in length. Alternatively, the linker is from about 2 about 30 atoms in length. In an embodiment, the linker is from about 4 to about 20 atoms in length. The linker may comprise a variety of heteroatoms that may be saturated or unsaturated, substituted or unsubstituted, linear or cyclic, or straight or branched. The chain of atoms defining the linker will typically be selected from the group consisting of carbon, oxygen, nitrogen, sulfur, selenium, silicon and phosphorous. In an alternative embodiment, the chain of atoms is selected from the group consisting of carbon, oxygen, nitrogen, sulfur and selenium. In an embodiment, the linker will comprise substantially carbon and oxygen atoms. In addition, the chain of atoms defining the linker may be substituted or unsubstituted with atoms other than hydrogen, including, but not limited to, hydroxy, keto ($=O$), or acyl, such as acetyl. Thus, the chain may optionally include one or more ether, thioether, selenoether, amide, or amine linkages between hydrocarbyl or substituted hydrocarbyl regions. Exemplary linkers include ethylene glycol and aminohexanoic acid. More specifically, a linker may be a

polyethylene glycol linker. Such a linker may be referred to as a heterobifunctional PEG linker or a homobifunctional PEG linker.

[0048] In certain embodiments, a linker further comprises one or more spacers. Spacers are known in the art. Non-limiting examples of spacers include 2-aminoethoxy-2-ethoxy acetic acid (AEEA) linkers, AEEEA linkers, and AEA linkers. In a specific embodiment, a linker further comprises one or more 2-aminoethoxy-2-ethoxy acetic acid (AEEA) linkers.

(d) exemplary compounds of the invention

[0049] In one exemplary embodiment, the compound will have the characteristics detailed above and will be defined by formula (I):



wherein:

R^1 is an imaging agent;

R^2 is independently selected from the group consisting of a treatment agent, hydrogen, hydroxyl, NH_2 , hydrocarbyl, and substituted hydrocarbyl;

X^1 and X^2 are independently selected from any amino acid residue;

$X^1_m-R-D-X^2_p$ together form a linear or cyclic peptide;

m is an integer from 1 to about 10;

n is an integer from 1 to about 10;

p is an integer from 1 to about 10; and

a dash (-) represent a covalent bond.

[0050] In an alternative embodiment, the compound will have formula (I) wherein:

n is from 1 to 5;

m is from 1 to 3;

p is from 1 to 3; and

X^1 and X^2 are selected from the group consisting of C, G, S, P, C, N, Q, D, E, K, R, T and H.

[0051] In a specific embodiment, X^1 is CG; m is 1; X^2 is SPC; p is 1; and n is 1. In another specific embodiment, the C of X^1 is D-cysteine and/or the C of X^2 is D-cysteine. In still another specific embodiment, the Y is D-tyrosine.

[0052] In certain embodiments, a cyclic peptide is [CGRDSPC] (SEQ ID NO:24), wherein the two cysteines cyclize the peptide by forming a disulfide bridge. The cysteines are both L-cysteine, both D-cysteine, or one is L-cysteine and one is D-cysteine. In other embodiments, one or more amino acid residues are inserted prior to or after the Y. In a specific embodiment, an amino acid residue is inserted after Y. In another specific embodiment, a Lys (K) is inserted after the Y.

[0053] In certain embodiments, the Y is halogenated. Halogens include fluorine, chlorine, bromine, iodine, and astatine. More specifically, the halogen is a radioisotope selected from the group consisting of ^{18}F , ^{75}Br , ^{77}Br , ^{122}I , ^{123}I , ^{124}I , ^{125}I , ^{129}I , ^{131}I , and ^{211}At .

[0054] In an exemplary embodiment, $[X^1_m\text{-R-D-}X^2_p]_n\text{-Y}$ is selected from the group consisting of:

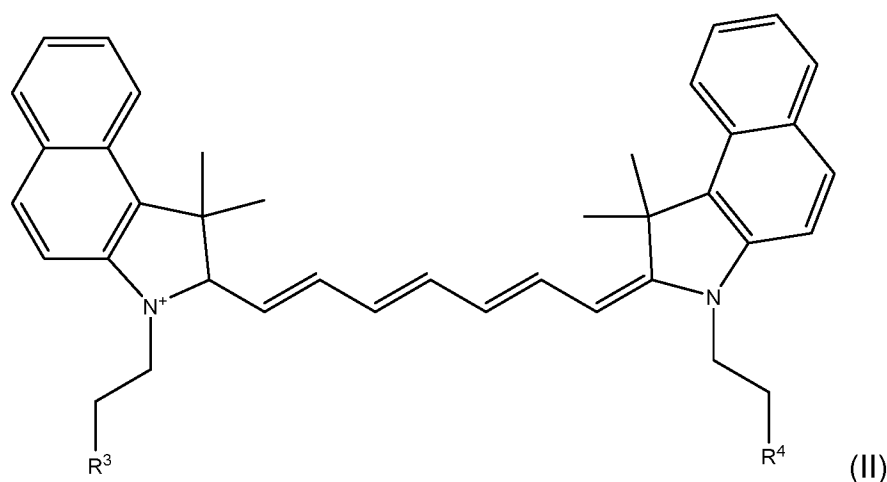
SEQ ID NO:	$[X^1_m\text{-R-D-}X^2_p]_n\text{-Y}$
8	$[_D\text{Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-Lys-Tyr}$
9	$[_D\text{Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-Tyr}$
10	$[_D\text{Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-Tyr-Lys}$
11	$[_D\text{Cys-Gly-Arg-Asp-Ser-Pro-}_D\text{Cys}]\text{-Tyr}$
12	$[\text{Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-Tyr}$
17	$\text{Gly-}[_D\text{Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-Tyr}$
18	$\text{Gly-}[_D\text{Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-}_D\text{Tyr}$
19	$\text{Gly-}[_D\text{Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-}_D\text{Tyr-Lys}$
20	$\text{Gly-}[\text{Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-}_D\text{Tyr-Lys}$
21	$\text{Gly-}[_D\text{Cys-Gly-Arg-Asp-Ser-Pro-}_D\text{Cys}]\text{-Tyr}$
22	$\text{Gly-}[\text{Cys-Gly-Arg-Asp-Ser-Pro-}_D\text{Cys}]\text{-Tyr}$
23	$\text{Gly-}[_D\text{Cys-Gly-Arg-Asp-Ser-Pro-}_D\text{Cys}]\text{-}_D\text{Tyr}$

[0055] In certain embodiments, R^2 is selected from the group consisting of hydroxyl and NH_2 . In a specific embodiment, R^2 is hydroxyl.

[0056] In an embodiment, R^1 is a carbocyanine dye or a derivative thereof. In another embodiment, R^1 is selected from the group consisting of Cypate (cypate 4), LS288, LS798, LS276, LS843, Cypate 3, and Cypate 2. In a specific embodiment, a derivative of a carbocyanine dye comprises an alkyne, azido (N_3), or a chelating agent. In still another specific embodiment, a derivative of a carbocyanine dye comprises a nanoparticle, a small organic molecule, a peptide, an organometallic, a metal chelate, a protein, a drug, an antibiotic, or a carbohydrate.

[0057] In each embodiment for compounds having formula (I), the compound may optionally comprise a linker, L^1 , that conjugates R^1 to X^1 . The compound may additionally comprise a linker, L^2 , that conjugates R^2 to Y . In addition, the compound may additionally comprise a linker, L^3 , that conjugates R^1 to X^2 . The compound may additionally comprise a linker, L^4 , that conjugates R^2 to X^1 . The compound may additionally comprise a linker, L^5 , that conjugates X^1 to X^2 . Furthermore, the compound may additionally comprise a linker, L^6 , that conjugates R^1 to R^2 . In a specific embodiment, L^1 is glycine. In another specific embodiment, L^1 is polyethylene glycol.

[0058] In another exemplary embodiment, the compound will have the characteristics detailed above and will be defined by formula (II):



wherein:

R^3 is selected from the group consisting of nanoparticles, small organic molecules, peptides, organometallics, metal chelates, proteins, drugs, antibiotics, and carbohydrates; and

R^4 is $[X^1_m-R-D-X^2_p]_n-Y-R^2$, wherein:

R^2 is independently selected from the group consisting of a hydrogen, hydroxyl, NH_2 , hydrocarbyl, and substituted hydrocarbyl;

X^1 and X^2 are independently selected from any amino acid residue;

$X^1_m-R-D-X^2_p$ together form a linear or cyclic peptide;

m is an integer from 1 to about 10;

n is an integer from 1 to about 10;

p is an integer from 1 to about 10; and

a dash (-) represent a covalent bond.

[0059] In an alternative embodiment, the compound will have formula (II) wherein:

n is from 1 to 5;

m is from 1 to 3;

p is from 1 to 3; and

X^1 and X^2 are selected from the group consisting of C, G, S, P, C, N, Q, D, E, K, R, T and H.

[0060] In a specific embodiment, X^1 is CG; m is 1; X^2 is SPC; p is 1; and n is 1. In another specific embodiment, the C of X^1 is D-cysteine and/or the C of X^2 is D-cysteine. In still another specific embodiment, the Y is D-tyrosine.

[0061] In certain embodiments, a cyclic peptide is [CGRDSPC] (SEQ ID NO:24), wherein the two cysteines cyclize the peptide by forming a disulfide bridge. The cysteines are both L-cysteine, both D-cysteine, or one is L-cysteine and one is D-cysteine. In other embodiments, one or more amino acid residues are inserted prior to or after the Y. In a specific embodiment, an amino acid residue is inserted after Y. In another specific embodiment, a Lys (K) is inserted after the Y.

[0062] In certain embodiments, the Y is halogenated. Halogens include fluorine, chlorine, bromine, iodine, and astatine. More specifically, the halogen is a

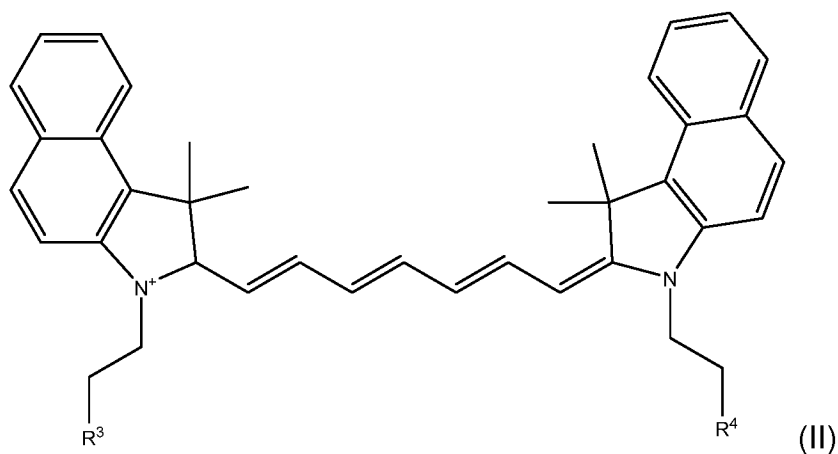
radioisotope selected from the group consisting of ^{18}F , ^{75}Br , ^{77}Br , ^{122}I , ^{123}I , ^{124}I , ^{125}I , ^{129}I , ^{131}I , and ^{211}At .

[0063] In an exemplary embodiment, $[\text{X}^1\text{-R-D-X}^2]_n\text{-Y}$ is selected from the group consisting of:

SEQ ID NO:	$[\text{X}^1\text{-R-D-X}^2]_n\text{-Y}$
8	$[\text{D-Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-Lys-Tyr}$
9	$[\text{D-Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-Tyr}$
10	$[\text{D-Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-Tyr-Lys}$
11	$[\text{D-Cys-Gly-Arg-Asp-Ser-Pro-D-Cys}]\text{-Tyr}$
12	$[\text{Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-Tyr}$
17	$\text{Gly-}[\text{D-Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-Tyr}$
18	$\text{Gly-}[\text{D-Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-D-Tyr}$
19	$\text{Gly-}[\text{D-Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-D-Tyr-Lys}$
20	$\text{Gly-}[\text{Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-D-Tyr-Lys}$
21	$\text{Gly-}[\text{D-Cys-Gly-Arg-Asp-Ser-Pro-D-Cys}]\text{-Tyr}$
22	$\text{Gly-}[\text{Cys-Gly-Arg-Asp-Ser-Pro-D-Cys}]\text{-Tyr}$
23	$\text{Gly-}[\text{D-Cys-Gly-Arg-Asp-Ser-Pro-D-Cys}]\text{-D-Tyr}$

[0064] In certain embodiments, R^2 is selected from the group consisting of hydroxyl and NH_2 . In a specific embodiment, R^2 is hydroxyl.

[0065] In other embodiments, the compound will have the characteristics detailed above and will be defined by formula (II):



wherein:

R³ is selected from the group consisting of nanoparticles, small organic molecules, peptides, organometallics, metal chelates, proteins, drugs, antibiotics, and carbohydrates; and

R⁴ is selected from the group consisting of:

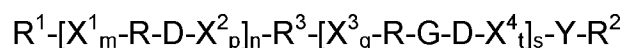
SEQ ID NO:	R ⁴
8	CONH-[D-Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Lys-Tyr-OH
8	CONH-[D-Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Lys-Tyr-NH ₂
9	CONH-[D-Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Tyr-OH
10	CONH-[D-Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Tyr-Lys-OH
11	CONH-[D-Cys-Gly-Arg-Asp-Ser-Pro-D-Cys]-Tyr-OH
12	CONH-[Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Tyr-OH
13	CONH-[Cys-Arg-Gly-Asp-Ser-Pro-Cys]-Tyr-OH
14	CONH-[D-Cys-Arg-Gly-Asp-Ser-Pro-Cys]-Tyr-OH
15	CONH-[D-Cys-Arg-Gly-Asp-Ser-Pro-D-Cys]-Tyr-OH
17	CONH-Gly-[D-Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Tyr-OH
18	CONH-Gly-[D-Cys-Gly-Arg-Asp-Ser-Pro-Cys]-D-Tyr-OH
19	CONH-Gly-[D-Cys-Gly-Arg-Asp-Ser-Pro-Cys]-D-Tyr-Lys-OH
20	CONH-Gly-[Cys-Gly-Arg-Asp-Ser-Pro-Cys]-D-Tyr-Lys-OH
21	CONH-Gly-[D-Cys-Gly-Arg-Asp-Ser-Pro-D-Cys]-Tyr-OH
22	CONH-Gly-[Cys-Gly-Arg-Asp-Ser-Pro-D-Cys]-Tyr-OH
23	CONH-Gly-[D-Cys-Gly-Arg-Asp-Ser-Pro-D-Cys]-D-Tyr-OH

[0066] In certain embodiments, the Y is halogenated. Halogens include fluorine, chlorine, bromine, iodine, and astatine. More specifically, the halogen is a radioisotope selected from the group consisting of ¹⁸F, ⁷⁵Br, ⁷⁷Br, ¹²²I, ¹²³I, ¹²⁴I, ¹²⁵I, ¹²⁹I, ¹³¹I, and ²¹¹At.

[0067] Alternatively, for each embodiment for compounds having formula (I) or formula (II), the compound may optionally comprise at least one cyclic peptide having a RGD motif. Generally, the cyclic peptide has from about 4 amino acid

residues to about 10 amino acid residues. In one embodiment, the cyclic peptide has 5 amino acid residues. In this embodiment, 3 of the amino acid residues will be the RGD motif and the other two amino acid residues may be selected from any amino acid residue detailed above. In an alternative embodiment, the cyclic peptide has 6 amino acid residues. In this embodiment, 3 of the amino acid residues will be the RGD motif and the other three may be selected from any amino acid residue detailed above.

[0068] In a further exemplary embodiment, the compound will have the characteristics detailed above and will be defined by formula (II):



wherein:

R^1 is an imaging agent;

R^2 is independently selected from the group consisting of a treatment agent, hydrogen, hydroxyl, NH_2 , hydrocarbyl, and substituted hydrocarbyl;

R^3 is a covalent bond or a linker;

X^1 , X^2 , X^3 and X^4 are independently selected from any amino acid residue;

$X^1_m-R-D-X^2_p$ together form a linear or cyclic peptide;

$X^3_q-R-G-D-X^4_t$ together form a cyclic peptide;

m is an integer from 1 to about 10;

n is an integer from 1 to about 10;

p is an integer from 1 to about 10;

q is an integer from about 1 to 5;

s is an integer from about 1 to 10;

t is an integer from about 1 to 5; and

a dash (-) represent a covalent bond.

[0069] In an alternative embodiment, the compound will have formula (II) wherein:

n is from 1 to 3;

m is from 1 to 3;

p is from 1 to 3;

q is 2 or 3;

s is from 1 to 3;

t is 2 or 3;

X¹ and X² are selected from the group consisting of G, S, N, Q, D, E, K, R, T, Y, C, P and H; and

X³ and X⁴ are selected from any amino acid residue.

[0070] In each embodiment for compounds having formula (II), the compound may optionally comprise a linker, L¹, that conjugates R¹ to X¹. In addition, the compound may additionally comprise a linker, L², that conjugates R² to Y.

[0071] Other exemplary compounds of the invention are illustrated in the Examples.

[0072] In addition, the compound(s) of the present invention can exist in tautomeric, geometric or stereoisomeric forms. The present invention contemplates all such compounds, including cis- and trans-geometric isomers, E- and Z-geometric isomers, R- and S-enantiomers, diastereomers, d-isomers, l-isomers, the racemic mixtures thereof and other mixtures thereof. Pharmaceutically acceptable salts of such tautomeric, geometric or stereoisomeric forms are also included within the invention. The terms "cis" and "trans", as used herein, denote a form of geometric isomerism in which two carbon atoms connected by a double bond will each have a hydrogen atom on the same side of the double bond ("cis") or on opposite sides of the double bond ("trans"). Some of the compounds described contain alkenyl groups, and are meant to include both cis and trans or "E" and "Z" geometric forms. Furthermore, some of the compounds described contain one or more stereocenters and are meant to include R, S, and mixtures of R and S forms for each stereocenter present.

[0073] In a further embodiment, the compound(s) of the present invention may be in the form of free bases or pharmaceutically acceptable acid addition salts thereof. The term "pharmaceutically-acceptable salts" are salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases. The nature of the salt may vary, provided that it is pharmaceutically acceptable. Suitable pharmaceutically acceptable acid addition salts of compounds for use in the present methods may be prepared from an inorganic acid or from an organic acid. Examples of such inorganic

acids are hydrochloric, hydrobromic, hydroiodic, nitric, carbonic, sulfuric and phosphoric acid. Appropriate organic acids may be selected from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, examples of which are formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucuronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, mesylic, 4-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethanesulfonic, benzenesulfonic, pantothenic, 2-hydroxyethanesulfonic, toluenesulfonic, sulfanilic, cyclohexylaminosulfonic, stearic, algenic, hydroxybutyric, salicylic, galactaric and galacturonic acid. Suitable pharmaceutically-acceptable base addition salts of compounds of use in the present methods include metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from N, N'-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, ethylenediamine, meglumine- (N-methylglucamine) and procaine.

[0074] As will be appreciated by a skilled artisan, the compound(s) of the present invention can be administered by a number of different means that will deliver an effective dose for either detection or treatment purposes. Such compositions can be administered orally, parenterally, by inhalation spray, rectally, intradermally, transdermally, or topically in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants, and vehicles as desired. Topical administration may also involve the use of transdermal administration such as transdermal patches or iontophoresis devices. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, or intrasternal injection, or infusion techniques. Formulation of drugs is discussed in, for example, Hoover, John E., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa. (1975), and Liberman, H. A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y. (1980).

[0075] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions, can be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation

may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed, including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are useful in the preparation of injectables. Dimethyl acetamide, surfactants including ionic and non-ionic detergents, and polyethylene glycols can be used. Mixtures of solvents and wetting agents such as those discussed above are also useful.

[0076] Solid dosage forms for oral administration may include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the compound is ordinarily combined with one or more adjuvants appropriate to the indicated route of administration. If administered *per os*, the compound can be admixed with lactose, sucrose, starch powder, cellulose esters of alkanoic acids, cellulose alkyl esters, talc, stearic acid, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulfuric acids, gelatin, acacia gum, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and then tableted or encapsulated for convenient administration. Such capsules or tablets can contain a controlled-release formulation as can be provided in a dispersion of active compound in hydroxypropylmethyl cellulose. In the case of capsules, tablets, and pills, the dosage forms can also comprise buffering agents such as sodium citrate, or magnesium or calcium carbonate or bicarbonate. Tablets and pills can additionally be prepared with enteric coatings.

[0077] For therapeutic purposes, formulations for parenteral administration can be in the form of aqueous or non-aqueous isotonic sterile injection solutions or suspensions. These solutions and suspensions can be prepared from sterile powders or granules having one or more of the carriers or diluents mentioned for use in the formulations for oral administration. The compounds can be dissolved in water, polyethylene glycol, propylene glycol, ethanol, corn oil, cottonseed oil, peanut oil,

sesame oil, benzyl alcohol, sodium chloride, and/or various buffers. Other adjuvants and modes of administration are well and widely known in the pharmaceutical art.

[0078] Liquid dosage forms for oral administration can include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions can also comprise adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

II. METHODS

[0079] The present invention encompasses a method for detecting, monitoring and/or treatment or prevention of a variety of integrin-mediated disorders in a subject. Specifically, the present invention encompasses a method for detecting a variety of integrin-mediated disorders in a subject.

[0080] In an aspect, the present invention encompasses a method for detecting expression of a $\beta 3$ subunit of integrin in a cell. The method comprises contacting a population of cells with a compound of the invention and detecting the presence of a signal emitted from the imaging agent of the compound of the invention in the population of cells, the signal being emitted from a cell expressing a $\beta 3$ subunit of integrin.

[0081] In another aspect, the present invention encompasses a method for detecting, monitoring and/or treatment or prevention of cancer. The method comprises administering an effective amount of a composition comprising a compound of the invention to a subject; and detecting the presence of a signal emitted from the imaging agent of the compound of the invention in the subject, wherein detection of signal above baseline indicates cancer. In a specific embodiment, the present invention encompasses a method for detecting cancer in a subject.

[0082] In still another aspect, the present invention encompasses a method for detecting, monitoring and/or treatment or prevention of pancreatic cancer. The method comprises administering an effective amount of a composition comprising a compound of the invention to a subject; and detecting the presence of a signal emitted from the imaging agent of the compound of the invention in the subject, wherein

detection of signal above baseline indicates pancreatic cancer. In a specific embodiment, the present invention encompasses a method for detecting pancreatic cancer in a subject.

[0083] In still yet another aspect, the present invention encompasses a method for detecting, monitoring and/or treatment or prevention of early stage pancreatic ductal adenocarcinoma (PDAC) and precursor pancreatic intraepithelial neoplasia (PanIN). The method comprises administering an effective amount of a composition comprising a compound of the invention to a subject; and detecting the presence of a signal emitted from the imaging agent of the compound of the invention in the subject, wherein detection of signal above baseline indicates early stage pancreatic ductal adenocarcinoma (PDAC) or pancreatic intraepithelial neoplasia (PanIN). In a specific embodiment, the present invention encompasses a method for detecting early stage pancreatic ductal adenocarcinoma (PDAC) and precursor pancreatic intraepithelial neoplasia (PanIN).

[0084] In yet still another aspect, the present invention encompasses a method for differentiating pancreatic ductal adenocarcinoma (PDAC) and precursor pancreatic intraepithelial neoplasia (PanIN) from pancreatitis. The method comprises administering an effective amount of a composition comprising a compound of the invention to a subject; and detecting the presence of a signal emitted from the imaging agent of the compound of the invention in the subject, wherein detection of signal above baseline indicates PDAC or PanIN and detection of a signal at or below baseline indicates pancreatitis.

[0085] In yet still another aspect, the present invention encompasses a method for differentiating pancreatic ductal adenocarcinoma (PDAC) and PanIN-3 from PanIN-1/2 and ductal hyperplasia/metaplasia. The method comprises administering an effective amount of a composition comprising a compound of the invention to a subject; and detecting the presence of a signal emitted from the imaging agent of the compound of the invention in the subject, wherein detection of signal above baseline indicates PDAC or PanIN-3 and detection of a signal at or below baseline indicates PanIN-1/2 or ductal hyperplasia/metaplasia.

[0086] In a different aspect, the present invention encompasses a method for detecting and/or monitoring circulating tumor cells (CTCs). The method comprises administering an effective amount of a composition comprising a compound of the invention to a subject; and detecting the presence of a signal emitted from the imaging agent of the compound of the invention in the subject, wherein detection of signal above baseline indicates the presence of CTCs. In a specific embodiment, the present invention encompasses a method for detecting CTCs in a subject.

[0087] By “treating, stabilizing, or preventing cancer” is meant causing a reduction in the size of a tumor or in the number of cancer cells, slowing or preventing an increase in the size of a tumor or cancer cell proliferation, increasing the disease-free survival time between the disappearance of a tumor or other cancer and its reappearance, preventing an initial or subsequent occurrence of a tumor or other cancer, or reducing an adverse symptom associated with a tumor or other cancer. In a desired embodiment, the percent of tumor or cancerous cells surviving the treatment is at least 20, 40, 60, 80, or 100% lower than the initial number of tumor or cancerous cells, as measured using any standard assay (e.g., caspase assays, TUNEL and DNA fragmentation assays, cell permeability assays, and Annexin V assays). Desirably, the decrease in the number of tumor or cancerous cells induced by administration of a compound of the invention is at least 2, 5, 10, 20, or 50-fold greater than the decrease in the number of non-tumor or non-cancerous cells. Desirably, the methods of the present invention result in a decrease of 20, 40, 60, 80, or 100% in the size of a tumor or in the number of cancerous cells, as determined using standard methods. Desirably, at least 20, 40, 60, 80, 90, or 95% of the treated subjects have a complete remission in which all evidence of the tumor or cancer disappears. Desirably, the tumor or cancer does not reappear or reappears after at least 5, 10, 15, or 20 years.

[0088] Suitable subjects include, but are not limited to, a human, a livestock animal, a companion animal, a lab animal, and a zoological animal. A subject may or may not be known to have a tumor. In one embodiment, the subject may be a rodent, e.g. a mouse, a rat, a guinea pig, etc. In another embodiment, the subject may be a livestock animal. Non-limiting examples of suitable livestock animals may include

pigs, cows, horses, goats, sheep, llamas and alpacas. In yet another embodiment, the subject may be a companion animal. Non-limiting examples of companion animals may include pets such as dogs, cats, rabbits, and birds. In yet another embodiment, the subject may be a zoological animal. As used herein, a "zoological animal" refers to an animal that may be found in a zoo. Such animals may include non-human primates, large cats, wolves, and bears. In a preferred embodiment, the animal is a laboratory animal. Non-limiting examples of a laboratory animal may include rodents, canines, felines, and non-human primates. In another preferred embodiment, the subject is a human.

[0089] The methods of the invention may be used to detect, monitor, treat or prevent a tumor derived from a neoplasm or a cancer. The neoplasm may be malignant or benign, the cancer may be primary or metastatic; the neoplasm or cancer may be early stage or late stage. Non-limiting examples of neoplasms or cancers that may be treated include acute lymphoblastic leukemia, acute myeloid leukemia, adrenocortical carcinoma, AIDS-related cancers, AIDS-related lymphoma, anal cancer, appendix cancer, astrocytomas (childhood cerebellar or cerebral), basal cell carcinoma, bile duct cancer, bladder cancer, bone cancer, brainstem glioma, brain tumors (cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, ependymoma, medulloblastoma, supratentorial primitive neuroectodermal tumors, visual pathway and hypothalamic gliomas, breast cancer, bronchial adenomas/carcinoids, Burkitt lymphoma, carcinoid tumors (childhood, gastrointestinal), carcinoma of unknown primary, central nervous system lymphoma (primary), cerebellar astrocytoma, cerebral astrocytoma/malignant glioma, cervical cancer, childhood cancers, chronic lymphocytic leukemia, chronic myelogenous leukemia, chronic myeloproliferative disorders, colon cancer, cutaneous T-cell lymphoma, desmoplastic small round cell tumor, endometrial cancer, ependymoma, esophageal cancer, Ewing's sarcoma in the Ewing family of tumors, extracranial germ cell tumor (childhood), extragonadal germ cell tumor, extrahepatic bile duct cancer, eye cancers (intraocular melanoma, retinoblastoma), gallbladder cancer, gastric (stomach) cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor, germ cell tumors (childhood extracranial, extragonadal,

ovarian), gestational trophoblastic tumor, gliomas (adult, childhood brain stem, childhood cerebral astrocytoma, childhood visual pathway and hypothalamic), gastric carcinoid, hairy cell leukemia, head and neck cancer, hepatocellular (liver) cancer, Hodgkin lymphoma, hypopharyngeal cancer, hypothalamic and visual pathway glioma (childhood), intraocular melanoma, islet cell carcinoma, Kaposi sarcoma, kidney cancer (renal cell cancer), laryngeal cancer, leukemias (acute lymphoblastic, acute myeloid, chronic lymphocytic, chronic myelogenous, hairy cell), lip and oral cavity cancer, liver cancer (primary), lung cancers (non-small cell, small cell), lymphomas (AIDS-related, Burkitt, cutaneous T-cell, Hodgkin, non-Hodgkin, primary central nervous system), macroglobulinemia (Waldenström), malignant fibrous histiocytoma of bone/osteosarcoma, medulloblastoma (childhood), melanoma, intraocular melanoma, Merkel cell carcinoma, mesotheliomas (adult malignant, childhood), metastatic squamous neck cancer with occult primary, mouth cancer, multiple endocrine neoplasia syndrome (childhood), multiple myeloma/plasma cell neoplasm, mycosis fungoides, myelodysplastic syndromes, myelodysplastic/myeloproliferative diseases, myelogenous leukemia (chronic), myeloid leukemias (adult acute, childhood acute), multiple myeloma, myeloproliferative disorders (chronic), nasal cavity and paranasal sinus cancer, nasopharyngeal carcinoma, neuroblastoma, non-Hodgkin lymphoma, non-small cell lung cancer, oral cancer, oropharyngeal cancer, osteosarcoma/malignant fibrous histiocytoma of bone, ovarian cancer, ovarian epithelial cancer (surface epithelial-stromal tumor), ovarian germ cell tumor, ovarian low malignant potential tumor, pancreatic cancer, pancreatic cancer (islet cell), paranasal sinus and nasal cavity cancer, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytoma, pineal astrocytoma, pineal germinoma, pineoblastoma and supratentorial primitive neuroectodermal tumors (childhood), pituitary adenoma, plasma cell neoplasia, pleuropulmonary blastoma, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell carcinoma (kidney cancer), renal pelvis and ureter transitional cell cancer, retinoblastoma, rhabdomyosarcoma (childhood), salivary gland cancer, sarcoma (Ewing family of tumors, Kaposi, soft tissue, uterine), Sézary syndrome, skin cancers (nonmelanoma, melanoma), skin carcinoma (Merkel cell), small cell lung

cancer, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, squamous neck cancer with occult primary (metastatic), stomach cancer, supratentorial primitive neuroectodermal tumor (childhood), T-Cell lymphoma (cutaneous), testicular cancer, throat cancer, thymoma (childhood), thymoma and thymic carcinoma, thyroid cancer, thyroid cancer (childhood), transitional cell cancer of the renal pelvis and ureter, trophoblastic tumor (gestational), unknown primary site (adult, childhood), ureter and renal pelvis transitional cell cancer, urethral cancer, uterine cancer (endometrial), uterine sarcoma, vaginal cancer, visual pathway and hypothalamic glioma (childhood), vulvar cancer, Waldenström macroglobulinemia, and Wilms tumor (childhood). In a specific embodiment, the neoplasm or cancer is pancreatic cancer.

[0090] The invention comprises, in part, imaging a subject. Non-limiting examples of modalities of imaging may include magnetic resonance imaging (MRI), ultrasound (US), computed tomography (CT), Positron Emission Tomography (PET), Single Photon Emission Computed Tomography (SPECT), and optical imaging (OI, bioluminescence and fluorescence). Radioactive molecular probes are traditionally imaged with PET, SPECT or gamma (γ) cameras, by taking advantage of the capability of these imaging modalities to detect the high energetic γ rays. In contrast, OI generally detects low energy lights (visible or near-infrared lights) emitted from bioluminescence or fluorescence probes.

[0091] As used herein, "baseline" may be the background signal. Alternatively, baseline may be no signal. In a specific embodiment, baseline is the signal detected in uninvolved tissue. A skilled artisan would be able to determine the baseline of a signal. By above is meant that the signal is greater than the baseline signal. For example, the signal may be at least 2% greater than baseline. For example, the signal may be at least 2%, at least 5%, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% greater than baseline. In a specific embodiment, the signal is > 20% above baseline. In other embodiments, the signal may be increased at least 2-fold over baseline. For example, the signal may be increased at least 2-fold, at least 5-fold, at

least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 30-fold, at least 35-fold, at least 40-fold, at least 45-fold, or at least 50-fold over baseline.

[0092] The term “signal” as used herein, refers to a signal derived from an imaging agent that can be detected and quantitated with regards to its frequency and/or amplitude. The signal may be an optical signal. The signal can be generated from one or more imaging agents of the present disclosure. In an embodiment, the signal may need to be the sum of each of the individual signals. In an embodiment, the signal can be generated from a summation, an integration, or other mathematical process, formula, or algorithm, where the signal is from one or more imaging agent. In an embodiment, the summation, the integration, or other mathematical process, formula, or algorithm can be used to generate the signal so that the signal can be distinguished from background noise and the like. It should be noted that signals other than the signal of interest can be processed and/or obtained in a similar manner as that of the signal of interest.

[0093] Using a method of the invention, microscopic lesions of cancer may be detected in a subject. Such lesions are generally not visible with current imaging techniques. Further the compounds of the disclosure may be used to guide needle biopsy, assess surgical margins, and detect occult metastatic disease in real time.

[0094] In certain aspects, a pharmacologically effective amount of a compound of the invention may be administered to a subject. Administration is performed using standard effective techniques, including peripherally (i.e. not by administration into the central nervous system) or locally to the central nervous system. Peripheral administration includes but is not limited to intravenous, intraperitoneal, subcutaneous, pulmonary, transdermal, intramuscular, intranasal, buccal, sublingual, or suppository administration. Local administration, including directly into the central nervous system (CNS) includes but is not limited to via a lumbar, intraventricular or intraparenchymal catheter or using a surgically implanted controlled release formulation.

[0095] Pharmaceutical compositions for effective administration are deliberately designed to be appropriate for the selected mode of administration, and pharmaceutically acceptable excipients such as compatible dispersing agents, buffers,

surfactants, preservatives, solubilizing agents, isotonicity agents, stabilizing agents and the like are used as appropriate. Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton Pa., 16Ed ISBN: 0-912734-04-3, latest edition, incorporated herein by reference in its entirety, provides a compendium of formulation techniques as are generally known to practitioners. It may be particularly useful to alter the solubility characteristics of the compounds useful in this discovery, making them more lipophilic, for example, by encapsulating them in liposomes or by blocking polar groups.

[0096] Effective peripheral systemic delivery by intravenous or intraperitoneal or subcutaneous injection is a preferred method of administration to a living patient. Suitable vehicles for such injections are straightforward. In addition, however, administration may also be effected through the mucosal membranes by means of nasal aerosols or suppositories. Suitable formulations for such modes of administration are well known and typically include surfactants that facilitate cross-membrane transfer. Such surfactants are often derived from steroids or are cationic lipids, such as N-[1-(2,3-dioleoyl)propyl]-N,N,N-trimethyl ammonium chloride (DOTMA) or various compounds such as cholesterol hemisuccinate, phosphatidyl glycerols and the like.

[0097] For therapeutic applications, a therapeutically effective amount of a composition of the invention is administered to a subject. A "therapeutically effective amount" is an amount of the therapeutic composition sufficient to produce a measurable biological tumor response (e.g., an immunostimulatory, an anti-angiogenic response, a cytotoxic response, or tumor regression). Actual dosage levels of active ingredients in a therapeutic composition of the invention can be varied so as to administer an amount of the active compound(s) that is effective to achieve the desired therapeutic response for a particular subject. The selected dosage level will depend upon a variety of factors including the activity of the therapeutic composition, formulation, the route of administration, combination with other drugs or treatments, tumor size and longevity, and the physical condition and prior medical history of the subject being treated. In some embodiments, a minimal dose is administered, and dose is escalated in the absence of dose-limiting toxicity. Determination and adjustment of a therapeutically

effective dose, as well as evaluation of when and how to make such adjustments, are known to those of ordinary skill in the art of medicine.

[0098] For diagnostic applications, a detectable amount of a composition of the invention is administered to a subject. A “detectable amount”, as used herein to refer to a diagnostic composition, refers to a dose of such a composition that the presence of the composition can be determined *in vivo* or *in vitro*. A detectable amount will vary according to a variety of factors, including but not limited to chemical features of the peptide being labeled, the imaging agent, labeling methods, the method of imaging and parameters related thereto, metabolism of the labeled drug in the subject, the stability of the label (e.g. the half-life of a radionuclide label), the time elapsed following administration of the compound prior to imaging, the route of drug administration, the physical condition and prior medical history of the subject, and the size and longevity of the tumor or suspected tumor. Thus, a detectable amount can vary and can be tailored to a particular application. After study of the present disclosure, and in particular the Examples, it is within the skill of one in the art to determine such a detectable amount. A detectable amount may be visible from about 1 to about 120 hours or more. For example, a detectable amount may be visible from about 1 to about 110 hours, or from about 1 to about 100 hours. Accordingly, a detectable amount may be visible at about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 16, about 24, about 36, about 48, about 60, about 72, about 84, about 96, about 108, or about 120 hours.

[0099] The frequency of dosing may be daily or once, twice, three times or more per week or per month, as needed as to effectively treat the symptoms. The timing of administration of the treatment relative to the disease itself and duration of treatment will be determined by the circumstances surrounding the case. Treatment could begin immediately, such as at the site of the injury as administered by emergency medical personnel. Treatment could begin in a hospital or clinic itself, or at a later time after discharge from the hospital or after being seen in an outpatient clinic. Duration of treatment could range from a single dose administered on a one-time basis to a life-long course of therapeutic treatments.

[0100] Although the foregoing methods appear the most convenient and most appropriate and effective for administration of peptide constructs, by suitable adaptation, other effective techniques for administration, such as intraventricular administration, transdermal administration and oral administration may be employed provided proper formulation is utilized herein.

[0101] In addition, it may be desirable to employ controlled release formulations using biodegradable films and matrices, or osmotic mini-pumps, or delivery systems based on dextran beads, alginate, or collagen.

[0102] Typical dosage levels can be determined and optimized using standard clinical techniques and will be dependent on the mode of administration.

[0103] In certain aspects, the methods of the invention may further comprise administering therapeutic agents not conjugated to a compound of the invention for neoplasms and cancer. Suitable therapeutic agents for neoplasms and cancers are known in the art, and will depend upon the type and stage of cancer. Summaries of cancer drugs, including information regarding approved indications, may be found via the National Cancer Institute at the National Institutes of Health (www.cancer.gov/cancertopics/druginfo/alphalist) and the FDA Approved Drug Product database (accessdata.fda.gov/scripts/cder/drugsatfda/).

DEFINITIONS

[0104] The term “detect” as used herein refers to diagnostic procedures and methods to image the compounds of the invention. These procedures include, but are not limited to, optical tomography, fluorescence endoscopy, imaging detection or measurement of or by fluorescence and imaging absorption, light scattering, acoustic, sonographic, magnetic resonance, or radiation means.

[0105] The term “hydrocarbyl” as used herein describe organic compounds or radicals consisting exclusively of the elements carbon and hydrogen. These moieties include alkyl, alkenyl, alkynyl, and aryl moieties. These moieties also include alkyl, alkenyl, alkynyl, and aryl moieties substituted with other aliphatic or cyclic hydrocarbon groups, such as alkaryl, alkenaryl and alkynaryl. Unless otherwise indicated, these moieties preferably comprise 1 to 20 carbon atoms.

[0106] The term “localized therapy” is a procedure for administering a compound of the invention directly into pathological tissue. Treatment in localized therapy may be accomplished by photo-, chemical-, or biological activation of the compound of the invention. Photoactivation may be conducted with light within a specific wavelength range. Chemical activation may be induced cytotoxicity. Biological activation may be initiated by physiological and molecular processes, including enzymatic activation of the compound.

[0107] The term “monitoring” as used herein refers to the continued or intermittent detection and may be combined with treatment for purposes including, but not limited to, ascertaining the progress or mechanism of pathology and efficacy of a particular treatment regime.

[0108] The term “substituted hydrocarbyl” moieties described herein are hydrocarbyl moieties which are substituted with at least one atom other than carbon, including moieties in which a carbon chain atom is substituted with a hetero atom such as nitrogen, oxygen, silicon, phosphorous, boron, sulfur, or a halogen atom. These substituents include halogen, carbocycle, aryl, heterocyclo, alkoxy, alkenoxy, alkynoxy, aryloxy, hydroxy, protected hydroxy, keto, acyl, acyloxy, nitro, amino, amido, nitro, cyano, thiol, ketals, acetals, esters and ethers.

[0109] The term “treat” or “treatment” includes partial or total inhibition of the progression of a particular disorder. For example when the disorder is cancer, treatment include partial or total inhibition of neoplasia growth, spreading or metastasis, as well as partial or total destruction of the neoplasia cells. Treatment also includes prevention of a neoplasia or related disorder. By way of further example when the disorder is inflammation, the term “treat” also includes partial or total inhibition of inflammation or an inflammation related disorder. Treatment also includes prevention of an inflammation or inflammation related disorder.

[0110] As various changes could be made in the above compounds, products and methods without departing from the scope of the invention, it is intended that all matter contained in the above description and in the examples given below, shall be interpreted as illustrative and not in a limiting sense.

EXAMPLES

[0111] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1. Improved RD targeting compounds.

[0112] Compare the performance of the different sequences: A variety of probes which have similar sequence compared to LS301 were designed and prepared (**Table 1**). The probes have different peptide sequences, D-, L- amino acid variations or different groups on C-terminus. The sequences of the probes were tested by LC-MS and the spectroscopy properties were recorded. The performances of the tumor targeting abilities were also tested *in vivo*. We discovered LS838 (**FIG. 1**) has better brightness after accumulation inside the cancer tumors and the targeting performance is similar to LS301. It was unexpected that LS838 would be brighter relative to LS301 and it is unknown to us why it does. However, the enhanced brightness allows the use of smaller amounts of material to achieve the same uptake kinetics and accumulation as LS301. **FIG. 5** depicts the accumulation of LS838 *in vivo* and **FIG. 6** graphically quantifies the intensity of LS838 in various organs. LS838 precisely accumulates in the tumor at 24 hours. LS838 labeled can be labeled with radionuclides such as radio-iodine, bromine, or fluorine for combined optical and nuclear imaging. The labeling does not alter the excellent tumor localizing properties. Notably, *in vivo* administration of LS747 and LS758, both of which have a Tyr adjacent to the cypate, led to rapid loss of retention in tumors. These results suggest that the position of the Tyr residue is important.

Table 1: Compounds prepared for performance comparison with LS301 peptide		
No.	Sequence	SEQ ID NO
LS301	Cypate-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Lys-OH	1
LS651	Cypate-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro- _D Cys)-Lys-OH	2
LS652	Cypate-Cyclo(Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Lys-OH	3
LS653	Cypate-Cyclo(Cys-Arg-Gly-Asp-Ser-Pro-Cys)-Lys-OH	4
LS654	Cypate-Cyclo(_D Cys-Arg-Gly-Asp-Ser-Pro-Cys)-Lys-OH	5
LS655	Cypate-Cyclo(_D Cys-Arg-Gly-Asp-Ser-Pro- _D Cys)-Lys-OH	6
LS747	Cypate-Tyr-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Lys-OH	7
LS748	Cypate-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Lys-Tyr-OH	8
LS758	Tyr-Cypate-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Lys-NH ₂	1
LS837	Cypate-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Lys-NH ₂	1
LS838	Cypate-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Tyr-OH	9
LS839	Cypate-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Tyr-Lys-OH	10
LS839-2	Cypate-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Lys-Tyr-NH ₂	8

[0113] Compare the performance of the LS301 derivative with fluorophores with different hydrophobicity: LS301 derivatives with fluorophores with different hydrophobicity were prepared to test if the hydrophobicity of the dye on the same peptide sequence will affect the targeting performance. Four of the NIR dyes with different hydrophobicity (**FIG. 2**) beside cypate were attached to the same GRD peptide (**Table 2**). These probes were tested *in vivo* to compare with LS301.

Table 2: Compounds prepared with fluorophores with different hydrophobicity		
No.	Sequence	SEQ ID NO
LS636	LS288-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Lys-OH	1
LS840	LS798-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Lys-OH	1
LS841	LS276-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Lys-OH	1
LS844	LS843-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Lys-OH	1

[0114] Multicolor LS301 derivatives for non-invasive molecular imaging: LS301 derivatives were prepared with different cyanine dyes (cypate 3 and cypate 2, **FIG. 3** and **FIG. 4**) which have emission wavelength (**Table 3**). These probes were tested *in vivo* and will allow the use of different wavelengths for the optical imaging.

Table 3: Compounds prepared with cyanine dyes with different emission wavelength		
No.	Sequence	SEQ ID NO
LS789	Cypate 3-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Lys-OH	1
LS858	Cypate 2-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Lys-OH	1

[0115] Multimodality LS301 derivatives for non-invasive molecular imaging: A variety of the multimodality LS301 derivatives readily available for radio-isotope labeling were designed and prepared (**Table 4**). These probes can be used for nuclear-imaging with the superior performance of targeted optical imaging, providing versatile application in different type of cancer diseases. The cold labeled probes were prepared and tested *in vivo* to compare with LS301. **FIG. 13** provides an example of derivatization of the cypate for incorporation of drugs, biologically active molecules and/or radio-halogens.

Table 4: Compounds prepared for multimodal imaging.

No.	Compound	Structures	SEQ ID NO
LS838		Cypate-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Tyr-OH	9
LS848	alkyne - LS301	alkyne-Cypate-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Lys-OH	1
LS859	Azido-LS301	N ₃ -Cypate-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Lys-OH	1
LS861	DTPA-LS301	DTPA-Cypate-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Lys-OH	1
LS862	DTPA-LS838	DTPA-Cypate-Cyclo(_D Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Tyr-OH	9

Example 2. Design and identification of LS838 as a pancreatic ductal adenocarcinoma (PDAC) imaging agent.

[0116] The current strategy to develop molecular probes with high binding affinity to a protein receptor overexpressed on tumor cells has allowed imaging of bulk tumor masses *in vivo*. Identifying gross tumors is straightforward in most cases. Unlike the bulk tumor, however, small tumors are surrounded by non-tumor tissue which also expresses the target receptor. Although the target protein is usually expressed at lower levels, the large surface area of the surrounding tissue increases the net background fluorescence, significantly decreasing the contrast between pathologic and uninvolved tissues. This concern was realized when previous attempts to use a dye-labeled RGD peptide or octreotate to target $\alpha\beta3$ integrin or somatostatin receptors, respectively, reportedly overexpressed in PDAC, were not successful in identifying early stage PDAC. It was concluded that what is needed is a secondary trapping mechanism that can longitudinally retain a molecular probe in malignant cells while it clears from non-target tissue.

[0117] A new fluorescent molecular probe, LS838 (**FIG. 7A**) selectively targets early and late stages of PDAC. LS838 is a small molecule (<1.6 kDa) consisting of a NIR fluorescent dye (cypate) and an octapeptide that is cyclized through a disulfide bond. Cypate has a biological clearance profile and spectral properties (absorption, emission, quantum yield) similar to the FDA-approved indocyanine green (ICG; **FIG. 7A**), which is not tumor selective and does not react with biomolecules for targeted delivery to tumors. The spectral properties of LS838 are suitable for NIR fluorescence imaging studies (excitation/emission 785/810 nm in serum and 20% aq. DMSO solution; molar absorptivity, ϵ , 240,000 M⁻¹; cm⁻¹; and fluorescence quantum yield, ψ , 10%). Cypate binds reversibly to the hydrophobic pocket of albumin, a source of nitrogen and energy for tumors. Unlike covalently dye-labeled albumin, cypate is released in tumors under mild acidic conditions as it traffics through the endosomal pathway. The released cypate is only transiently retained in tumors before efflux, limiting the tumor-to-background contrast.

[0118] To overcome this problem, the use of peptides as a tumor-trapping mechanism was explored. The highly reducing environment of tumor cells is known to reduce disulfide bonds to thiols, which can trans-thiolate with cysteine-containing intracellular proteins to trap the molecular probe. Therefore, a variety of disulfide-containing peptides were conjugated to cypate and the compounds were screened in mice with advanced stage PDAC. Results showed that LS838 was selectively retained in tumors for over 48 h, longer than the other peptide conjugates. Structure-tumor retention analysis revealed that the unnatural D-Cysteine linked to cypate confers high stability on the molecular probe because of its resistance to degradation by proteases. Replacement of the D-Cysteine with the natural L-Cysteine residue abrogates this retention.

[0119] NIR fluorescence microscopy of LS838 in diverse tumor cells showed punctate intracellular fluorescence typical of receptor-mediated endocytosis (**FIG. 8**) and that the fluorescence uptake was barely visible in non-tumor cells (not shown). At early time points, LS838 is largely in the lysosomes, but it translocates to the cytosol in a time-dependent manner. Western blot, proteomic analysis, and blocking

studies in cells with diverse inhibitors of albumin endocytosis point to albumin-mediated endocytosis (data not shown). This mechanism of uptake was further supported *in vitro* and *in vivo* by co-incubation of Alexa 680 labeled bovine serum albumin (BSA) with LS838, which showed colocalization of the two fluorophores in cancer cells at early time points, followed by divergence of the signals, indicating retention of LS838 and efflux of the Alexa 680.

Example 3. LS838 selectively detects PDAC and precursor pancreatic intraepithelial neoplasia (PanIN) cells with high accuracy, and distinguishes these lesions from acute and chronic pancreatitis.

[0120] LS838, genetically engineered KPC mice (p48-CRE/LSL-Kras^{G12D}/p53^{Flox/+}; activate KRAS and inactivate p53), and noninvasive fluorescence molecular tomography (FMT) were used to delineate pancreatic lesions. A high-speed FMT system was developed for quantitative imaging of molecular processes *in vivo*. Using quantitative FMT, results show that intravenous (i.v.) administration of LS838 allowed the detection of PDAC/PanIN lesions, which can be differentiated from acute and chronic pancreatitis (**Fig. 9A-E**). Reconstructed images 10 mm from the dorsal sides are shown for the different mice models. NIR fluorescence in the pancreatic region was drastically lower in both pancreatitis and Matrigel control models compared to that in PDAC and early neoplasia. The pancreas from different animal models was excised and imaged *ex vivo*. For the orthotopically implanted PDAC, fluorescence in the pancreas head was seen within a week of implantation, and the intensity increased by a factor of two within 2 weeks post-implant (**FIG. 9A-B**). Unlike the focal orthotopic tumor in the pancreas, the spontaneous model (PanIN/KPC) showed diffuse fluorescence in the pancreas (**FIG. 9C**). This is not surprising because this model readily transforms the majority of the pancreas into fully malignant tissue by the time the animals are 3-4 months of age. In contrast, LS838 fluorescence was barely detectable in the chronic and acute pancreatitis models, as well as in the control orthotopic Matrigel implant (**FIG. 9D-E**).

[0121] Histologic validation of the presence of pancreatic tumor cells in the LS838 fluorescent regions was performed by comparing the NIR fluorescence in tissue

sections with hematoxylin and eosin (H&E) stained tissues (**FIG. 10**). The results confirmed the accurate detection of PDAC and precursor PDAC cells by LS838. Importantly, *ex vivo* tissue analysis of the chronic pancreatitis model showed significant fluorescence in a few areas of the pancreas which appears to correlate with PanIN-3 lesions that are known to progress to PDAC (**FIG. 10D**).

[0122] To determine the cell type(s) that take up LS838 *in vivo*, mice bearing established PDAC tumors stably transfected with a KP mCher⁺ fluorescence reporter were treated with LS838. Tumor tissue was harvested and dissociated by collagenase digestion to obtain single-cell suspensions for analysis by flow cytometry. Using mCherry to identify PDAC tumor cells, it was found that uptake by these malignant cells was 100 to 1,000 times higher than in other cell types (**FIG. 11A**). Modest uptake was also seen in tumor infiltrating macrophages, but these macrophages were also labeled with mCherry derived from the tumor cells themselves, suggesting this fluorescence was a product of phagocytosis of PDAC cells (**FIG. 11B**).

Example 4. NIR fluorescence laparoscopy using LS838 accurately detects PDAC and premalignant neoplasia and delineates these lesions from pancreatitis.

[0123] For laparoscopy, the Karl Storz rigid, straightforward telescope was modified so that it was capable of imaging in both visible (color; RGB) and NIR fluorescence modes. In the RGB mode, an endoscopic cold light fountain (Xenon Nova 175) with adjustable light intensity was used as the light source. The light cable was coupled to the illumination channel on a 0-degree Hopkins straightforward telescope. Images were captured by an NIR-sensitive CCD camera (Fluorivivo) with an RGB Bayer filter. A telescope coupler with a focus ring focused the CCD camera on the image formation plane at the back of the telescope. In the fluorescence mode, the cold light fountain source was replaced by a 780-nm excitation laser source with an incident light of 5 mW reaching the tissue. An 800-nm long-pass emission filter was placed behind the telescope in a slit of a custom-made adapter designed to couple the macro lens to the telescope. Images were captured using the Fluorivivo CCD camera with a quantum efficiency of 30% at 800 nm. Using this setup, the four murine models were investigated to provide validity to this approach. A midline incision was made to expose the

peritoneal cavity of the mouse and the cavity was imaged at 90 degrees with a fixed distance of 4 cm. As shown in **FIG. 12**, the pancreas can be readily visualized by the conventional color image, but the LS838 NIR fluorescence selectively highlighted tumor tissues. Fluorescence in uninvolved pancreas and acute pancreatitis was below the detection limit of our device. Particularly exciting is the differential fluorescence in the involved and uninvolved pancreas, suggesting the ability to use this method to guide needle biopsy, assess surgical margins, and detect occult metastatic disease in real time. The detection sensitivity of the endoscope is low relative to a commercial system recently developed by Karl Storz, the Image1H3-Z NIR fluorescence endoscope.

[0124] The encouraging results support the development of LS838 for detection of pancreatic lesions. In addition to delivering optical imaging agents and potentially drugs to PDAC lesions, the presence of tyrosine in LS838 structure allows for future radiolabeling of the molecule for noninvasive nuclear imaging applications.

Example 5. Synthesize and characterize LS838 analogues to understand the mechanism of LS838 uptake and retention in PDAC relative to uninvolved pancreatic cells.

[0125] The unprecedented retention of LS838 in pancreatic cells undergoing oncogenic transformation and PDAC cells requires further understanding of the retention mechanism. This is more so because the compound is not trapped in cells undergoing inflammation – as in acute or wound healing models. When some cells lit up in the chronic pancreatitis model, these cells were in the late stage of PanIN, thereby identifying the onset of PDAC. These studies were carried out in different animal models of pancreatic cancer with similar outcomes. The objectives of this study are to determine the trapping mechanism of LS838 in PDAC/PanIN tissues, improve the biodistribution profile of the compound so that fluorescence in adjacent organs and uninvolved pancreas will be minimal within an hour post injection of the agent, and formulate the compound for ease of administration and reduced uptake in non-target tissues.

[0126] Determine the effects of dyes and peptides on the uptake and retention of LS838 in PDAC/PanIN. Data strongly suggests albumin-mediated facilitated transport of LS838 into PDAC/PanIN-2/3 cells.

[0127] *Dye effect:* The hypothesis is that the binding of cypate to albumin facilitates the internalization of LS838 in PDAC/PanIN lesions. In a previous study, the binding of some carbocyanine dyes to albumin was compared. It was found that ICG and cypate possessed the strongest binding constant (K) to albumin, with values of $554,000 \text{ M}^{-1}$ and $556,000 \text{ M}^{-1}$ respectively, followed by LS276 ($135,000 \text{ M}^{-1}$); LS288 bound the weakest ($12,100 \text{ M}^{-1}$). The structures of LS288 and LS276 are shown in **FIG. 7B-C**. It was also shown that conjugation of peptides to the dyes decreases the K by a factor of ~ 10 . Using the same method, we determined the K value of LS838 to be $86,000 \text{ M}^{-1}$.

[0128] By conjugating dyes with disparate albumin-binding constants to the same peptide sequence, this will validate the role of the dyes in the uptake, and possible retention, of the molecular probes. This will allow for the design of alternative compounds to LS838, if needed. The functionalizable dyes (Cypate, LS288, and LS276) will be conjugated to the LS838 peptide (denoted as GRD peptide; note that this is not the conventional RGD peptide).

[0129] For cell studies, each of the LS288 and LS276 conjugates of the RGD peptides ($1 \mu\text{M}$) will be incubated with the established PDAC cells. Flow cytometry will be used to quantitatively determine the rate of internalization by calculating the slope of a plot of fluorescence vs. time in 1000 cells (0.5, 1, 2, 4, and 8 h post incubation). Intracellular retention will be determined by washing the cells after 24 h incubation (when cytosolic fluorescence can be seen in cells treated with LS838). The cells will be incubated in fresh medium and the efflux rate (rate of fluorescence decrease with time at 0.5, 1, 2, 4, and 8 h post incubation after washing) will be determined by NIR flow cytometry using 1000 cells. The results will be compared with that of LS838 to establish the role of dyes in PDAC uptake and retention. By using a rate constant to report uptake and retention, bias in data analysis caused differences in the brightness (quantum yield $\times$ molar absorptivity) of each dye will be avoided. It is

expected that the rate of internalization will correlate with the dye-albumin binding affinity, but that the retention rate will be similar if this parameter is mediated by the peptide (the same peptide is used) or aggresome (cell type effect; see below).

Statistically significant differences in retention rates will indicate that the dyes also play a role in this parameter. This information will be used to optimize the molecular probe by combining features that favor fast internalization with those that promote high intracellular retention. If these parameters conflict, high retention rate over internalization rate will be chosen to achieve high fluorescence in tumor cells compared to non-tumor cells.

[0130] *Peptide effect:* Data shows that cypate dye alone internalizes in tumor cells, but then rapidly effluxes when the cells are transferred to a new culture medium. A similar trend is observed *in vivo*, where preferential uptake in PDAC relative to uninvolved pancreas is not observed. It was also found that replacement of D-cysteine with L-cysteine abrogated retention of LS838 in PDAC. Similarly, use of other cypate-labeled disulfide cyclic octapeptides, such as octreotate, did not achieve statistically significant contrast between PDAC and the surrounding pancreas. These results suggest that the peptide plays an important role in the selective retention of LS838 in PDAC tissue. Permutation of the arginine-aspartic acid sequence results in a loss of PDAC-selective uptake. It has been shown that cypate binds to the hydrophobic pockets of albumin, irrespective of the source (human, murine, or bovine). The reduction in binding constant upon conjugation with peptides suggests that the peptides perturb the albumin-dye interaction. Thus, the primary goal of this study is to determine the role of the peptide in tumor cell internalization or retention. Based on preliminary data, this question can be addressed by preparing the following cypate-labeled peptides (underlined amino acids are changes made to LS838): cyclo(DCys-Gly-Arg-Asp-Ser-Pro-DCys)-Tyr-OH (SEQ ID NO:11); cyclo(Cys-Gly-Arg-Asp-Ser-Pro-Cys)-Tyr-OH (SEQ ID NO:12); cyclo(Cys-Arg-Gly-Asp-Ser-Pro-Cys)-Tyr-OH (SEQ ID NO:13); cyclo(DCys-Arg-Gly-Asp-Ser-Pro-Cys)-Tyr-OH (SEQ ID NO:14); and cyclo(DCys-Arg-Gly-Asp-Ser-Pro-DCys)-Tyr-OH (SEQ ID NO:15). The peptides will be prepared and labeled with cypate at the N-terminus. The albumin binding constants will be determined as

described above to assess the perturbation of the dye-albumin interaction, which could affect cell uptake. Using the internalization and retention rates described above, whether loss of contrast is due to poor uptake or poor retention in tumors will be determined. The data will allow the determination if loss of PDAC selectivity is caused by a poor internalization rate due to perturbation of the dye-albumin binding. In that case, spacers will be incorporated, such as various lengths of polyethyleneglycols, between the dye and the peptide to minimize this perturbation. If the loss of uptake is caused by a high efflux rate, the intracellular target of LS838 will be determined, as described below.

[0131] Determine the mechanism of selective trapping of LS838 in pancreatic lesions. Data indicate that LS838 rapidly internalizes in the pancreas within 1 h post injection, but then remains primarily in the tumor cells (up to 100 h) relative to non-tumor cells. Cellular studies suggest that LS838 undergoes time-dependent translocation from the lysosome to the cytosol in cancer cells, but remains in the lysosomes in non-tumor cells before efflux from the cells. This trafficking profile was not observed with the control probes nor with the cypate dye alone, which showed transient retention in tumor cells before efflux in a similar manner as non-tumor cells. Proteomics studies show the association of LS838 with a protein determined to possess a significant albumin peptide sequence. Western blot analysis indicated the association of LS838 fluorescence with α -actinin-4 protein and a fatty acid receptor protein. Therefore, to understand why LS838 is able to be selectively retained by pancreatic tumors, two hypotheses will be tested.

[0132] *Hypothesis 1: A dysfunction in the clearance of LS838 from cancer cells will decrease the efflux rate, thereby increasing the selective and longitudinal retention of the imaging agent in cancer cells.* Data show that a significant amount of LS838 is retained in the lysosomes for over 24 h in tumor cells, but lies in a perinuclear inclusion in normal cells. Aggresomes are microtubule-catalyzed aggregates of lysosomes that are used to degrade particularly stable and aggregated proteins. It is postulated that LS838 forms stable aggregates with albumin which may require the formation of aggresomes for their removal. It will be evaluated if tumors have a lower

ability to form aggresomes because of microtubule dysfunction, leading to prolonged retention in tumor cells compared to normal cells. If LS838 induces aggresome formation in tumor cells, but not fibroblasts will first be determined. Using the ProteoStat Aggresome detection kit, an assay for aggresome formation will be performed using literature methods. It will also be determined if aggresomes form *in vivo* using a literature method. LS838 will be injected into mice and they will be euthanized 24 h post injection. Aggresome formation will be determined by staining muscle, skin, and tumors for anti-ubiquitin protein. Aggresome formation will be confirmed by TEM visualization of perinuclear inclusions. Control studies with LS838N and cypate will be conducted. The hypothesis will be positively tested by demonstrating that the rate of aggresome formation in PDAC cells is slower than in non-tumor pancreatic cells, with a $p \leq 0.05$.

[0133] *Hypothesis 2: Longitudinal retention of LS838 in cancer cells is mediated by trans-thiolation with intracellular biomolecules via the stable (unnatural) D-cysteine amino acid linked to cypate.* Data show that LS838 can be internalized in tumors, but rapidly clears before attaining significant tumor-to-background contrast if any one of the D-cysteine, lysine, or aspartic acid residues is replaced with different amino acids. In one set of proteomic data, which was confirmed by Western blot, α -actinin-4 protein (~104 kDa) and fatty acid receptor proteins (~37 kDa; 15 kDa) were identified as potential intracellular targets for LS838. It is expected that under the high reducing intracellular environment of cancer cells, the reduced thiol groups of α -actinin-4 can cross-link with the thiol from the D-cysteine of LS838 peptide. It is likely that the hydrophobic cypate dye interacts with the fatty acid receptor, providing sufficient resident time in cells for trans-thiolation to occur.

[0134] A proteomic approach will be utilized to identify the intracellular biomolecules that bind strongly with LS838 in cancer cells. The cells will be trypsinized and lysed, and then the lysed protein will be fractionated and analyzed for NIR fluorescence. Compared to non-treated cell protein distributions along the anionic exchange column, bound proteins will be slightly shifted in their anionic charge in the treated cells. The combination of anionic shift and overlap in NIR fluorescence will help identify candidate proteins for binding affinity. Proteins shifted in anionic charge by

LS838, but not shifted by cypate, will be used to identify candidates for proteins involved in retention. The identified proteins that match the above criteria will then be analyzed for binding affinity using a competitive binding assay. To normalize the data, the binding affinity will be expressed as apparent binding constants. Confirmation of a protein's retention effect will be determined by pre-incubating tumor cells with competitive inhibitors of the protein. It is expected that there will be a significant loss of LS838 retention in tumor cells, which will be expressed as percent inhibition. Additionally, siRNA may be used to knockout/down the target protein to establish functional validation of the retention mechanism. All experiments will be conducted with cypate and dye-labeled peptide derivatives.

[0135] Determination of the cytotoxicity of LS838: To assess the cytotoxicity of LS838, commercially available normal (non-tumor) human primary pancreatic cells (T0199 from ABM), and non-tumor cell lines from the two major excretion organs, the kidneys (HK-2) and liver (Fa2N-4) will be used. Primary pancreatic adenocarcinoma (BXPC3) or other cancer cell lines will also be used to assess potential toxicity in tumors. The cytotoxicity tests will be performed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Based on a previous study, it is expected that the molecular probes will have a lethal dose (LD50) >100 μ M in cells, which is orders of magnitude above what is expected to be used for *in vivo* imaging. The NIR confocal microscope (FV1000, Olympus) will be used to assess the intracellular distribution of the probes. All the methods for these assays are standard.

[0136] LS838 currently clears through the liver. While this is not a problem in the proposed laparoscopy, the excretion route to the kidneys can be altered by conjugating positively charged moieties ($-^+NMe_3$) or nonionic groups (e.g. PEG) to the free carboxylic acid group of cypate. It was found that conjugation of small molecules (<500 Da) does not affect LS838 binding to tumors. In the unlikely event that the cyanine dyes are photo-unstable, they will be replaced with NIR pyrrolopyrrole cyanine dyes (PPCy), which are highly photostable and have exceptionally high fluorescence quantum yields (up to 80%).

Example 6. Determine the role of Kras-induced oncogenic transformation on the selective uptake and retention of LS838 by PDAC/PanIN-2/3.

[0137] The detection and differentiation of advanced lesions (PanIN-3 and PDAC) from more indolent disease (PanIN-1/2) and ductal hyperplasia/metaplasia would inform more aggressive treatment decisions. It will also be determined if activated Kras-mediated oncogenic transformation of non-malignant pancreatic cells to PDAC drives specific uptake and retention of LS838 in malignant cells. Information on *in vivo* imaging methods is summarized in **Example 7**.

[0138] Determine the specificity and sensitivity of LS838 in detecting early PanIN and PDAC lesions: Data show that LS838 identifies advanced PDAC tumors and microscopic PanIN3⁺ lesions. However, it is not yet known the earliest stage of disease that LS838 can differentiate from non-tumor tissue. This is an important clinical question, as the ability to non-invasively detect early PanIN lesions would allow for increased surveillance in these individuals without further major surgical procedures. In particular, the detection and differentiation of advanced lesions such as PanIN3 and PDAC from more indolent disease, i.e. PanIN1-2 and ductal hyperplasia/metaplasia, would inform more aggressive treatment decisions. Thus, the disease stages detected by LS838 will be defined.

[0139] Mouse models for PDAC, indolent and aggressive PanIN, and chronic pancreatitis will be used in these studies. While LS838 is visible in histologic sections, this signal is significantly reduced during tissue processing for standard immunohistochemistry, making co-localization with cell type specific markers in tissue sections difficult. To overcome this barrier, KPC-Y mice (p48-CRE/LSL-Kras^{G12D}/p53^{Flox/+}; YFP+) will be employed. This model will allow the specific detection of LS838⁺/YFP⁺ transformed cells even in very early PanIN lesions. Using KPC-Y mice aged to 2, 3 or 4 months of age, LS838 signal intensity will be assessed using a combination of non-invasive quantitative FMT (**FIG. 9**) *in vivo* and high resolution *ex vivo* imaging of the pancreas to assess the distribution of LS838 in these early stage tissues. Using YFP-positivity to identify transformed cells (Kras^{G12D}+ cells), the spectrum of LS838 retention in premalignant and malignant cells will be assessed by

flow cytometry. Additionally, co-localization of LS838 and YFP+ will be analyzed in frozen tissue sections, and fluorescence intensity in individual PanIN-1, -2, -3 and PDAC lesions (using MetaMorf) will be determined and compared to histology as the Gold Standard. Together, these data will determine at what stage of PDAC progression LS838 is selectively retained in pre-malignant cells and when disease is discernable by non-invasive imaging.

[0140] To determine if LS838 selective uptake and retention is increased as Kras-transformed pancreas cells move from indolent to progressive disease, pancreatitis-induced disease progression in p48-CRE/LSL-Kras^{G12D}/LSL-YFP mice will be analyzed. Without the loss of p53, the majority of these mice will have stable early stage PanIN disease that will not progress to PDAC. However in the context of Caerulein induced pancreatitis, disease will progress stochastically. LS838 signal intensity will be assessed using a combination of non-invasive quantitative FMT and *ex vivo* imaging of the pancreas in 2-month-old p48-CRE/LSL-Kras^{G12D}/LSL-YFP and control (p48-negative) mice treated with Caerulein (50mg/kg x3/week) or vehicle for 1 and 3 months. Pancreatic tissues from these mice will be analyzed by flow cytometry and immunofluorescence microscopy for LS838 in YFP+ cells, as described above. Nine mice will be analyzed for each group at each stage. These experiments will determine if LS838 selective uptake and retention is increased as transformed cells move from indolent to progressive disease during pancreatitis.

[0141] Determine if Kras-induced oncogenic transformation leads to LS838 selective uptake and retention: Activating mutations in Kras are present in >90% of pancreatic adenocarcinomas, but are often also found in indolent hyperplastic or dysplastic pancreas tissue. To determine if oncogenic transformation by activated Kras drives malignant cell specific uptake and retention of LS838, the effects of Kras mutation on LS838 retention *in vitro* will be assessed. To accomplish this goal, normal pancreatic ductal epithelial cells from LSL-Kras^{G12D} mice will be isolated using standard protocols. To rearrange wild-type Kras into oncogenic Kras^{G12D}, isolated pancreatic epithelial cells will be infected with Adenovirus encoding CRE and GFP or GFP only (as control). About 48 h after infection, GFP+ cells will be FACS sorted for

use. The uptake and retention of LS838 will be assessed in Kras^{G12D+} and negative pancreatic epithelial cells. The data will be compared to cell lines isolated from late stage KPC mice. Both imaging and flow cytometry will be used to determine uptake and retention every 3 h for 36 h. These data are important because they will determine if oncogenetic transformation alone is sufficient to induce specific LS838 uptake and retention. Direct correlation will provide an indirect method to report the expression level of Kras necessary to stimulate oncogenic processes in cells.

[0142] The sensitivity and specificity for detecting cancer and neoplasia will be analyzed. The relative contrast of tumor to surrounding healthy tissue will be measured for each tumor to determine specificity of the molecular probe and sensitivity of the endoscope. The relative contrast of tumor to surrounding healthy tissue will also be measured for each tumor to determine specificity and sensitivity of the molecular probe. The statistical significance of tumor-specific contrast will be analyzed using Student's t-test with $\alpha = 0.05$ and $P < 0.05$ considered significant. It is expected that LS838 will produce high sensitivity (>90%) and specificity (>90%) for PDAC and will distinguish these lesions from pancreatitis.

[0143] Kras expression may not correlate with the uptake of LS838. In that case, the results will indicate the earliest PanIN cell phenotype detected by LS838. An alternative hypothesis is that the uptake of LS838 may correlate with an increase in the proliferation state of transforming pancreatic cells. In this case, the relationship between LS838 uptake and the metabolic status of tumors will be determined by correlating LS838 uptake with uptake of [¹⁸F]-2-fluoro-deoxyglucose, a reporter of glucose metabolism, using PET.

Example 7. Determine the accuracy of detecting PDAC/PanIN in human patient-derived PDAC and mouse models that recapitulate different stages of PDAC.

[0144] Genetic models of PDAC progression in mice as well as acute and chronic pancreatitis models will be used for this study. This model will be used to assess the translation of findings using LS838 in mouse to human models of PDAC. It is expected that LS838 will accurately characterize pancreatic lesions and potentially identify occult metastatic disease at the time of surgery.

[0145] Determination of the accuracy of imaging PDAC and chronic pancreatitis mouse models using LS838: PDAC, premalignant neoplasia, and chronic pancreatitis models will be employed in this study. The optimal injection dose to obtain the best tumor/normal tissue contrast will be determined. It is expected that tumors <0.5 mm diameter will be detected. LS838 and a nonspecific control [LS838N: Cypate-cyclo(Tyr-Gly-Arg-Asp-Ser-Pro-D-Cys)-Lys-NH₂] (SEQ ID NO:16), will be administered intravenously (i.v.) using 10 nmol of the molecular probe dissolved in PBS containing 2% mouse serum albumin per 25 g mouse (this concentration was used in preliminary studies, but will be varied to optimize dosage: 1, 5, 10, and 20 nmol/25 g mouse). We will use KPC mice at 3 different stages of PDAC development: (a) PanIN-1/2 – 1-2 months; PanIN-2/3 – 2-3 months; and advanced PDAC - >4 months. The animals will first be imaged longitudinally and noninvasively with the fluorescence molecular tomography (FMT) system. A side view will be used to image the pancreas using the LICOR planar NIR imaging system. Non-invasive imaging will be performed at 0.5, 1, 4, 8, 18, 24, 48, 72, and 96 h after injection to determine the longitudinal retention of the agent in PDAC. Imaging will be stopped when fluorescence in the tumor and adjacent pancreas are similar or indistinguishable by the imaging systems. Noninvasive FMT will first be used to image the mice at 1, 4, 8, 24, and 48 h post injection before exposing the pancreas by skin incision and gentle retraction with blunt dissection. Using a Karl Storz Image1H3-Z color-NIR fluorescence endoscope, endoscopy of the pancreas will be simulated to determine the detection and delineation of pancreatic lesions. A cholangiopancreatroscope will be used to identify areas of high fluorescence intensity (**FIG. 12**), a feature that is useful for image-guided surgery. Acquired images will be processed via the controlling software to measure the fluorescence intensity from areas identified as tumor or normal. These values will be used to establish thresholds for tumor detection. Receiver Operating Characteristic curve (ROC) analysis will be used to determine the threshold fluorescence contrast for detecting PDAC cells. Based on preliminary data, it is expected that >20% NIR fluorescence in PDAC cells relative to the intensity in uninvolved pancreas will serve as an internal control to delineate this lesion. Suspicious areas, including highly fluorescent and non-fluorescent pancreatic tissues

with and without obvious tumors, will be excised and snap-frozen for cryosectioning. Frozen tissues will be examined by fluorescence microscopy and data will be referenced to histopathology as the gold standard.

[0146] Determination of biodistribution of the molecular probes: A simple method for fluorescence-based biodistribution studies will be used. Briefly, after completing endoscopy at the time points indicated above, mice will be euthanized by cervical dislocation. Aliquots of blood and all major organs (pancreas, heart, kidney, lung, spleen, stomach, intestine, muscle, kidney, adrenals, liver, skin, bone, and brain) will be harvested, washed with PBS, dried and weighed. The organs and a small glass vial containing a known volume of blood will be imaged with the LICOR NIR planar imaging system. Using a reference tissue model (a known concentration of the molecular probe is injected into similar tissues from untreated mice) as fiducial markers, the relative uptake of the probe in various tissues will be determined. This approach will allow normalization of differences in the optical properties of tissue. The imaging system has software that calculates the mean fluorescence count plus or minus standard error of mean within any chosen area on the fluorescence image. The relative uptake as percent fluorescence per gram tissue relative to the pancreas will be reported. Organs will be processed for histopathology. Tissue classification as PDAC/PanIN vs. pancreatitis by LS838 fluorescence will be compared with histopathology to determine the diagnostic accuracy of our approach.

[0147] Determination of the detection of microscopic PDAC using LS838-aided NIR laparoscopy to improve the diagnostic yield of pancreatic biopsies: A potential advantage of using LS838 is the potential to detect microscopic PDAC cells when visual inspection or white light is not capable of identifying these lesions. LS838-aided biopsy of the pancreas will be simulated by using early stage PDAC in the KPC mouse model, when a significant number of cells are in the PanIN-2/3 stage, as reported by the KPC-Y model. Based on the point of highest contrast between PDAC and uninvolved pancreas as determined above, the NIR laparoscope will be utilized to identify PDAC cells, (as described above) to simulate tissue biopsy (n = 9). Resected tissue will be analyzed and scored by histopathology. It is expected that the NIR

fluorescence-aided tissue sample collection will improve the yield of pancreatic biopsy with accuracy approaching 100% relative to conventional biopsy.

[0148] Determination of the distribution and uptake of new molecular probes synthesized in **Example 5** by PDAC: New compounds will be prepared as described in **Example 5**. When they become available, the optimized imaging time point and injected dose of LS838 will be used to determine the *in vivo* biodistribution and retention of the new compounds. Specifically, these parameters will be determined for LS276 and LS288 dye-labeled analogues of LS838 (role of dye in retention), as well as for two molecular probes from the modified peptide analogues of LS838 based on the *in vitro* results (role of peptide in retention). Thus, in addition to LS838, four new molecular probes will be imaged and the data compared with those of LS838. The animals will be imaged at 1, 4, and 24 h post injection before excising their organs for biodistribution studies, as described above. It is expected that the *in vivo* results will confirm the findings of the cell studies. Together, the information can be used to optimize the structural features of LS838 for rapid uptake and retention in tumors, with the highest contrast between tumor and uninvolved pancreas within 4 h post injection.

[0149] Determination of the potential use of LS838 fluorescence to detect PDAC from human patients: The PCBC SCC has established >30 PDAC lines from resected pancreatic cancer specimens and has developed heterotopic and orthotopic PDXs (Patient Derived Xenografts) in NOD-SCID mice. PDXs and their derivatives are attractive model systems. PDXs are typically generated by subcutaneous implantation of human tumors directly from patients (via biopsy or surgery) into immunodeficient mice (generally mice based on the non-obese diabetic – severe combined immunodeficient, or NOD-SCID, background). Tumors can then be passaged serially in NOD-SCID mice via heterotopic (subcutaneously) or orthotopic (into the originating organ) transplantation and used as a model system for various aspects of cancer research. Some advantages of PDXs and their derivatives include: (1) their human tumor origin, (2) the ability to correlate findings in PDX models with the clinical outcomes of the patients from which the PDXs and derivatives were established, and

(3) the potential to use PDXs to develop translational oncology model systems to study, for example, metastatic biology and to evaluate novel therapeutics.

[0150] 27 mice each of heterotopic and orthotopic PDX bearing NOD-SCID mice from the PCBC SCC will be obtained for this study. The accuracy of identifying PDX in both models (n=9 for each model) will be determined as described above using LS838 and the non-PDAX specific control compound, LS838N. Imaging will commence when tumor size reaches ≥ 5 mm (determined by caliper measurement for the heterotopic model and established growth rate for the orthotopic model). After administering the imaging agent (10 nmol/20 g mouse), longitudinal FMT imaging will be performed at 0.5, 1, 4, 8, 18, and 24 h. At 24 h, laparoscopy will be conducted as described above and the animal will be euthanized for biodistribution analysis and histopathology. Because LS838 is intended to be used in patients scheduled to undergo surgery or exploratory laparoscopy, another set of 9 mice will be used for laparoscopy and biodistribution/histology to simulate clinical settings. A similar study will be conducted with LS838N. Data will be processed and analyzed as described above. It is expected that LS838 will have similar accuracy in detecting PDX as mouse PDAC. The results will provide preclinical data to determine potential translation of the compound in humans. The sensitivity and specificity (accuracy) will be calculated as well as positive and negative predictive values of PDAC/PanIN detection using LS838 based on the histology data.

[0151] Data strongly supports the feasibility of using LS838 to delineate PDAC and premalignant neoplasia from pancreatitis. LS838 fluorescence may not correlate with presence of disease determined by histology. In this case, YFP fluorescence from the KPC-YFP model will be used to determine early PDAC lesions. The quantitative accuracy of FMT system and NIR laparoscope may be affected by differences in tissue optical properties. Since LS838 has a tyrosine residue, the compound could be radiolabeled with ^{125}I for SPECT imaging and biodistribution studies (note that thyroid uptake of iodine will be considered in data analysis).

[0152] The methodologies described herein result in the following, non-limiting innovations:

[0153] 1: A novel imaging agent, LS838, has been developed which detects PDAC and PanIN 2/3 lesions with high accuracy. A new uptake mechanism involves facilitated uptake mediated by the energy needs of these metabolically active cells, followed by intracellular trapping under the highly reducing environment of these cells.

[0154] 2: Application of LS838 to distinguish PDAC and transforming PanIN from chronic pancreatitis with high accuracy represents significant progress in the management of this disease.

[0155] 3: The NIR fluorescence allows detection of microscopic lesions, which are not visible with current clinical imaging techniques. In addition, the fluorescence of LS838 is brighter than LS301, allowing us to use smaller amounts of the material to achieve the same uptake kinetics as LS301.

[0156] 4: LS838 can be radiolabeled at a tyrosine residue for combined intravital fluorescence microscopy and noninvasive PDAC imaging. Unlike LS301, the tyrosine within LS838 allows the labeling of LS838 with radionuclides such as fluorine-18, iodine-123, I-124, I-125, and I-131. This allows the imaging of cancer in the human body noninvasively using nuclear imaging methods, and then the use of optical methods to guide tissue biopsy, surgery, and assessment of surgical margins. This combination of noninvasive and invasive methods opens new opportunity for the use of this compound. The placement of the tyrosine is important in the retention of LS838 in tumors.

[0157] 5: Conjugation of chemotherapeutics to the free carboxylic acid group of LS838 will allow highly selective treatment of PDAC with minimal off-target effect on the healthy pancreatic cells.

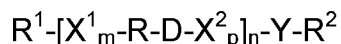
[0158] 6: LS838 selectively remains in diverse tumors without significant loss of fluorescence over time.

[0159] 7: The high specificity of LS838 for cancer cells in the presence of healthy white blood cells creates a unique opportunity to use the same agent for detecting circulating tumor cells (CTCs) without resorting to additional tagging steps with multiple expensive antibodies.

CLAIMS

What is claimed is:

1. A compound having the formula:



wherein:

R^1 is a carbocyanine dye or derivative thereof;

R^2 is selected from the group consisting of a treatment agent, hydrogen, hydroxyl, NH_2 , hydrocarbyl, and substituted hydrocarbyl;

$X^1_m-R-D-X^2_p$ together form a linear or cyclic peptide;

X^1 and X^2 are independently selected from any amino acid residue;

m is an integer from 1 to about 10;

n is an integer from 1 to about 10;

p is an integer from 1 to about 10; and

a dash (-) represent a covalent bond.

2. The compound of claim 1, wherein X^1 and X^2 are independently selected from the group consisting of G, S, N, Q, D, E, K, R, T, C, H, and P.
3. The compound of any of the preceding claims, wherein R^2 is a treatment agent selected from the group consisting of a drug and a hormone.
4. The compound of any of the preceding claims, wherein R^2 is a hydroxyl.
5. The compound of any of the preceding claims, further comprising a polyethylene glycol moiety conjugated to the carbocyanine dye.
6. The compound of any of claim 1-4, further comprising a nanoparticle, small organic molecule, peptide, organometallic, metal chelate, protein, drug, antibiotic, or carbohydrate conjugated to the carbocyanine dye.
7. The compound of claim 6, wherein the drug is a chemotherapeutic agent.

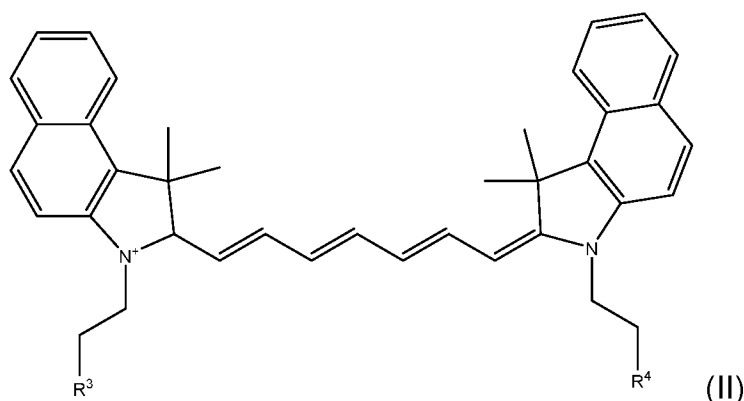
8. The compound of any of claim 1-4, further comprising a radio-halogen conjugated to the carbocyanine dye.
9. The compound of any of the preceding claims, wherein the Y is radiolabeled.
10. The compound of claim 9, wherein the radiolabel is fluorine or iodine radionuclide.
11. The compound of any of the preceding claims, further comprising a linker, L^1 , that conjugates R^1 to X^1 .
12. The compound of any of the preceding claims, further comprising a linker, L^2 , that conjugates R^2 to Y.
13. The compound of claim 11 or 12, wherein the linker is selected from the group consisting of an amino acid residue, a hydrocarbyl and a substituted hydrocarbyl.
14. The compound of any of the preceding claims, wherein:
 - n is from 1 to 5;
 - m is from 1 to 3;
 - p is from 1 to 3; and
 - X^1 and X^2 are selected from the group consisting of C, G, S, P, C, N, Q, D, E, K, R, T and H.
15. The compound of any of the preceding claims, wherein the cyclic peptide has from about 4 amino acid residues to about 10 amino acid residues.
16. The compound of any of the preceding claims, wherein:
 - X^1 is C-G;
 - m is 1;
 - X^2 is S-P-C;
 - p is 1; and
 - n is 1.

17. The compound of claim 16, wherein R^2 is a hydroxyl.
18. The compound of any of the preceding claims, wherein one or more of the C residues are $_D$ Cys.
19. The compound of any of the preceding claims, wherein a C residue is linked to R^1 and wherein the C residue is $_D$ Cys.
20. The compound of any of the preceding claims, wherein $[X^1_m-R-D-X^2_p]_n-Y$ is selected from the group consisting of:

SEQ ID NO:	$[X^1_m-R-D-X^2_p]_n-Y$
8	$[_D\text{Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-Lys-Tyr}$
9	$[_D\text{Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-Tyr}$
10	$[_D\text{Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-Tyr-Lys}$
11	$[_D\text{Cys-Gly-Arg-Asp-Ser-Pro-}_D\text{Cys}]\text{-Tyr}$
12	$[\text{Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-Tyr}$
17	$\text{Gly-}[_D\text{Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-Tyr}$
18	$\text{Gly-}[_D\text{Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-}_D\text{Tyr}$
19	$\text{Gly-}[_D\text{Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-}_D\text{Tyr-Lys}$
20	$\text{Gly-}[\text{Cys-Gly-Arg-Asp-Ser-Pro-Cys}]\text{-}_D\text{Tyr-Lys}$
21	$\text{Gly-}[_D\text{Cys-Gly-Arg-Asp-Ser-Pro-}_D\text{Cys}]\text{-Tyr}$
22	$\text{Gly-}[\text{Cys-Gly-Arg-Asp-Ser-Pro-}_D\text{Cys}]\text{-Tyr}$
23	$\text{Gly-}[_D\text{Cys-Gly-Arg-Asp-Ser-Pro-}_D\text{Cys}]\text{-}_D\text{Tyr}$

21. The compound of any of the preceding claims, wherein the carbocyanine dye is selected from the group consisting of Cypate (cypate 4), LS288, LS798, LS276, LS843, Cypate 3, and Cypate 2.
22. The compound of claim 21, wherein the carbocyanine dye comprises a nanoparticle, a small organic molecule, a peptide, an organometallic, a metal chelate, a protein, a drug, an antibiotic, or a carbohydrate.

23. A compound having formula (II):



wherein:

R^3 is selected from the group consisting of nanoparticles, small organic molecules, peptides, organometallics, metal chelates, proteins, drugs, antibiotics, and carbohydrates; and

R^4 is $[X^1_m-R-D-X^2_p]_n-Y-R^2$, wherein:

R^2 is independently selected from the group consisting of a hydrogen, hydroxyl, NH_2 , hydrocarbyl, and substituted hydrocarbyl;

X^1 and X^2 are independently selected from any amino acid residue;

$X^1_m-R-D-X^2_p$ together form a linear or cyclic peptide;

m is an integer from 1 to about 10;

n is an integer from 1 to about 10;

p is an integer from 1 to about 10; and

a dash (-) represent a covalent bond.

24. The compound of claim 23, wherein n is from 1 to 5; m is from 1 to 3; p is from 1 to 3; and X^1 and X^2 are selected from the group consisting of C, G, S, P, C, N, Q, D, E, K, R, T and H.

25. The compound of claim 23, wherein X^1 is CG; m is 1; X^2 is SPC; p is 1; and n is 1.

26. The compound of claim 25, wherein the C of X^1 is D-cysteine and/or the C of X^2 is D-cysteine.

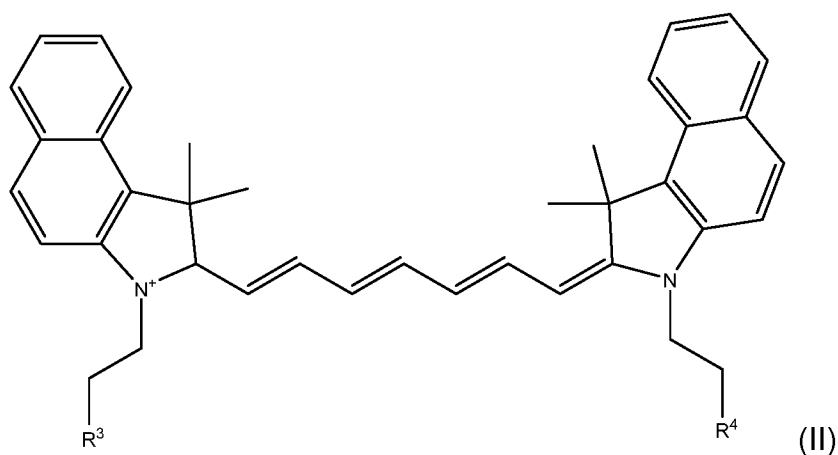
27. The compound of any of claims 23-26, wherein the Y is D-tyrosine.
28. The compound of claim 23, wherein $X^1_m\text{-R-D-X}^2_p$ is a cyclic peptide have the sequence [CGRDSPC] (SEQ ID NO:24), wherein the two cysteines cyclize the peptide by forming a disulfide bridge.
29. The compound of claim 28, wherein the cysteines are both L-cysteine, both D-cysteine, or one is L-cysteine and one is D-cysteine.
30. The compound of any of claims 23-29, wherein one or more amino acid residues are inserted prior to or after the Y.
31. The compound of claim 30, wherein a Lys (K) is inserted after the Y.
32. The compound of any of claims 23-31, wherein the Y is halogenated.
33. The compound of claim 32, wherein the halogen is a radioisotope selected from the group consisting of ^{18}F , ^{75}Br , ^{77}Br , ^{122}I , ^{123}I , ^{124}I , ^{125}I , ^{129}I , ^{131}I , and ^{211}At .
34. The compound of claim 23, wherein $[X^1_m\text{-R-D-X}^2_p]_n\text{-Y}$ is selected from the group consisting of:

SEQ ID NO:	$[X^1_m\text{-R-D-X}^2_p]_n\text{-Y}$
8	[$^{\text{D}}$ Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Lys-Tyr
9	[$^{\text{D}}$ Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Tyr
10	[$^{\text{D}}$ Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Tyr-Lys
11	[$^{\text{D}}$ Cys-Gly-Arg-Asp-Ser-Pro- $^{\text{D}}$ Cys]-Tyr
12	[Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Tyr
17	Gly-[$^{\text{D}}$ Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Tyr
18	Gly-[$^{\text{D}}$ Cys-Gly-Arg-Asp-Ser-Pro-Cys]- $^{\text{D}}$ Tyr
19	Gly-[$^{\text{D}}$ Cys-Gly-Arg-Asp-Ser-Pro-Cys]- $^{\text{D}}$ Tyr-Lys
20	Gly-[Cys-Gly-Arg-Asp-Ser-Pro-Cys]- $^{\text{D}}$ Tyr-Lys
21	Gly-[$^{\text{D}}$ Cys-Gly-Arg-Asp-Ser-Pro- $^{\text{D}}$ Cys]-Tyr
22	Gly-[Cys-Gly-Arg-Asp-Ser-Pro- $^{\text{D}}$ Cys]-Tyr

23	Gly-[_D Cys-Gly-Arg-Asp-Ser-Pro- _D Cys]- _D Tyr
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35. The compound of any of claims 23-34, wherein R² is hydroxyl.

36. A compound having formula (II):



wherein:

R³ is selected from the group consisting of nanoparticles, small organic molecules, peptides, organometallics, metal chelates, proteins, drugs, antibiotics, and carbohydrates; and

R⁴ is selected from the group consisting of:

SEQ ID NO:	R ⁴
8	CONH-[_D Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Lys-Tyr-OH
8	CONH-[_D Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Lys-Tyr-NH ₂
9	CONH-[_D Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Tyr-OH
10	CONH-[_D Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Tyr-Lys-OH
11	CONH-[_D Cys-Gly-Arg-Asp-Ser-Pro- _D Cys]-Tyr-OH
12	CONH-[Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Tyr-OH
13	CONH-[Cys-Arg-Gly-Asp-Ser-Pro-Cys]-Tyr-OH
14	CONH-[_D Cys-Arg-Gly-Asp-Ser-Pro-Cys]-Tyr-OH
15	CONH-[_D Cys-Arg-Gly-Asp-Ser-Pro- _D Cys]-Tyr-OH
17	CONH-Gly-[_D Cys-Gly-Arg-Asp-Ser-Pro-Cys]-Tyr-OH

18	CONH-Gly-[^D Cys-Gly-Arg-Asp-Ser-Pro-Cys]- ^D Tyr-OH
19	CONH-Gly-[^D Cys-Gly-Arg-Asp-Ser-Pro-Cys]- ^D Tyr-Lys-OH
20	CONH-Gly-[Cys-Gly-Arg-Asp-Ser-Pro-Cys]- ^D Tyr-Lys-OH
21	CONH-Gly-[^D Cys-Gly-Arg-Asp-Ser-Pro- ^D Cys]-Tyr-OH
22	CONH-Gly-[Cys-Gly-Arg-Asp-Ser-Pro- ^D Cys]-Tyr-OH
23	CONH-Gly-[^D Cys-Gly-Arg-Asp-Ser-Pro- ^D Cys]- ^D Tyr-OH

37. The compound of claim 36, wherein the Y is halogenated.
38. The compound of claim 37, wherein the halogen is a radioisotope selected from the group consisting of ¹⁸F, ⁷⁵Br, ⁷⁷Br, ¹²²I, ¹²³I, ¹²⁴I, ¹²⁵I, ¹²⁹I, ¹³¹I, and ²¹¹At.
39. A method for detecting expression of a $\beta 3$ subunit of integrin in a cell, the method comprising:
- contacting a population of cells with a compound of claim 1-38; and
 - detecting the presence of a signal emitted from the carbocyanine dye of the compound of claim 1-38 in the population of cells, the signal being emitted from a cell expressing a $\beta 3$ subunit of integrin.
40. A method for detecting cancer, the method comprising:
- administering a composition comprising an effective amount of a compound of claim 1-38 to a subject; and
 - detecting the presence of a signal emitted from the carbocyanine dye of the compound of claim 1-38 in the subject, wherein detection of signal above baseline indicates cancer.
41. A method for detecting pancreatic cancer, the method comprising:
- administering a composition comprising an effective amount of a compound of claim 1-38 to a subject; and
 - detecting the presence of a signal emitted from the carbocyanine dye of the compound of claim 1-38 in the subject, wherein detection of signal above baseline indicates pancreatic cancer.

42. A method for detecting early stage pancreatic ductal adenocarcinoma (PDAC) or precursor pancreatic intraepithelial neoplasia (PanIN), the method comprising:
- b) administering a composition comprising an effective amount of a compound of claim 1-38 to a subject; and
 - b) detecting the presence of a signal emitted from the carbocyanine dye of the compound of claim 1-38 in the subject, wherein detection of signal above baseline indicates early stage pancreatic ductal adenocarcinoma (PDAC) or precursor pancreatic intraepithelial neoplasia (PanIN).
43. A method for differentiating pancreatic ductal adenocarcinoma (PDAC) and precursor pancreatic intraepithelial neoplasia (PanIN) from pancreatitis, the method comprising:
- a) administering a composition comprising an effective amount of a compound of claim 1-38 to a subject; and
 - b) detecting the presence of a signal emitted from the carbocyanine dye of the compound of claim 1-38 in the subject, wherein detection of signal above baseline indicates PDAC or PanIN and detection of a signal at or below baseline indicates pancreatitis.
44. A method for differentiating pancreatic ductal adenocarcinoma (PDAC) and PanIN-3 from PanIN-1/2 and ductal hyperplasia/metaplasia, the method comprising:
- c) administering a composition comprising an effective amount of a compound of claim 1-38 to a subject; and
 - c) detecting the presence of a signal emitted from the carbocyanine dye of the compound of claim 1-38 in the subject, wherein detection of signal above baseline indicates PDAC or PanIN-3 and detection of a signal at or below baseline indicates PanIN-1/2 or ductal hyperplasia/metaplasia.
45. The method of any of claims 39-44, wherein the method detects microscopic or small lesions unable to be detected via standard methods.

- 46. The method of any of claims 39-44, wherein the cancer is treated with the proviso that the compound is conjugated to a drug or therapeutic radionuclide.
- 47. The method of any of claims 39-44, wherein the baseline is the signal emitted in uninvolved tissue.
- 48. The method of claim 47, wherein a signal of greater than 20% above baseline indicates disease.
- 49. The method of any of claims 39-44, wherein the signal is detected about 1 to about 100 hours after administration of the compound.

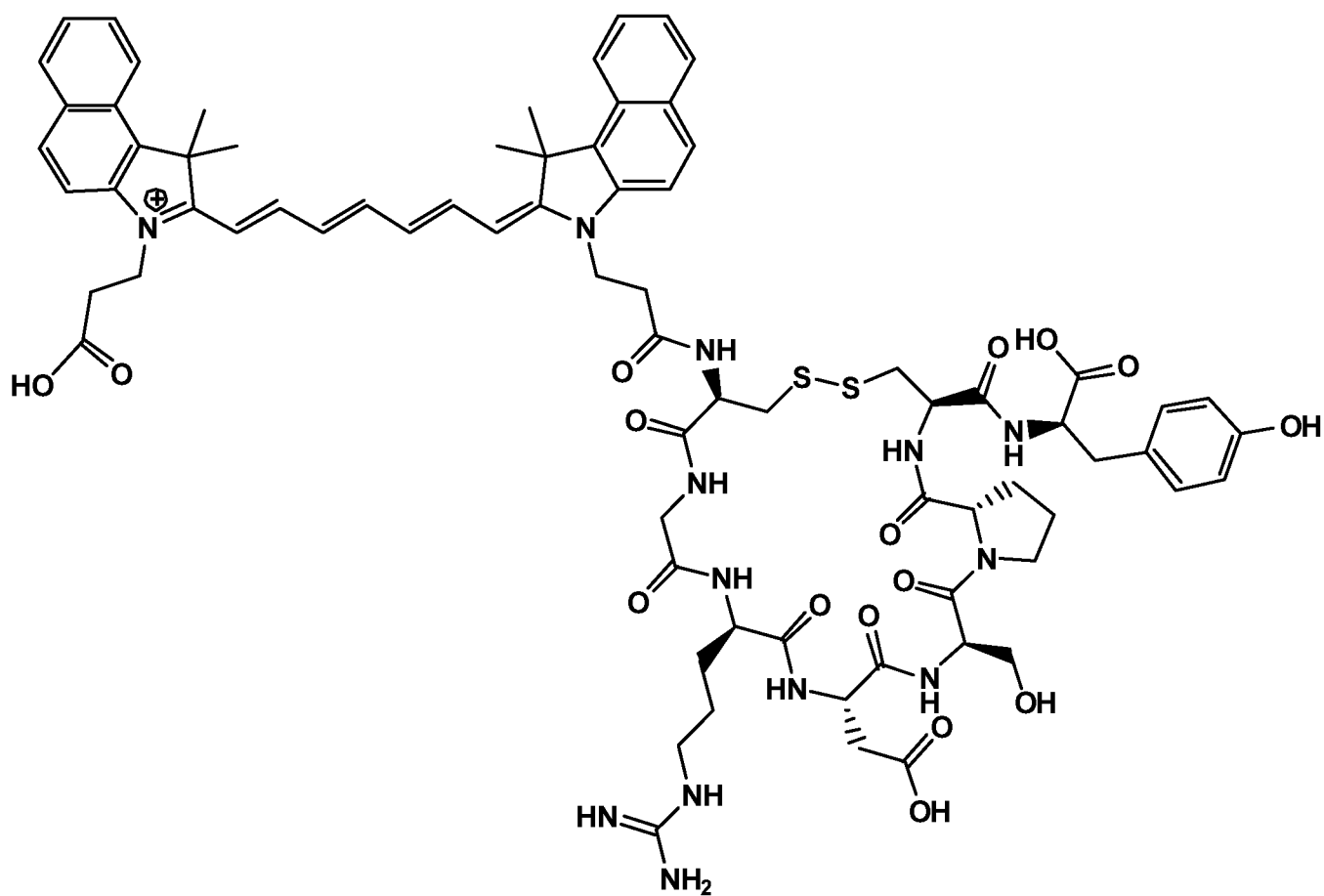


FIG. 1

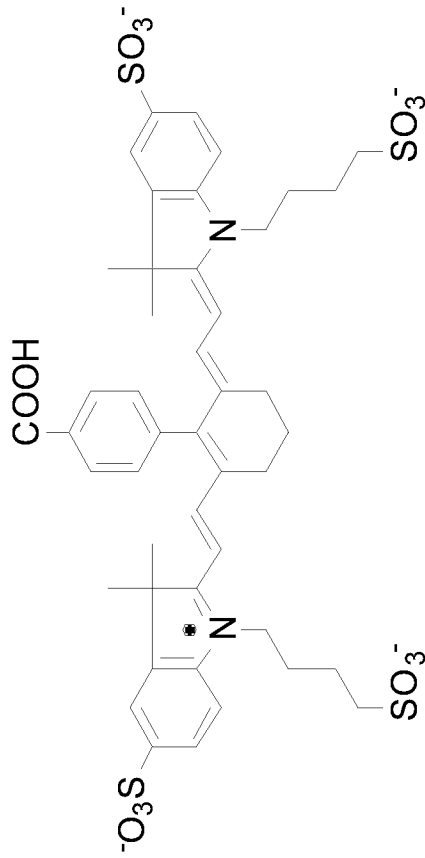


FIG. 2A

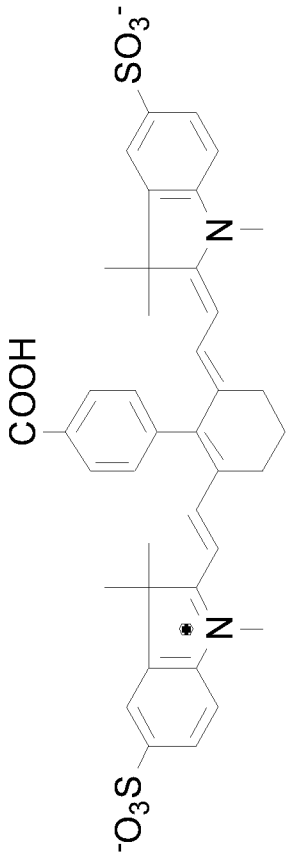


FIG. 2B

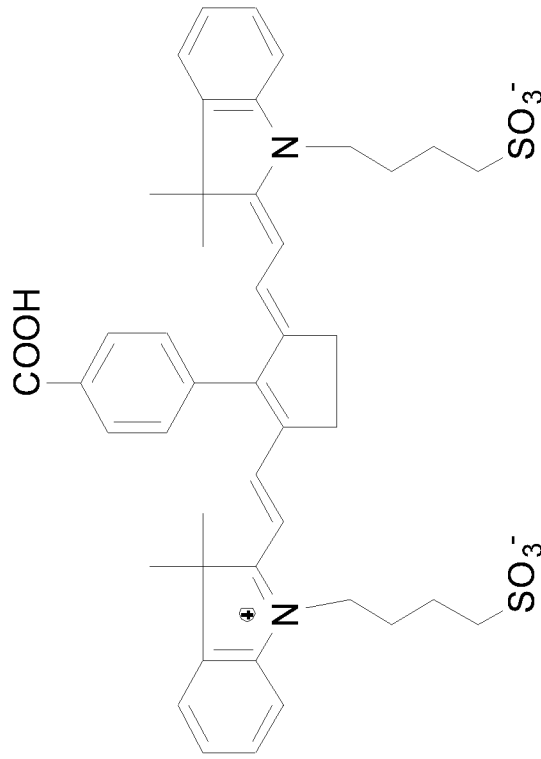


FIG. 2C

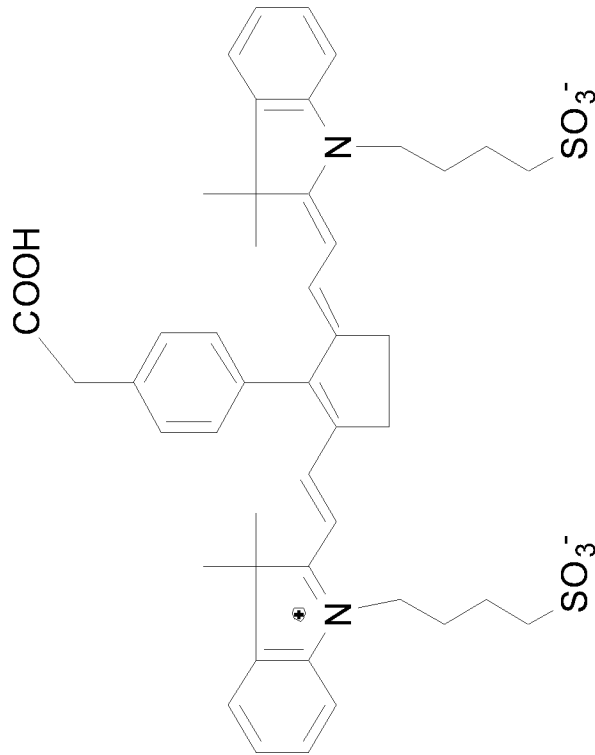
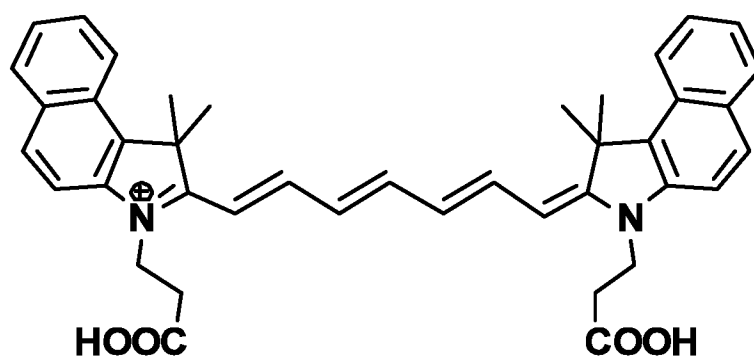
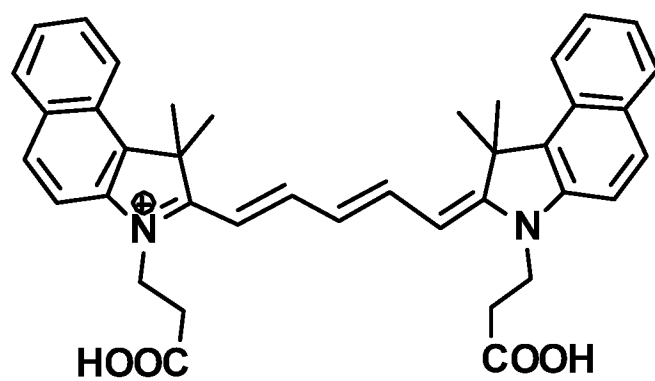
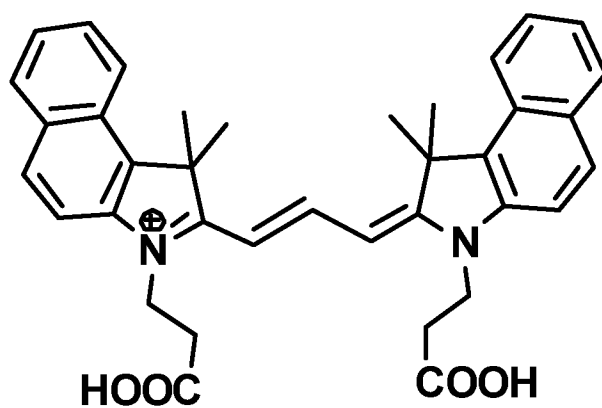
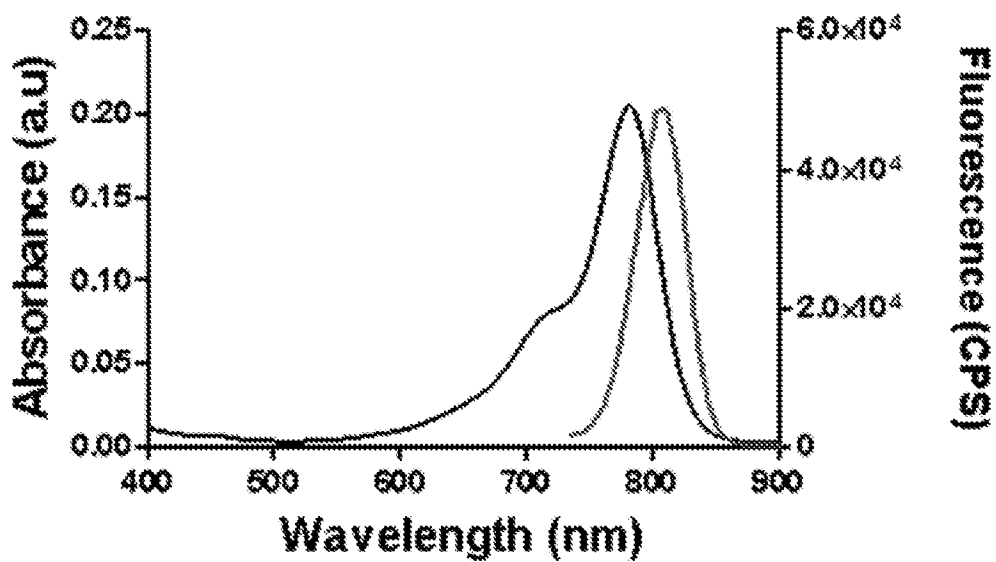
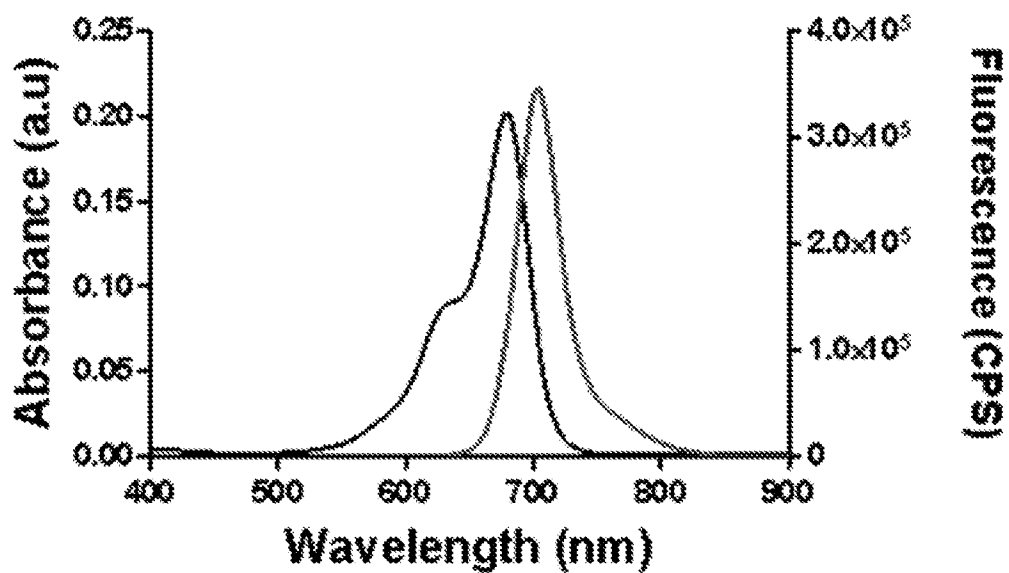


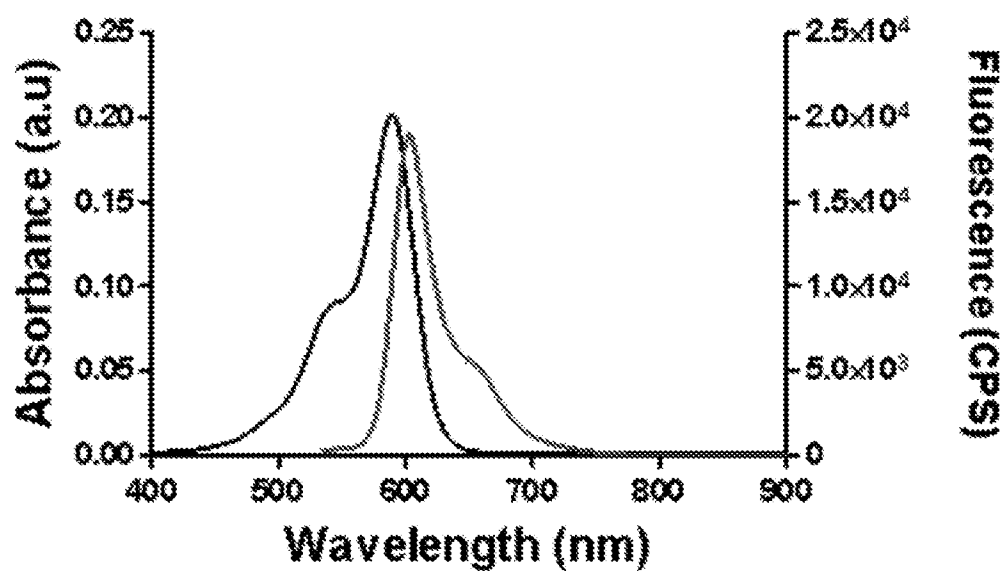
FIG. 2D

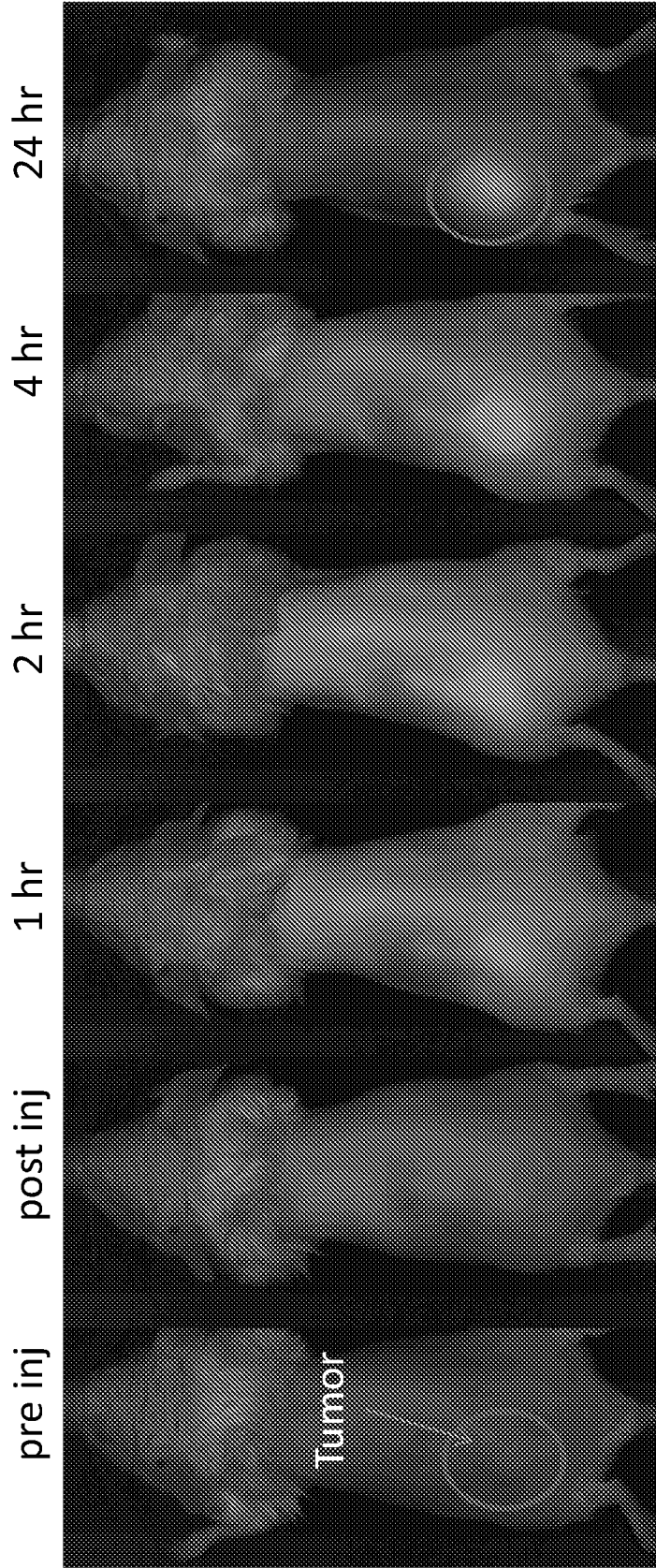
**FIG. 3A****FIG. 3B****FIG. 3C**

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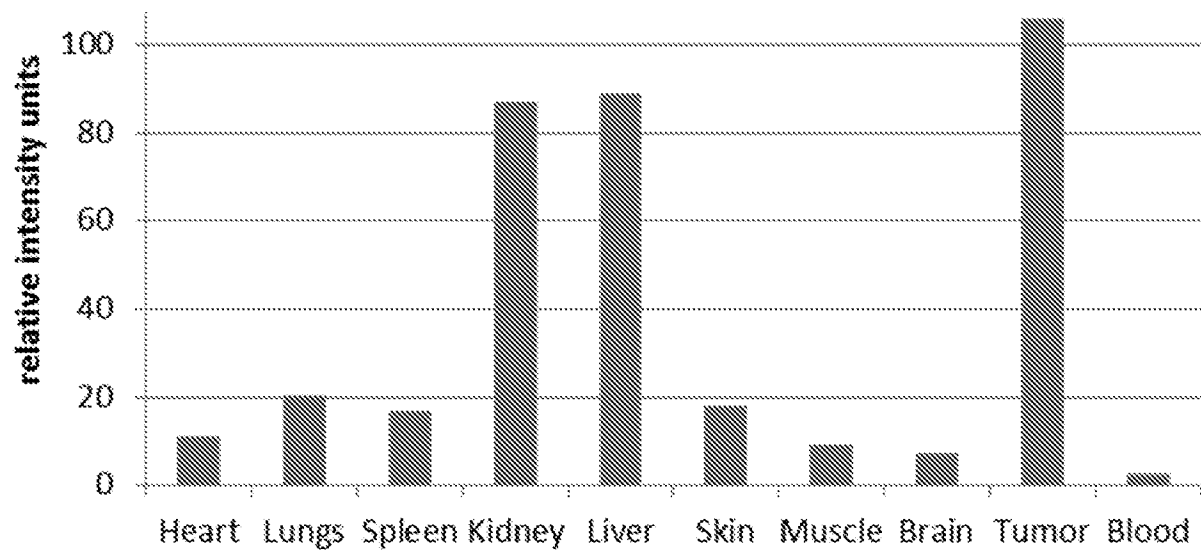
**FIG. 4A****FIG. 4B**

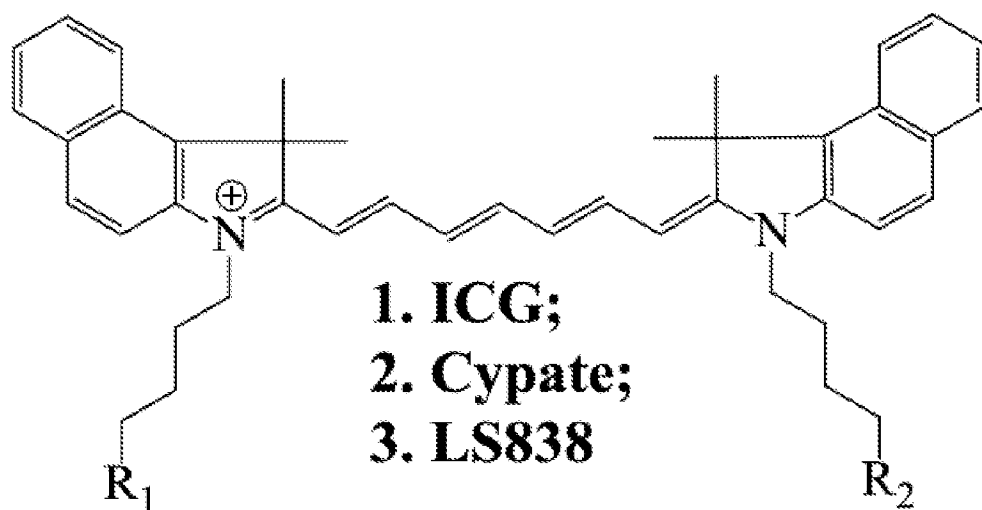
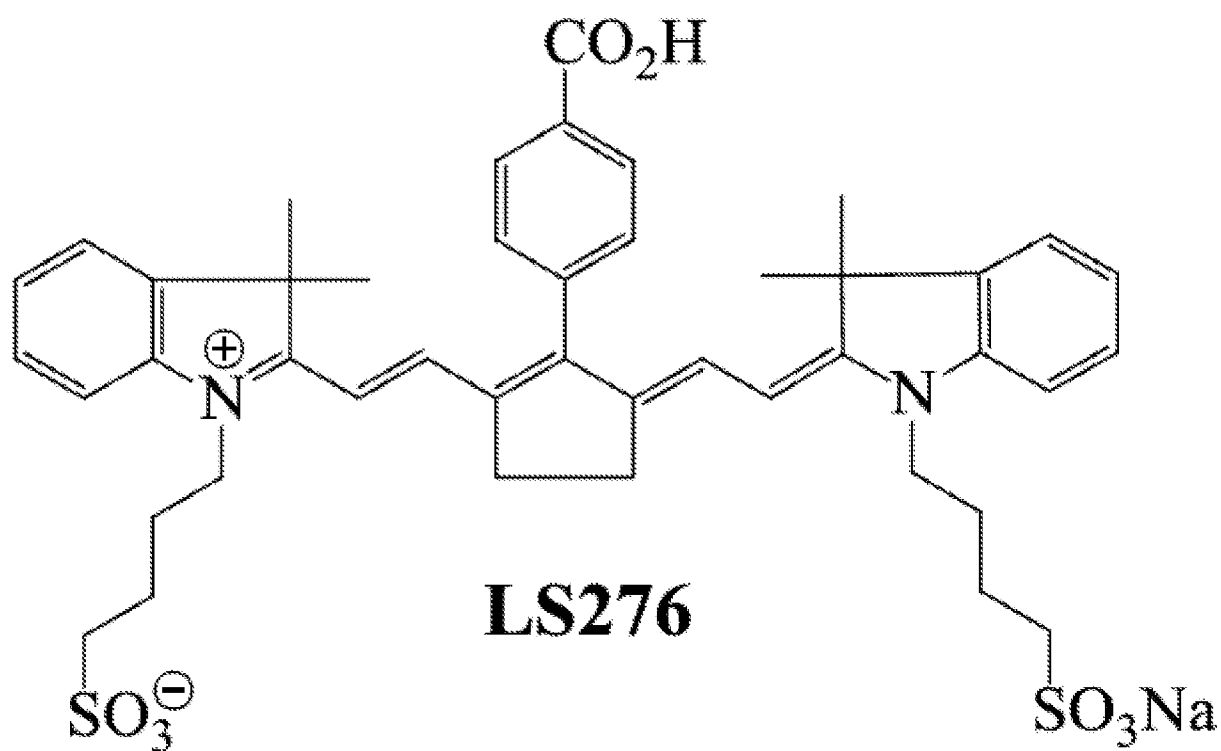
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**FIG. 4C**

**FIG. 5**

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**FIG. 6**

**FIG. 7A****FIG. 7B**

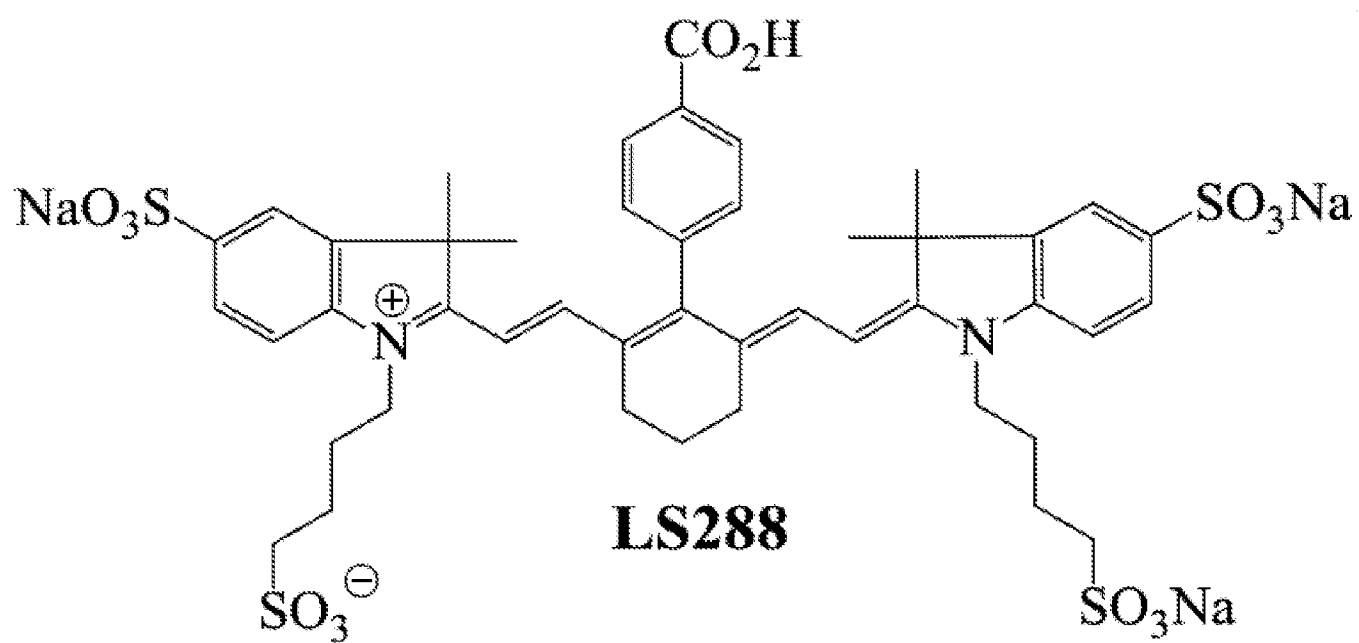


FIG. 7C

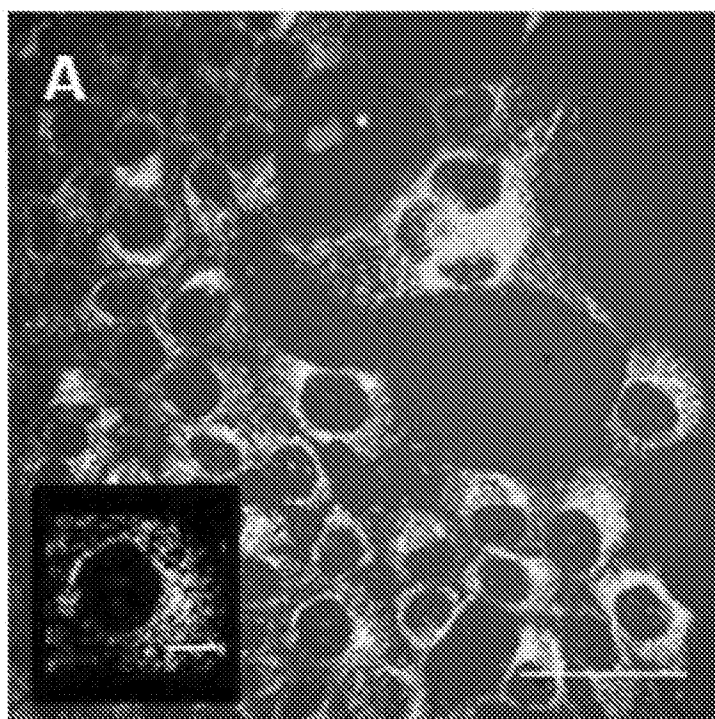


FIG. 8A

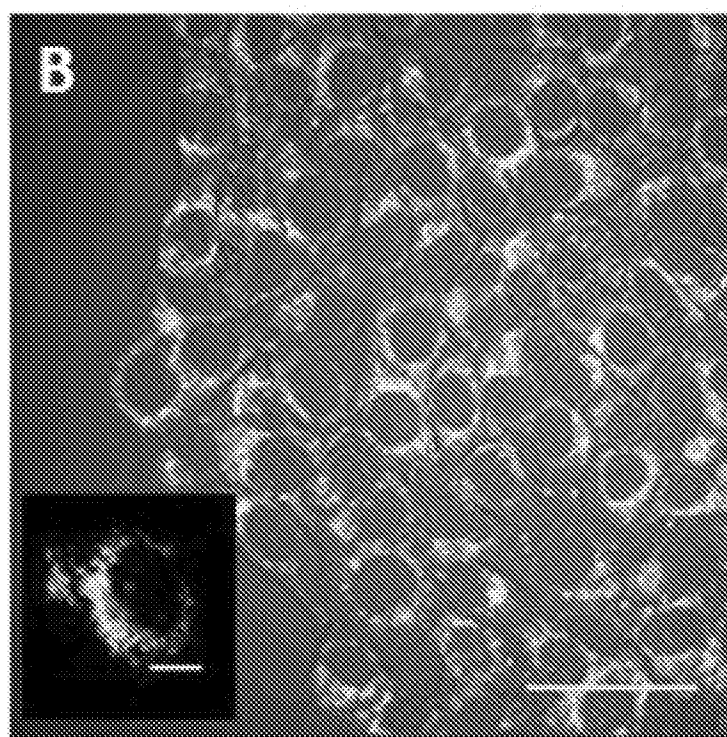
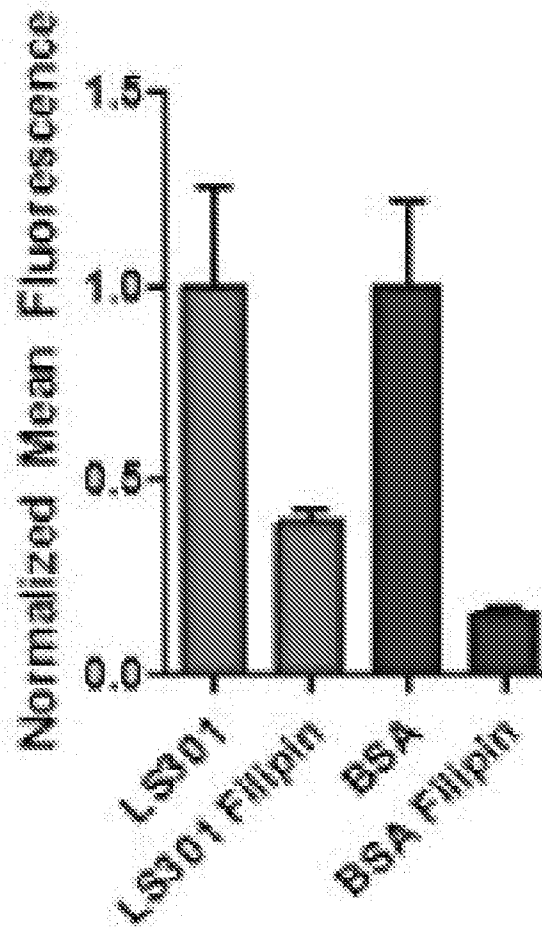
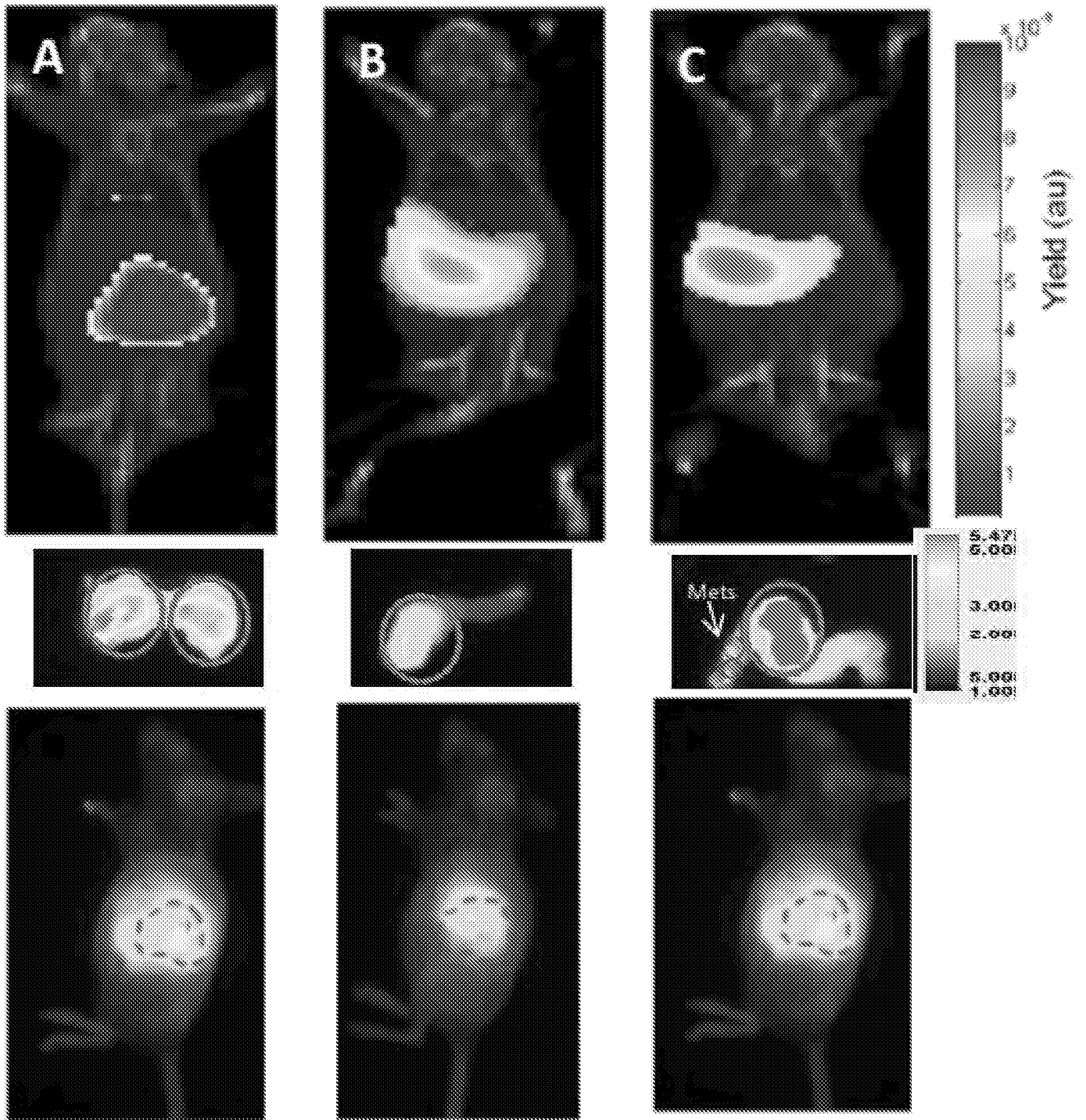
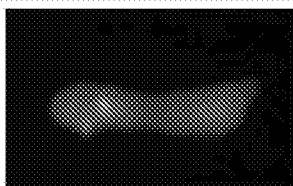
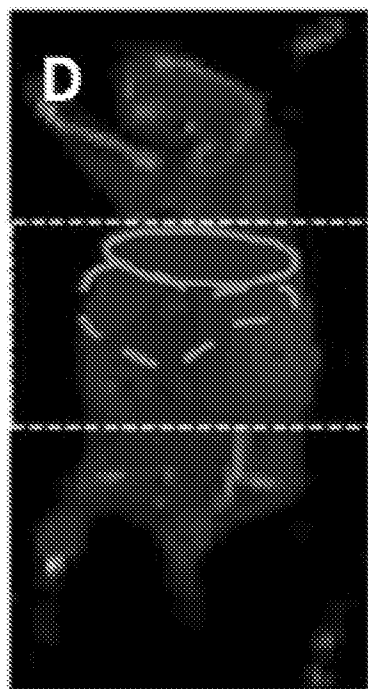
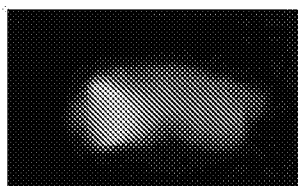
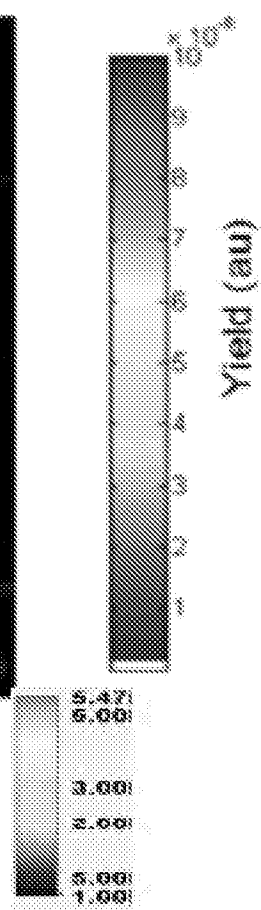
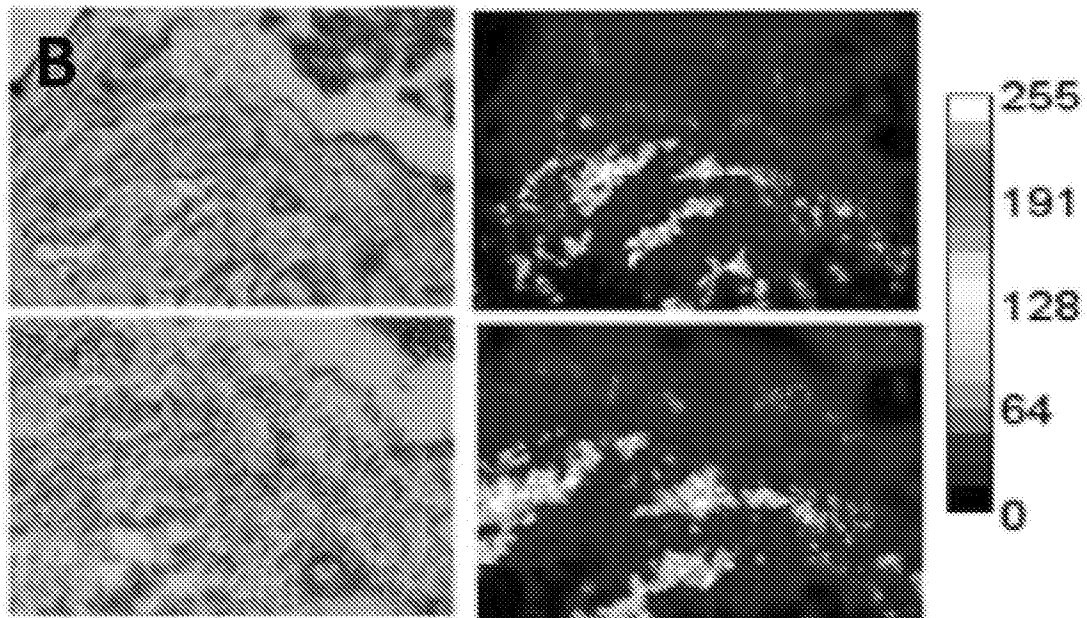
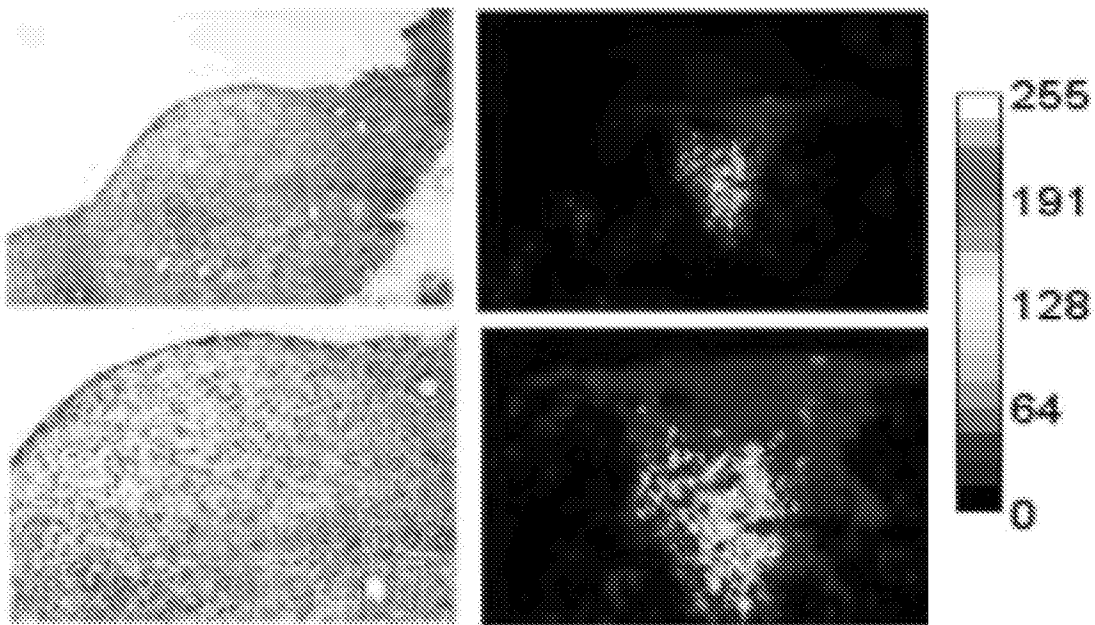


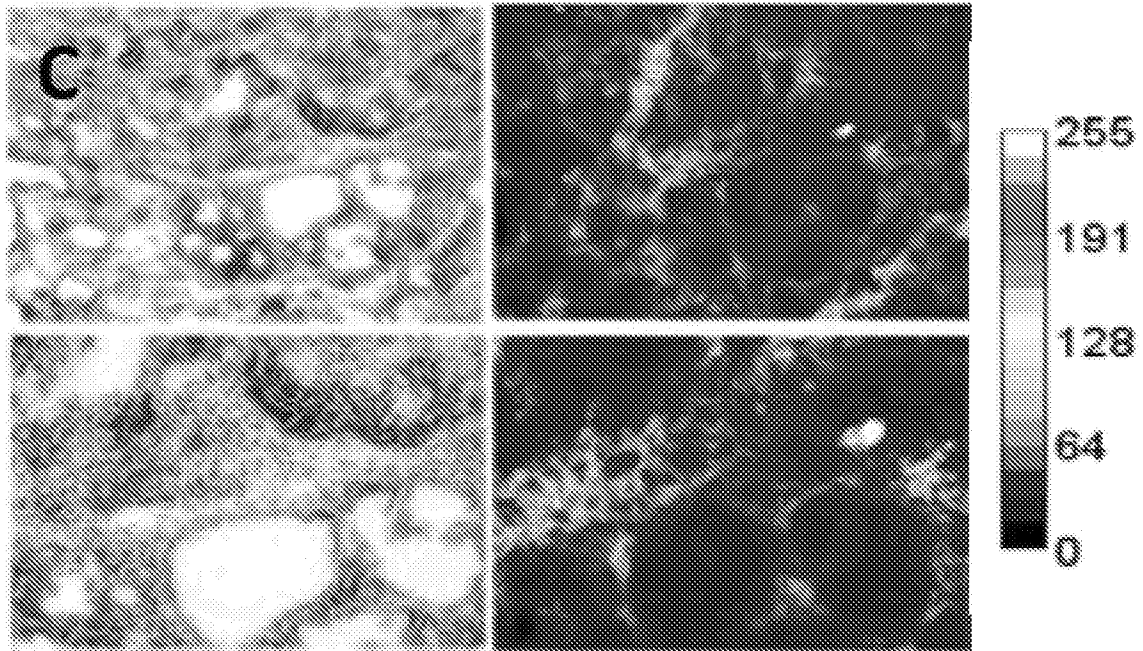
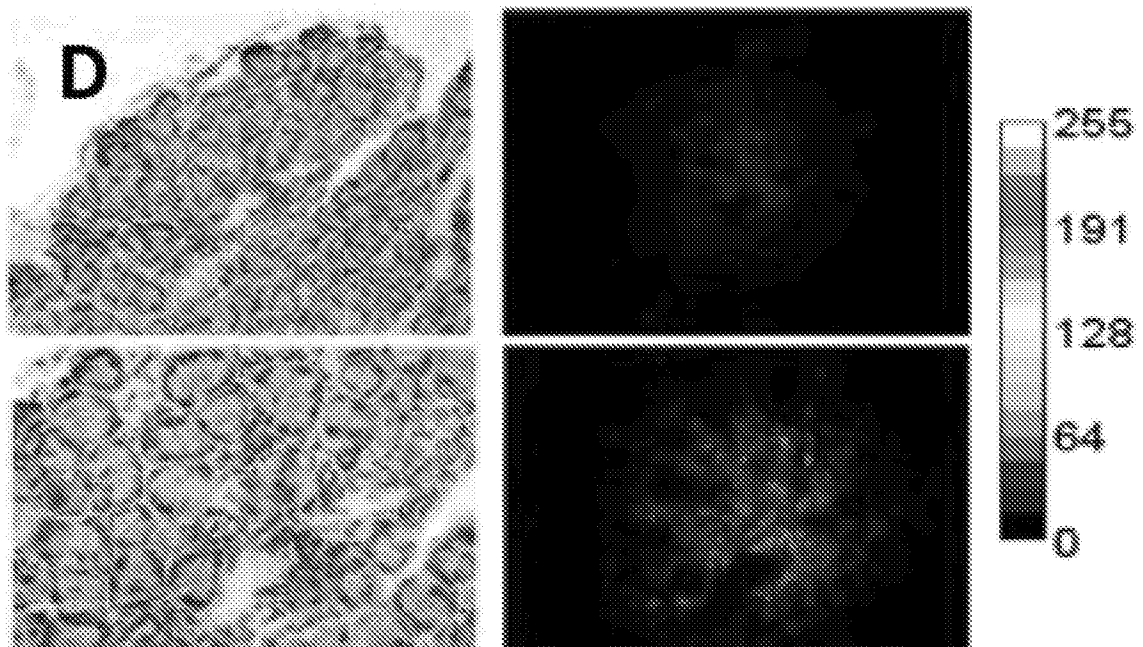
FIG. 8B

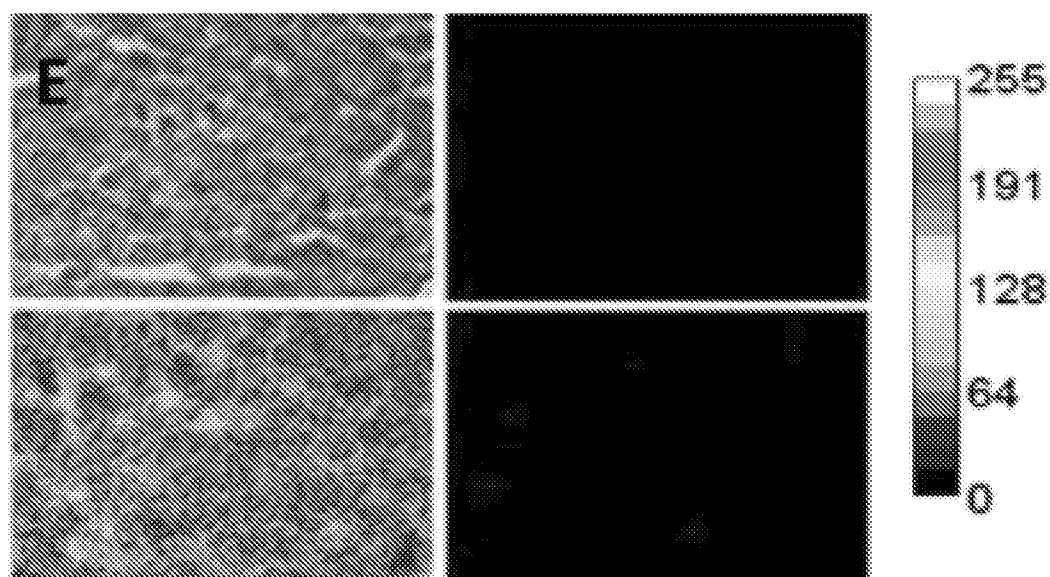
**FIG. 8C**



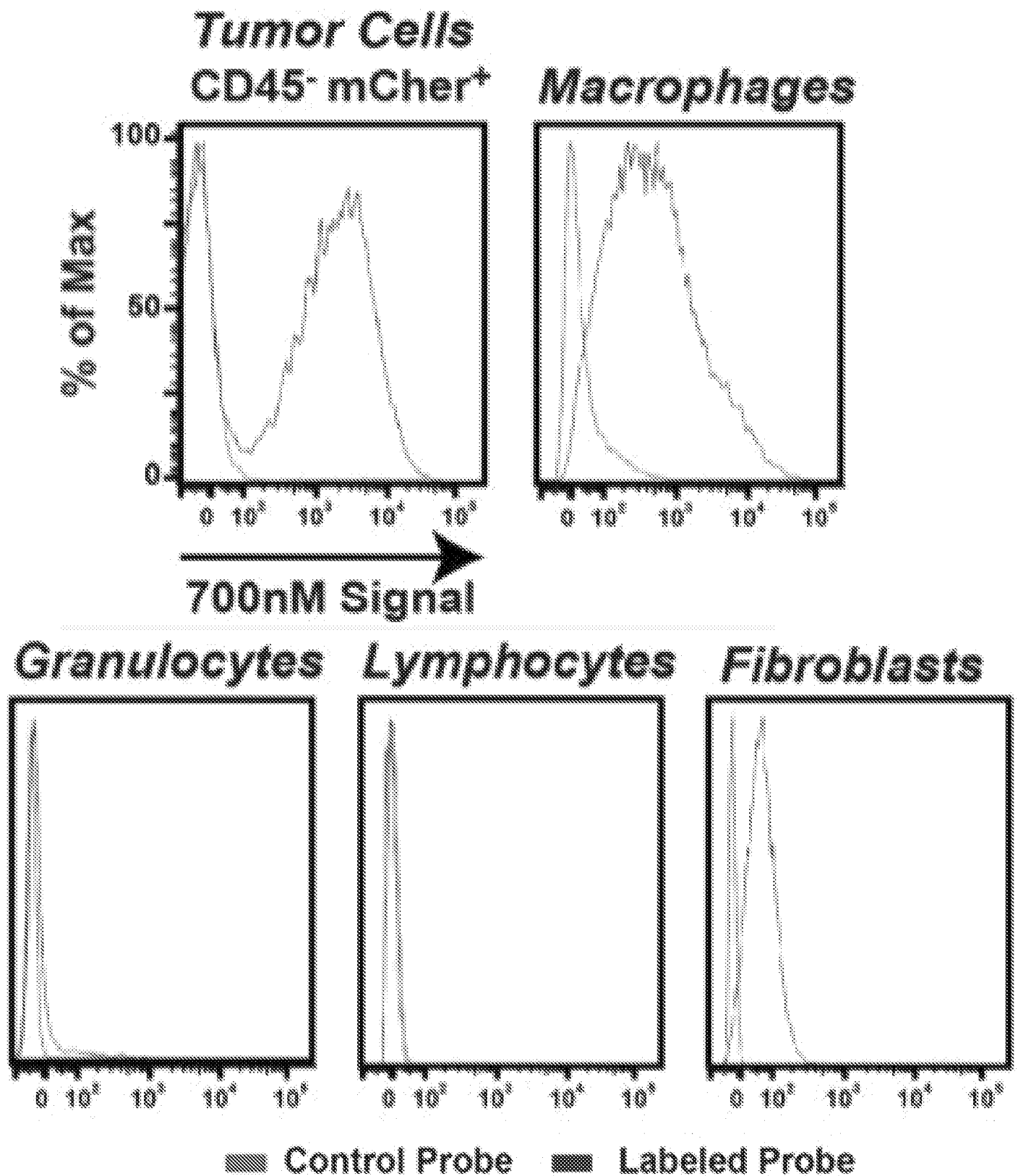
**FIG. 9D****FIG. 9E**

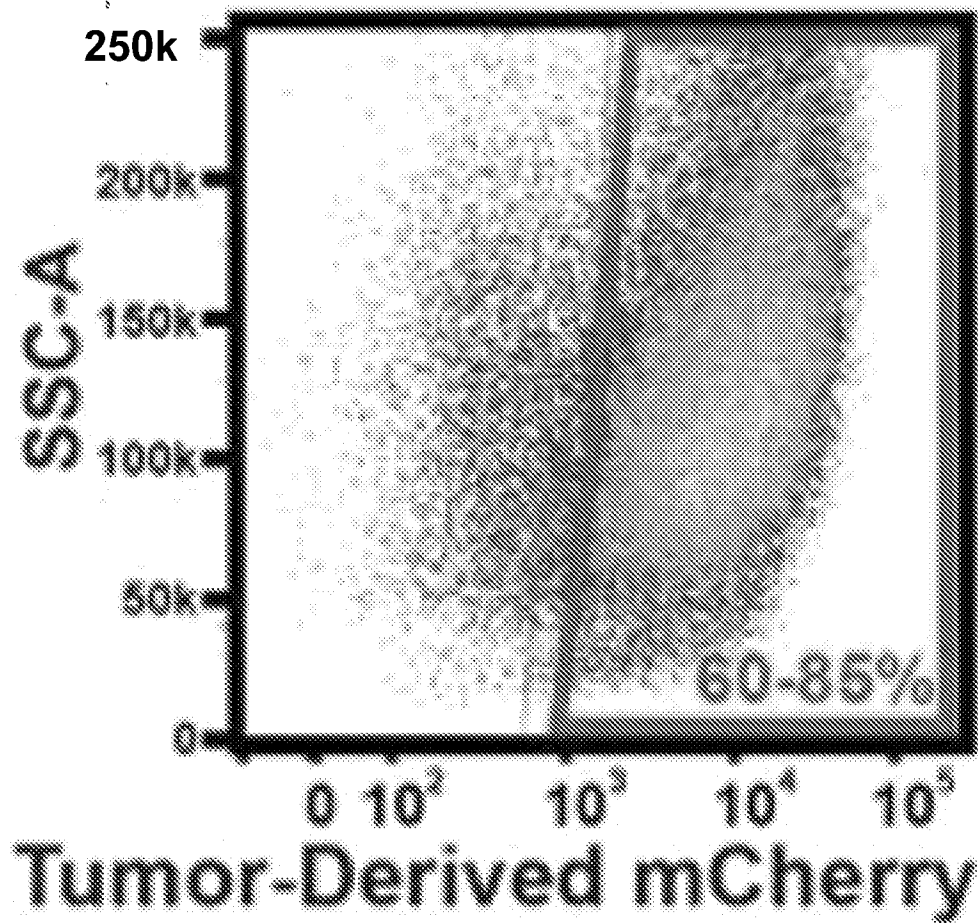


**FIG. 10C****FIG. 10D**

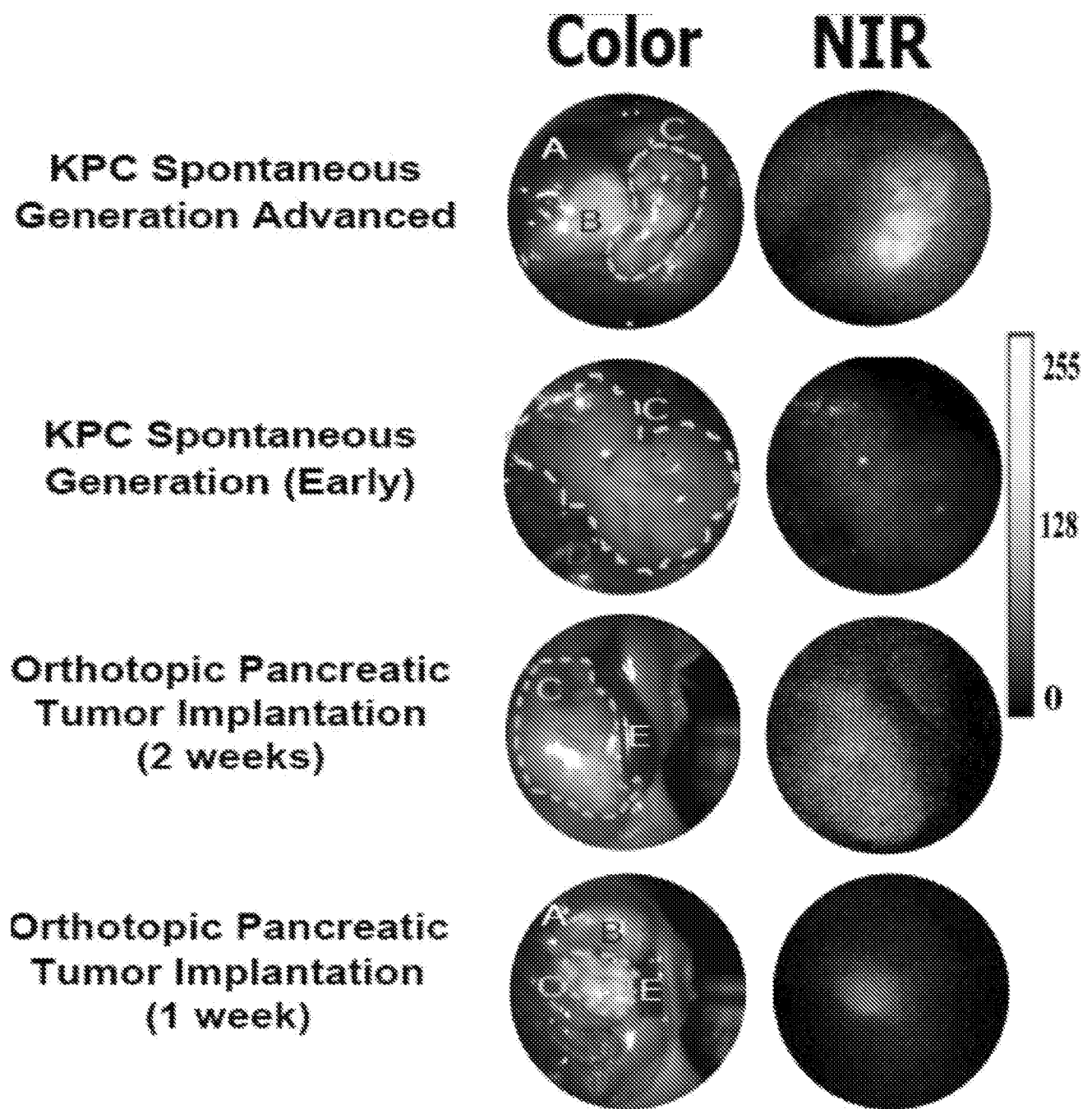
**FIG. 10E**

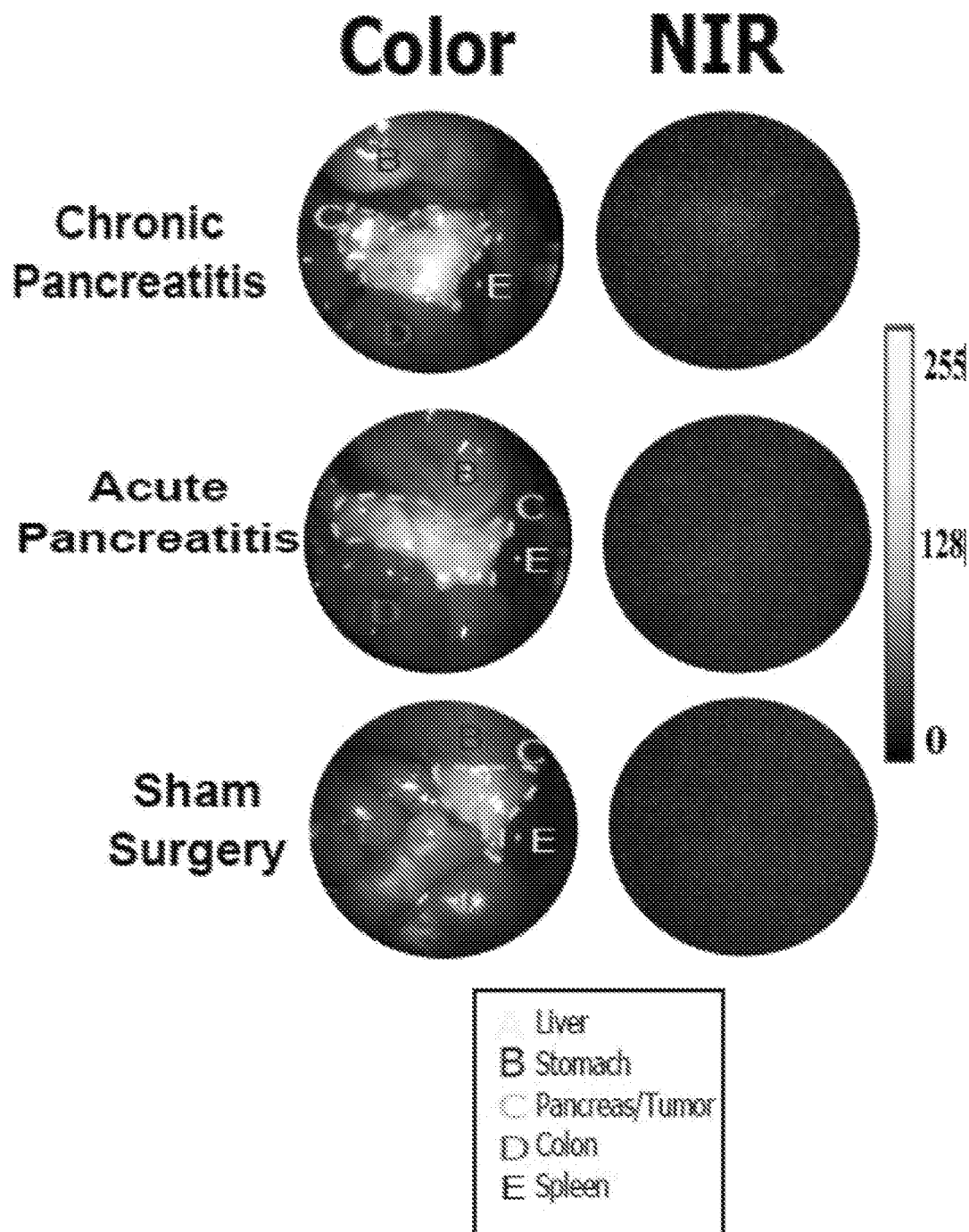
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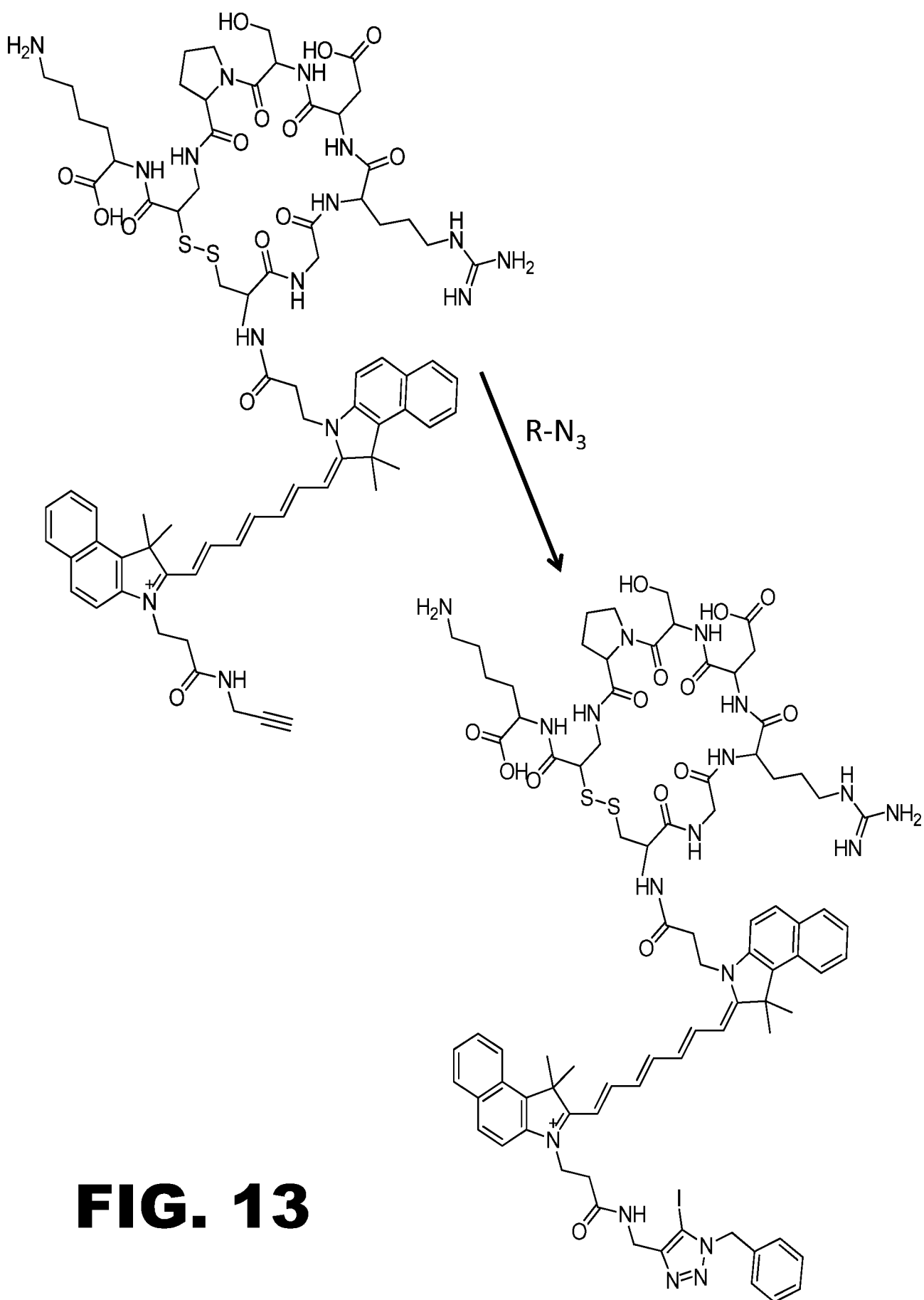
**FIG. 11A**

**FIG. 11B**

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**FIG. 12A**

**FIG. 12B**



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/30893

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 7/64, 4/00; A61K 38/08, 38/12, 38/03 (2016.01)

CPC - C07K 7/64; A61K 38/08, 38/12, 38/04

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) Classifications: C07K 7/64, 4/00; A61K 38/08, 38/12, 38/03 (2016.01)

CPC Classifications: C07K 7/64; A61K 38/08, 38/12, 38/04

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

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

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA); Google Scholar; EBSCO; PubMed:

Keywords: inhibit, antagonist, block, bind, integrin, SPCKY, DSPKY, 'pro_cys_lys_tyr', 'ser_pro_lys_tyr', cypate, peptide, tyrosine, mutate, modified, add, substituted, carbocyanine, GRD, heptapeptide, LS301

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2009/0028788 A1 (ACHILEFU) 29 January 2009; Figures 11C, 11D; paragraphs [0008], [0044], [0046]-[0052], [0104]	1-2, 3/1-2, 23-24, 27/23-24
Y	(COOPER, S) Polyurethane Biomaterials; Abstract of video lecture; presented 9 November 2011; online: <URL: http://hdl.handle.net/1853/42056 >; downloaded from <URL: https://smartechn.gatech.edu/handle/1853/42056 > (accessed 22 July 2016); abstract	1-2, 3/1-2, 23-24, 27/23-24
A	WO 2013/112554 A1 (WASHINGTON UNIVERSITY) 1 August 2013; paragraph [0072]	25-26, 27/25-26, 28-29, 34, 36-38
A	(YE, Y. et al.) Integrin Targeting for Tumor Optical Imaging. Theranostics; 2011; Vol. 1; pg. 102-126. Entire document	1-2, 3/1-2, 23-26, 27/23-26, 28-29, 34, 36-38
A	(ACHILEFU, S. et al.) Synergistic effects of light-emitting probes and peptides for targeting and monitoring integrin expression. Proc. Natl. Acad. Sci. USA; 31 May 2005; Vol. 102 (22); pg. 7976-7981. Entire document.	1-2, 3/1-2, 23-26, 27/23-26, 28-29, 34, 36-38

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

28 July 2016 (28.07.2016)

Date of mailing of the international search report

09 SEP 2016

Name and mailing address of the ISA/

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P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/30893

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-22, 30-33, 35, 39-49
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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