



(51) International Patent Classification:
A61B 5/00 (2006.01)

(21) International Application Number:
PCT/US2010/053574

(22) International Filing Date:
21 October 2010 (21.10.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/279,522 21 October 2009 (21.10.2009) US

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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, 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, IS, JP, KE, KG, KM, KN, KP,

[Continued on next page]

(54) Title: PAA NANOPARTICLES FOR ENHANCEMENT OF TUMOR IMAGING

(57) Abstract: A composition comprising PAA nanoparticles containing a post loaded tetrapyrrolic photosensitizer and an imaging agent and methods for making and using same.

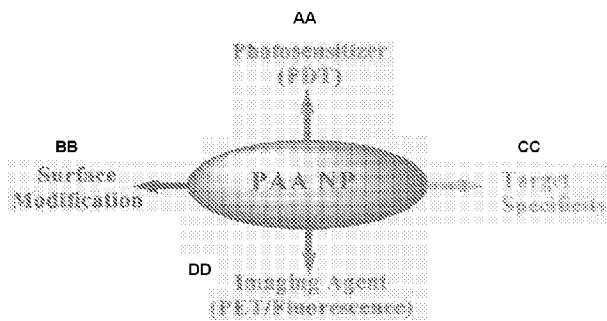


FIGURE 15



KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, 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.

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, ML, MR, NE, SN, TD, TG).

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ,

Published:

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

PAA NANOPARTICLES FOR ENHANCEMENT OF TUMOR IMAGING**5 STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR
DEVELOPMENT**

[0001] This invention was made with United States Government support under Grant Numbers CA19358 and CA114053 awarded by the National Institute of Health. The United States Government has certain rights in the invention.

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CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application claims the benefit under 35 U.S.C. §119(e) of U.S. Provisional Application No. 61/279,522, filed October 21, 2009, which application is incorporated by reference herein in its entirety.

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BACKGROUND OF THE INVENTION

[0003] Nanoscience is being developed in conjunction with advanced medical science for further precision in diagnosis and treatment. Nanoplatfroms and nanovectors that deliver a therapeutic or imaging agent for biomedical applications show promise for cancer diagnosis
20 and therapy. Therapeutic examples include nanoparticle containing PDT agents, folate receptor-targeted, boron containing dendrimers for neutron capture and nanoparticle-directed thermal therapy.

[0004] Nanoparticles have had disadvantages when considered for use in photodynamic therapy (PDT). In particular, certain nanoparticles have no relatively large
25 knowledge base on cancer imaging, PDT, chemical sensing, stability and biodegradation. (2) have in *in-vivo* toxicity. (3) Have short plasma circulation time without surface modification and unstable or uncontrollable biodegradation and bioelimination rates (4) Have problems associated with scale-up and are not storage stabile over extended periods. And (5) have additional limitations including relative difficulty in incorporating hydrophobic compounds,
30 leaching of small hydrophilic components unless they are "anchored", and unknown limitation on bulk tumor permeability because of hydrogel swelling.

[0005] A major challenge of cancer therapy is preferential destruction of malignant cells with sparing of normal tissue. Critical for successful eradication of malignant disease are early detection and selective ablation of the malignancy. Photodynamic therapy (PDT) is

a clinically effective and still evolving locally selective therapy for cancers. The utility of PDT has been demonstrated with various photosensitizers for multiple types of disease. It is FDA approved for early and late stage lung cancer, obstructive esophageal cancer, high-grade dysplasia associated with Barrett's esophagus, age-related macular degeneration and actinic keratoses. PDT employs tumor localizing photosensitizers that produce reactive singlet oxygen upon absorption of light which is believed to be responsible for the destruction of the tumor. Subsequent oxidation-reduction reactions also can produce superoxide anions, hydrogen peroxide and hydroxyl radicals which contribute to tumor ablation⁴. Photosensitizers have been designed which localize relatively specifically to certain subcellular structures such as mitochondria, which are highly sensitive targets. On the tumor tissue level, direct photodynamic tumor cell kill, destruction of the tumor supporting vasculature and possibly activation of the innate and adaptive anti-tumor immune system interact to destroy the malignant tissue⁶. The preferential killing of the targeted cells (e.g. tumor), rather than adjacent normal tissues, is essential for PDT, and the preferential target damage achieved in clinical applications is a major driving force behind the use of the modality. The success of PDT relies on development of tumor-avid molecules that are preferentially retained in malignant cells but cleared from normal tissues.

[0006] In efforts to develop effective photosensitizers with the required photophysical characteristics, compounds having a tetrapyrrolic core ring were used. Usually, chlorophyll-a and bacteriochlorophyll-a were used as intermediates in synthesis. Extensive QSAR studies on a series of the alkyl ether derivatives of pyropheophorbide-a (660 nm) led to selection of HPPH (hexyl ether derivative), now in promising Phase II clinical trials. Photosensitizer development now extends to purpurinimide (700 nm) and bacteriopurpurinimide (780-800 nm) series with high singlet oxygen producing capability. Long wavelength absorption is important for treating large deep seated tumors, because longer wavelength light increases penetration and minimizes the number of optical fibers needed for light delivery within the tumor.

[0007] Various efforts have been made to target tumor cells so that an agent may destroy the tumor cells while sparing normal cells. Such systems are reliant upon specific receptors and as such must reach receptor location. This is a disadvantage since even though the agent may reach the targeted cell, it may not be effective unless the particular receptor is reached and bound.

[0008] Multiple, complementary techniques for tumor detection, including magnetic resonance, scintigraphic and optical imaging are under active development. Each approach

has particular strengths and advantages. Optical imaging includes measurement of absorption of endogenous molecules (e. g. hemoglobin) or administered dyes, detection of bioluminescence in preclinical models, and detection of fluorescence from endogenous fluorophores or from targeted exogenous molecules. Fluorescence, the emission of absorbed light at a longer wavelength, can be highly sensitive: a typical cyanine dye with a lifetime of 0.6 nsec can emit up to 1032 photons/second/mole. A sensitive optical detector can image <103 photons/second. Thus even with low excitation power, low levels of fluorescent molecular beacons can be detected. A challenge is to deliver the dyes selectively and in high enough concentration to detect small tumors. Use of ICG alone to image hypervascular or “leaky” angiogenic vessels around tumors has been disappointing, due to its limited intrinsic tumor selectivity. Multiple approaches have been employed to improve optical probe-localization, including administering it in a quenched form that is activated within tumors, or coupling it to antibodies or small molecules such as receptor ligands. Recent studies have focused on developing dye conjugates of small bioactive molecules, to improve rapid diffusion to target tissue and use combinatorial and high throughput strategies to identify, optimize, and enhance *in vivo* stability of the new probes. Some peptide analogs of ICG derivatives have moderate tumor specificity and are entering pre-clinical studies. However, none of these compounds are designed for both tumor detection and therapy. It is important to develop targeting strategies that cope with the heterogeneity of tumors *in vivo*, where there are inconsistent and varying expressions of targetable sites.

[0009] Photosensitizers (photosensitizer) generally fluoresce and their fluorescence properties *in vivo* has been exploited for the detection of early-stage cancers in the lung, bladder and other sites. For treatment of early disease or for deep seated tumors the fluorescence can be used to guide the activating light. However, photosensitizer are not optimal fluorophores for tumor detection for several reasons: (i) They have low fluorescence quantum yields (especially the long wavelength photosensitizers related to bacteriochlorins). Efficient photosensitizer tend to have lower fluorescence efficiency (quantum yield) than compounds designed to be fluorophores, such as cyanine dyes because the excited singlet state energy emitted as fluorescence is instead transferred to the triplet state and then to molecular oxygen. (ii) They have small Stokes shifts. Porphyrin-based photosensitizer have a relatively small difference between the long wavelength absorption band and the fluorescence wavelength (Stokes shift), which makes it technically difficult to separate the fluorescence from the excitation wavelength. (iii) Most photosensitizer have relatively short fluorescent wavelengths, < 800 nm, which are not optimal for detection deep in tissues.

[0010] Attempts have been made to develop bifunctional conjugates that use tumor-avid photosensitizer to target the NIR fluorophores to the tumor. The function of the fluorophore is to visualize the tumor location and treatment site. The presence of the photosensitizer allows subsequent tumor ablation. The optical imaging allows the clinician performing PDT to continuously acquire and display patient data in real-time. This “see and treat” approach may determine where to treat superficial carcinomas and how to reach deep-seated tumors in sites such as the breast, lung and brain with optical fibers delivering the photo-activating light. A similar approach was also used for developing potential PDT/MRI conjugates in which HPPH was conjugated with Gd(III)DTPA. Due to a significant difference between imaging and therapeutic doses, the use of a single molecule that includes both modalities is problematic.

[0011] Positron emission tomography (PET) is a technique that permits non-invasive use of radioisotope labeled molecular imaging probes to image and assay biochemical processes at the level of cellular function in living subjects²⁰. PET predominately has been used as a metabolic marker, without specific targeting to malignancies. Recently, there has been growing use of radiolabeled peptide ligands to target malignancies. Currently, PET is important in clinical care and is a critical component in biomedical research, supporting a wide range of applications, including studies of tumor hypoxia, apoptosis and angiogenesis²¹. For targeting, a long circulation time may be desirable, as it can increase delivery of the agent into tumors. HPPH and the iodobenzyl pheophorbide-a have plasma half lives ~25 h. The long radiological half life of ^{124}I is well matched to the pheophorbides; it permits sequential imaging with time for clearance from normal tissue. Labeling techniques with radioiodine are well defined with good yield and radiochemical purity²². Despite the complex decay scheme of ^{124}I which results in only 25% abundance of positron (compared with 100% positron emission of ^{18}F), *in vivo* quantitative imaging with ^{124}I labeled antibodies has been successfully carried out under realistic conditions using a PET/CT scanner. A variety of biomolecules have been labeled with ^{124}I . We have devised a coupling reaction which rapidly and efficiently links ^{124}I to a tumor-avid photosensitizer²³⁻²⁵, and used the conjugate to target and image murine breast tumor and its metastasis to lung. Acquisition of clinical PET images can be slow, but combination PET-CT scanners allow real time guidance of therapeutic interventions. Also, new developments in tracking may permit real time interventions guided by PET data sets.

BRIEF SUMMARY OF THE INVENTION

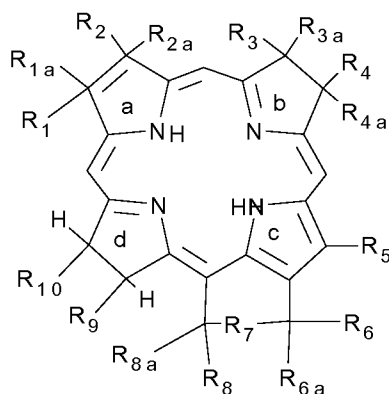
[0012] The present invention relates to polyacrylic acid (PAA) nanoparticles containing a photosensitizer and an imaging enhancing agent.

[0013] In accordance with the invention, therapeutic and imaging potential of
5 encaphotosensitizerululated, post-loaded and covalently linked photosensitizer-nanoparticles have been evaluated. In PAA nanoparticle the post-loading efficiency showed enhanced *in vitro/in vivo* therapeutic and imaging potential. PAA nanoparticle have core matrixes that can readily incorporate molecular or small nanoparticle payloads, and can be prepared in 10-150 nm sizes, with good control of size distributions. The surfaces of nanoparticles can be readily
10 functionalized, to permit attachment of targeting ligands, and both are stable to singlet oxygen (1O_2) produced during photodynamic therapy (PDT). PAA-nanoparticles, i.e. poly(acrylic acid) nanoparticles, have the *advantages* of (1) A relatively large knowledge base on cancer imaging, PDT, chemical sensing, stability and biodegradation. (2) No known *in-vivo* toxicity. (3) Long plasma circulation time without surface modification, but with
15 biodegradation and bioelimination rates controllable *via* the type and amount of selective cross-linking (introduced during polymerization inside reverse micelles). (4) Scale-up to 400g material has been demonstrated, as well as storage stability over extended periods. Limitations have included relative difficulty in incorporating hydrophobic compounds, leaching of small hydrophilic components unless they are "anchored", and unknown
20 limitation on bulk tumor permeability because of hydrogel swelling.

[0014] In accordance with the invention, photosensitizers have several very desirable properties as therapeutic agents deliverable by PAA nanoparticles. In particular, (1) Only a very small fraction of administered targeted non-photodynamic drug makes it to tumor sites and the remainder can cause systemic toxicity. However, PDT provides dual selectivity in
25 that the photosensitizer is inactive in the absence of light and is innocuous without photoactivation. Thus the photosensitizer contained by the nanoparticle can be locally activated at the site of disease. (2) PDT effects are due to production of singlet oxygen, which, in accordance with the compounds and methods of the invention, can readily diffuse from the pores of the nanoparticle. Thus, in contrast to chemotherapeutic agents, release of
30 encaphotosensitizerululated drug from the nanoparticle, is not necessary. Instead, stable nanoparticles with long plasma residence times can be used, which increases the amount of drug delivered to the tumors. (3) PDT is effective regardless of the intracellular location of the photosensitizer. While mitochondria are a principal target of singlet oxygen, photosensitizer incorporated in lysosomes are also active the photodynamic process causes

rupture of the lysosomes with release of proteolytic enzymes and redistribution of the photosensitizer within the cytoplasm. nanoparticle platforms also provide significant advantages for PDT: (1) High levels of imaging agents can be combined with the photosensitizer in the nanoparticle permitting a “see and treat” approach, with fluorescence
 5 image guided placement of optical fibers to direct the photoactivating light to large or subsurface tumors, or to early non clinically evident disease. (2) It is possible to add targeting moieties, such as cRGD or F3 peptide to the nanoparticle so as to increase the selective delivery of the photosensitizer. (3) The nanoparticle can carry large numbers of photosensitizers, and their surface can be modified to provide the desired hydrophilicity for
 10 optimal plasma pharmacokinetics. Thus, they can deliver high levels of photosensitizer to tumors, reducing the amount of light necessary for tumor cure.

[0015] The photosensitizer is preferably a tetrapyrrolic photosensitizer having the structural formula:



or a pharmaceutically acceptable derivative thereof, wherein:

R₁ and R₂ are each independently substituted or unsubstituted alkyl, substituted or
 20 unsubstituted alkenyl, -C(O)R_a or -COOR_a or -CH(CH₃)(OR_a) or -CH(CH₃)(O(CH₂)_nXR_a)
 where R_a is hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl,
 substituted or unsubstituted alkynyl, or substituted or unsubstituted cycloalkyl; where R₂ may
 be -CH=CH₂, -CH(OR₂₀)CH₃, -C(O)Me, -C(=NR₂₁)CH₃ or -CH(NHR₂₁)CH₃

where X is an aryl or heteroaryl group;

n is an integer of 0 to 6;

where R₂₀ is methyl, butyl, heptyl, docetyl or 3,5-bis(trifluoromethyl)-benzyl; and

R₂₁ is 3,5-bis(trifluoromethyl)benzyl;

R_{1a} and R_{2a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form a covalent bond;

R₃ and R₄ are each independently hydrogen or substituted or unsubstituted alkyl;

R_{3a} and R_{4a} are each independently hydrogen or substituted or unsubstituted alkyl, or
5 together form a covalent bond;

R₅ is hydrogen or substituted or unsubstituted alkyl;

R₆ and R_{6a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form =O;

R₇ is a covalent bond, alkylene, azaalkyl, or azaaraalkyl or =NR₂₀ where R₂₀ is 3,5-
10 bis(tri-fluoromethyl)benzyl or -CH₂X-R¹ or -YR¹ where Y is an aryl or heteroaryl group;

R₈ and R_{8a} are each independently hydrogen or substituted or unsubstituted alkyl or together form =O;

R₉ and R₁₀ are each independently hydrogen, or substituted or unsubstituted alkyl and R₉ may be -CH₂CH₂COOR² where R² is an alkyl group that may optionally substituted with
15 one or more fluorine atoms;

each of R₁-R₁₀, when substituted, is substituted with one or more substituents each independently selected from Q, where Q is alkyl, haloalkyl, halo, photosensitizer, pseudohalo, or -COOR_b where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, araalkyl, or OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl or CONR_dR_e where
20 R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or =NR_h where R_h is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or is an amino acid residue;

each Q is independently unsubstituted or is substituted with one or more substituents
25 each independently selected from Q₁, where Q₁ is alkyl, haloalkyl, halo, photosensitizer, pseudohalo, or -COOR_b where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, araalkyl, or OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl or CONR_dR_e where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or NR_fR_g where R_f and R_g are each independently
30 hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or =NR_h where R_h is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or is an amino acid residue.

[0016] The photosensitizer may be conjugated with an image enhancing agent prior to incorporation into the nanoparticle, after incorporation into the nanoparticle or the photosensitizer and/or image enhancing agent may chemically bound to the nano particle

and/or one or more of the photosensitizer and image enhancing agent may be physically bound to the nanoparticle.

[0017] Imaging enhancing agents may be for essentially any imaging process, e.g. Examples of such imaging enhancing agents are discussed in the background of the invention previously discussed and in the list of references incorporated by reference herein as background art.

[0018] It is to be understood that other agents may be incorporated into the nanoparticle such as tumor targeting moieties and tumor inhibiting or tumor toxic moieties.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Figure 1. (A): *In vivo* photosensitizing efficacy of HPPH-CD conjugate **1** in C3H mice bearing RIF tumors (10 mice/ group) at variable drug doses. The tumors were exposed to light (135J/cm²/75mW/cm²) at 24h post-injection. **(B):** Localization of the conjugate **1** in a live mouse 24 h after injection (drug dose 0.3 μ mole/kg). The light treatment parameters are not optimized (in progress) [Without PAA nanoparticle]

Figure 2. Whole body images of BALB/c mice bearing Colon26 tumors with PAA nanoparticles formulations (HPPH and cyanine dye (CD) were post-loaded in 2 to 1 ratio). The CD concentration was kept constant (0.3 μ mol/kg) at the images were obtained at variable time points. **A** = 24 h, **B** = 48 h and **C** = 72 h post injection (λ_{ex} : 785 nm; λ_{Em} : 830 nm). L = Low and H = High.

Figure 3. *In vivo* PDT efficacy of HPPH and CD post loaded in a ratio of 2:1 and 4:1 in PAA and ORMOSIL nanoparticles. Note: HPPH dose: 0.47 μ mol/kg in PAA nanoparticles and 0.78 μ mol/kg in ORMOSIL nanoparticles.

Figure 4. Slow release of HPPH and CD from PAA nanoparticles (post loaded in 2:1 ratio) after several washes with 1% HSA.

Figure 5. Comparative *in vivo* imaging at variable time points of BALB/c mice bearing Colon26 tumors with HPPH-CD conjugate **1** and CD-conjugated with PAA nanoparticles/post;-loaded with HPPH. The nanoparticles were more tumor specific. (Mouse 1)

Figure 6. Panel 1 (4T1 tumors): Primary (PT) and metastasized tumors (MT) dissected. **Panel 2** (4T1 tumors): PET imaging of the dissected primary and metastasized tumors. **Panel 3** (BALB/C mouse bearing 4T1 tumor): Whole body PET imaging. The tumor

metastasis in lung was clearly observed. **Panel 4:** The position of the lung is shown by the transmission scan using ^{57}Co source in mice with no lung metastasis. **Panel 5:** (BALB/C mouse bearing Colo-26 (non-metastatic tumor): Whole body imaging by PET. A high accumulation of the ^{124}I - photosensitizer in tumor is clearly observed without any significant accumulation in lungs (injected dose: 100 μCi). T = Tumor, PT = Primary tumor; MT = Metastatic tumor.

Figure 7. *In vivo* biodistribution of ^{18}F -FDG (100 μCi , half-life 2 h) at 110 min and ^{124}I -photosensitizer **2** (100 μCi , half-life 4.2 d) at 48h in BALB/c mice bearing Colon 26 tumor (3 mice/group). Tumor-uptake was similar for both agents. However, the higher uptake of FDG over ^{124}I -photosensitizer **2** in normal organs is clearly evident.

Figure 8. Comparative *in vivo* PET imaging (72 h post injection) and biodistribution (24h, 48h and 72h postinjection) of ^{124}I -labeled photosensitizer **2** with and without PAA nanoparticles in BALB/c mice bearing Colon26 tumors (see the text). [Biodistribution of PET imaging agent **2**: No PAA , with PAA].

Figure 9. Fluorescence intensity of cells targeted by F3- targeted (A series), F3-Cys targeted (B series) and nontargeted nanoparticles (F series) in nucleolin rich MDA-MB-435 cell lines.

Figure 10. Fluorescence (left) & Live/dead cell assay (right) of HPPH conjugated PAA nanoparticles + or - F3-Cys peptide incubated for 15 min with MDA-MB- 435 cells.

Figure 11. Confocal images showing the target-specificity of F3-Cys peptide in 9L Glioma tumor cells. Left: F3-Cys PEG Rhodamine-PAA nanoparticles (9L cells). Right: PEG Rhodamine-PAA nanoparticles (9L Cells)

Figure 12. *In vivo* biodistribution of ^{14}C -labeled HPPH, and ^{14}C -labeled HPPH post-loaded into PAA nanoparticles in BALB/c mice bearing Colon26 tumors. ^{14}C -labeled photosensitizer (3.8 $\mu\text{Ci}/0.2\text{ mL}$) were administered to 12 mice/group. At 24, 48, 72h after injection, three mice/time-point were sacrificed. The organs of interest were removed and the radioactivity was measured The raw data were converted to counts/ gram of tissue.

Figure 13. *In vivo* biodistribution of iodinated photosensitizer using variable sizes of PAA nanoparticles at 24, 48 and 72h post injection. Left: 531-ME Post-Loaded into 30 nm PAA Nanoparticles. Right: Biodistribution of 531-ME post pre-treatment with 150 nm PAA Nanoparticles.

Figure 14 shows the structural formula of HPPH.

Figure 15 is a diagram of Multifunctional PAA Nanoparticles.

Figure 16 shows flow diagrams of methods to make the PAA nanoparticles of the invention

DETAILED DESCRIPTION OF THE INVENTION

5 [0019] Photosensitizers (photosensitizer) generally fluoresce and their fluorescence properties *in vivo* has been exploited for the detection of early-stage cancers in the lung, bladder and other sites. For treatment of early disease or for deep seated tumors the fluorescence can be used to guide the activating light. However, photosensitizer are not optimal fluorophores for tumor detection for several reasons: (i) They have low fluorescence
10 quantum yields (especially the long wavelength photosensitizers related to bacteriochlorins). Efficient photosensitizer tend to have lower fluorescence efficiency (quantum yield) than compounds designed to be fluorophores, such as cyanine dyes because the excited singlet state energy emitted as fluorescence is instead transferred to the triplet state and then to molecular oxygen. (ii) They have small Stokes shifts. Porphyrin-based photosensitizer have a
15 relatively small difference between the long wavelength absorption band and the fluorescence wavelength (Stokes shift), which makes it technically difficult to separate the fluorescence from the excitation wavelength. (iii) Most photosensitizer have relatively short fluorescent wavelengths, < 800 nm, which are not optimal for detection deep in tissues.

[0020] We have previously shown that certain tumor-avid photosensitizer(s) (e. g.,
20 HPPH) conjugated with NIR absorbing fluorophore(s) (non-tumor specific cyanine dyes) can be used as bifunctional agents for tumor-imaging by fluorescence and phototherapy (PDT). Here, HPPH was used as a vehicle to deliver the imaging agent to tumor. The limitation of this approach was that the conjugate exhibited significantly different dose requirements for the two modalities. The imaging dose was approximately 10-fold lower than the
25 phototherapeutic dose (Fig. 1), which could be due to a part of the singlet oxygen (a key cytotoxic agent responsible for the destruction of the tumors) produced on exciting the photosensitizer being quenched by the fluorophore leading to its photo-destruction. Exposing the tumor at 780 nm (excitation wavelength for the cyanine dye) produced *in vivo* emission at 860 nm and, as expected, no significant photobleaching of the fluorophore (CD) or the
30 photosensitizer (HPPH) was observed.

[0021] For investigating the utility of PAA nanoparticles three different approaches were used. First HPPH and the cyanine dye (fluorophore) were post-loaded in variable ratios (HPPH to CD: 1:1; 2:1; 3:1 and 4:1 molar concentrations). In brief, HPPH was postloaded to PAA nanoparticles first. Free HPPH was removed by spin filtration and then cyanine dye was

postloaded. It was spin-filtered again, washed several times with 1% bovine calf serum and the concentration was measured. The 2:1 formulations produce the best tumor imaging and long-term tumor cure in BALB/c mice bearing Colon26 tumors. This formulation contained in a single dose the therapeutic dose of HPPH (0.47 $\mu\text{mol/kg}$) and the imaging dose of Cyanine dye (0.27 $\mu\text{mol/kg}$), which were similar to the components used alone for tumor imaging and therapy, but with much more tumor selectivity (skin to tumor ratio of HPPH was 4:1 instead of 2:1 without nanoparticles). Under similar treatment parameters the Ormosil nanoparticles showed a significantly reduced response (imaging and PDT, not shown). The stability of the drugs in PAA nanoparticle was established by repeated washing with aqueous bovine calf serum through Amicon centrifugal filter units with a 100KDa or larger cut off membrane and drug in the filtrate was measured spectrophotometrically. The comparative *in vivo* PDT efficacy of the ORMOSIL and PAA formulations, their tumor imaging potential and stability (*in vitro* release kinetics) is shown in **Figs. 2-4**, which clearly illustrate the advantages of PAA nanoparticles in reducing the therapeutic dose by almost 8- fold without diminishing the tumor-imaging potential and also avoiding the Tween-80 formulation required for the HPPH-CD conjugate **1**. In the 2nd approach the HPPH CD conjugate **1** was post-loaded to PAA nanoparticles, which certainly enhanced the tumorimaging, but the therapeutic dose was still 10-fold higher (similar to the HPPH CD conjugate, **Fig. 5**). In the 3rd approach the cyanine dye was conjugated peripherally to the PAA nanoparticles first and then HPPH was post loaded. Again, compared to HPPH-CD conjugate **1**, the PAA formulation showed enhanced tumor-specificity (imaging) (**Fig.5**).

Effect of nanoparticles on tumor selectivity

[0022] A photosensitizer (photosensitizer) with increased selectivity and longer wavelength could be a more suitable candidate for brain and deeply seated tumors (especially breast, brain and lung). The evolution of light sources and delivery systems is also critical to the progression of photodynamic therapy (PDT) in the medical field. Two different techniques: interstitial and intracavitary light delivery have been used for treatment of brain tumors. Powers using interstitial PDT on patients with recurrent brain tumors showed that the majority of patients had tumor recurrence within two months of treatment. However, it was later observed that treatment failures appeared to occur outside the region of the effective light treatment. Chang et al reported an effective radius of tumor cell kill in 22 glioma patients of 8 mm compared with the 1.5 cm depth of necrosis noted by Pierria with the intracavitary illumination method. It is believed that tumor resection is important so that the numbers of tumor cells remaining to treat are minimized. With stereotactic implantation of

fibers for interstitial PDT there is no cavity to accommodate swelling and a considerable volume of necrotic tumor which causes cerebral edema. However, cerebral edema can be readily controlled with steroid therapy. Compared to chemotherapy and radiotherapy, patients with brain tumors treated with PDT have definitely shown long-term survival, whereas glioma patients treated with adjuvant chemotherapy or radiotherapy do not seem to show additional benefits. as On the basis of our preliminary data, the $\alpha v \beta 3$ targeted nanoparticles may improve tumor-selectivity and PDT outcome.

PET imaging and PDT: PAA nanoparticles decreased the liver uptake of the ^{124}I -photosensitizer (PET imaging agent) and enhanced the tumor-specificity

[0023] Our initial investigation with an ^{124}I -labeled photosensitizer **2** indicates its *in vivo* PDT efficacy and capability of detecting tumors 104-106 (RIF, Colon26, U87, GL261, pancreatic tumor xenograft) and tumor metastases (BALB/c mice bearing orthotopic 4T1 (breast) tumors) (**Fig 6**). Interestingly, compared to ^{18}F FDG photosensitizer **2** showed enhanced contrast in most of the tumors including those where ^{18}F FDG-PET provides limited imaging potential (e.g., brain, lung and pancreatic tumors). See **Fig. 7** for comparative biodistribution. This is the first report showing the utility of porphyrin-based compounds as a “BIFUNCTIONAL AGENT” for imaging breast tumor and tumor metastasis. Similar to most nanoparticles, PAA nanoparticle accumulate in liver and spleen. Their clearance rate from most organs is significantly faster than Ormosil nanoparticle and they do not show long-term organ toxicity. Even tumor-avid porphyrinbased photosensitizer exhibit high uptake in liver and spleen, but are non-toxic until exposed to light. The photosensitizer clear from the system quickly (days) without organ toxicity. However, radioactive photosensitizer such as the ^{124}I -labeled analog **2** (superior to ^{18}F -FDG in PET-imaging of lung, brain, breast and pancreas tumors) with a $T_{1/2}$ of 4.2 days could cause radiation damage to normal organs. Based on the observation of high uptake of PAA nanoparticles in liver and spleen (below) we postulated that saturating the organs with the non-toxic PAA nanoparticles before injecting the PET agent might reduce uptake and radiation damage by ^{124}I - imaging agent. For proof-of principle blank PAA nanoparticles were first injected (i.v.) into mice bearing Colon26 tumors followed 24 h later by i.v. ^{124}I -analog (100- 50 μCi). The mice were imaged at 24, 48 and 72h post injection and biodistribution studies were performed at each time point summarized in **Figure 8** (only 72h images shown).

[0024] The presence of PAA nanoparticles made a remarkable difference in tumor contrast with brain, lung and pancreatic tumors). See **Fig. 7** for comparative biodistribution.

PAA nanoparticles can be targeted to nucleolin with F3-Cys:

[0025] F3-targeted nanoparticles were prepared using two kinds of F3 peptides: F3 peptide conjugated to nanoparticle *via* one of the 8 lysines available in its sequence and F3-Cys peptide conjugated to nanoparticle *via* cysteine. Cysteine capped nanoparticles served as non-targeted control. Three 25 mg batches of each type of nanoparticle contained: 2.6, 5.1 and 7.7 mg F3, (**A3-A5**) respectively; 2.7, 5.3 and 8 mg F3-Cys (**B3-B5**) respectively, and 0.29, 0.58 and 0.87 mg Cys (**C3-C5**) respectively. The fluorescence intensity from PAA nanoparticle incubated *in vitro* with nucleolin positive MDA-MB-435 cells is shown in **Fig. 9**. The F3-Cys conjugated nanoparticles show considerably higher binding efficiency than non-targeted nanoparticles, while F3 conjugated nanoparticles do not. Conjugation via a cysteine link preserves the specificity of F3 peptide for nucleolin. In addition excess cysteine on the nanoparticles help photosensitizer to minimize the non-specific binding. Additional experiments (not shown) suggested that the amount of F3-Cys peptide (5.3 mg/25mg nanoparticle) used for B4 nanoparticles was optimal.

[0026] ***Optical properties of post-loaded PAA nanoparticles.*** The absorption spectrum of PAA nanoparticles post-loaded with both HPPH and cyanine dye (even at 0.5 mg/ml), clearly shows characteristic signatures for both the photosensitizer and dye, without aggregation-induced broadening, while the fluorescence spectrum shows strong signals from both components.

HPPH conjugated PAA nanoparticles with F3-Cys peptide at the outer surface show targeted specificity:

[0027] F3-mediated specificity is retained in the presence of conjugated HPPH. F3 targeted nanoparticles did not, indicating that F3-mediated specificity is retained in the presence of conjugated HPPH. F3 targeted nanoparticles did not accumulate in the nucleus. On activation of cells with light at 660 nm only F3-targeted nanoparticle caused cell kill (**Fig 11**). Cell internalization of F3- targeted nanoparticles was confirmed by fluorescence confocal microscopy.

HPPH conjugated PAA nanoparticles with F3-Cyspeptide at the outer surface show targeted specificity:

[0028] The specificity of targeted nanoparticles was tested by fluorescent imaging (**Fig. 10**). F3 targeted HPPH conjugated PAA nanoparticle specifically bound to MDA-MB-435 cells (expressing nucleolin) while non-targeted nanoparticles did not, indicating that F3-mediated specificity is retained in the presence of conjugated HPPH. F3 targeted nanoparticles did not accumulate in the nucleus. On activation of cells with light at 660 nm

only F3-targeted nanoparticle caused cell kill (Fig 11). Cell internalization of F3-targeted nanoparticles was confirmed by fluorescence confocal microscopy.

F3-Cys shows target-specificity in 9L glioma cells:

[0029] Similar to F3-cys, a pegylated form of F3-Cys PEG on PAA nanoparticles also
5 showed remarkable target-specificity in 9L rat glioma cells which also expresses nucleolin,
Fig 11. (Note: HPPH is replaced with a Rhodamine moiety).

Biodistribution studies: PAA nanoparticle Enhances tumor uptake of HPPH:

[0030] The biodistribution of 14C-HPPH and 14C-HPPH post-loaded PAA
nanoparticle was performed in BALB/c mice bearing Colon26 tumors at 24, 48 and 72 h post
10 injection (3 mice/time point) and the results are summarized in **Fig. 12**. As can be seen
presence of PAA nanoparticles made a significant increase in tumor uptake with reduced
uptake in other organs.

Size of PAA nanoparticles made remarkable difference in tumor-enhancement:

[0031] The biodistribution of 124I-photosensitizer was investigated using variable
15 sizes of nanoparticles either injecting the nanoparticles first and then administering the
labeled photosensitizer or postloading the labeled photosensitizer to PAA nanoparticles and
then perform in vivo biodistribution in mice at 24, 48 and 72 h. The results summarized in
Figure 13 clearly indicate that the size of PAA nanoparticles makes a significant impact in
tumor enhancement. Experiments related to in vivo PDT efficacy of these formulations are
20 currently in progress.

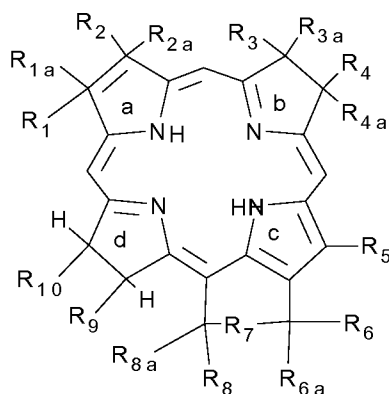
[0032] This invention shows the utility of porphyrin-based compounds in a
“bifunctional agent” for imaging breast tumor and tumor metastasis. Similar to most
nanoparticles, PAA nanoparticle accumulate in liver and spleen. Their clearance rate from
most organs is significantly faster than Ormosil nanoparticle and they do not show long-term
25 organ toxicity. Even tumor-avid porphyrin based photosensitizer exhibit high uptake in liver
and spleen, but are non-toxic until exposed to light. The photosensitizer clear from the system
quickly (days) without organ toxicity. However, radioactive photosensitizer such as the 124I-
labeled analog **2** (superior to 18F-FDG in PET-imaging of lung, brain, breast and pancreas
tumors) with a $T_{1/2}$ of 4.2 days could cause radiation damage to normal organs. Based on the
30 observation of high uptake of PAA nanoparticles in liver and spleen (below) we postulated
that saturating the organs with the non-toxic PAA nanoparticles before injecting the PET
agent might reduce uptake and radiation damage by 124I- imaging agent. For proof-of
principle blank PAA nanoparticles were first injected (i.v.) into mice bearing Colon26 tumors
followed 24 h later by i.v. 124I-analog (100-150 μ Ci). The mice were imaged at 24, 48 and

72h post injection and biodistribution studies were performed at each time point summarized in **Figure 8** (only 72h images shown).

[0033] The presence of PAA nanoparticles makes a remarkable difference in tumor contrast with significantly reduced uptake in spleen and liver and improved tumor-uptake/contrast at 24, 48 and 72 h post injection (3 mice/group Similar studies (tumor-imaging and PDT efficacy) in which the labeled photosensitizer is post-loaded to variable sizes. Similar studies (tumor-imaging and PDT efficacy) in which the labeled photosensitizer is post-loaded to variable sizes PAA nanoparticles are currently in progress.

What is claimed is:

1. A composition comprising PAA nanoparticles containing a tetrapyrrolic photosensitizer and an imaging agent.
2. The composition of claim 1 wherein the tetrapyrrolic photosensitizer has the structural formula:



10

or a pharmaceutically acceptable derivative thereof, wherein:

- R_1 and R_2 are each independently substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, $-C(O)R_a$ or $-COOR_a$ or $-CH(CH_3)(OR_a)$ or $-CH(CH_3)(O(CH_2)_nXR_a)$ where R_a is hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, or substituted or unsubstituted cycloalkyl; where R_2 may be $-CH=CH_2$, $-CH(OR_{20})CH_3$, $-C(O)Me$, $-C(=NR_{21})CH_3$ or $-CH(NHR_{21})CH_3$

15

where X is an aryl or heteroaryl group;

n is an integer of 0 to 6;

where R_{20} is methyl, butyl, heptyl, docetyl or 3,5-bis(trifluoromethyl)-benzyl; and

20

R_{21} is 3,5-bis(trifluoromethyl)benzyl;

R_{1a} and R_{2a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form a covalent bond;

R_3 and R_4 are each independently hydrogen or substituted or unsubstituted alkyl;

R_{3a} and R_{4a} are each independently hydrogen or substituted or unsubstituted alkyl, or

25

together form a covalent bond;

R_5 is hydrogen or substituted or unsubstituted alkyl;

R_6 and R_{6a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form =O;

R_7 is a covalent bond, alkylene, azaalkyl, or azaaraalkyl or =NR₂₀ where R₂₀ is 3,5-bis(tri-fluoromethyl)benzyl or -CH₂X-R¹ or -YR¹ where Y is an aryl or heteroaryl group;

5 R_8 and R_{8a} are each independently hydrogen or substituted or unsubstituted alkyl or together form =O;

R_9 and R_{10} are each independently hydrogen, or substituted or unsubstituted alkyl and R_9 may be -CH₂CH₂COOR² where R² is an alkyl group that may optionally substituted with one or more fluorine atoms;

10 each of R_1 - R_{10} , when substituted, is substituted with one or more substituents each independently selected from Q, where Q is alkyl, haloalkyl, halo, photosensitizer pseudohalo, or -COOR_b where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, araalkyl, or OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl or CONR_dR_e where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or
15 NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or =NR_h where R_h is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or is an amino acid residue;

each Q is independently unsubstituted or is substituted with one or more substituents each independently selected from Q₁, where Q₁ is alkyl, haloalkyl, halo, photosensitizer pseudohalo, or -COOR_b where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, araalkyl, or OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl or CONR_dR_e where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or =NR_h where R_h is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or is an amino acid residue.
25

3. The composition of claim 2 wherein the photosensitizer is post loaded onto the nanoparticle after nanoparticle formation.

30 4. The composition of claim 3 wherein the imaging agent is a cyanine dye.

5. The composition of claim 3 wherein the imaging agent is a ¹²⁴I labeled compound.

6. The composition of claim 3 wherein the imaging agent is a PET, fluorescence or MR imaging agent.

7. The composition of claim 6 wherein the nanoparticle contains a targeting moiety.

5

8. The composition of claim 7 wherein the targeting moiety is a peptide, folic acid or a carbohydrate.

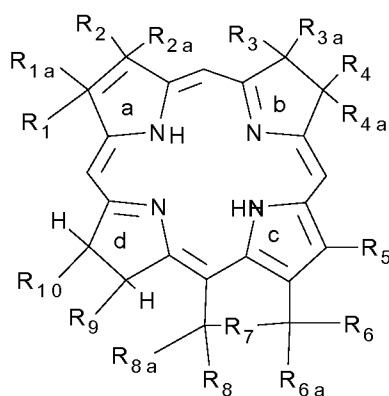
9. A method for imaging comprising:

10 injecting PAA nanoparticles containing post loaded photosensitizer into a subject,
injecting an imaging agent, and
imaging the subject at 24, 48 and 72 h post injections.

10. A method for making PAA nanoparticle's containing a photosensitizer and an
15 imaging agent by post loading a photosensitizer and a fluorophore onto a preprepared PAA nanoparticle.

11. The method of claim 10 where the photosensitizer has the structural formula:

20



or a pharmaceutically acceptable derivative thereof, wherein:

25 R_1 and R_2 are each independently substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, $-C(O)R_a$ or $-COOR_a$ or $-CH(CH_3)(OR_a)$ or $-CH(CH_3)(O(CH_2)_nXR_a)$ where R_a is hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl,

substituted or unsubstituted alkynyl, or substituted or unsubstituted cycloalkyl; where R_2 may be $-\text{CH}=\text{CH}_2$, $-\text{CH}(\text{OR}_{20})\text{CH}_3$, $-\text{C}(\text{O})\text{Me}$, $-\text{C}(=\text{NR}_{21})\text{CH}_3$ or $-\text{CH}(\text{NHR}_{21})\text{CH}_3$

where X is an aryl or heteroaryl group;

n is an integer of 0 to 6;

5 where R_{20} is methyl, butyl, heptyl, docecyl or 3,5-bis(trifluoromethyl)-benzyl; and

R_{21} is 3,5-bis(trifluoromethyl)benzyl;

R_{1a} and R_{2a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form a covalent bond;

R_3 and R_4 are each independently hydrogen or substituted or unsubstituted alkyl;

10 R_{3a} and R_{4a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form a covalent bond;

R_5 is hydrogen or substituted or unsubstituted alkyl;

R_6 and R_{6a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form $=\text{O}$;

15 R_7 is a covalent bond, alkylene, azaalkyl, or azaaraalkyl or $=\text{NR}_{20}$ where R_{20} is 3,5-bis(tri-fluoromethyl)benzyl or $-\text{CH}_2\text{X}-\text{R}^1$ or $-\text{YR}^1$ where Y is an aryl or heteroaryl group;

R_8 and R_{8a} are each independently hydrogen or substituted or unsubstituted alkyl or together form $=\text{O}$;

R_9 and R_{10} are each independently hydrogen, or substituted or unsubstituted alkyl and

20 R_9 may be $-\text{CH}_2\text{CH}_2\text{COOR}^2$ where R^2 is an alkyl group that may optionally substituted with one or more fluorine atoms;

each of R_1 - R_{10} , when substituted, is substituted with one or more substituents each independently selected from Q, where Q is alkyl, haloalkyl, halo, photosensitizer pseudohalo, or $-\text{COOR}_b$ where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, araalkyl, or OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl or CONR_dR_e where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or $=\text{NR}_h$ where R_h is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or is an amino acid residue;

30 each Q is independently unsubstituted or is substituted with one or more substituents each independently selected from Q_1 , where Q_1 is alkyl, haloalkyl, halo, photosensitizer pseudohalo, or $-\text{COOR}_b$ where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, araalkyl, or OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl or CONR_dR_e where R_d and R_e are each independently hydrogen, alkyl,

alkenyl, alkynyl, cycloalkyl, or aryl, or NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or $=\text{NR}_h$ where R_h is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or is an amino acid residue.

- 5 12. A method for making PAA nanoparticle's by conjugating a fluorophore to a PAA nanoparticle and then postloading a photosensitizer to the PAA nanoparticle.

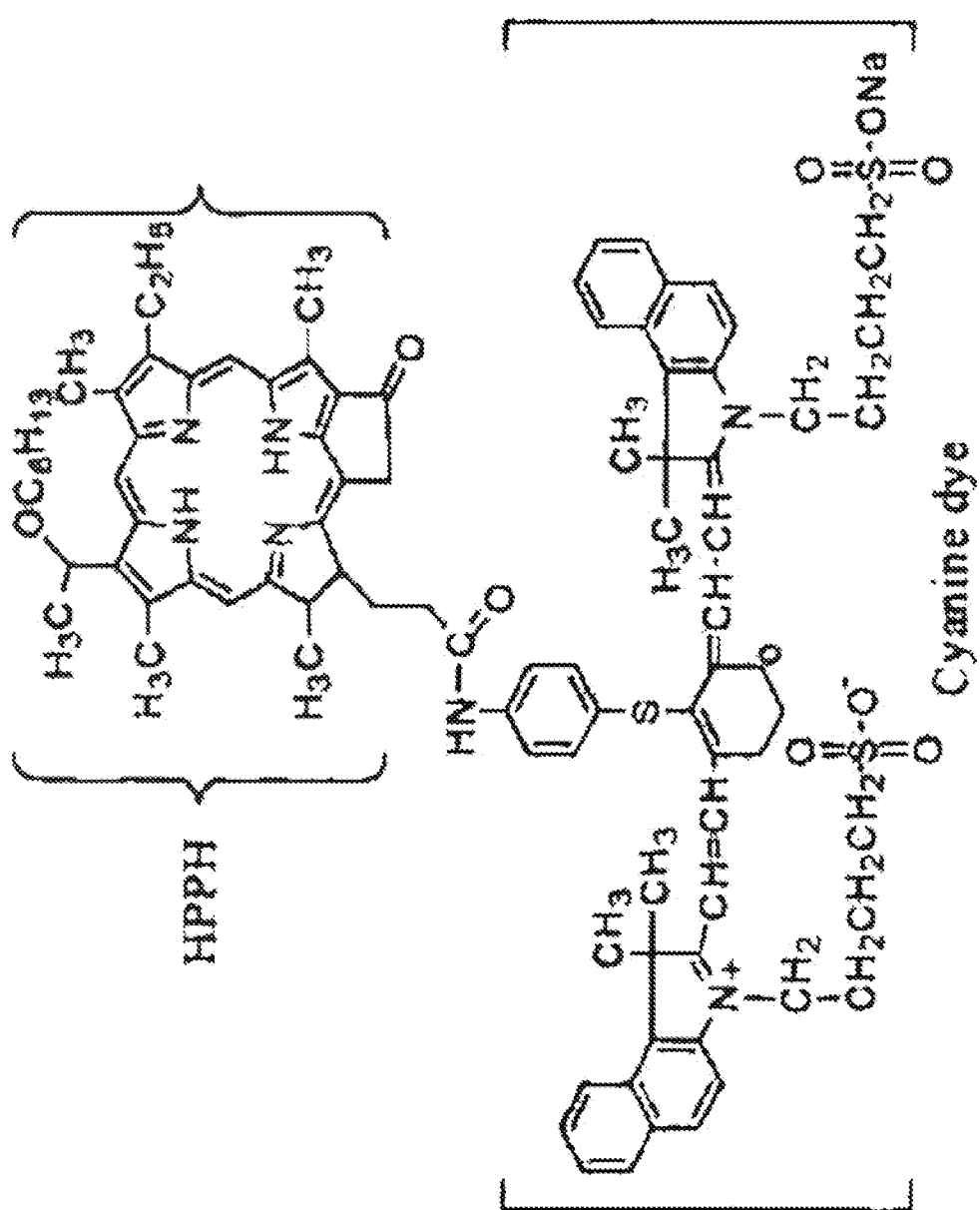


Fig. 1

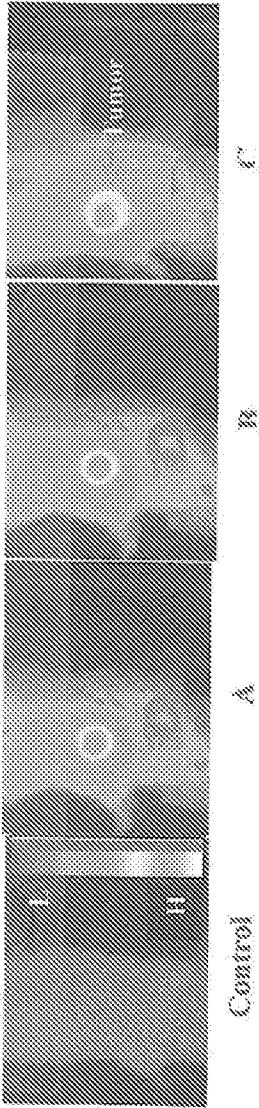


FIGURE 2

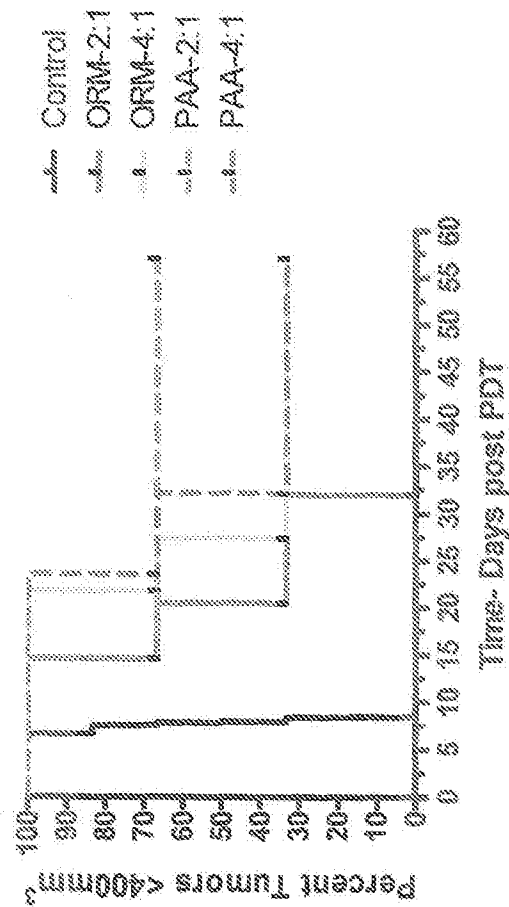


FIGURE 3

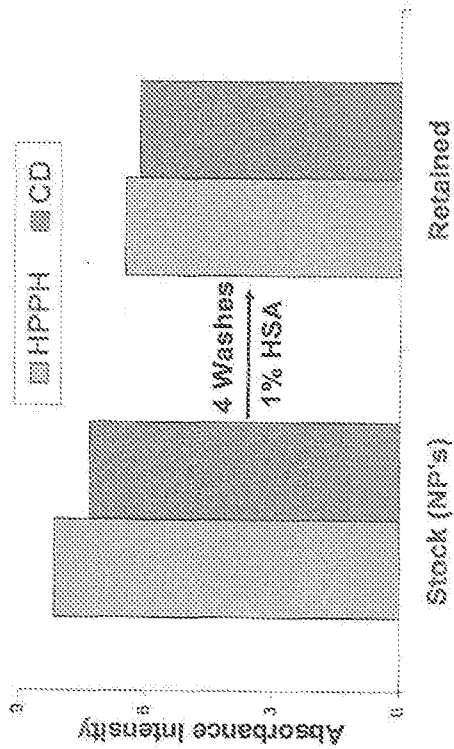


FIGURE 4

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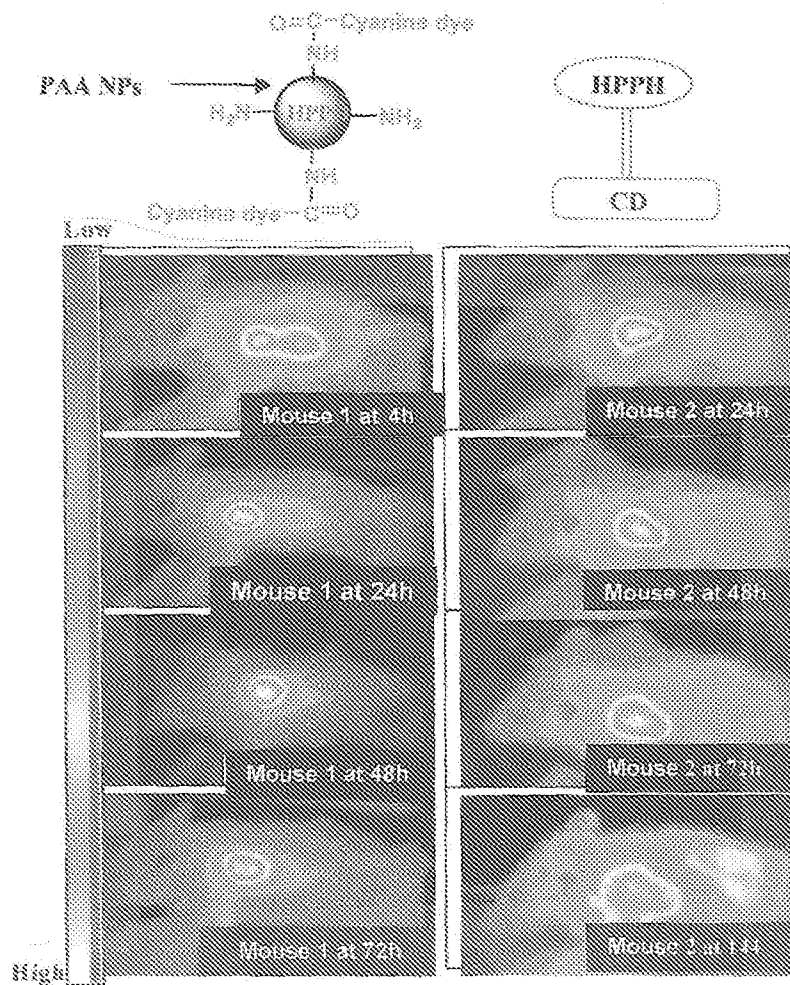


FIGURE 5

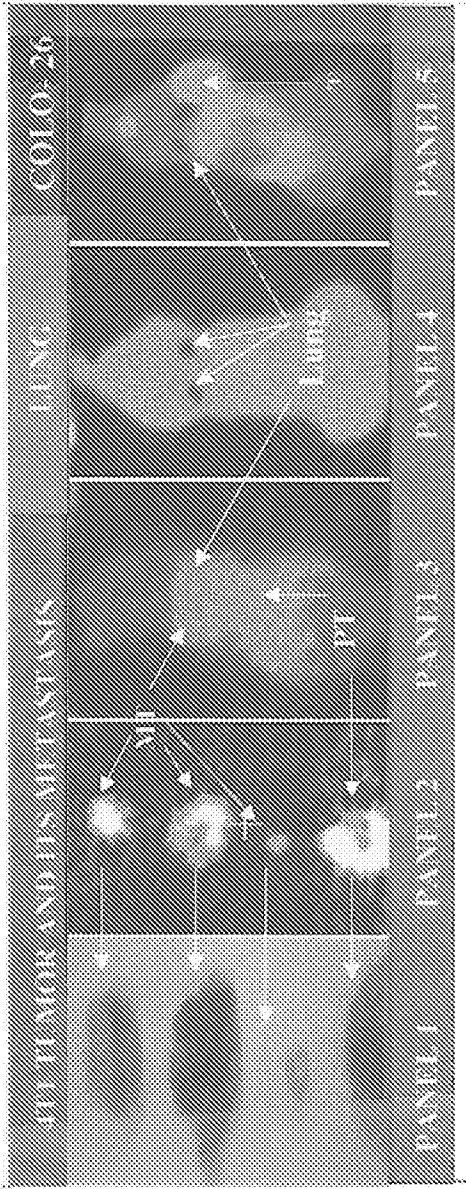


FIGURE 6

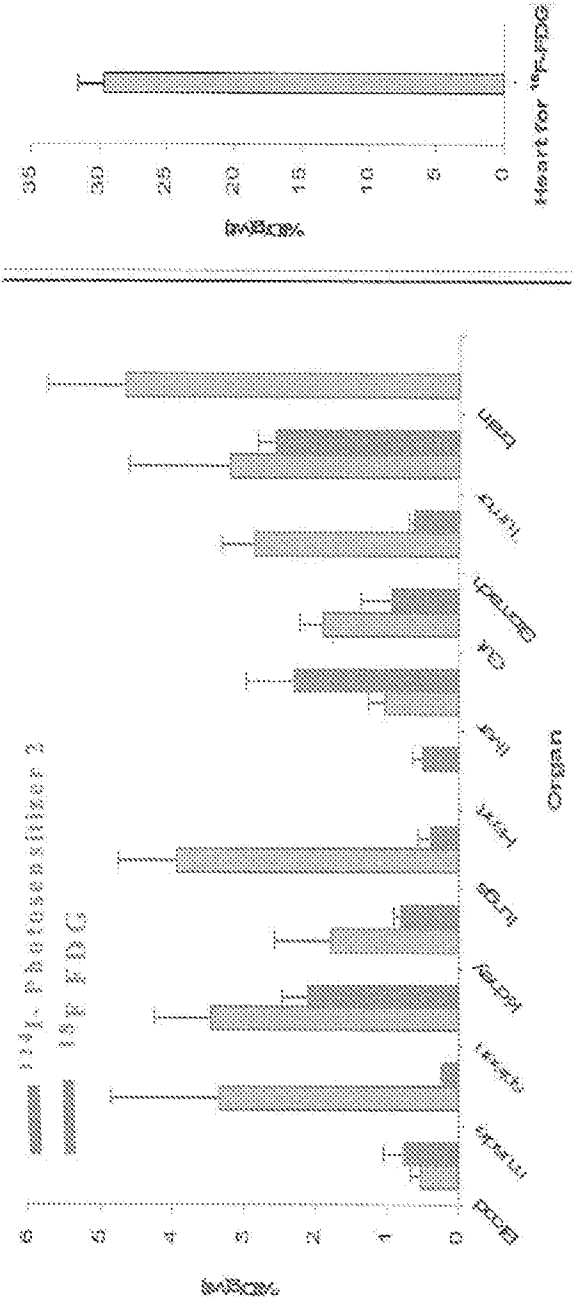


FIGURE 7

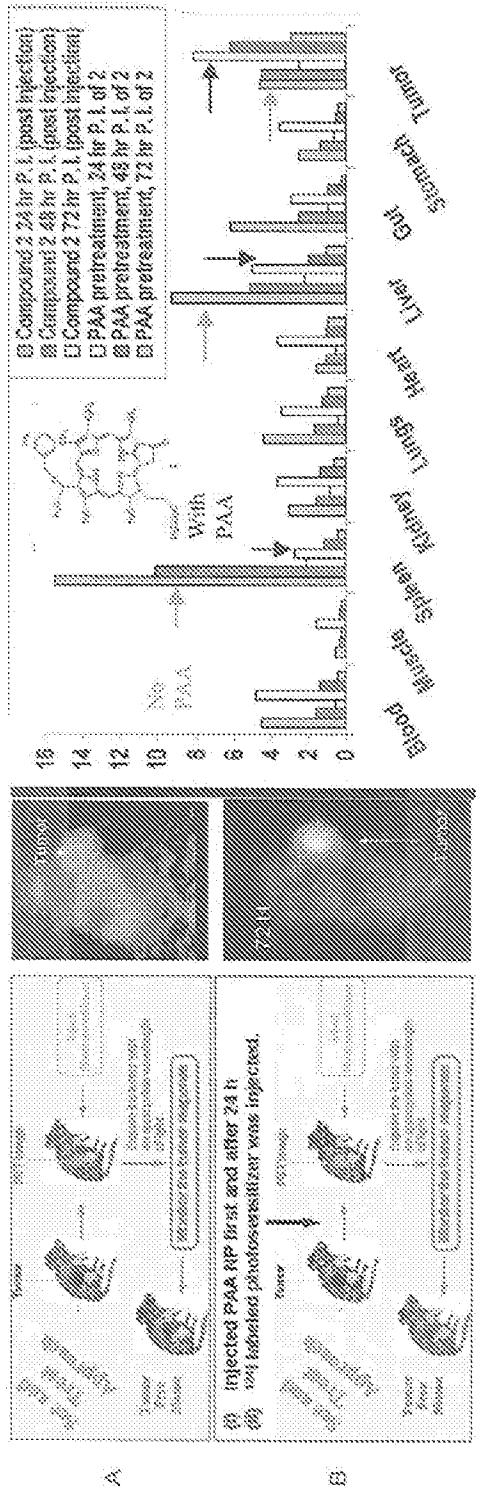


FIGURE 8

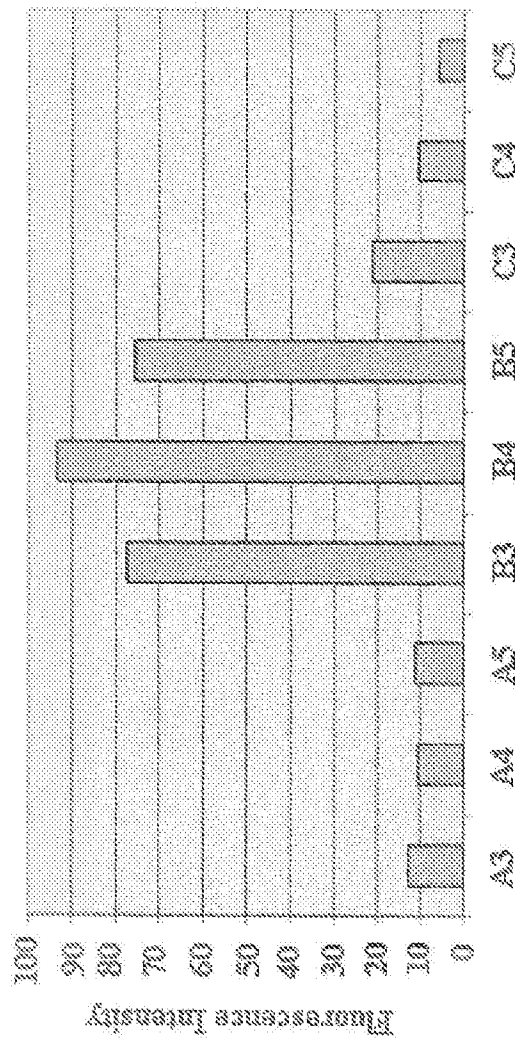


FIGURE 9

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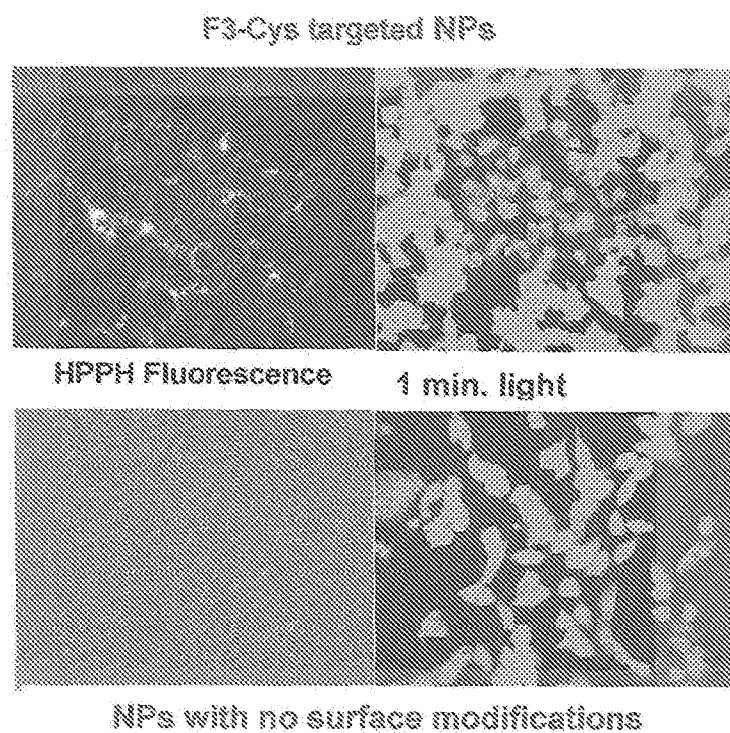


FIGURE 10

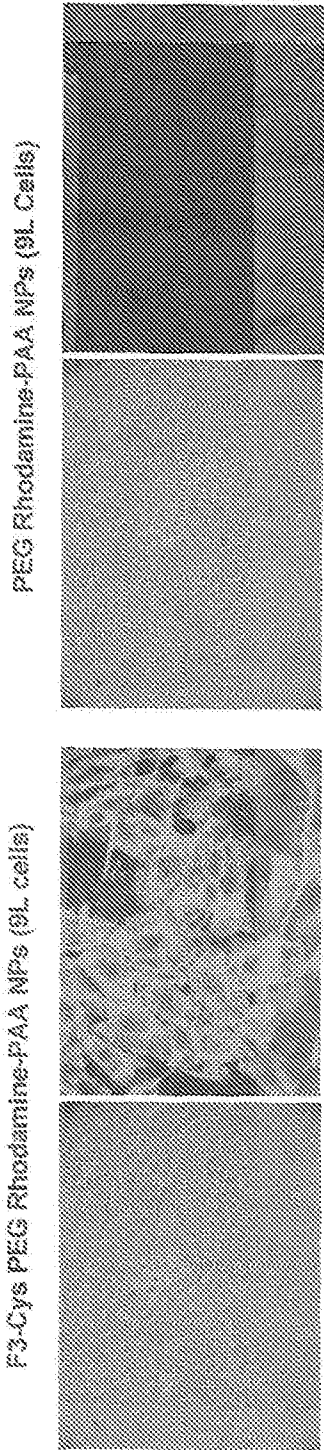


FIGURE 11

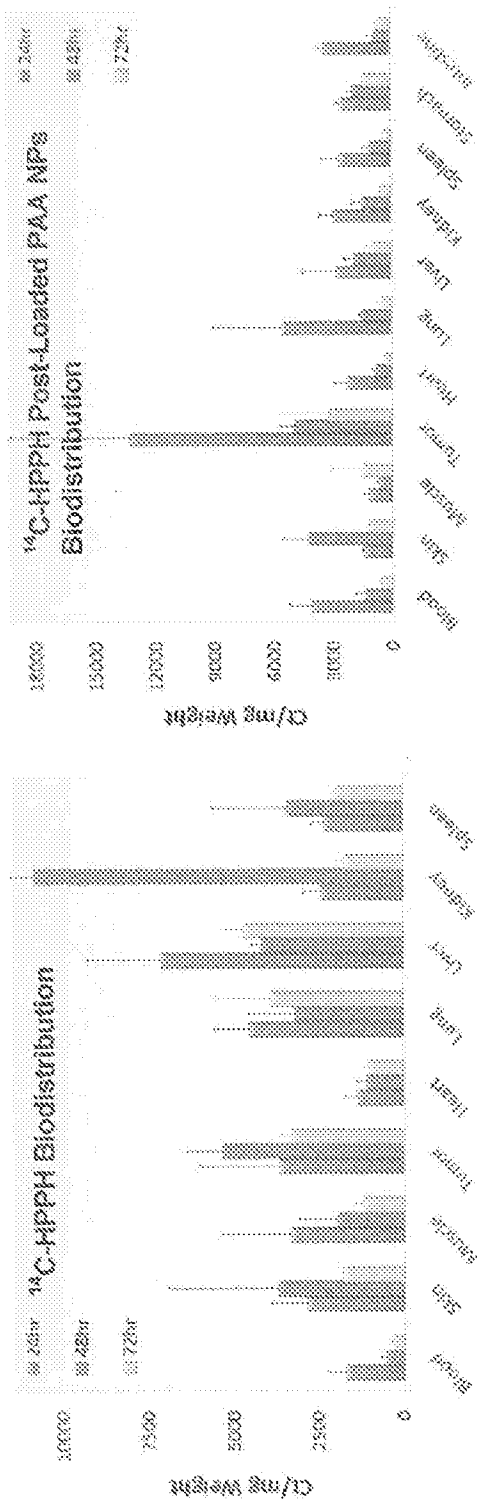


FIGURE 12

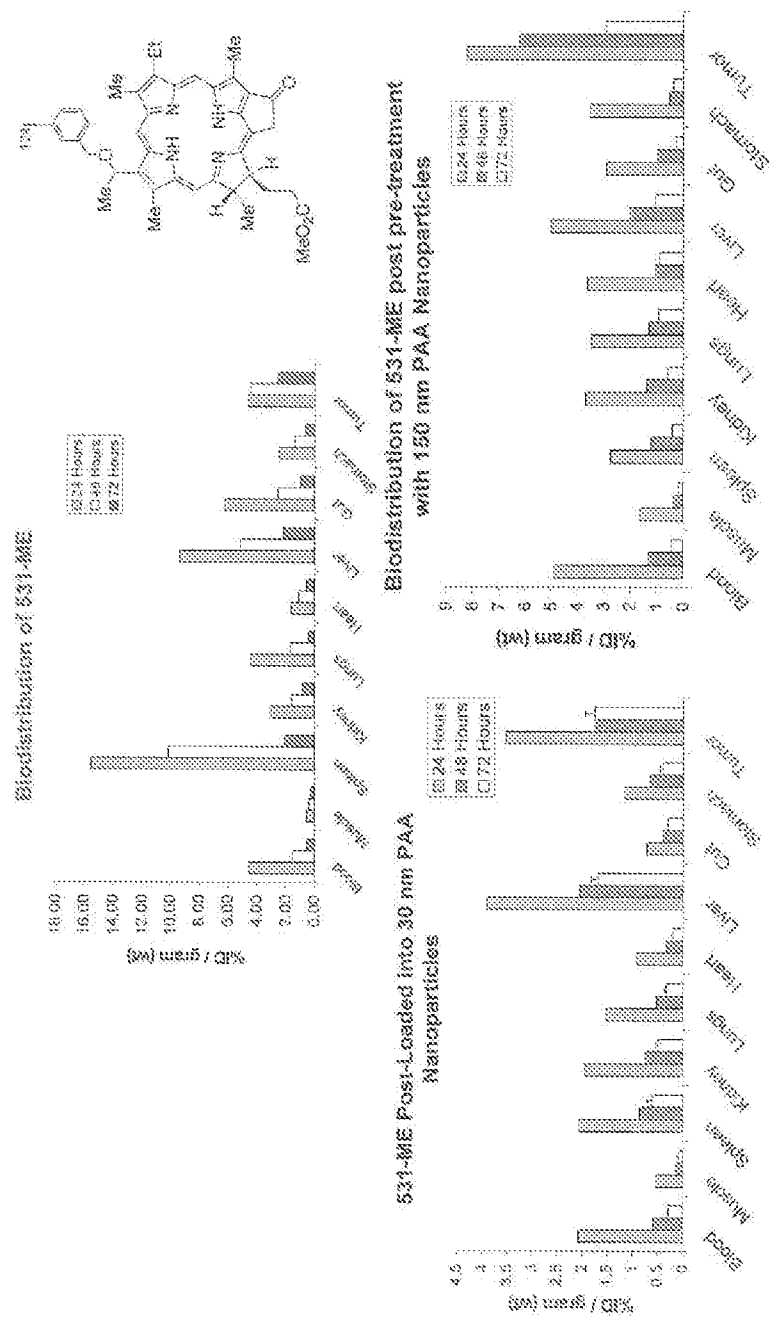


FIGURE 13

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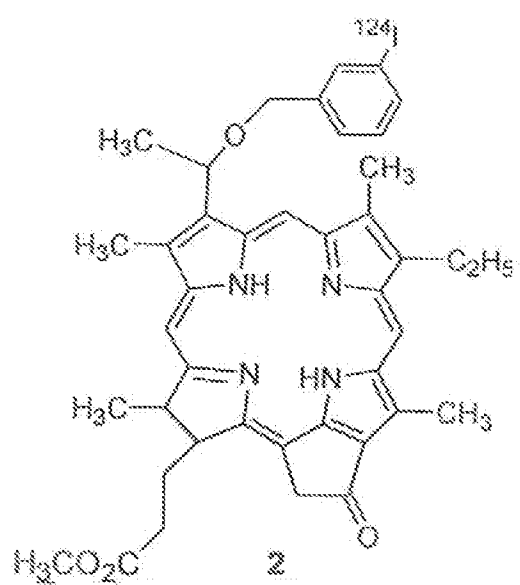


FIGURE 14

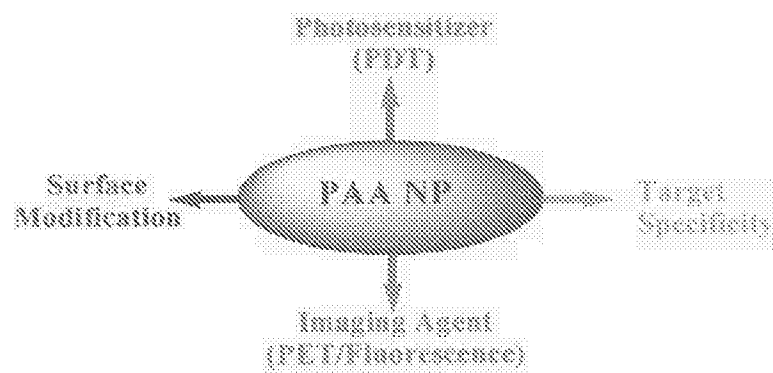
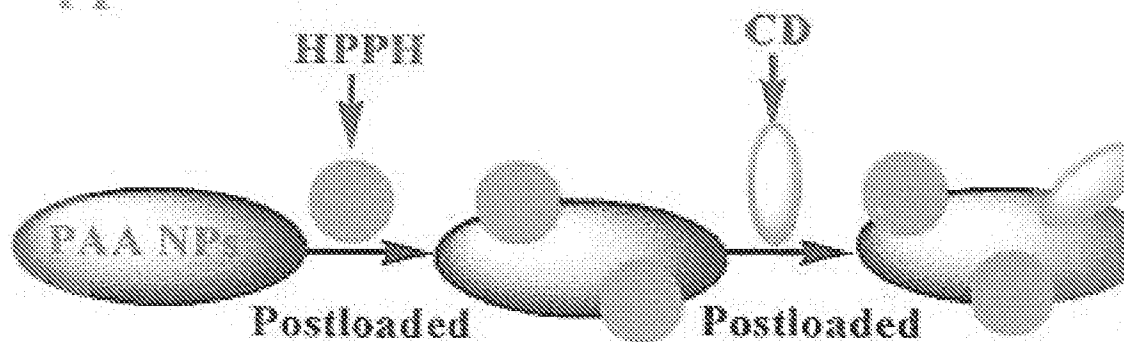


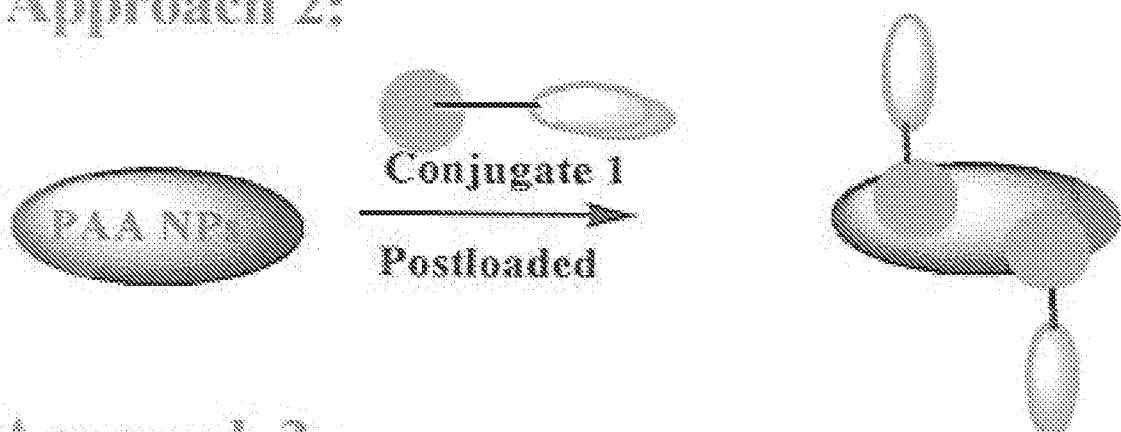
FIGURE 15

Approach 1:

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Approach 2:



Approach 3:

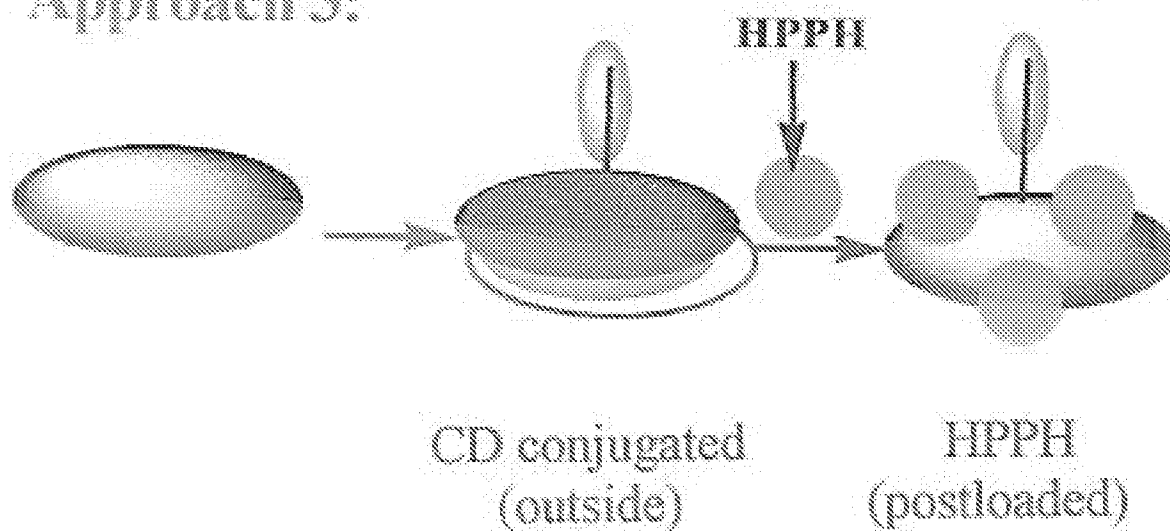


FIGURE 16

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 10/53574

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61B 5/00 (2010.01)

USPC - 424/9.61 ; 424.9.362

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)

USPC- 424/9.61; 424.9.362

IPC(8): A61B 5/00 (2010.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC- 424/9.3; 514/279; 514/410; 540/145; 424/9.6; 534/14; 534/15; 424/1.11; 534/11; 536/17.4 (keywords below)

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

PubWEST (PGPB, USPT, USOC, EPAB, JPAB), Google Scholar/Patents: polyacrylic acid nanoparticles imaging agents tumor cyanine photosensitizer tetrapyrrolic pdt PET PAA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2009/105209 A1 (PANDEY et al) 27 August 2009 (27.08.2009) para [0011], [0013], [0023], [0025]-[0027], [0029], [0031], [0034], [0054], [0057], [0066], [0072]-[0073], [0077]-[0078], [0082]-[0085]; pg 11, ln 25-33; pg 12, ln 1-16; Fig 25	1-12
Y	WO2009/061473 A1 (NEUMANN et al) 14 May 2009 (14.05.2009) para [0019]	1-12
Y	US 2006/0258629 A1 (FREEMAN) 16 November 2006 (16.11.2006) para [0172]-[0173]	9

☐ Further documents are listed in the continuation of Box C.



* 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

16 December 2010 (16.12.2010)

Date of mailing of the international search report

29 DEC 2010

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

专利名称(译)	PAA纳米粒子用于增强肿瘤成像		
公开(公告)号	EP2437653A1	公开(公告)日	2012-04-11
申请号	EP2010825681	申请日	2010-10-21
[标]申请(专利权)人(译)	健康研究股份有限公司 密歇根大学		
申请(专利权)人(译)	健康研究，INC. 研究基金会纽约州立大学 MICHIGAN OFFICE技术转让的大学董事会		
当前申请(专利权)人(译)	健康研究，INC. 研究基金会纽约州立大学 MICHIGAN OFFICE技术转让的大学董事会		
[标]发明人	PANDEY RAVINDRA K GUPTA ANURAG SAJJAD MUNAWWAR KOPELMAN RAOUL		
发明人	PANDEY, RAVINDRA, K. GUPTA, ANURAG SAJJAD, MUNAWWAR KOPELMAN, RAOUL		
IPC分类号	A61B5/00 A61K41/00 A61K47/48 A61K49/00 A61K51/04 A61K51/12 B82Y15/00		
CPC分类号	A61K41/0071 A61K47/60 A61K47/64 A61K49/0002 A61K49/0032 A61K49/0052 A61K49/0093 A61K51/0451 A61K51/1255 B82Y15/00		
优先权	61/279522 2009-10-21 US		
其他公开文献	EP2437653A4 EP2437653B1		
外部链接	Espacenet		

摘要(译)

包含PAA纳米颗粒的组合物，所述PAA纳米颗粒含有负载后的四吡咯光敏剂和显像剂，以及制备和使用它们的方法。