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(54) **PAA NANOPARTICLES FOR ENHANCEMENT OF TUMOR IMAGING**

PAA-NANOPARTIKEL FÜR ERWEITERTE TUMOR-BILDGEBUNG

NANOPARTICULES PAA PERMETTANT D'AMÉLIORER L'IMAGERIE TUMORALE

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Description

BACKGROUND OF THE INVENTION

[0001] Nanoscience is being developed in conjunction with advanced medical science for further precision in diagnosis and treatment. Nanoplatforms and nanovectors that deliver a therapeutic or imaging agent for biomedical applications show promise for cancer diagnosis and therapy. Therapeutic examples include nanoparticle containing PDT agents, folate receptor-targeted, boron containing dendrimers for neutron capture and nanoparticle-directed thermal therapy.

[0002] Nanoparticles have had disadvantages when considered for use in photodynamic therapy (PDT). In particular, certain nanoparticles have no relatively large 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, leaching of small hydrophilic components unless they are "anchored", and unknown limitation on bulk tumor permeability because of hydrogel swelling.

[0003] 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.

[0004] In efforts to develop effective photosensitizers with the required photophysical characteristics, compounds haying a tetrapyrollic core ring were used. Usually, chlorophyll-a and bacteriochlorophyll-a were used as intermediates in sythesis. 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 bacteriopurpurinimde (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.

[0005] 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.

[0006] 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 mission 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.

[0007] 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.

[0008] 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.

[0009] ROSS. B. ET AL.: ,PHOTONIC AND MAGNETIC NANOEXPLORERS FOR BIOMEDICAL USE: FROM SUBCELLULAR IMAGING TO CANCER DIAGNOSTIC AND THERAPY, vol. 5331, 2004, pages 76-83,discloses PAA (polyacrylamide) nanoparticles comprising superparamagnetic iron oxide crystals, photofrin and being conjugated to RGD peptides which are used for photodynamic therapy followed by MRI imaging.

[0010] 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 18F), *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

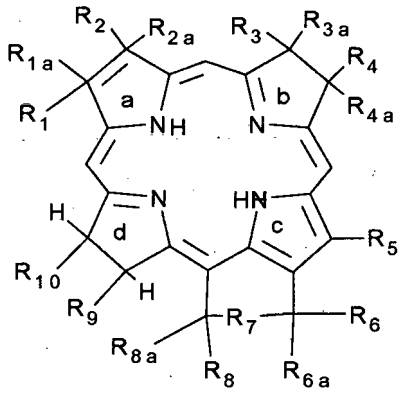
[0011] The present invention relates to polyacrylic acid (PAA) nanoparticles containing a photosensitizer and an imaging enhancing agent.

[0012] In accordance with the invention, therapeutic and imaging potential of encaphotosensitizerulated, 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 functionalized, to permit attachment of targeting ligands, and both are stable to singlet oxygen (102) 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 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 limitation on bulk tumor permeability because of hydrogel swelling.

[0013] In accordance with the invention, photosensi-

tizers 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 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 encaphotosensitizerulated 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 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 optimal plasma pharmacokinetics. Thus, they can deliver high levels of photosensitizer to tumors, reducing the amount of light necessary for tumor cure.

[0014] The photosensitizer is preferably a tetrapyrollic photosensitizer having the structural formula:



or a phamaceutically 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 $-CH(NHR_{21})CH_3$

where X is an aryl or heteroaryl group;

n is an integer of 0 to 6;

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;

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 =O;

R_7 is a covalent bond, alkylene, azaalkyl, or azaaraalkyl or $=NR_{20}$ where R_{20} is 3,5-bis(tri-fluoromethyl)benzyl or $-CH_2X-R^1$ or - 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 =O;

R_9 and R_{10} are each independently hydrogen, or substituted or unsubstituted alkyl and R_9 may be $-CH_2CH_2COOR$ 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, photosensitizereudohalo, 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 Rg 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 Q_1, where Q_1 is alkyl, haloalkyl, halo, photosensitizereudohalo, 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 Rg 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.

[0015] 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.

[0016] 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 herein as background art.

[0017] 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

[0018]

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 μmole/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 μmol/kg) at the images were obtained at variable time points. **A** = 24 h, **B** = 48 h and **C** = 72 h post injection (λex: 785 nm; λEm: 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 μmol/kg in PAA nanoparticles and 0.78 μmol/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 57Co 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 124I- 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 18F-FDG (100 μCi, half-life 2 h) at 110 min and 124I-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 124I-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 124I-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 μCi/0.2 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

[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 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.

[0020] We have previously shown that certain tumor-avid photosensitizer(s) (e. g., 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 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 photodestruction. 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 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 μmol/kg) and the imaging dose of Cyanine dye (0.27 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 124I-photosensitizer (PET imaging agent) and enhanced the tumor-specificity

[0023] Our initial investigation with an 124I-labeled photosensitizer **2** indicates its *in vivo* PDT efficacy and capability of detecting tumors104-106 (RIF, Colon26, U87, GL261, pancreatic tumor xenograft)) and tumor metastases (BALB/c mice bearing orthotopic 4T1 (breast) tumors) (**Fig 6**). Interestingly, compared to 18F FDG photosensitizer 2 showed enhanced contrast in most of the tumors including those where 18F 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 124I-labeled analog 2 (superior to 18F-FDG in PET-imaging of lung, brain, breast and pancreas tumors) with a T½ 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 nontoxic 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 (100-50μCi). 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 nontargeted 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 helphotosensitizer 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 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 nontargeted 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 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 biodistbiodistribution 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 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 1241-photosensitizer was investigated using variable sizes of nanoparticles either injecting the nanoparticles first and then administrating 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 currently in progress.

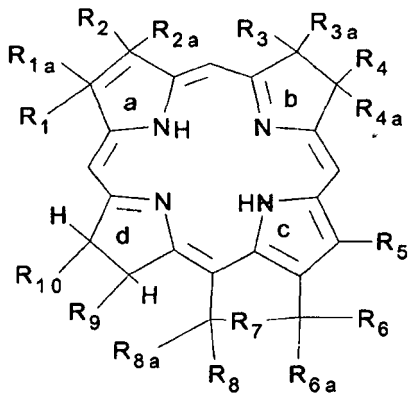
[0032] This invention shows the utility of porphyrin-based compounds in a "bifunctional agent" for imaging breast tumor and tumor metastasis. Similar to most nanoparticles, PAA nanoparticle accumulate in liver and spleen. Their clearance rate from most organs is significantly faster than Ormosil nanoparticle and they do not show long-term 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 T½ 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 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 (100-150μCi). 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.

Claims

1. A composition comprising polyacrylic acid (PAA) nanoparticles containing a post loaded tetrapyrollic photosensitizer that is post loaded onto the nanoparticle after nanoparticle formation and an imaging agent.

2. The composition of claim 1 wherein the post loaded tetrapyrollic photosensitizer has the structural formula:



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 $-CH(NHR_{21})CH_3$
where X is an aryl or heteroaryl group;
n is an integer of 0 to 6;
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;

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 =O;
R_7 is a covalent bond, alkylene, azaalkyl, or azaaraalkyl or $=NR_{20}$ where R_{20} is 3,5-bis(trifluoromethyl)benzyl or-CH_2X-R^1 or-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 =O;
R_9 and R_{10} are each independently hydrogen, or substituted or unsubstituted alkyl and R_9 may be -$CH_2CH_2COOR^2$ 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, photosensitizereudohalo, 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 Rg 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 Q_1, where Q_1 is alkyl, haloalkyl, halo, photosensitizereudohalo, 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 Rg 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.

3. The composition of one of claims 1 and 2 wherein the imaging agent is a cyanine dye.

4. The composition of claims 1 and 2 wherein the imaging agent is a ^{124}I labeled compound.

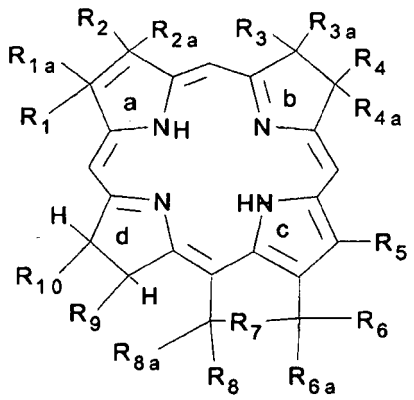
5. The composition of claims 1 and 2 wherein the imaging agent is a PET, fluorescence or MR imaging agent.

6. The composition of one of claims 1 to 5 wherein the nanoparticle contains a targeting moiety.

7. The composition of claim 6 wherein the targeting moiety is a peptide, folic acid or a carbohydrate.

8. A method for making polyacrylic acid (PAA) nanoparticles containing a photosensitizer and an imaging agent by postloading a photosensitizer and a fluorophore onto a preprepared polyacrylic acid (PAA) nanoparticle.

9. The method of claim 8 where the photosensitizer has the structural formula:



or a phamaceutically 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 -$CH(NHR_{21})CH_3$
where X is an aryl or heteroaryl group;
n is an integer of 0 to 6;
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;
R_{3a} and R_{4a} are each independently hydrogen

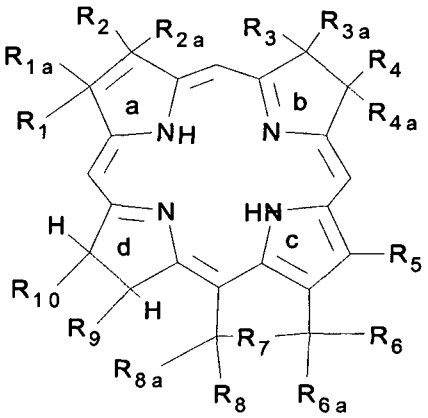
or substituted or unsubstituted alkyl, or together form a covalent bond;
R_5 is hydrogen or substituted or unsubstituted alkyl;
R_6 and R_{6a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form =O;
R_7 is a covalent bond, alkylene, azaalkyl, or azaaraalkyl or $=NR_{20}$ where R_{20} is 3,5-bis(trifluoromethyl)benzyl or $-CH_2X-R^1$ or $-YR^1$ 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 =O;
R_9 and R_{10} are each independently hydrogen, or substituted or unsubstituted alkyl and R_9 may be $-CH_2CH_2COOR^2$ 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, photosensitizereudohalo, 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 Rg 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 Q_1, where Q_1 is alkyl, haloalkyl, halo, photosensitizereudohalo, 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 Rg 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.

10. A method for making polyacrylic acid (PAA) nanoparticles by conjugating a fluorophore to a polyacrylic acid (PAA) nanoparticle and then postloading a photosensitizer to the polyacrylic acid (PAA) nanoparticle.

Patentansprüche

1. Zusammensetzung umfassend Polyacrylsäure-(PAA-) Nanopartikel, welche einen nachträglich geladenen Tetrapyrrol-Photosensibilisator, der nach der Nanopartikelbildung nachträglich auf den Nanopartikel geladen wird, und ein Mittel für die Bildgebung enthalten.

2. Zusammensetzung nach Anspruch 1, wobei der nachträglich geladene Tetrapyrrol-Photosensibilisator die folgende Strukturformel hat:



oder ein pharmazeutisch annehmbares Derivat davon ist, wobei:

R_1 und R_2 jeweils unabhängig substituiertes oder unsubstituiertes Alkyl, substituiertes oder unsubstituiertes Alkenyl, $-C(O)R_a$ oder $-COOR_a$ oder $CH(CH_3)(OR_a)$ oder $-CH(CH_3)(O(CH_2)_nX-R_a)$, sind, wobei R_a Wasserstoff, substituiertes oder unsubstituiertes Alkyl, substituiertes oder unsubstituiertes Alkenyl, substituiertes oder unsubstituiertes Alkynyl oder substituiertes oder unsubstituiertes Cycloalkyl ist, wobei R_2 $-CH=CH_2$, $-CH(OR_{20})CH_3$, $=C(O)Me$, $-C(=NR_{21})CH_3$ oder $-CH(NHR_{21})CH_3$ sein kann,
wobei X eine Aryl- oder Heteroarylgruppe ist,
n eine ganze Zahl von 0 bis 6 ist,
wobei R_{20} Methyl, Butyl, Heptyl, Dodecyl oder 3,5-Bis(trifluormethyl)-benzyl ist, und R_{21} 3,5-Bis(trifluormethyl)benzyl ist,
R_{1a} und R_{2a} jeweils unabhängig Wasserstoff oder substituiertes oder unsubstituiertes Alkyl sind oder zusammen eine kovalente Bindung bilden,
R_3 und R_4 jeweils unabhängig Wasserstoff oder substituiertes oder unsubstituiertes Alkyl sind,
R_{3a} und R_{4a} jeweils unabhängig Wasserstoff oder substituiertes oder unsubstituiertes Alkyl sind oder zusammen eine kovalente Bindung

bilden,
R_5 Wasserstoff oder substituiertes oder unsubstituiertes Alkyl ist,
R_6 und R_{6a} jeweils unabhängig Wasserstoff oder substituiertes oder unsubstituiertes Alkyl sind oder zusammen =O bilden,
R_7 eine kovalente Bindung, Alkylen, Azaalkyl oder Azaaraalkyl oder $=NR_{20}$ ist, wobei R_{20} ein 3,5-Bis(trifluormethyl)benzyl oder $-CH_2X-R^1$ oder $-YR^1$ ist, wobei Y eine Aryl- oder Heteroarylgruppe ist,
R_8 und R_{8a} jeweils unabhängig Wasserstoff oder substituiertes oder unsubstituiertes Alkyl sind oder zusammen =O bilden,
R_9 und R_{10} jeweils unabhängig Wasserstoff oder subsituiertes oder unsubstituiertes Alkyl sind und R_9 $-CH_2CH_2COOR^2$ sein kann, wobei R^2 eine Alkylgruppe ist, die wahlweise mit einem oder mehreren Fluoratomen substituiert werden kann,
jedes von R_1-R_{10}, wenn substituiert, mit einem oder mehreren Substituenten substituiert wird, unabhängig ausgewählt aus Q, wobei Q Alkyl, Halogenalkyl, Halogen, Photosensibilisatoreudohalogen oder $-COOR_b$ ist, wobei R_b Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl, Aryl, Heteroaryl, Araalkyl oder OR_c ist, wobei R_c Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl oder Aryl oder $CONR_dR_e$ ist, wobei R_d und R_e jeweils unabhängig Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl oder Aryl oder NR_fR_g sind, wobei R_f und R_g jeweils unabhängig Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl oder Aryl oder $=NR_h$ sind, wobei R_h Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl oder Aryl oder ein Aminosäurerest ist,
jedes Q unabhängig mit einem oder mehreren Substituenten unsubstituiert oder substituiert wird, jeder unabhängig ausgewählt aus Q_1, wobei Q_1 Alkyl, Halogenalkyl, Halogen, Photosensibilisatoreudohalogen oder $-COOR_b$ ist, wobei R_b Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl, Aryl, Heteroaryl, Araalkyl oder OR_c ist, wobei R_c Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl oder Aryl oder $CONR_dR_e$ ist, wobei R_d und R_e jeweils unabhängig Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl oder Aryl oder NR_fR_g sind, wobei R_f und R_g jeweils unabhängig Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl oder Aryl oder $=NR_h$ sind, wobei R_h Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl oder Aryl oder ein Aminosäurerest ist.

3. Zusammensetzung nach einem der Ansprüche 1 und 2, wobei das Mittel für die Bildgebung ein Cyaninfarbstoff ist.

4. Zusammensetzung nach den Ansprüchen 1 und 2, wobei das Mittel für die Bildgebung eine ^{124}I-markierte Verbindung ist.

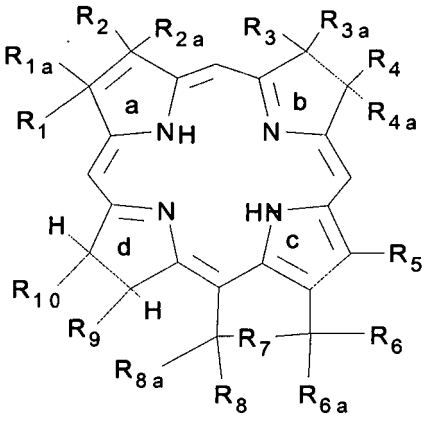
5. Zusammensetzung nach den Ansprüchen 1 und 2, wobei das Mittel für die Bildgebung ein PET-, Fluoreszenz- oder MR-Bildgebungsmittel ist.

6. Zusammensetzung nach einem der Ansprüche 1 bis 5, wobei der Nanopartikel eine zielende Komponente aufweist.

7. Zusammensetzung nach Anspruch 6, wobei die zielende Komponente ein Peptid, eine Folsäure oder ein Kohlenhydrat ist.

8. Verfahren zur Herstellung von Polyacrylsäure-(PAA-) Nanopartikeln, welche einen Photosensibilisator und ein Mittel für die Bildgebung enthalten, indem ein Photosensibilisator und ein Fluorophor auf einen zuvor hergestellten Polyacrylsäure- (PAA-) Nanopartikel nachträglich geladen werden.

9. Verfahren nach Anspruch 8, wobei der Photosensibilisator die folgende Strukturformel hat:



oder ein pharmazeutisch annehmbares Derivat davon, wobei:

R_1 und R_2 jeweils unabhängig substituiertes oder unsubstituiertes Alkyl, substituiertes oder unsubstituiertes Alkenyl, $-C(O)R_a$ oder $-COOR_a$ oder $CH(CH_3)(OR_a)$ oder $-CH(CH_3)(O(CH_2)_nX-R_a)$, sind, wobei R_a Wasserstoff, substituiertes oder unsubstituiertes Alkyl, substituiertes oder unsubstituiertes Alkenyl, substituiertes oder unsubstituiertes Alkynyl oder substituiertes oder unsubstituiertes Cycloalkyl ist, wobei R_2 $-CH=CH_2$, $-CH(OR_{20})CH_3$, $-C(O)Me$, $-C(=NR_{21})CH3$ oder-$CH(NHR_{21})CH_3$ sein kann,
wobei X eine Aryl- oder Heteroarylgruppe ist,
n eine ganze Zahl von 0 bis 6 ist,

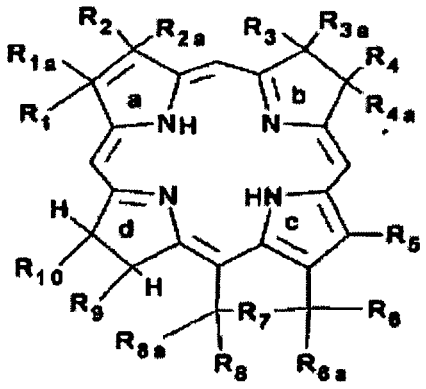
wobei R_{20} Methyl, Butyl, Heptyl, Dodecyl oder 3,5-Bis(trifluormethyl)-benzyl ist, und
R_{21} 3,5-Bis(trifluormethyl)benzyl ist,
R_{1a} und R_{2a} jeweils unabhängig Wasserstoff oder substituiertes oder unsubstituiertes Alkyl sind oder zusammen eine kovalente Bindung bilden,
R_3 und R_4 jeweils unabhängig Wasserstoff oder substituiertes oder unsubstituiertes Alkyl sind,
R_{3a} und R_{4a} jeweils unabhängig Wasserstoff oder substituiertes oder unsubstituiertes Alkyl sind oder zusammen eine kovalente Bindung bilden,
R_5 Wasserstoff oder substituiertes oder unsubstituiertes Alkyl ist,
R_6 und R_{6a} jeweils unabhängig Wasserstoff oder substituiertes oder unsubstituiertes Alkyl sind oder zusammen =O bilden,
R_7 eine kovalente Bindung, Alkylen, Azaalkyl oder Azaaraalkyl oder $=NR_{20}$ ist, wobei R_{20} ein 3,5-Bis(trifluormethyl)benzyl oder $-CH_2X-R^1$ oder $-YR^1$ ist, wobei Y eine Aryl- oder Heteroarylgruppe ist,
R_8 und R_{8a} jeweils unabhängig Wasserstoff oder substituiertes oder unsubstituiertes Alkyl sind oder zusammen =O bilden,
R_9 und R_{10} jeweils unabhängig Wasserstoff oder subsituiertes oder unsubstituiertes Alkyl sind und R_9 $-CH_2CH_2COOR^2$ sein kann, wobei R^2 eine Alkylgruppe ist, die wahlweise mit einem oder mehreren Fluoratomen substituiert werden kann,
jedes von R_1-R_{10}, wenn substituiert, mit einem oder mehreren Substituenten substituiert wird, unabhängig ausgewählt aus Q, wobei Q Alkyl, Halogenalkyl, Halogen, Photosensibilisatoreudohalogen oder $-COOR_b$ ist, wobei R_b Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl, Aryl, Heteroaryl, Araalkyl oder OR_c ist, wobei R_c Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl oder Aryl oder $CONR_dR_e$ ist, wobei R_d und R_e jeweils unabhängig Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl oder Aryl oder NR_fR_g sind, wobei R_f und R_g jeweils unabhängig Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl oder Aryl oder $=NR_h$ sind, wobei R_h Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl oder Aryl oder ein Aminosäurerest ist,
jedes Q unabhängig mit einem oder mehreren Substituenten unsubstituiert oder substituiert wird, jeder unabhängig ausgewählt aus Q_1, wobei Q_1 Alkyl, Halogenalkyl, Halogen, Photosensibilisatoreudohalogen oder $-COOR_b$ ist, wobei R_b Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl, Aryl, Heteroaryl, Araalkyl oder OR_c ist, wobei R_c Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl oder Aryl oder $CONR_dR_e$ ist, wobei R_d und R_e jeweils unabhängig Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl oder Aryl oder NR_fR_g sind, wobei R_f und R_g jeweils unabhängig Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl oder Aryl oder $=NR_h$ sind, wobei R_h Wasserstoff, Alkyl, Alkenyl, Alkynyl, Cycloalkyl oder Aryl oder ein Aminosäurerest ist.

10. Verfahren zur Herstellung von Polyacrylsäure- (PAA-) Nanopartikeln durch Konjugieren eines Fluorophors an einen Polyacrylsäure- (PAA-) Nanopartikel und dann nachträgliches Laden eines Photosensibilisators auf den Polyacrylsäure- (PAA-) Nanopartikel.

Revendications

1. Composition comprenant des nanoparticules d'acide polyacrylique (PAA) contenant un photosensibilisateur tétrapyrollique chargé postérieurement, qui est chargé postérieurement sur la nanoparticule après la formation de la nanoparticule, et un agent d'imagerie.

2. Composition selon la revendication 1, dans laquelle le photosensibilisateur tétrapyrollique chargé postérieurement a la formule développée suivante:



ou l'un de ses dérivés pharmaceutiquement acceptables, dans laquelle:

R_1 et R_2 représentent chacun indépendamment un groupe alkyle substitué ou non substitué, alcényle substitué ou non substitué, $-C(O)R_a$ ou $-COOR_a$ ou $-CH(CH_3)(OR_a)$ ou $-CH(CH_3)(O(CH_2)_nXRa)$, où R_a est un atome d'hydrogène, un groupe alkyle substitué ou non substitué, alcényle substitué ou non substitué, alcynyle substitué ou non substitué, ou cycloalkyle substitué ou non substitué; où R_2 peut être $-CH=CH_2$, $-CH(OR_{20})CH_3$, -C(O)Me, $-C(=NR_{21})CH_3$ ou $-CH(NHR_{21})CH_3$;

où X est un groupe aryle ou hétéroaryle;
n est un nombre entier de 0 à 6;
où R_{20} est un groupe méthyle, butyle, heptyle, dodécyle ou 3,5-bis(trifluorométhyl)benzyle; et R_{21} représente le 3,5-bis(trifluorométhyl)benzyle;
R_{1a} et R_{2a} représentent chacun indépendamment un atome d'hydrogène ou un groupe alkyle substitué ou non substitué, ou forment ensemble une liaison covalente;
R_3 et R_4 représentent chacun indépendamment un atome d'hydrogène ou un groupe alkyle substitué ou non substitué,
R_{3a} et R_{4a} représentent chacun indépendamment un atome d'hydrogène ou un groupe alkyle substitué ou non substitué, ou forment ensemble une liaison covalente;
R_5 est un atome d'hydrogène ou un groupe alkyle substitué ou non substitué;
R_6 et R_{6a} représentent chacun indépendamment un atome d'hydrogène ou un groupe alkyle substitué ou non substitué, ou forment ensemble =O;
R_7 est une liaison covalente, un groupe alkylène, azaalkyle ou azaaralkyle ou $=NR_{20}$, où R_{20} représente le 3,5-bis(trifluorométhyl)benzyle ou $-CH_2X-R^1$ ou YR^1, où Y est un groupe aryle ou hétéroaryle;
R_8 et R_{8a} représentent chacun indépendamment un atome d'hydrogène ou un groupe alkyle substitué ou non substitué, ou forment ensemble =O;
R_9 et R_{10} représentent chacun indépendamment un atome d'hydrogène ou un groupe alkyle substitué ou non substitué et R_9 peut être $-CH_2CH_2COOR^2$, où R^2 est un groupe alkyle qui peut être facultativement substitué par un ou plusieurs atomes de fluor;
chacun des radicaux R_1-R_{10}, lorsqu'ils sont substitués, est substitué par un ou plusieurs substituants choisis chacun indépendamment parmi Q, où Q est un groupe alkyle, halogénoalkyle, halogéno, photosensibilisateur-eudo-halogéno, ou $-COOR_b$, où R_b est un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle, aryle, hétéroaryle, aralkyle, ou OR_c, où R_c est un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle ou aryle, ou $CONR_dR_e$, où R_d et R_e représentent chacun indépendamment un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle ou aryle, ou NR_fR_g, où R_f et Rg représentent chacun indépendamment un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle ou aryle, ou $=NR_h$, où R_h est un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle ou aryle, ou est un résidu d'acide aminé;
chaque Q est indépendamment non substitué ou est substitué par un ou plusieurs substituants choisis chacun indépendamment parmi Q_1, où Q_1 est un groupe alkyle, halogénoalkyle, halogéno, photosensibilisateur-eudo-halogéno, ou $-COOR_b$, où R_b est un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle, aryle, hétéroaryle, aralkyle, ou OR_c, où R_c est un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle ou aryle, ou $CONR_dR_e$, où R_d et R_e représentent chacun indépendamment un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle ou aryle, ou NR_fR_g, où R_f et Rg représentent chacun indépendamment un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle ou aryle, ou $=NR_h$, où R_h est un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle ou aryle, ou est un résidu d'acide aminé.

3. Composition selon l'une des revendications 1 et 2, dans laquelle l'agent d'imagerie est un colorant cyanine.

4. Composition selon les revendications 1 et 2, dans laquelle l'agent d'imagerie est un composé marqué au ^{124}I.

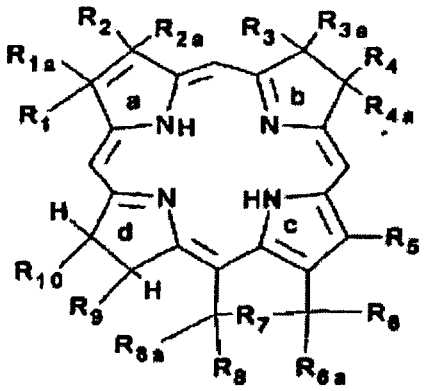
5. Composition selon les revendications 1 et 2, dans laquelle l'agent d'imagerie est un agent d'imagerie de PET, florescence ou RM.

6. Composition selon l'une des revendications 1 et 5, dans laquelle la nanoparticule contient un groupement de ciblage.

7. Composition selon la revendication 6, dans laquelle le groupement de ciblage est un peptide, l'acide folique ou un glucide.

8. Procédé de fabrication de nanoparticules d'acide polyacrylique (PAA) contenant un photosensibilisateur et un agent d'imagerie par chargement postérieur d'un photosensibilisateur et d'un fluorophore sur une nanoparticule d'acide polyacrylique (PAA) préparée au préalable.

9. Procédé selon la revendication 8, où le photosensibilisateur a la formule développée suivante:



ou l'un de ses dérivés pharmaceutiquement acceptables, dans laquelle:

R_1 et R_2 représentent chacun indépendamment un groupe alkyle substitué ou non substitué, alcényle substitué ou non substitué, $-C(O)R_a$ ou $-COOR_a$ ou $-CH(CH_3)(OR_a)$ ou $-CH(CH_3)(O(CH_2)_nXRa)$, où R_a est un atome d'hydrogène, un groupe alkyle substitué ou non substitué, alcényle substitué ou non substitué, alcynyle substitué ou non substitué ou cycloalkyle substitué ou non substitué; où R_2 peut être $-CH=CH_2$, $-CH(OR_{20})CH_3$, -C(O)Me, $-C(=NR_{21})CH_3$ ou $-CH(NHR_{21})CH_3$;
où X est un groupe aryle ou hétéroaryle;
n est un nombre entier de 0 à 6;
où R_{20} est un groupe méthyle, butyle, heptyle, dodécyle ou 3,5-bis(trifluorométhyl)benzyle; et R_{21} représente le 3,5-bis(trifluorométhyl)benzyle;
R_{1a} et R_{2a} représentent chacun indépendamment un atome d'hydrogène ou un groupe alkyle substitué ou non substitué, ou forment ensemble une liaison covalente;
R_3 et R_4 représentent chacun indépendamment un atome d'hydrogène ou un groupe alkyle substitué ou non substitué,
R_{3a} et R_{4a} représentent chacun indépendamment un atome d'hydrogène ou un groupe alkyle substitué ou non substitué, ou forment ensemble une liaison covalente;
R_5 est un atome d'hydrogène ou un groupe alkyle substitué ou non substitué;
R_6 et R_{6a} représentent chacun indépendamment un atome d'hydrogène ou un groupe alkyle substitué ou non substitué, ou forment ensemble =O;
R_7 est une liaison covalente, un groupe alkylène, azaalkyle ou azaaralkyle ou $=NR_{20}$, où R_{20} représente le 3,5-bis(trifluorométhyl)benzyle ou $-CH_2X-R^1$ ou $-YR^1$, où Y est un groupe aryle ou hétéroaryle;
R_8 et R_{8a} représentent chacun indépendamment un atome d'hydrogène ou un groupe alkyle substitué ou non substitué, ou forment ensemble =O;
R_9 et R_{10} représentent chacun indépendamment un atome d'hydrogène ou un groupe alkyle substitué ou non substitué et R_9 peut être $-CH_2CH_2COOR^2$, où R^2 est un groupe alkyle qui peut être facultativement substitué par un ou plusieurs atomes de fluor;
chacun des radicaux R_1-R_{10}, lorsqu'ils sont substitués, est substitué par un ou plusieurs substituants choisis chacun indépendamment parmi Q, où Q est un groupe alkyle, halogénoalkyle, halogène, photosensibilisateur-eudo-halogéno, ou $-COOR_b$, où R_b est un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle, aryle, hétéroaryle, aralkyle, ou OR_c, où R_c est un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle ou aryle, ou $CONR_dR_e$, où R_d et R_e représentent chacun indépendamment un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle ou aryle, ou NR_fR_g, où R_f et Rg représentent chacun indépendamment un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle ou aryle, ou $=NR_h$, où R_h est un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle ou aryle, ou est un résidu d'acide aminé;
chaque Q est indépendamment non substitué ou est substitué par un ou plusieurs substituants choisis chacun indépendamment parmi Q_1, où Q_1 est un groupe alkyle, halogénoalkyle, halogéno, photosensibilisateur-eudo-halogéno, ou $-COOR_b$, où R_b est un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle, aryle, hétéroaryle, aralkyle, ou OR_c, où R_c est un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle ou aryle, ou $CONR_dR_e$, où R_d et R_e représentent chacun indépendamment un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle ou aryle, ou NR_fR_g, où R_f et R_g représentent chacun indépendamment un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle ou aryle, ou $=NR_h$, où R_h est un atome d'hydrogène, un groupe alkyle, alcényle, alcynyle, cycloalkyle ou aryle, ou est un résidu d'acide aminé.

10. Procédé de fabrication de nanoparticules d'acide polyacrylique (PAA) par conjugaison d'un fluorophore à une nanoparticule d'acide polyacrylique (PAA) puis chargement postérieur d'un photosensibilisateur sur la nanoparticule d'acide polyacrylique (PAA).

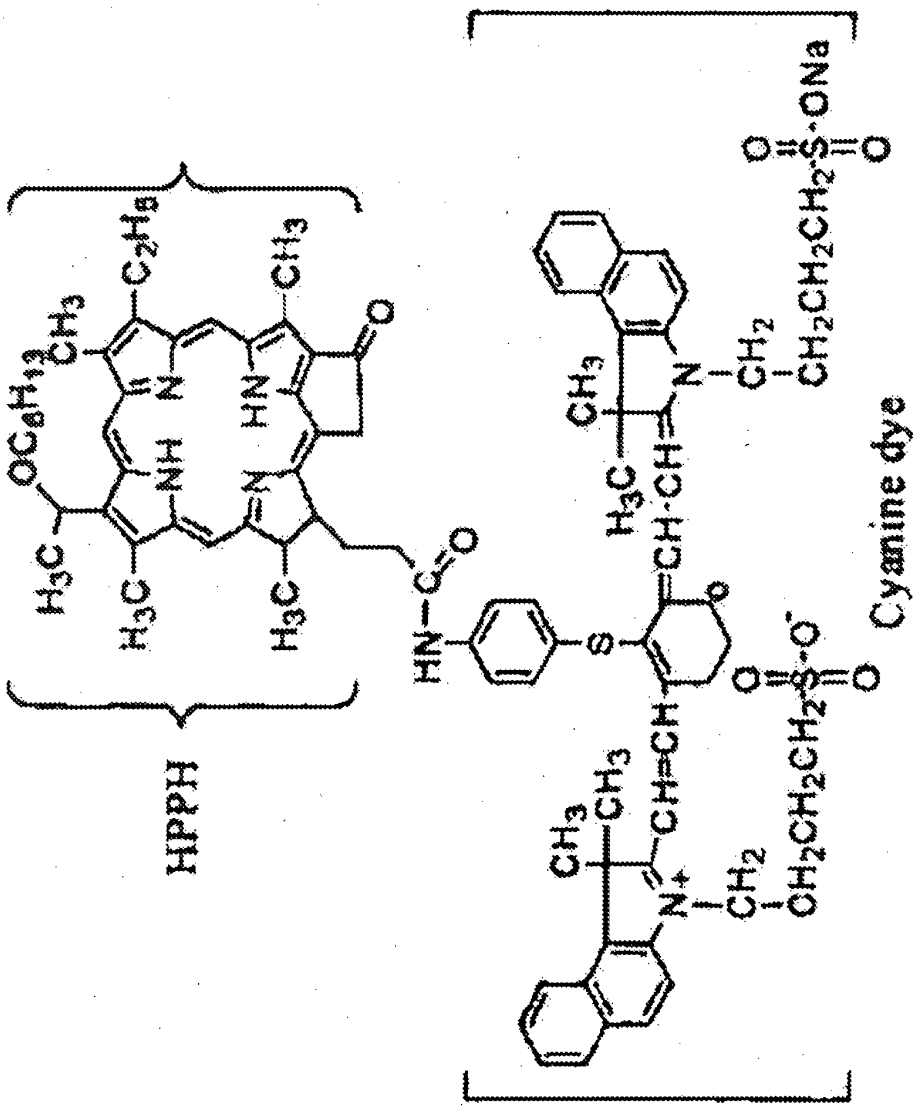


Fig. 1

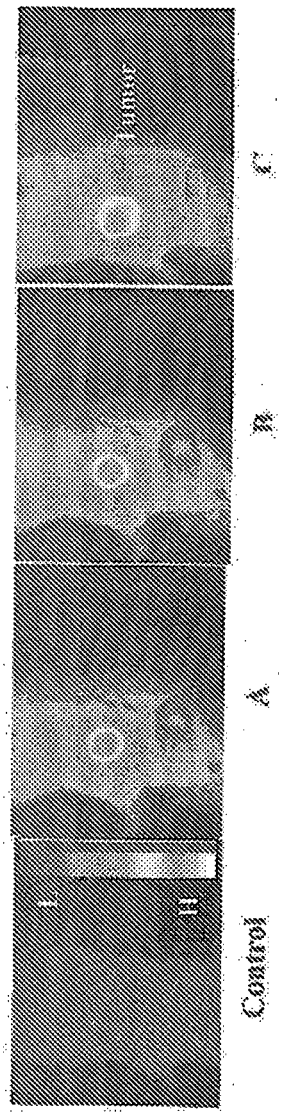


FIGURE 2

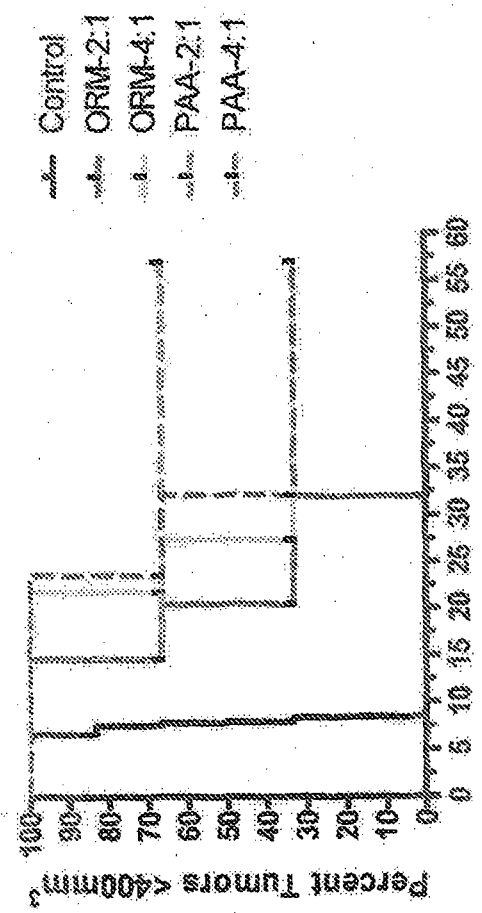

Control
ORM-2:1
ORM-4:1
PAA-2:1
PAA-4:1
Percent Tumors <400mm³
100
90
80
70
60
50
40
30
20
10
0
0 5 10 15 20 25 30 35 40 45 50 55 60
Time- Days post PDT

FIGURE 3

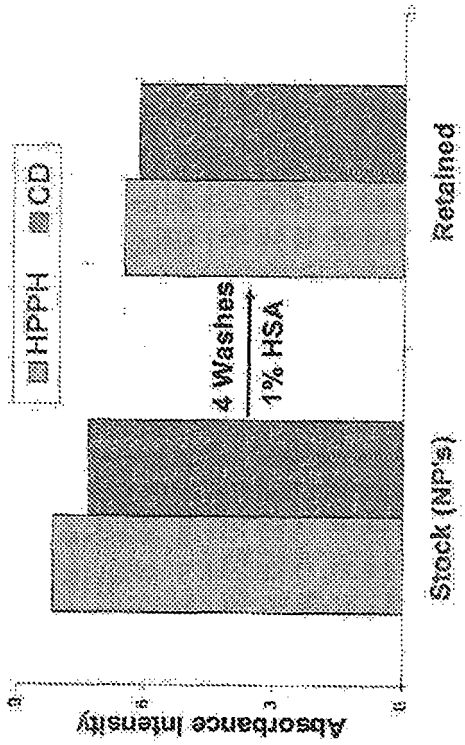

HPPH
CD
4 Washes
1% HSA
Absorbance Intensity
Stock (NP's)
Retained

FIGURE 4

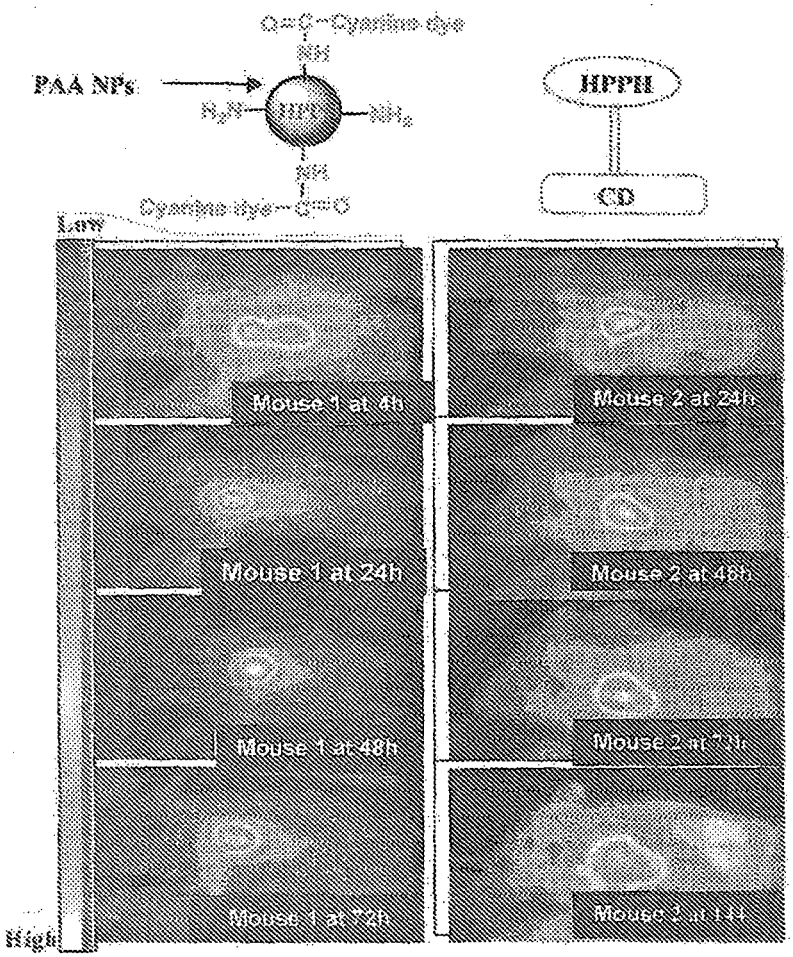

PAA NPs
HPPH
CD
Low
High
Mouse 1 at 4h
Mouse 1 at 24h
Mouse 1 at 48h
Mouse 1 at 72h
Mouse 2 at 24h
Mouse 2 at 48h
Mouse 2 at 72h

FIGURE 5

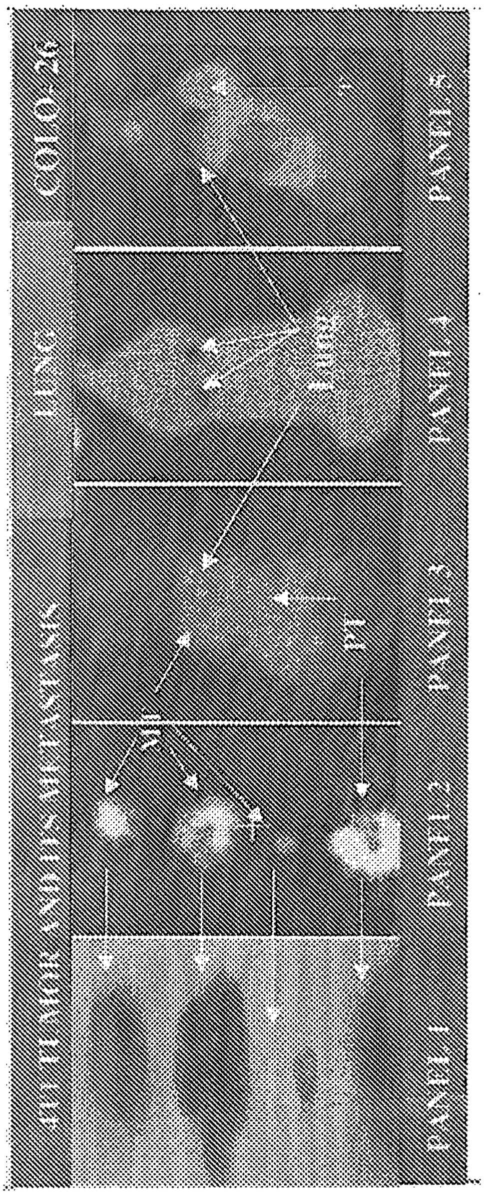

4T1 TUMOR AND ITS METASTASIS
LUNG
COLO-26
MT
PT
PANEL 1
PANEL 2
PANEL 3
PANEL 4
PANEL 5

FIGURE 6

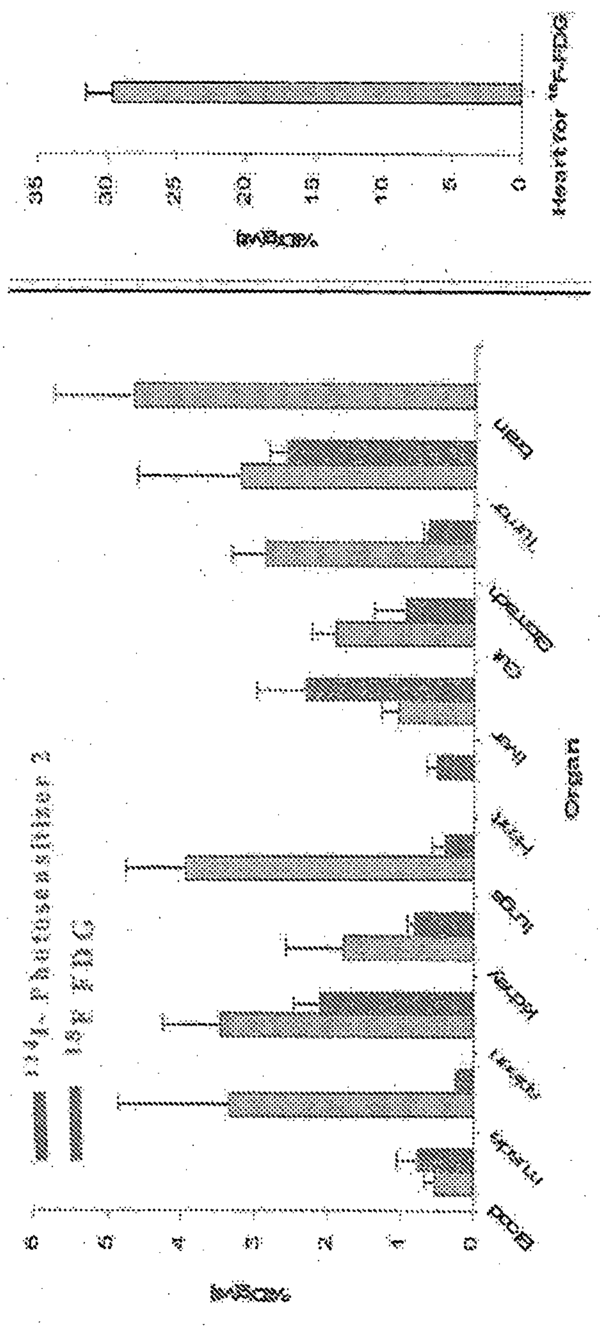


FIGURE 7

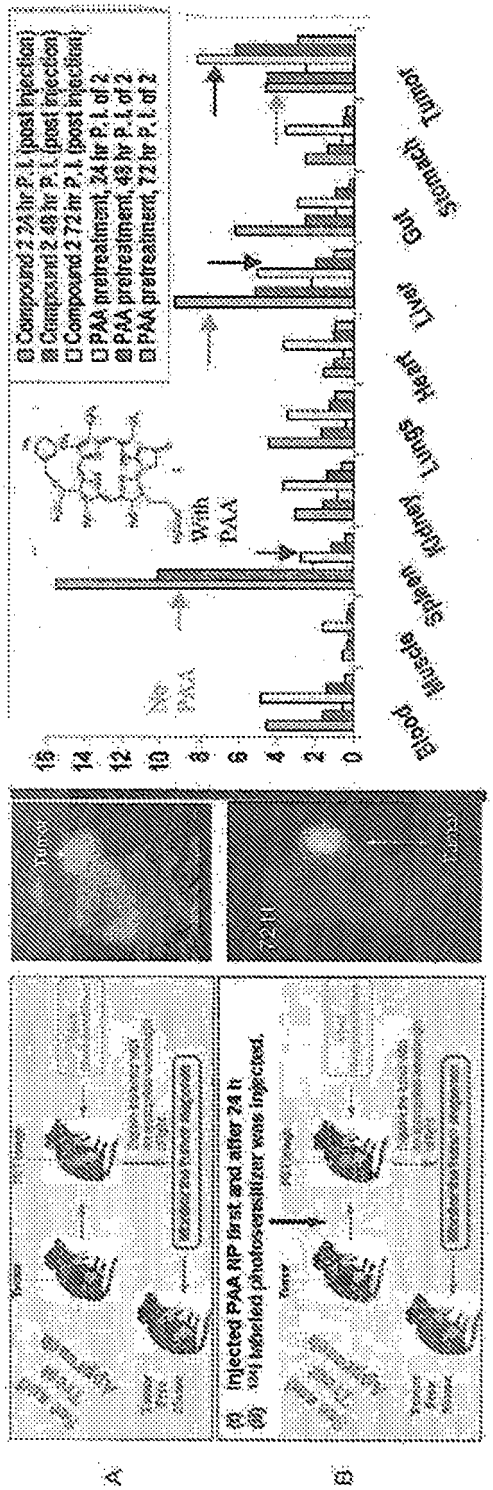

A
B
Injected PAA RP first and after 24 h
Compound 2 24 hr P.I. (post injection)
Compound 2 48 hr P.I. (post injection)
Compound 2 72 hr P.I. (post injection)
PAA pretreatment, 24 hr P.I. of 2
PAA pretreatment, 48 hr P.I. of 2
PAA pretreatment, 72 hr P.I. of 2
With PAA
Blood
Muscle
Spleen
Kidney
Lungs
Heart
Liver
Gut
Stomach
Tumor

FIGURE 8

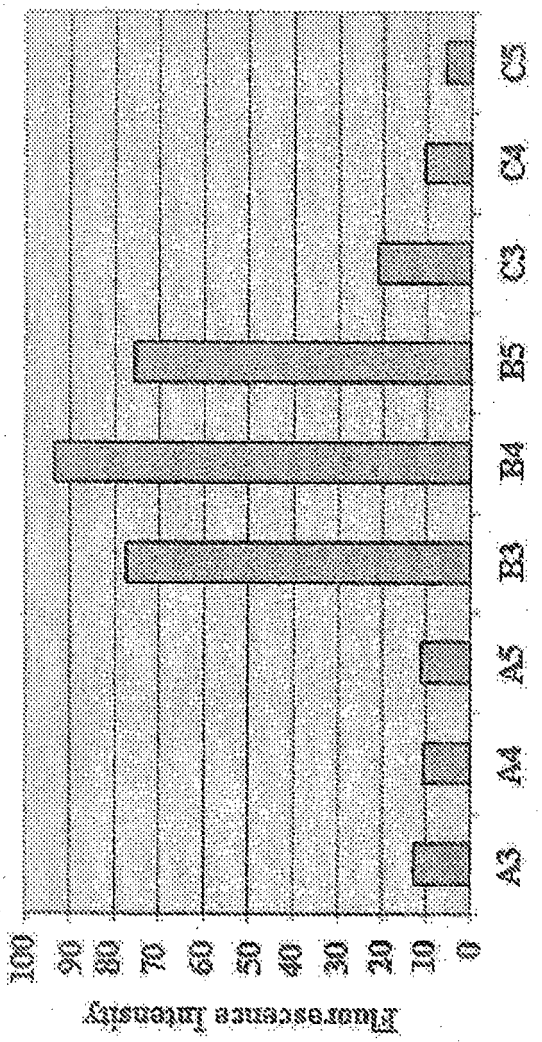

Fluorescence Intensity
100
90
80
70
60
50
40
30
20
10
0
A3
A4
A5
B3
B4
B5
C3
C4
C5

FIGURE 9

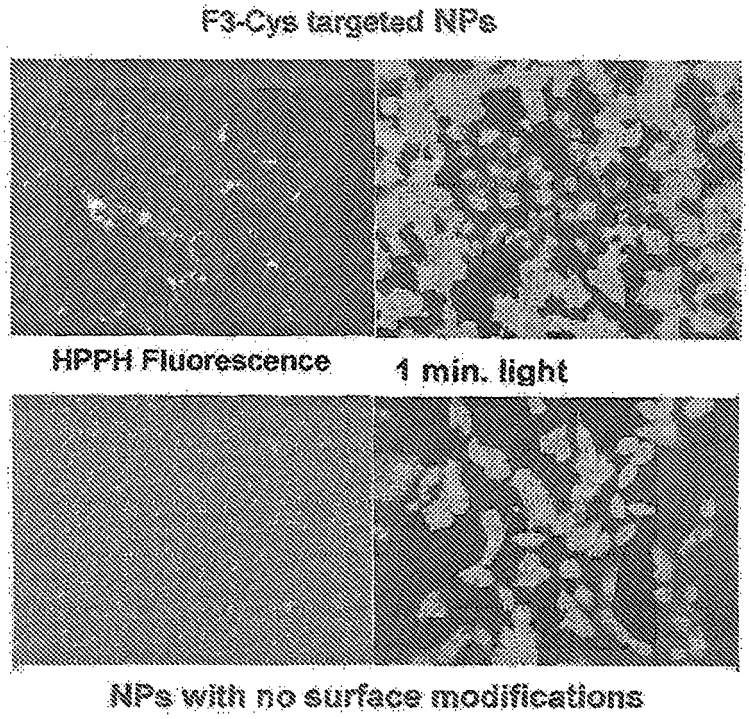

F3-Cys targeted NPs
HPPH Fluorescence
1 min. light
NPs with no surface modifications

FIGURE 10

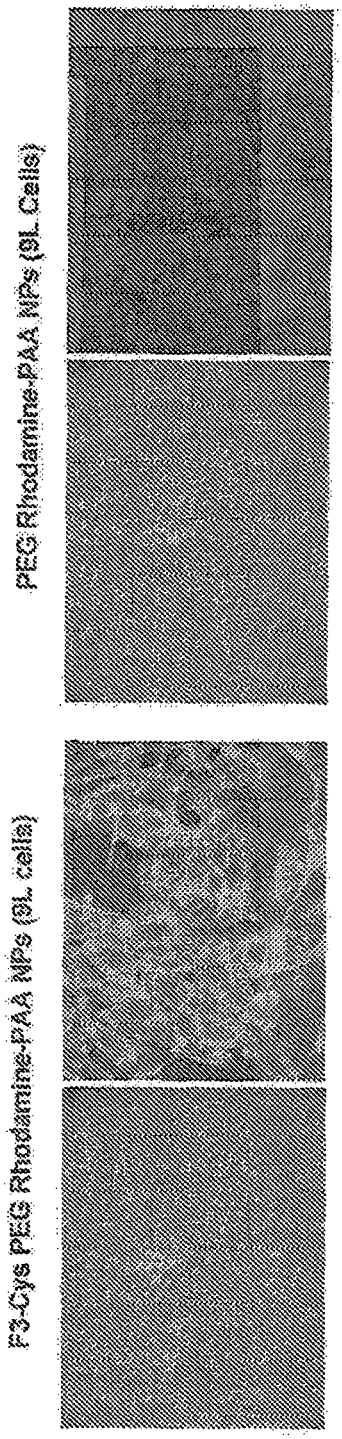

F3-Cys PEG Rhodamine-PAA NPs (9L cells)
PEG Rhodamine-PAA NPs (9L Cells)

FIGURE 11

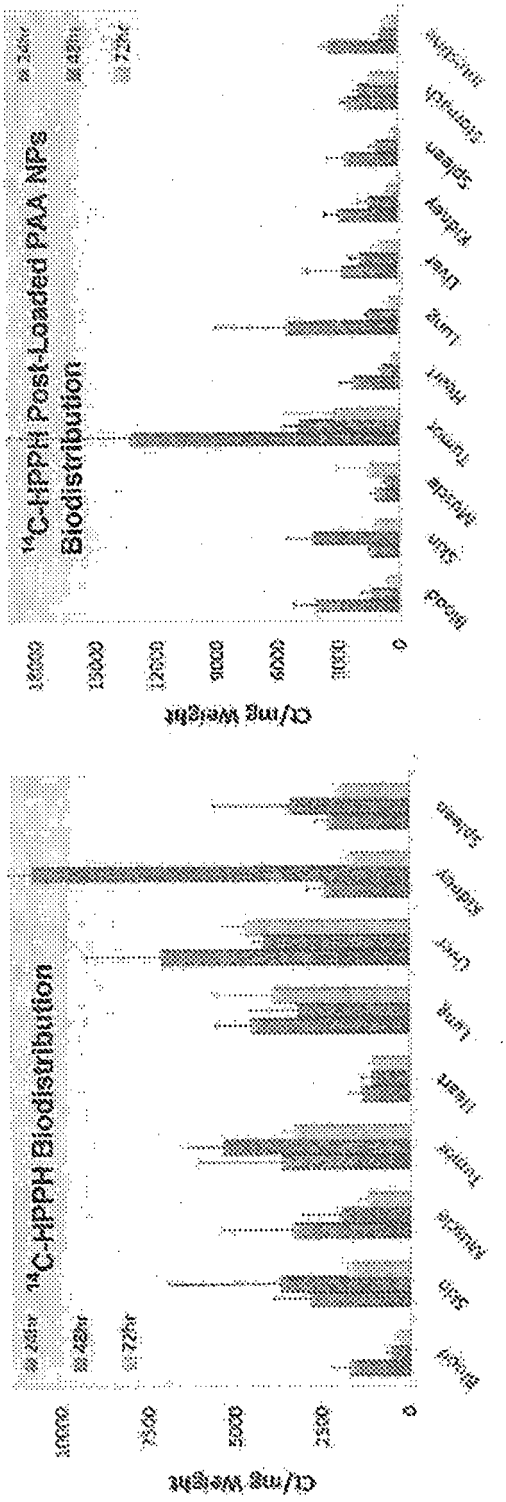

^{14}C-HPPH Post-Loaded PAA NPs
Biodistribution
24hr
48hr
72hr
Cl/mg Weight
Blood
Skin
Muscle
Tumor
Heart
Lung
Liver
Spleen
Stomach
Intestine
^{14}C-HPPH Biodistribution
24hr
48hr
72hr
Cl/mg Weight
Blood
Skin
Muscle
Tumor
Heart
Lung
Liver
Spleen
Stomach
Intestine

FIGURE 12

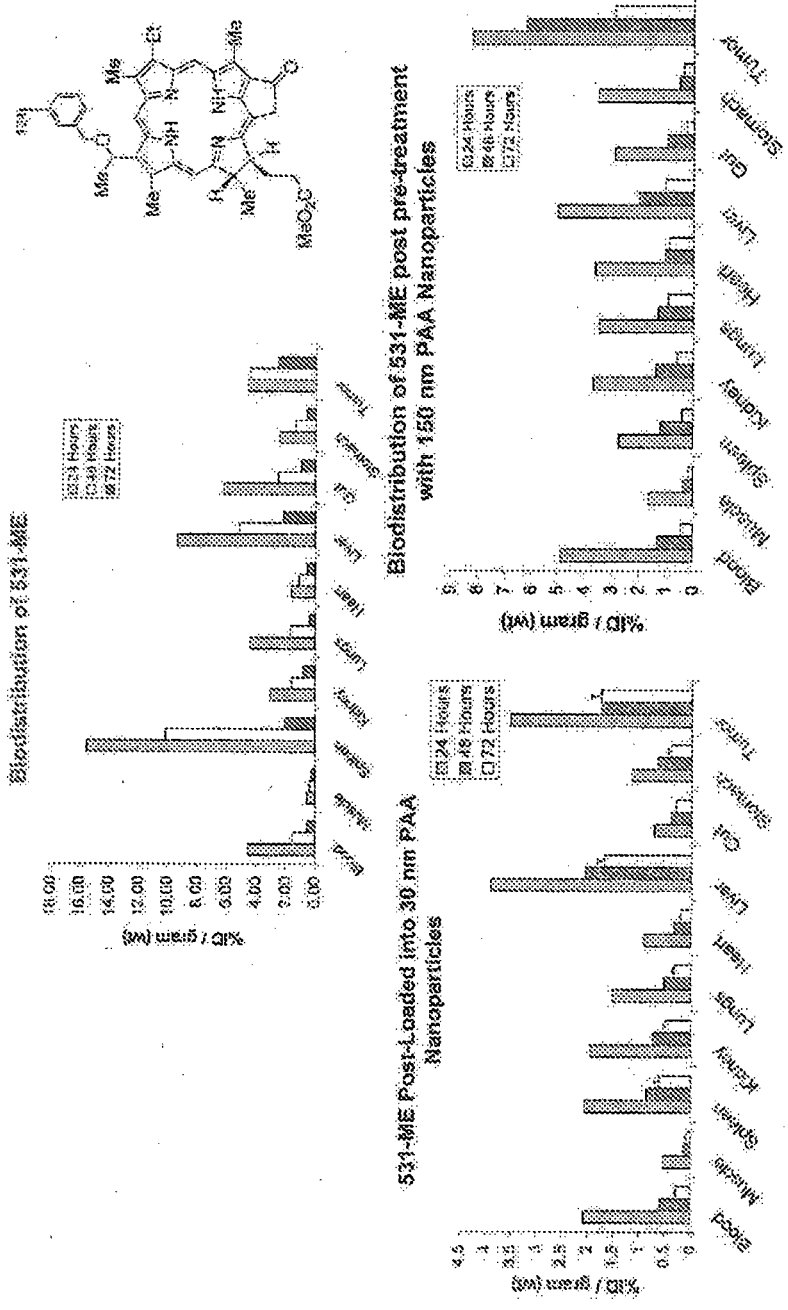

Biodistribution of 531-ME
Biodistribution of 531-ME post pre-treatment with 150 nm PAA Nanoparticles
531-ME Post-Loaded into 30 nm PAA Nanoparticles
%ID / gram (wt)
24 Hours
48 Hours
72 Hours
Blood
Muscle
Spleen
Kidney
Lungs
Heart
Liver
GI
Stomach
Tumor

FIGURE 13

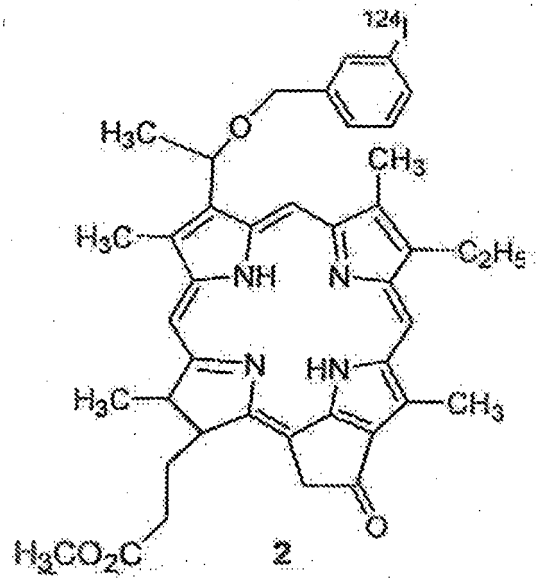


FIGURE 14

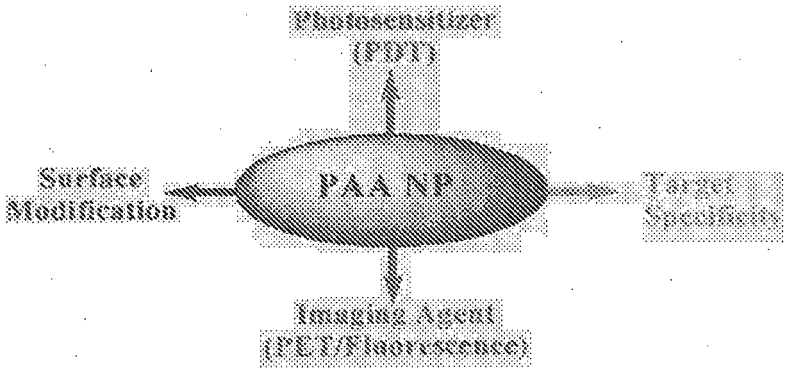

Photosensitizer
(PDT)
Surface
Modification
PAA NP
Imaging Agent
(PET/Fluorescence)

FIGURE 15

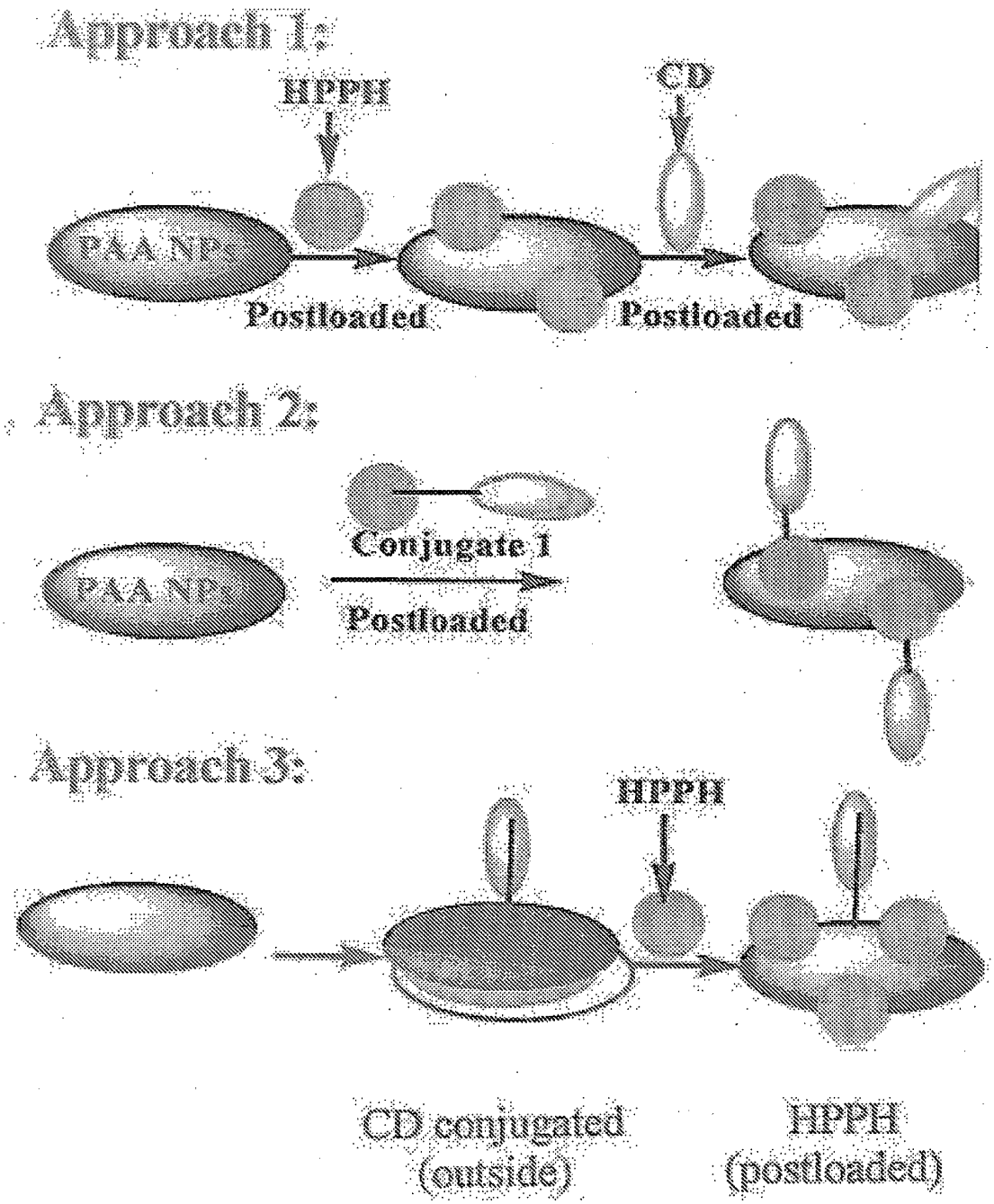

Approach 1:
HPPH
CD
PAA NPs
Postloaded
Postloaded
Approach 2:
Conjugate 1
PAA NPs
Postloaded
Approach 3:
HPPH
CD conjugated
(outside)
HPPH
(postloaded)

FIGURE 16

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Non-patent literature cited in the description

- **ROSS. B. et al.** *PHOTONIC AND MAGNETIC NANOEXPLORERS FOR BIOMEDICAL USE: FROM SUBCELLULAR IMAGING TO CANCER DIAGNOSTIC AND THERAPY,* 2004, vol. 5331, 76-83 **[0009]**

专利名称(译)	PAA纳米粒子用于增强肿瘤成像		
公开(公告)号	EP2437653B1	公开(公告)日	2014-07-23
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 A61K47/48 A61K41/00 A61K49/00 A61K51/12 A61K51/04 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 EP2437653A1		
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。