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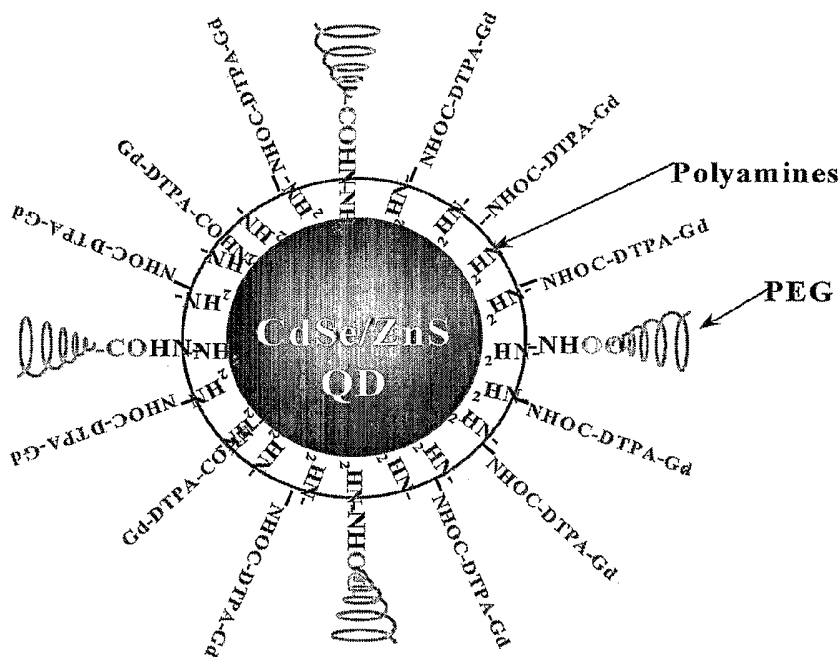
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[Continued on next page]

(54) Title: MULTIMODALITY NANOSTRUCTURES, METHODS OF FABRICATION THEREOF, AND METHODS OF USE THEREOF



(57) Abstract: Briefly described, embodiments of this disclosure include multimodality nanostructures, methods of forming the multimodality nanostructures, and methods of using the multimodality nanostructures.

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**MULTIMODALITY NANOSTRUCTURES, METHODS OF FABRICATION
THEREOF, AND METHODS OF USE THEREOF**

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to copending U.S. provisional patent application entitled "MULTIMODALITY NANOSTRUCTURES, METHODS, FABRICATION THEREOF, AND METHODS OF USE THEREOF" filed on April 20, 2004 and accorded serial number 60/563,865, which is entirely incorporated herein by reference.

**STATEMENT REGARDING FEDERALLY SPONSORED
RESEARCH OR DEVELOPMENT**

The U.S. government may have a paid-up license in embodiments of this disclosure and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of grants awarded by the U.S. NIH R01 GM60562 of the U.S. Government.

BACKGROUND

Recent advances in bioanalytical sciences and bioengineering have led to the development of DNA chips, miniaturized biosensors, and microfluidic devices. In addition, applications benefiting from fluorescent labeling include medical (and non-medical) fluorescence microscopy, histology, flow cytometry, fundamental cellular and molecular biology protocols, fluorescence in situ hybridization, DNA sequencing, immunoassays, binding assays, and separation. These enabling technologies have substantially impacted many areas in biomedical research, such as gene expression profiling, drug discovery, and clinical diagnostics.

Imaging techniques such as fluorescence reflectance imaging, fluorescence mediated tomography, bioluminescence imaging, intravital microscopy, X-ray computed tomography (CT), magnetic resonance imaging (MRI), ultrasound (ULT), single photon emission computed tomography (SPECT) positron emission tomography (PET), and the like, can be used to study various biological systems and events. However, each imaging technique has strengths and weaknesses (*e.g.*, cost, sensitivity, resolution, safety, imaging depth, and the like). Therefore, there is a need

in the industry for more accurate, sensitive, and broader method of detection that combines the strengths of the imaging techniques.

SUMMARY

Briefly described, embodiments of this disclosure include multimodality nanostructures, methods of forming the multimodality nanostructures, and methods of using the multimodality nanostructures. One exemplary multimodality nanostructure, among others, includes at least one nanospecies having a first detectable functionality and a second molecule having a second detectable functionality. The second molecule is attached to the nanospecies. The first detectable functionality and the second detectable functionality are different.

One exemplary method of preparing a multimodality nanostructure, among others, includes: providing a nanospecies having a first detectable functionality; and disposing a second molecule to the nanospecies, wherein the second molecule has a second detectable functionality, and wherein the first detectable functionality and the second detectable functionality are different.

One exemplary method of detecting a target in a subject, among others, includes: providing a nanostructure as described above, introducing the nanostructure to a subject, and determining the presence of the target in the subject corresponding to the probe by detecting the first detectable functionality of the nanospecies and the second detectable functionality of the second molecule.

BRIEF DESCRIPTION OF THE DRAWINGS

Many aspects of the disclosure can be better understood with reference to the following drawings. The components in the drawings are not necessarily to scale, emphasis instead being placed upon clearly illustrating the principles of the present disclosure. Moreover, in the drawings, like reference numerals designate corresponding parts throughout the several views.

FIG. 1 illustrates an embodiment of a multimodality nanostructure.

FIG. 2 illustrates an embodiment of a multimodality nanostructure.

FIG. 3 illustrates an embodiment of a multimodality nanostructure.

FIG. 4 illustrates an embodiment of a multimodality nanostructure.

FIG. 5 illustrates an embodiment of a multimodality nanostructure.

FIG. 6 illustrates an embodiment of a multimodality nanostructure.

FIG. 7 illustrates an embodiment of a multimodality nanostructure.

FIG. 8 illustrates an embodiment of a multimodality nanostructure.

FIG. 9 illustrates an embodiment of a multimodality nanostructure.

FIG. 10 illustrates an embodiment of a multimodality nanostructure.

FIG. 11 illustrates an embodiment of a multimodality nanostructure.

FIG. 12 illustrates an embodiment of a multimodality nanostructure.

FIG. 13 illustrates an embodiment of a multimodality nanostructure.

FIG. 14 illustrates an embodiment of a multimodality nanostructure.

FIG. 15 illustrates an embodiment of a multimodality nanostructure.

FIG. 16 illustrates that the polyamines enhance the fluorescence intensity of the multimodality nanostructure.

FIG. 17 is a graph that illustrates the degree of modification of amines on an embodiment of the multimodality nanostructure shown in FIG. 16.

FIG. 18 illustrates MRI images before and after injection of the multimodality nanostructure shown in FIG. 16 into a mouse and a fluorescence image after injection.

DETAILED DESCRIPTION

In accordance with the purpose(s) of the present disclosure, as embodied and broadly described herein, embodiments of the present disclosure, in one aspect, relate to multimodality nanostructures (hereinafter nanostructures), methods of fabricating the nanostructures, and methods of using the nanostructures. The nanostructures are distinguishable because they include components having at least two different detectable functionalities, and therefore, can be individually detected using two or more imaging techniques. The imaging techniques can include, but is not limited to, optical systems, magnetic systems, x-ray systems, nuclear systems, positron emission tomography (PET) imaging systems, ultrasound systems, and the like. In particular, the imaging techniques can include, but are not limited to, fluorescence mediated tomography, bioluminescence imaging, intravital microscopy, X-ray computed tomography (CT), magnetic resonance imaging (MRI), ultrasound (ULT), single photon emission computed tomography (SPECT), PET, and combinations thereof.

The nanostructures can be used in many areas such as, but not limited to, multimodality imaging contrast agents (*in vitro* and *in vivo*), biosensing, biolabeling, gene expression studies, protein studies, medical diagnostics, diagnostic libraries, microfluidic systems, delivery vehicles, nano-tweezers (*e.g.*, the magnetic property

can be used for nano-tweezers movement, while the optical property can be used for imaging, detection, and/or visualization) self-assembly, and lithography and patterning.

In addition, the nanostructures can be modified (*e.g.*, attach biomolecule probes to the nanostructure) so that the nanostructures interact with certain target molecules, which allow detection of the target molecules using one or more imaging techniques. For example, the nanostructures in combination with spectral imaging can be used for multiplexed, optical imaging and detection of genes, proteins, and small-molecule drugs in single living cells, while MRI, PET, and the like, allow imaging of magnetic and radioactive compounds. Since each imaging technique has strengths and weaknesses, using multimodality nanostructures can be used to combine two or more of the strengths of the imaging techniques.

Embodiments of the nanostructure include, but are not limited to, a nanospecies (*e.g.*, quantum dots, metal particles and magnetic particles) having an inherently detectable functionality (*e.g.*, detectable using one or more of the imaging techniques mentioned above) and at least one contrasting agent having an inherent detectable functionality different than the inherent functionality of the nanospecies. For example, the nanostructure can include a nanospecies with a first detectable functionality, while also having a contrasting agent (*e.g.*, atoms, ions, molecules, nanostructures) disposed (directly or indirectly) thereon having a second detectable functionality that is different than the first detectable functionality. Another example includes a nanospecies with the first detectable functionality, while also having a first contrasting agent and a second contrasting agent each disposed (directly or indirectly) on the nanospecies. The first contrasting agent and the second contrasting agent have a second and a third detectable functionality, respectively. The first, the second, and the third detectable functionality are each different from one another, but two may be the same in some embodiments. A few illustrative embodiments are disclosed in FIGS. 1 through 15 and in Examples 1-11 below.

The detectable functionality of the nanospecies and the contrasting agent can each include, but are not limited to, optical functionality, a magnetic functionality, an x-ray functionality, and a radioactive functionality. As mentioned above, the detectable functionality can be detected using two or more imaging techniques. The imaging technique can include, but is not limited to, optical imaging systems (*e.g.*, fluorescence, luminescence, and scattering), magnetic imaging systems, x-ray

imaging systems, nuclear imaging systems, positron emission tomography (PET) imaging systems, ultrasound imaging systems, and combinations of such imaging systems. The functionality can be measured simultaneously or sequentially using one or more of the imaging systems.

The nanospecies can include, but is not limited to, semiconductor (semiconductor quantum dots), metal, and metal oxide nanoparticles (*e.g.*, iron, gold, silver, copper, titanium, nickel, platinum, palladium, oxides thereof (*e.g.*, Cr₂O₃, Co₃O₄, NiO, MnO, Fe₂O₃, Fe₃O₄, FePt, FeCo, FeAl, FeCoAl, CoFe₂O₄, and MnFeO₄), alloys thereof, oxynitrides of each, clusters of each, cluster composites of each, and combinations thereof), metalloid and metalloid oxide nanoparticles, the lanthanide series metal nanoparticles, and combinations thereof. In an embodiment, the nanospecies is a single structure, while in other embodiments, the nanospecies includes two or more structures (*e.g.*, clusters, agglomerates, and the like).

The diameter of the nanostructure including contrast agents and other components can vary significantly depending on the particular application. In general, the diameter of the nanostructure is about 1 to 100 nm, about 2 to 50 nm, and about 2 to 30 nm.

In general, the size of the nanospecies depends on the nanospecies, the presence of a coating(s) on the nanospecies, the size and number of the contrast agents as well as the presence of other components such as bio-compatibility compounds and probes. Typically, when the nanospecies is a quantum dot, the diameter of the quantum dot is about 1 nanometer (nm) to 30 nm, about 1 to 25 nm, about 1 to 20 nm, about 1 to 15 nm, and about 1 to 10 nm. When the nanospecies is a metal, a metal oxide, a metalloid and metalloid oxide nanoparticle, or a lanthanide series metal nanoparticle, the diameter is about 1 to 100 nm, about 1 to 80 nm, about 1 to 60 nm, about 1 to 40 nm, or about 1 to 20 nm. The diameter of the clusters and/or agglomerates of the nanospecies varies depending on the number of nanospecies and the presence of compounds on the nanospecies.

In particular, semiconductor quantum dots are described in more detail below and in U.S. Patent 6,468,808 and International Patent Application WO 03/003015, both of which are incorporated herein by reference. Furthermore, the magnetic nanoparticles (*e.g.*, those having magnetic, paramagnetic, and superparamagnetic properties) can include, but are not limited to, cobalt, iron nanoparticles, iron composite nanoparticles, oxides of each, alloys, clusters of each, or combinations

thereof. In particular, the magnetic nanoparticles can include superparamagnetic iron oxide nanoparticles (*e.g.*, iron oxide nanoparticles such as Fe_3O_4 , Fe_2O_3). Additional paramagnetic compounds can include, but are not limited to, Cr, Mn, Fe, Cu, Eu, Gd, Dy, oxides thereof, or combinations thereof.

In an embodiment, the nanospecies can be covered in part, substantially covered, or completely covered by one or more layers of a material, such as a polymer or other compound. In an embodiment, the nanospecies can have a hydrophobic protection structure that encapsulates the nanospecies, while in another embodiment, the nanospecies is covered in a polyamine layer (*e.g.*, polylysine, polyarginine, chitosan, polyallylamine, polyethylenimine, etc.). In general, the polyamine layer includes a plurality of monomer units. The hydrophobic protection structure includes a capping ligand and/or an amphiphilic copolymer (*e.g.*, amphiphilic block copolymers, amphiphilic random copolymers, amphiphilic alternating copolymers, amphiphilic periodic copolymers, or combinations thereof). The layer or layers can be about 0.5 to 10 nm thick. Additional details are provided below.

The contrasting agent, a bio-compatibility compound, a probe, a linker, and/or a chelator compound can be attached directly to the nanospecies and/or attached to the layer disposed on the nanospecies. In addition, a probe (*e.g.*, a polynucleotide, a polypeptide, a therapeutic agent, and/or a drug) can be attached to the nanospecies or the layer disposed on the nanospecies. The contrasting agent, the bio-compatibility compound, the probe, and/or the chelator compound, can be attached directly to the layer and/or via one or more linkers. The contrasting agent, the bio-compatibility compound, the probe, and/or the chelator compound can be attached in series via one or more linkers. Numerous embodiments of how the nanostructures can be configured is described below, but the following is only an illustrative list and other configurations are intended to be covered by this disclosure.

In an embodiment, the bio-compatibility compound can be attached directly or indirectly with the nanospecies and/or the hydrophobic protection layer. In still another embodiment, the nanospecies can include the bio-compatibility compound and a probe. The bio-compatibility compound and/or the probe are substantially disposed (*e.g.*, attached to the surface of the hydrophobic protection structure and/or attached within the hydrophobic protection structure) on the hydrophobic protection structure.

In another embodiment, the nanostructure can include two or more nanospecies and/or two or more types of nanospecies. In addition, the nanostructure can include a hydrophobic protection structure having two or more polymers (*e.g.*, two or more block copolymers). Further, the nanostructure can include multiple nanospecies and/or multiple polymers (*e.g.*, block copolymers). In addition, the nanostructure can include two or more different types of probes having different functions. Furthermore, the nanospecies and the polymers (*e.g.*, block copolymers) can be assembled into micro and macro structures.

In still another embodiment, the nanospecies can have a ligand coating layer (original ligands). For different types of compound depositions, the original ligands can be kept, partially replaced, or completely replaced by other types of ligands. The original and/or new ligands can include small molecules (*e.g.*, trioctyl phosphine oxide, hexadecylamine, hexadecene, mercaptoacetic acid, oleic acid, citric acid, and derivatives thereof) or polymers (*e.g.*, polymers, copolymers, surfactants, lipid, or derivatives thereof), which can adsorb nanoparticles and also provide a linkage for the contrasting agent, the bio-compatibility compound, the probe, and the chelator compound. The ligands and the nanospecies can interact through interactions such as, but not limited to, covalent bonds, hydrophobic interactions, hydrophilic interactions, or pi-stacking, *etc.*, depending on the surface of nanospecies and the molecular structure of ligands.

In another embodiment, the nanospecies can be overcoated with another polymer on top of their original ligands, through interactions such as, but not limited to, hydrophobic interactions, hydrophilic interactions, covalent bond *etc.*, then the compound is adsorbed onto the polymer via chelating, hydrophobic interactions, hydrophilic interactions, pi-stacking, covalent bond, combinations thereof, and the like.

Exemplary polymers for the overcoat can include those described above. For example, the overcoat polymer can include, but is not limited to, amphiphilic polymers, detergent and/or a lipid structure including detergent derivatives and lipid derivatives. The amphiphilic polymer can include, but is not limited to hydrocarbons and DTPA modified poly(acrylic acid), poly(maleic acid), poly(maleic anhydride), and the like. The detergents can include, but are not limited to, AOT, brij family, Igepal family, triton family, SDS, or derivatives of each. In particular, the detergents can include, dioctyl sulfosuccinate sodium salt, polyethylene glycol dodecyl ether,

(octylphenoxy) polyethoxyethanol, octylphenyl-polyethylene glycol, t-octylphenoxypolyethoxyethanol, polyethylene glycol tert-octylphenyl ether, 4-(1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol, dodecyl sulfate sodium salt, or glycolic acid ethoxylate octyl ether. Further, the block copolymer can include lipids such as, but not limited to, lipid-PEG, natural lipids, synthetic lipids, sphingolipids, or derivatives of each.

In particular, the block copolymer can include an ABC triblock structure having a poly-butylacrylate segment, a poly-ethylacrylate segment, or a poly-methacrylic acid segment, for example. The block copolymer can include a diblock and/or triblock copolymer having two or more different poly-aliphatic-acrylate segments. In addition, the block copolymer can include a diblock and/or triblock copolymer having two or more poly-alkyl-acrylate segments.

In addition, the block copolymer can be used with, or in some embodiments replaced with, a detergent and/or a lipid. For example, the detergents can include, but are not limited to, AOT, brij family, Igepal family, triton family, SDS, or derivatives of each. In particular, the detergents can include, dioctyl sulfosuccinate sodium salt, polyethylene glycol dodecyl ether, octylphenoxy polyethoxyethanol, octylphenyl-polyethylene glycol, t-octylphenoxypolyethoxyethanol, polyethylene glycol tert-octylphenyl ether, 4-(1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol, dodecyl sulfate sodium salt, or glycolic acid ethoxylate octyl ether. Further, the block copolymer can include lipids such as, but not limited to, lipid-PEG, natural lipids, synthetic lipids, sphingolipids, and derivatives of each.

The thickness of each layer disposed on nanospecies can vary significantly depending on the particular application. In general, the thickness is about 0.5 to 20 nm, about 0.5 to 15 nm, about 0.5 to 10 nm, and about 0.5 to 5 nm.

As mentioned above, the contrast agents, bio-compatibility compound, chelator compound, linkers, and probes, can be linked to the nanospecies using any stable physical and/or chemical association to the nanospecies directly or indirectly by any suitable means. For example, the component can be linked to the nanospecies using a covalent link, a non-covalent link, an ionic link, and a chelated link, as well as being absorbed or adsorbed onto the nanospecies. In addition, the component can be linked to the nanospecies through hydrophobic interactions, hydrophilic interactions, charge-charge interactions, π -stacking interactions, combinations thereof, and like interactions.

The bio-compatibility compound can include compounds such as, but not limited to, polyethylene glycol (MW about 500 to 50,000 and 1000 to 10,000); polypropylene glycol 500 (MW of about 50,000), dextran, and derivatives such as amino-dextran and carboxy-dextran, and polysaccharides. The bio-compatibility compound can be attached directly or indirectly with the nanospecies and/or a layer (*e.g.*, a hydrophobic protection layer or a polyamine layer) disposed on the nanospecies. The amount bio-compatibility compound disposed on the nanospecies can range widely depending on the application.

The chelator compound can include, but is not limited to, natural chelators and synthetic chelators. The natural chelators include, but are not limited to, carbohydrates (*e.g.*, polysaccharides), organic acids with more than one coordination group, lipids, steroids, amino acids, peptides, phosphates, nucleotides, tetrapyrroles, ferrioxamines, ionophores (*e.g.*, gramicidin, monensin, and valinomycin), and phenolics. The synthetic chelators include, but are not limited to, ammonium citrate dibasic, ammonium oxalate monohydrate, ammonium tartrate dibasic, ammonium tartrate dibasic solution, pyromellitic acid, calcium citrate tribasic tetrahydrate, ethylene glycol-bis(2-aminoethylether)-N,N,N,N-tetra acetic acid, sodium glycocholate, ammonium citrate dibasic, calcium citrate tribasic tetrahydrate, magnesium citrate tribasic, potassium citrate, sodium citrate monobasic, lithium citrate tribasic, sodium citrate tribasic, citric acid, N,N-dimethyldecylamine-N-oxide, N,N-dimethyldodecylamine-N-oxide, ammonium citrate dibasic, ammonium tartrate dibasic, ethylenediaminetetraacetic acid diammonium salt, potassium D-tartrate monobasic, N,N-dimethyldecylamine-N-oxide, N,N-dimethyldodecylamine-N-oxide, ethylenediaminetetraacetic acid dipotassium salt dihydrate, sodium tartrate dibasic, ethylenediaminetetraacetic acid, ethylenediaminetetraacetic acid disodium salt dihydrate, ethylenediaminetetraacetic acid, ethylenediaminetetraacetic acid tetrasodium salt hydrate, ethylenediaminetetraacetic acid tripotassium salt, ethylene glycol-bis(2-aminoethylether)-N,N,N,N-tetra acetic acid, ethylene glycol-bis(2-aminoethylether)-N,N,N,N-tetra acetic acid, potassium oxalate, sodium oxalate, ethylene glycol-bis(2-aminoethylether)-N,N,N,N-tetra acetic acid, ethylenediaminetetraacetic acid, ethylenediaminetetraacetic acid diammonium salt, ethylenediaminetetraacetic acid dipotassium salt dihydrate, ethylenediaminetetraacetic acid disodium salt dihydrate, ethylenediaminetetraacetic acid disodium salt dihydrate, ethylenediaminetetraacetic acid, ethylenediaminetetraacetic acid tetrasodium salt

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The amount of chelator compound disposed on the nanospecies can range widely depending on the particular application, but should not substantially alter the signal of the nanospecies and/or the physical properties of the nanostructure. It should be noted that the larger the number of chelator compounds disposed on the nanospecies, the stronger the signal from the compound chelated by the chelator compound.

The linker can include a functional group (*e.g.*, an amine group) on the layer disposed on the nanospecies and/or the linker can include a separate compound attached to the nanospecies or the layer at one end and a chelator compound, contrast agent, bio-compatibility compound, or another linker at the other end. The linker can include functional groups such as, but not limited to, amines, carboxylic acids, hydroxyls, thios, and combinations thereof. The linker can include compounds such as, but not limited to, DTPA, EDTA, DOPA, EGTA, NTA, and combinations thereof. In an embodiment, the linker and the chelator compound are the same, but in other embodiments they can be different. The percentage of linkers attached to the chelator compound, contrast agent, and/or another linker can be about 0.1 to 100%.

As indicated above, the nanostructure can include quantum dots such as, but not limited to, luminescent semiconductor quantum dots. In general, quantum dots include a core and a cap, however, uncapped quantum dots can be used as well. The "core" is a nanometer-sized semiconductor. While any core of the IIA-VIA, IIIA-VA, or IVA-IVA, IVA-VIA semiconductors can be used in the context of the present disclosure, the core must be such that, upon combination with a cap, a luminescent quantum dot results. A IIA-VIA semiconductor is a compound that contains at least one element from Group IIA and at least one element from Group VIA of the periodic table, and so on. The core can include two or more elements. In one embodiment, the core is a IIA-VIA, IIIA-VA, or IVA-IVA semiconductor that ranges in size from about 1 nm to about 40 nm, about 1 nm to 30, about 1 nm to 20 nm, about 1 nm to 10 nm. In another embodiment, the core is more preferably a IIA-VIA semiconductor and ranges in size from about 2 nm to about 10 nm. For example, the core can be CdS, CdSe, CdTe, ZnSe, ZnS, PbS, PbSe, or an alloy.

The "cap" is a semiconductor that differs from the semiconductor of the core and binds to the core, thereby forming a surface layer on the core. The cap can be such that, upon combination with a given semiconductor core a luminescent quantum dot results. The cap should passivate the core by having a higher band gap than the core. In one embodiment, the cap is a IIA-VIA semiconductor of high band gap. For example, the cap can be ZnS or CdS. Combinations of the core and cap can include, but are not limited to, the cap is ZnS when the core is CdSe or CdS, and the cap is CdS when the core is CdSe. Other exemplary quantum dots include, but are not limited to, CdS, ZnSe, CdSe, CdTe, CdSe_xTe_{1-x}, InAs, InP, PbTe, PbSe, PbS, HgS,

HgSe, HgTe, CdHgTe, and GaAs. The cap is about 0.1 to 10 nm, about 0.1 to 5 nm, and about 0.1 to 2 nm.

The wavelength emitted (*i.e.*, color) by the quantum dots can be selected according to the physical properties of the quantum dots, such as the size and the material of the nanocrystal. Quantum dots are known to emit light from about 300 nanometers (nm) to 2000 nm (*e.g.*, UV, near IR, and IR). The colors of the quantum dots include, but are not limited to, red, blue, green, and combinations thereof. The color or the fluorescence emission wavelength can be tuned continuously. The wavelength band of light emitted by the quantum dot is determined by either the size of the core or the size of the core and cap, depending on the materials which make up the core and cap. The emission wavelength band can be tuned by varying the composition and the size of the QD and/or adding one or more caps around the core in the form of concentric shells.

The synthesis of quantum dots is well known and is described in U.S. Patent Nos. 5,906,670; 5,888,885; 5,229,320; 5,482,890; 6,468,808; 6,306,736; 6,225,198, *etc.*, International Patent Application WO 03/003015, (all of which are incorporated herein by reference) and in many research articles. The wavelengths emitted by quantum dots and other physical and chemical characteristics have been described in US Patent 6,468,808 and International Patent Application WO 03/003015 and will not be described in any further detail. In addition, methods of preparation of quantum dots are described in US Patent 6,468,808 and International Patent Application WO 03/003015 and will not be described any further detail.

In an embodiment, the quantum dot can be substantially coated or coated with a polymer. The polymer can include, but is not limited to, a polyamine, a capping ligand, a hydrophobic protection layer, and combinations thereof. In particular, the polyamine can include, but is not limited to, polylysine. The coverage of the quantum dot with the polymer can range from about 1 to 100%. The thickness of the polymer layer can range from about 0.5 to 50 nm.

In another embodiment, the quantum dot can be capped with a capping ligand, which forms a layer on the quantum dot, and have a polymer layer disposed on the capping ligand. The capping ligand can include compounds such as, but not limited to, an $O=PR_3$ compound, an $O=PHR_2$ compound, an $O=PHR_1$ compound, a H_2NR compound, a HNR_2 compound, a NR_3 compound, a HSR compound, a SR_2 compound, and combinations thereof. "R" can be a C_1 to C_{18} hydrocarbon such as,

but not limited to, linear hydrocarbons, branched hydrocarbons, cyclic hydrocarbons, substituted hydrocarbons (*e.g.*, halogenated), saturated hydrocarbons, unsaturated hydrocarbons, and combinations thereof. Preferably, the hydrocarbon is a saturated linear C₄ to C₁₈ hydrocarbon, a saturated linear C₆ to C₁₈ hydrocarbon, and a saturated linear C₁₈ hydrocarbon. A combination of R groups can be attached to P, N, or S. In particular, the capping ligand can be selected from, but is not limited to, tri-octylphosphine oxide, stearic acid, and octyldecyl amine, oleic acid, and derivatives thereof.

The polymer can include di- and/or tri-block copolymers. In addition, the polymer can be amphiphilic. Further, the polymer can include hydrocarbon side chains such as, but not limited to, 1-18 carbon alkyl side chains and combinations thereof. In particular, the block copolymer can include an ABC triblock structure having a polybutylacrylate segment, a polyethylacrylate segment, and a polymethacrylic acid segment, for example.

The contrast agent having functionality different than the first functionality of the nanospecies can include, but is not limited to, quantum dots (for embodiments where the nanospecies is not a quantum dot), magnetic compounds, paramagnetic compounds, radioisotopes, fluorescent compounds, organic dyes, and combinations thereof. The quantum dots, magnetic compounds, and paramagnetic compounds are described above in regard to nanospecies. It should be noted that in one embodiment the nanospecies could be a quantum dot, while in another embodiment the contrast agent is a quantum dot. The semiconductor, metal, and metal oxide nanoparticles, metalloid and metalloid oxide nanoparticles, the lanthanide series metal nanoparticles, magnetic compounds, paramagnetic compounds, and radioisotopes, can each be used as a nanospecies or a contrast agent. The particular configuration depends, at least in part, upon the application and detection system(s) used. The amount of contrast agent disposed on the nanospecies depends, at least in part, upon the strength of signal desired, the layers, if any, disposed on the nanospecies, and the imaging detection system. In addition, the amount of contrast agent disposed on the nanospecies should not interfere with the desired signal from the nanospecies or other chemical properties.

The radioisotopes can include, but are not limited to, indium (¹¹¹In), yttrium (⁹⁰Y), ⁹⁹Tc (technetium), ²⁰¹Tl (thallium), ⁶⁷Ga (gallium), ¹²³I (iodine), ¹³¹I (iodine),

³²P (phosphorus), ¹⁵³Sm (samarium), ¹⁸⁶Re, ¹⁸⁸Re (rhenium), ¹⁶⁵Dy (dysprosium), ¹⁶⁶Ho (holmium), and combinations thereof.

The fluorescent compounds (*e.g.*, organic dyes) can include, but are not limited to, fluorescein, Pyrene, Coumarin, BODIPY, Oregon green, Rhodamine, and Texas Red, or cyanine dyes. Additional dyes can be found from commercial websites, such as Molecular Probes (www.probes.com).

The fluorescent compounds can include, but are not limited to, ,1,5 IAEDANS; 1,8-ANS; 4-Methylumbelliferone; 5-carboxy-2,7-dichlorofluorescein; 5-carboxyfluorescein (5-FAM); 5-Carboxynaphthofluorescein; 5-carboxytetramethylrhodamine (5-TAMRA); 5-FAM (5-carboxyfluorescein); 5-HAT (hydroxy tryptamine); 5-hydroxy tryptamine (HAT); 5-ROX (carboxy-X-rhodamine); 5-TAMRA (5-carboxytetramethylrhodamine); 6-carboxyrhodamine 6G; 6-CR 6G; 6-JOE; 7-amino-4-methylcoumarin; 7-aminoactinomycin D (7-AAD); 7-hydroxy-4-methylcoumarin; 9-amino-6-chloro-2-methoxyacridine; ABQ; Acid Fuchsin; ACMA (9-amino-6-chloro-2-methoxyacridine); Acridine Orange; Acridine Red; Acridine Yellow; Acriflavin; Acriflavin Feulgen SITSa; Aequorin (Photoprotein); AFPs - AutoFluorescent Protein - (Quantum Biotechnologies); Alexa Fluor 350™; Alexa Fluor 430™; Alexa Fluor 488™; Alexa Fluor 532™; Alexa Fluor 546™; Alexa Fluor 568™; Alexa Fluor 594™; Alexa Fluor 633™; Alexa Fluor 647™; Alexa Fluor 660™; Alexa Fluor 680™; Alizarin Complexon; Alizarin Red; Allophycocyanin (APC); AMC, AMCA-S; AMCA (Aminomethylcoumarin); AMCA-X; Aminoactinomycin D; Aminocoumarin; Aminomethylcoumarin (AMCA); Anilin Blue; Anthrocyll stearate; APC (Allophycocyanin); APC-Cy7; APTRA-BTC; APTS; Astrazon Brilliant Red 4G; Astrazon Orange R; Astrazon Red 6B; Astrazon Yellow 7 GLL; Atabrine; ATTO-TAG™ CBQCA; ATTO-TAG™ FQ; Auramine; Auroposphine G; Auroposphine; BAO 9 (Bisaminophenyloxadiazole); BCECF (high pH); BCECF (low pH); Berberine Sulphate; Beta Lactamase; Bimane; Bisbenzamide; Bisbenzimidazole (Hoechst); bis-BTC; Blancophor FFG; Blancophor SV; BOBO™ -1; BOBO™ -3; Bodipy 492/515; Bodipy 493/503; Bodipy 500/510; Bodipy 505/515; Bodipy 530/550; Bodipy 542/563; Bodipy 558/568; Bodipy 564/570; Bodipy 576/589; Bodipy 581/591; Bodipy 630/650-X; Bodipy 650/665-X; Bodipy 665/676; Bodipy Fl; Bodipy FL ATP; Bodipy Fl-Ceramide; Bodipy R6G SE; Bodipy TMR; Bodipy TMR-X conjugate; Bodipy TMR-X, SE; Bodipy TR; Bodipy TR ATP; Bodipy TR-X SE; BO-PRO™ -1; BO-PRO™ -3; Brilliant Sulphoflavin FF;

BTC; BTC-5N; Calcein; Calcein Blue; Calcium Crimson™; Calcium Green; Calcium Green-1 Ca²⁺ Dye; Calcium Green-2 Ca²⁺; Calcium Green-5N Ca²⁺; Calcium Green-C18 Ca²⁺; Calcium Orange; Calcofluor White; Carboxy-X-rhodamine (5-ROX); Cascade Blue™; Cascade Yellow; Catecholamine; CCF2 (GeneBlazer); CFDA; chlorophyll; chromomycin A; chromomycin A; CL-NERF; CMFDA; Coumarin Phalloidin; C-phycoyanine; CPM Methylcoumarin; CTC; CTC Formazan; Cy2™; Cy3.1 8; Cy3.5™; Cy3™; Cy5.1 8; Cy5.5™; Cy5™; Cy7™; cyclic AMP Fluorosensor (FiCRhR); Dabcyl; Dansyl; Dansyl Amine; Dansyl Cadaverine; Dansyl Chloride; Dansyl DHPE; Dansyl fluoride; DAPI; Dapoxyl; Dapoxyl 2; Dapoxyl 3' DCFDA; DCFH (dichlorodihydrofluorescein diacetate); DDAO; DHR (dihydrohodamine 123); Di-4-ANEPPS; Di-8-ANEPPS (non-ratio); DiA (4-Di-16-ASP); dichlorodihydrofluorescein Diacetate (DCFH); DiD - Lipophilic Tracer; DiD (DiIC18(5)); DIDS; Dihydrohodamine 123 (DHR); DiI (DiIC18(3)); Dinitrophenol; DiO (DiOC18(3)); DiR; DiR (DiIC18(7)); DM-NERF (high pH); DNP; dopamine; DTAF; DY-630-NHS; DY-635-NHS; ELF 97; Eosin; Erythrosin; Erythrosin ITC; ethidium bromide; ethidium homodimer -1 (EthD-1); Euchrysin; EukoLight; Europium (III) chloride; EYFP; Fast Blue; FDA; Feulgen (Pararosaniline); FIF (Formaldehyde-Induced Fluorescence); FITC; Flazo Orange; Fluo-3; Fluo-4; Fluorescein (FITC); Fluorescein Diacetate; Fluoro-Emerald; Fluoro-Gold (Hydroxystilbamidine); Fluor-Ruby; FluorX; FM 1-43™; FM 4-46; Fura Red™ (high pH); Fura Red™/Fluo-3; Fura-2; Fura-2/BCECF; Genacryl Brilliant Red B; Genacryl Brilliant Yellow 10GF; Genacryl Pink 3G; Genacryl Yellow 5GF; GeneBlazer (CCF2); Gloxalic Acid; Granular blue; Haematoporphyrin; Hoechst 33258; Hoechst 33342; Hoechst 34580; HPTS; Hydroxycoumarin; Hydroxystilbamidine (FluoroGold); Hydroxytryptamine; Indo-1, high calcium; Indo-1, low calcium; Indodicarbocyanine (DiD); Indotricarbocyanine (DiR); Intrawhite Cf; JC-1; JO-JO-1; JO-PRO-1; LaserPro; Laurodan; LDS 751 (DNA); LDS 751 (RNA); Leucophor PAF; Leucophor SF; Leucophor WS; Lissamine Rhodamine; Lissamine Rhodamine B; Calcein/Ethidium homodimer; LOLO-1; LO-PRO-1; Lucifer Yellow; Lyso Tracker Blue; Lyso Tracker Blue-White; Lyso Tracker Green; Lyso Tracker Red; Lyso Tracker Yellow; LysoSensor Blue; LysoSensor Green; LysoSensor Yellow/Blue; Mag Green; Magdala Red (Phloxin B); Mag-Fura Red; Mag-Fura-2; Mag-Fura-5; Mag-Indo-1; Magnesium Green; Magnesium Orange; Malachite Green; Marina Blue; Maxilon Brilliant Flavin 10 GFF; Maxilon Brilliant Flavin 8 GFF; Merocyanin;

Methoxycoumarin; Mitotracker Green FM; Mitotracker Orange; Mitotracker Red; Mitramycin; Monobromobimane; Monobromobimane (mBBr-GSH); Monochlorobimane; MPS (Methyl Green Pyronine Stilbene); NBD; NBD Amine; Nile Red; Nitrobenzoxadidole; Noradrenaline; Nuclear Fast Red; Nuclear Yellow; Nylosan Brilliant Iavin E8G; Oregon Green; Oregon Green 488-X; Oregon Green™; Oregon Green™ 488; Oregon Green™ 500; Oregon Green™ 514; Pacific Blue; Pararosaniline (Feulgen); PBF1; PE-Cy5; PE-Cy7; PerCP; PerCP-Cy5.5; PE-TexasRed [Red 613]; Phloxin B (Magdala Red); Phorwite AR; Phorwite BKL; Phorwite Rev; Phorwite RPA; Phosphine 3R; PhotoResist; Phycoerythrin B [PE]; Phycoerythrin R [PE]; PKH26 (Sigma); PKH67; PMIA; Pontochrome Blue Black; POPO-1; POPO-3; PO-PRO-1; PO-PRO-3; Primuline; Procion Yellow; Propidium Iodid (PI); PyMPO; Pyrene; Pyronine ; Pyronine B; Pyrozal Brilliant Flavin 7GF; QSY 7; Quinacrine Mustard; Red 613 [PE-TexasRed]; Resorufin; RH 414; Rhod-2; Rhodamine; Rhodamine 110; Rhodamine 123; Rhodamine 5 GLD; Rhodamine 6G; Rhodamine B; Rhodamine B 200; Rhodamine B extra; Rhodamine BB; Rhodamine BG; Rhodamine Green; Rhodamine Phallicidine; Rhodamine Phalloidine; Rhodamine Red; Rhodamine WT; Rose Bengal; R-phycoyanine; R-phycoerythrin (PE); S65A; S65C; S65L; S65T; SBFI; Serotonin; Sevron Brilliant Red 2B; Sevron Brilliant Red 4G; Sevron Brilliant Red B; Sevron Orange; Sevron Yellow L; SITS; SITS (Primuline); SITS (stilbene isothiosulphonic acid); SNAFL calcein; SNAFL-1; SNAFL-2; SNARF calcein; SNARF1; Sodium Green; SpectrumAqua; SpectrumGreen; SpectrumOrange; Spectrum Red; SPQ (6-methoxy-N-(3-sulfopropyl)quinolinium); Stilbene; Sulphorhodamine B can C; Sulphorhodamine Extra; SYTO 11; SYTO 12; SYTO 13; SYTO 14; SYTO 15; SYTO 16; SYTO 17; SYTO 18; SYTO 20; SYTO 21; SYTO 22; SYTO 23; SYTO 24; SYTO 25; SYTO 40; SYTO 41; SYTO 42; SYTO 43; SYTO 44; SYTO 45; SYTO 59; SYTO 60; SYTO 61; SYTO 62; SYTO 63; SYTO 64; SYTO 80; SYTO 81; SYTO 82; SYTO 83; SYTO 84; SYTO 85; SYTOX Blue; SYTOX Green; SYTOX Orange; Tetracycline; Tetramethylrhodamine (TRITC); Texas Red™; Texas Red-X™ conjugate; Thiadicarbocyanine (DiSC3); Thiazine Red R; Thiazole Orange; Thioflavin 5; Thioflavin S; Thioflavin TCN; Thiolyte; Thiozole Orange; Tinopol CBS (Calcofluor White); TMR; TO-PRO-1; TO-PRO-3; TO-PRO-5; TOTO-1; TOTO-3; TriColor (PE-Cy5); TRITC TetramethylRhodamineIsoThioCyanate; True Blue; TruRed; Ultralite; Uranine B; Uvitex SFC; WW 781; X-Rhodamine; XRITC; Xylene

Orange; Y66F; Y66H; Y66W; YO-PRO-1; YO-PRO-3; YOYO-1; YOYO-3, Sybr Green, Thiazole orange (interchelating dyes), or combinations thereof.

As mentioned above, the nanostructure can be attached to a probe molecule. The probe molecule can be any molecule capable of being linked to the nanostructure either directly or indirectly via one or more linkers. The probe molecule can be attached by any stable physical or chemical association to the nanostructure directly or indirectly by any suitable means.

In one embodiment, the probe molecule has an affinity for one or more target molecules (*e.g.*, cancer cell) for which detection (*e.g.*, determining the presence of and/or proximal position within the vessel (body)) is desired. If, for example, the target molecule is a nucleic acid sequence, the probe molecule should be chosen so as to be substantially complementary to the target molecule sequence, such that the hybridization of the target and the probe occurs. The term “substantially complementary,” means that the probe molecules are sufficiently complementary to the target sequences to hybridize under the selected reaction conditions.

The probe molecule and the target molecule can include, but are not limited to, polypeptides (*e.g.*, protein such as, but not limited to an antibody (monoclonal or polyclonal)), nucleic acids (both monomeric and oligomeric), polysaccharides, sugars, fatty acids, steroids, purines, pyrimidines, drugs (*e.g.*, small compound drugs), ligands, or combinations thereof.

Use of the phrase “polypeptide” or “protein” is intended to encompass a protein, a glycoprotein, a polypeptide, a peptide, and the like, whether isolated from nature, of viral, bacterial, plant, or animal (*e.g.*, mammalian, such as human) origin, or synthetic, and fragments thereof. A preferred protein or fragment thereof includes, but is not limited to, an antigen, an epitope of an antigen, an antibody, or an antigenically reactive fragment of an antibody.

Use of the phrase “nucleic acid” is intended to encompass DNA and RNA, whether isolated from nature, of viral, bacterial, plant or animal (*e.g.*, mammalian, such as a human) origin, synthetic, single-stranded, double-stranded, comprising naturally or non-naturally occurring nucleotides, or chemically modified.

In addition, the probe can also include, but is not limited to, a drug, a therapeutic agent, radiological agent, a small molecule drug, and combinations thereof, that can be used to treat the target molecule and/or the associated disease and condition of interest. The drug, therapeutic agent, and radiological agent can be

selected based on the intended treatment as well as the condition and/or disease to be treated. In an embodiment, the nanostructure can include two or more probes used to treat a condition and/or disease.

The following is a nonlimiting illustrative list of probes that can be used:

ProleukinTM, CampathTM, PanretinTM, ZylloprimTM, HexalenTM, EthyolTM,
 ArimidexTM, TrisenoxTM, ElsparTM, TICE BCGTM, TargretinTM, BlenoxaneTM,
 BusulfexTM, MyleranTM, MethosarbTM, XelodaTM, ParaplatinTM, BCNU, BiCNUTM,
 Gliadel WaferTM, CelebrexTM, LeukeranTM, Platino1TM, Leustatin,-2-CdATM, Cytoxan,
 NeosarTM, Cytoxan InjectionTM, Cytoxan TabletTM, Cytosar-UTM, DepoCytTM, DTIC-
 DomeTM, CosmegenTM, AranespTM, DanuoXomeTM, DaunorubicinTM, CerubidineTM,
 OntakTM, ZinecardTM, TaxotereTM, Adriamycin, RubexTM, Adriamycin PFS
 Injectionintravenous InjectionTM, DoxilTM, DromostanoloneTM, MasteroneTM, Elliott's
 B SolutionTM, EllenceTM, epogenTM, EmcytTM, EtopophosTM, VepesidTM,
 AromasinTM, NeupogenTM, FUDRTM, FludaraTM, AdrucilTM, FaslodexTM,
 GemzarTM, MylotargTM, Zoladex ImplantTM, ZoladrexTM, HydreaTM, ZevalinTM,
 IdamycinTM, IFEXTM, GleevecTM, Roferon-ATM, Intron ATM, CamptosarTM, FemaraTM,
 Wellcovorin, LeucovorinTM, LeucovorinTM, ErgamisolTM, CeeBUTM, MustargenTM,
 MegaceTM, AlkeranTM, PurinetholTM, MesnexTM, MethotrexateTM, UvadexTM,
 MutamycinTM, MitozytrexTM, LysodrenTM, NovatroneTM, Durabolin-50TM,
 VerlumaTM, NeumegaTM, EloxatinTM, PaxeneTM, TaxolTM, ArediaTM, Adagen
 (Pegademase Bovine)TM, OncasparTM, NeulastaTM, NipentTM, VercyteTM, MithracinTM,
 PhotofrinTM, MatulaneTM, AtabrineTM, ElitekTM, RituxanTM, ProkineTM, ZanosarTM,
 SclerosolTM, NolvadexTM, TemodarTM, VumonTM, TeslacTM, ThioguanineTM,
 ThioplexTM, HycamtinTM, FarestonTM, BexxarTM, HerceptinTM, VesanoideTM, Uracil
 Mustard CapsulesTM, ValstarTM, VelbanTM, OncovinTM, NavelbineTM, and ZometaTM.

In an embodiment, the nanostructure can include at least two different types of probes, one being a targeting probe that targets certain cells or compounds associated with a condition and/or disease, while the second probe is a drug used to treat the disease. In this manner, the nanostructure acts as a detection component, a delivery component to the cells of interest, and a delivery component for the treatment agent. The detection of the nanospecies can be used to ensure the delivery of the nanostructure to its intended destination as well as the quantity of nanostructures delivered to the destination.

FIG. 1 illustrates an embodiment of a multimodality nanostructure 10a. The multimodality nanostructure 10a includes, but is not limited to, a quantum dot 12, a polyamine layer 14, a chelator 18, a contrast agent 22, and a bio-compatibility agent or compound 24. The chelator 18 is attached to the polyamine layer 14 via a linker 16 (e.g., an amine function group of the polyamine layer 14). In addition, the bio-compatibility compound 24 is attached to the polyamine layer 14 via the linker 16. In another embodiment, the linker for the chelator 18 and the bio-compatibility compound 24 are different. In another embodiment, the multimodality nanostructure 10a can include two or more contrast agents with different functionalities.

For example, a specific example would include a QD coated with polylysine, where PEG-COOH and DTPA are conjugated to the amines, and a paramagnetic and/or an isotopic compound is chelated by the DTPA.

FIG. 2 illustrates an embodiment of a multimodality nanostructure 10b. The multimodality nanostructure 10b includes, but is not limited to, a quantum dot 12, a capping ligand 32, a polymer layer 34, a chelator 18, a contrast agent 22, and a bio-compatibility compound 24. The chelator 18 is attached to the polymer layer 34 via a linker 16. In addition, the bio-compatibility compound 24 is attached to the polymer layer 34 via the linker 16. In another embodiment, the linker for the chelator 18 and the bio-compatibility compound 24 are different. In another embodiment, the multimodality nanostructure 10b can include two or more contrast agents with different functionalities.

For example, a specific example would be include a hydrophobic ligand protected QD that is overcoated with a layer of amphiphilic polymer, where the PEG and DTPA are conjugated to the amphiphilic polymer. A paramagnetic and/or an isotopic compound is chelated by the DTPA. Another example includes a hydrophobic ligand protected QD that is overcoated with a mixture of lipid-PEG and lipid-DTPA. A paramagnetic and/or an isotopic compound is chelated by the DTPA.

FIG. 3 illustrates an embodiment of a multimodality nanostructure 10c. The multimodality nanostructure 10c includes, but is not limited to, a quantum dot 12, a capping ligand 32, a chelator 18, a contrast agent 22, and a bio-compatibility compound 24. The bio-compatibility compound 24 is attached to the capping ligand 32 via the linker 16. The chelator 18 is attached via the linker 16 to the bio-compatibility compound 24 (e.g., a PEG compound with having an amine function group). In another embodiment, the linker for the chelator 18 and the bio-

compatibility compound 24 are different. In another embodiment, the multimodality nanostructure 10c can include two or more contrast agents with different functionalities.

For example, a QD is coated with PEG modified polylysine, where the other termini of the PEG chelating reagent is conjugated (*e.g.*, DTPA, EDTA, and NTA). A paramagnetic or an isotopic compound is chelated by the chelating compound.

FIG. 4 illustrates an embodiment of a multimodality nanostructure 10d. The multimodality nanostructure 10d includes, but is not limited to, a quantum dot 12, a capping layer 32, a chelator 18, a contrast agent 22, and a bio-compatibility compound 24. The chelator 18 is attached to the capping layer 32 via a linker 16. In addition, the bio-compatibility compound 24 is attached to the capping layer 32 via the linker 16. In another embodiment, the linker for the chelator 18 and the bio-compatibility compound 24 are different. In another embodiment, the multimodality nanostructure 10d can include two or more contrast agents with different functionalities.

For example, an amine-modified DTPA or NTA and thio- or amine-modified PEG co-adsorbed on the surface of a QD. A paramagnetic or an isotopic compound is chelated by the chelating reagent.

FIG. 5 illustrates an embodiment of a multimodality nanostructure 10e. The multimodality nanostructure 10e includes, but is not limited to, a quantum dot 12, a capping ligand 32, a polymer layer 34, a chelator 18, a contrast agent 22, and a bio-compatibility compound 24. The bio-compatibility compound 24 is attached to the capping ligand 32 via the linker 16. The chelator 18 is attached via the linker 16 to the bio-compatibility compound 24 (*e.g.*, a PEG compound with having an amine function group). In another embodiment, the linker for the chelator 18 and the bio-compatibility compound 24 are different. In another embodiment, the multimodality nanostructure 10e can include two or more contrast agents with different functionalities.

FIG. 6 illustrates an embodiment of a multimodality nanostructure 10f. The multimodality nanostructure 10f includes, but is not limited to, a magnetic nanoparticle, a capping ligand 32, a polymer layer 34, a dye 38, and a bio-compatibility compound 24. The dye 38 is attached to the polymer layer 34 via a linker 16 and in another embodiment, the dye 38 is absorbed onto the polymer layer 34. In addition, the bio-compatibility compound 24 is attached to the polymer layer

34 via the linker 16. In another embodiment, the linker for the dye 38 and the bio-compatibility compound 24 are different. In another embodiment, the multimodality nanostructure 10f can include two or more contrast agents with different functionalities.

FIG. 7 illustrates an embodiment of a multimodality nanostructure 10g. The multimodality nanostructure 10g includes, but is not limited to, a magnetic nanoparticle 36 (in another embodiment a paramagnetic nanoparticle), a capping ligand 32, a polymer layer 34, a chelator 18, a contrast agent 22 (*e.g.*, a radioactive compound), and a bio-compatibility compound 24. The chelator 18 is attached to the polymer layer 34 via a linker 16. In addition, the bio-compatibility compound 24 is attached to the polymer layer 34 via the linker 16. In another embodiment, the linker for the chelator 18 and the bio-compatibility compound 24 are different. In another embodiment, the multimodality nanostructure 10g can include two or more contrast agents with different functionalities.

FIG. 8 illustrates an embodiment of a multimodality nanostructure 10h. The multimodality nanostructure 10h includes, but is not limited to, a magnetic nanoparticle 36 (in another embodiment a paramagnetic nanoparticle), a capping ligand 32, a polymer layer 34, a chelator 18, a contrast agent 22, and a bio-compatibility compound 24. The bio-compatibility compound 24 is attached to the capping ligand 32 via the linker 16. The chelator 18 is attached via the linker 16 to the bio-compatibility compound 24 (*e.g.*, a PEG compound with having an amine function group). In another embodiment, the linker for the chelator 18 and the bio-compatibility compound 24 are different. In another embodiment, the multimodality nanostructure 10h can include two or more contrast agents with different functionalities.

FIG. 9 illustrates an embodiment of a multimodality nanostructure 10i. The multimodality nanostructure 10i includes, but is not limited to, a magnetic nanoparticle 36 (in another embodiment a paramagnetic nanoparticle), a capping ligand 32, a polymer layer 34, a dye 38, and a bio-compatibility compound 24. The dye 38 is attached to the polymer layer 34 via a linker 16 and in another embodiment, the dye 38 is absorbed onto the polymer layer 34. In addition, the bio-compatibility compound 24 is attached to the polymer layer 34 via the linker 16. In another embodiment, the linker for the dye 38 and the bio-compatibility compound 24 are

different. In another embodiment, the multimodality nanostructure 10i can include two or more contrast agents with different functionalities.

FIG. 10 illustrates an embodiment of a multimodality nanostructure 10j. The multimodality nanostructure 10j includes, but is not limited to, a metallic nanoparticle 38, a capping ligand 32, a polymer layer 34, a chelator 18, a contrast agent 22, and a bio-compatibility compound 24. The chelator 18 is attached to the polymer layer 34 via a linker 16. In addition, the bio-compatibility compound 24 is attached to the polymer layer 34 via the linker 16. In another embodiment, the linker for the chelator 18 and the bio-compatibility compound 24 are different. In another embodiment, the multimodality nanostructure 10j can include two or more contrast agents with different functionalities.

FIG. 11 illustrates an embodiment of a multimodality nanostructure 10k. The multimodality nanostructure 10k includes, but is not limited to, a metallic nanoparticle 38, a capping ligand 32, a polymer layer 34, a chelator 18, a contrast agent 22, and a bio-compatibility compound 24. The bio-compatibility compound 24 is attached to the capping ligand 32 via the linker 16. The chelator 18 is attached via the linker 16 to the bio-compatibility compound 24 (*e.g.*, a PEG compound with having an amine function group). In another embodiment, the linker for the chelator 18 and the bio-compatibility compound 24 are different. In another embodiment, the multimodality nanostructure 10k can include two or more contrast agents with different functionalities.

FIG. 12 illustrates an embodiment of a multimodality nanostructure 10l. The multimodality nanostructure 10l includes, but is not limited to, a metallic nanoparticle 38, a capping ligand 32, a polymer layer 34, a dye 38, and a bio-compatibility compound 24. The dye 38 is attached to the polymer layer 34 via a linker 16 and in another embodiment, the dye 38 is absorbed onto the polymer layer 34. In addition, the bio-compatibility compound 24 is attached to the polymer layer 34 via the linker 16. In another embodiment, the linker for the dye 38 and the bio-compatibility compound 24 are different. In another embodiment, the multimodality nanostructure 10l can include two or more contrast agents with different functionalities.

FIG. 13 illustrates an embodiment of a multimodality nanostructure 10m. The multimodality nanostructure 10m includes, but is not limited to, a metallic nanoparticle 38, a chelator 18, a contrast agent 22, and a bio-compatibility compound 24. The chelator 18 is attached to the metallic nanoparticle 38 via a linker 16. In

addition, the bio-compatibility compound 24 is attached to the metallic nanoparticle 38 via the linker 16. In another embodiment, the linker for the chelator 18 and the bio-compatibility compound 24 are different. In another embodiment, the multimodality nanostructure 10m can include two or more contrast agents with different functionalities.

FIG. 14 illustrates an embodiment of a multimodality nanostructure 10n. The multimodality nanostructure 10n includes, but is not limited to, a metallic nanoparticle 38, a dye 38, and a bio-compatibility compound 24. The dye 38 is attached to the metallic nanoparticle 38 via a linker 16. In addition, the bio-compatibility compound 24 is attached to the metallic nanoparticle 38 via the linker 16. In another embodiment, the linker for the chelator 18 and the bio-compatibility compound 24 are different. In another embodiment, the multimodality nanostructure 10n can include two or more contrast agents with different functionalities.

Examples

Now having described the embodiments of the nanostructure in general, example 1-12 describes some embodiments of the nanostructure. While embodiments of nanostructures are described in connection with examples 1-12 and the corresponding text and figures, there is no intent to limit embodiments of the nanospecies to these descriptions. On the contrary, the intent is to cover all alternatives, modifications, and equivalents included within the spirit and scope of embodiments of the present disclosure.

Example 1: A Gd-Quantum Dot (QD) nanostructure

PEG and Gd-DTPA graphed polylysine can be prepared using literature procedures with some modifications (*e.g.*, by changing the PEG and DTPA substitution degree) (See, Review of Scientific Instruments (2003) 74, 4158-4163; Biophysical Journal 75, 1998, 2163-2169; Review of Scientific Instruments (2003) 74, 4012-4020; and Hypertension 2001, 38, 1158-1161, which are included herein by reference). It should be noted that the chelating reagents are not limited to DTPA could include DOPA, EDTA, and the like). The PEG and Gd-DTPA graphed polylysine can be titrated into QD-mercaptoacetic acid solution at a controlled ratio (*i.e.*, until the solution become optically clear and essentially aggregates free examined by fluorescence microscopy). The multiple amine groups quickly adsorb onto QD surface whereas PEG chains and Gd-DTPA each face outward to the

solution. The resulting nanostructure has dual fluorescent and magnetic functionalities.

Example 2: A Gd-QD nanostructure

To prepare lipid-DTPA-Gd coated QDs, DSPE-DTPA and DSPE-mPEG are mixed in a controlled ratio and are dissolved in a basic solution (above about a pH of 7). QDs are purified and suspended in dichloromethane. The two solutions are mixed together and sonicated with a probe sonicator. After formation of an optically clear QD solution and removal of the excess lipid ligands, the pH of the QD solution is adjusted to 5 using an acid (*e.g.*, HCl), followed by addition of $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$. The Gd^{3+} -labeled QDs are purified with a filtration column. The resulting nanostructure also has dual fluorescent and magnetic properties.

Example 2.1

To prepare lipid-DTPA-Gd coated QDs, Gd-QD DSPE-DTPA and DSPE-mPEG are mixed in a controlled ratio in chloroform and added to QD-chloroform solution. After slow vacuum drying, water or aqueous buffer are added to the lipid and particle dry film. Agitation or sonication lead to formation of an optically clear QD solution. After removal of the excess lipid ligands, the pH of the QD solution is adjusted to 5 using an acid (*e.g.*, HCl), followed by addition of $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$. The Gd^{3+} -labeled QDs are purified with a filtration column. The resulting nanostructure also has dual fluorescent and magnetic properties.

Example 3: A Gd-QD nanostructure

To prepare DTPA-Gd coated QDs, DSPE-PEG-NH₂ are modified at the NH₂ end with DTPA using EDAC as a crosslinker, or using DTPA anhydride. The purified product is dissolved in a basic solution (above about a pH of 7). The QDs are purified and suspended in dichloromethane. The two solutions are mixed together and sonicated with a probe sonicator. After formation of an optically clear QD solution and removal of the excess lipid ligands, the pH of the QD solution is adjusted to about 5 using an acid (*e.g.*, HCl), followed by addition of $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$. The Gd^{3+} -labeled QDs are purified with a filtration column. The resulting nanostructure also has dual fluorescent and magnetic properties.

Example 3.1 Gd-QD

To prepare DTPA-Gd coated QDs, DSPE-PEG-NH₂ are mixed QDs in chloroform solution. After slow vacuum drying, water or aqueous buffer are added to the lipid and particle dry film. Agitation or sonication lead to formation of an optically clear QD solution. After removal of the excess lipid ligands, diethylenetriaminepentaacetic acid dianhydride is used to modify free amine groups into DTPA. Excess (unbound) DTPA are purified out by filtration columns or dialysis. The pH of the QD solution is then adjusted to 5 using an acid (e.g., HCl), followed by addition of GdCl₃·6H₂O. The Gd³⁺-labeled dots are purified with a filtration column. The resulting nanostructure also has dual fluorescent and magnetic properties.

Example 4: A Gd-QD nanostructure

To prepare DTPA-Gd coated QDs, amphiphilic polymer poly(maleic anhydride alt-1-tetradecene) is first react with diamines, such as 2,2'-(ethylenedioxy)diethylamine, then modified into DTPA using diethylenetriaminepentaacetic acid dianhydride. To solublize the QDs, the purified polymer can be either mixed with QDs in organic solvents (e.g., chloroform, ethanol, or solvent mixtures) then dried and suspended into aqueous solution, or the purified polymer can be suspended in aqueous solution, after addition of QDs in dichloromethane and sonication, the dots are suspended in aqueous solution. The polymer coated QDs are then mixed with the Gd³⁺ solution and purified with filtration column or centrifugation. The resulting nanostructure also has dual fluorescent and magnetic properties.

Example 5: ⁶⁷Gd, ¹¹¹In or ⁹⁰Y QD nanostructure

Based on similar procedures described above (the DTPA coated QDs are described in example 1-4, except Gd³⁺ is added), ⁶⁷Gd, ¹¹¹In, ⁹⁰Y and other isotopic agents such as those described above can be chelated to the QD surface, leading to radioactive-fluorescent probes.

Example 6: ¹¹¹In or ⁹⁰Y labeled magnetic nanoparticle nanostructure

The hydrophobic magnetic nanoparticles are prepared as described in the literature (See, J. Am. Chem. Soc. 2001, 123, 12798-12801; Chem Commun., 2003, 927-934; J. Am. Chem. Soc. 2004, 126, 273-279; Nano Lett. 2004 4(1); 187-190; J. Am. Chem. Soc. 2002; 124, 8204-8205; and J. Phys. Chem. B. ; 2001; 105, 7913-

7919). The ligand and polymer coating procedures are described in Example 1-4. ^{67}Gd , ^{111}In , and ^{90}Y , *etc* can be chelated to the surface of magnetic nanoparticles leading to magnetic-radioactive dual probes.

Example 7: ^{111}In or ^{90}Y and Gd^{3+} labeled QD nanostructure

^{111}In , ^{90}Y , Gd^{3+} can be co-chelated to DTPA or DOTA functionalized QDs, leading to triple-modality probes.

Example 8:

Magnetic nanoparticles solubilized with polymers, lipids, detergents, small ligands, are covalently linked to organic dyes using crosslinking reagents such as EDAC, Sulfo-SMCC, and the like (see, Piercenet.com and *Nature*, 424 (6950): 810-816 Aug 14 2003) to form a magnetic-optical dual probe nanostructure.

Example, metallic nanoparticles (REF *J. Mater. Chem.*, 2002, 12, 2671–2674, CHEM. COMMUN., 2002, 1334–1335, *Chem. Commun.*, 2000, 183–184, J. AM. CHEM. SOC. 2003, 125, 14280-14281, *Chem. Mater.* 2004, 16, 3513-3517, J. AM. CHEM. SOC. 2004, 126, 8648-8649) solubilized with polymers, lipids, detergents, small ligands, are modified on the surface using procedures discussed in Example 1-8 and to form a magnetic-optical dual probe nanostructure or a radioactive-optical dual probe nanostructure, or a tri-functional nanostructure.

Example 9:

Magnetic nanoparticles are linked to QDs through electrostatic attraction, hydrophobic interaction, or covalent bond using crosslinking reagents such as EDAC, Sulfo-SMCC, and the like to form a magnetic-optical dual probe nanostructure (a satellite structure). In addition, magnetic particles can be attached to QD's.

Example 10:

The multimodality nanostructures can be linked to polynucleotides, polypeptides, genes, proteins, antibodies, small-molecule drugs, PEG, dextran, and the like, for *in vivo* targeting and imaging.

Example 11:

The magnetic-optical dual probe nanostructure can be used as a nano-tweezer, where the magnetic property provides a handle for movements and the optical property serves as a tracer. Exemplary structures include a magnetic particle core tagged with dyes on the surface, a magnetic particle core tagged with QDs on the surface, a QD tagged with Gd^{3+} on the surface, and the like. The nano-tweezers are much smaller than the traditional optical tweezers (See, *Nature*, 424 (6950): 810-816

Aug 14 2003; *Annu Rev Bioph Biom* 23: 247-285 1994; and *P Natl Acad Sci USA* 94 (10): 4853-4860 May 13 1997, which are included herein by reference) and magnetic tweezers (See, *Review of Scientific Instruments* (2003) 74, 4158-4163; *Biophysical Journal* 75, 1998, 2163–2169; *Review of Scientific Instruments* (2003) 74, 4012-4020; and *Hypertension* 2001, 38, 1158-1161).

Example 12:

Fluorescent quantum dots (QDs) are of considerable current interest for *in vivo* molecular imaging due to their unique optical properties in comparison with organic dyes, such as multicolor emission, large absorption coefficients across a wide spectral range, very high levels of brightness and photostability. However, this technology is unlikely to find broad uses in large animals limited by the intrinsic tissue penetration problem of fluorescence imaging. To address this problem, a multimodality QD probe that combines the strengths of multiple imaging modalities is described and examined. Due to the QDs' large surface area and our ability to deposit multiple other functionalities (*e.g.*, magnetic contrast agents, isotopes, therapeutic drugs and *etc.*), this technology is expected to open new opportunities in ultra-sensitive and multiplexed imaging of molecular targets in living cells and animals, as well as in development of traceable delivery vehicles for therapeutics.

FIG. 15 shows the design of a magnetic-optical dual modality probe. This multifunctional structure was designed and prepared by using semiconductor QDs as the fluorescent probe and structural scaffold for assembly of Gd-DTPA (Diethylenetriamine pentaacetic acid-Gadolinium). To prove the concept, QDs were coated with thioglycolic acid and transferred into water following literature procedures. This ligand exchange method is easy to carry, but accompanied by a significant drop in quantum yield (typically by 90%). To restore the fluorescence and add additional imaging modalities, polylysine (PL) with an average of 340 monomer units were modified with poly(ethylene glycol) (PEG) and DTPA-Gd to a certain degree. Upon mixing with the QDs, the PEG grafted PL bind to QD surface tightly and lead to formation of highly-stable single nanoparticles (at least 100 times more stable than untreated thioglycolic acid QDs).

The polyamines enhance the fluorescence intensity of thioglycolic acid QDs by up to 10 times, as shown in FIG. 16. Although not intending to be bound by theory, the fluorescence enhancement is likely to result from the direct interaction of the primary amines with the unsaturated Cd and Zn atoms on the QD surface, and

therefore reduces the possibility of charge carrier surface recombination induced by surface defects. It should also be noted, the current approach increases QD fluorescence instantly, which could lead to new development of nanoparticle-based fluorescence sensors.

As shown in FIG. 17, PL with approximately 40% of its primary amines changed to amides still activate QD very efficiently, but higher degree of modification leads to less fluorescence enhancement. Therefore, in typical assays, 3% of amines were grafted with PEG chains for better solubility and biocompatibility, and 30% with DTPA-Gd for magnetic contrast. This elaborate design leaves 67% of the primary amines intact, which are sufficient for fluorescence enhancement. The CdSe/ZnS QDs used in this report emit at 630 nm (~5 nm diameter) were mixed with PEG-PL-Gd in a molar ratio of 1:10. After purification with ultracentrifuge, the free polymers left in the supernatant was again quantified with Ninhydrin test in comparison with the original amount of polymer added into QDs. The results show that each nanoparticle is attached with 4.87 grafted polymers on average, corresponding to 497 Gd ions. It is well documented in the literature that DTPA-Gd immobilized on macromolecules and nanoparticles provides better T1 contrast than free DTPA-Gd³⁺ of the same concentration due to faster water exchange rate. Based on this mechanism, Gd-DTPA has been linked to biocompatible polymers (such as polylysine, polysaccharide, PEG, poly(glutamic acid) and *etc*), and natural macromolecular carriers (such as albumin), for MR imaging.

The stability of the dual-modality probe was performed. As aforementioned, the QD nanoparticle is highly water-soluble and bright due to the presence of the grafted polymer, which allows it to be dialyzed over an extended period of time without particle aggregation. After three days of dialysis, the PEG-PL-DTPA-Gd polymer coated QDs remain as a strong T1 contrast agent; however, in the control experiment (PL-PEG coated QDs mixed with free DTPA-Gd of the same concentration), only fluorescence is detectable, but not the T1 magnetic contrast. The results indicate that the probe is stable for *in vivo* molecular imaging, since most specific targeting results are accomplished within a few hours, and for PEG based long circulating probes, the images are often obtained in less than 48 hours. To demonstrate the feasibility of using QD-Gd as a dual modality probe, we injected 50 μ l of 50 nM QD-Gd conjugate into mice subcutaneously. MR imaging of mice before

and after injection on a clinical Philips 3T imager using standard protocols clearly marked the injection site (FIG. 18). In parallel, QD fluorescence was also detectable with a true color CCD camera and co-localized with the MR image.

In summary, the use of grafted polymer not only leads to formation of highly stable and bright QD nanoparticles, but also allows immobilization of paramagnetic compounds (such as Gd) and isotopes (*e.g.*, ^{111}In , ^{90}Y , $^{99\text{m}}\text{Tc}$, ^{67}Ga and etc) to their surface. Due to the large surface area of nanoparticles and the versatility of chelating chemistry, tri-modality probe combining optical, magnetic and isotopic properties can be further developed. The QDs offer significant advantages over organic dyes in optical properties, whereas Gd as a T1 contrast reagent (brightening) could provide better signal/noise ratio than the SPIO T2 contrast agents (darkening effect). By linking PEG termini with targeting probes, this technology is expected to open new opportunities in ultrasensitive and multiplexed molecular imaging. For example, MRI and PET allow examination of tumors in orthotropic organs, while fluoresce is ideal for high-resolution and multicolor biopsy studies. Improvements using DTPA functionalized amphiphilic polymers and specific targeting studies are underway and will be reported in clinical journals.

It should be emphasized that the above-described embodiments of the present disclosure are merely possible examples of implementations, and are merely set forth for a clear understanding of the principles of this disclosure. Many variations and modifications may be made to the above-described embodiment(s) of the disclosure without departing substantially from the spirit and principles of the disclosure. All such modifications and variations are intended to be included herein within the scope of this disclosure and protected by the following claims.

CLAIMS

Therefore the following is claimed:

1. A multimodality nanostructure, comprising:
 - at least one nanospecies having a first detectable functionality; and
 - a second molecule having a second detectable functionality, wherein the second molecule is attached to the nanospecies, and wherein the first detectable functionality and the second detectable functionality are different.
2. The nanostructure of claim 1, wherein the first detectable functionality and the second detectable functionality each are individually selected from the following: an optical functionality, a magnetic functionality, an x-ray functionality, and a radioactive functionality.
3. The nanostructure of claim 1, further comprising a bio-compatibility compound attached to the nanospecies.
4. The nanostructure of claim 3, wherein the bio-compatibility compound is a polyethylene glycol molecule having a molecular weight of about 500 to 50,000.
5. The nanostructure of claim 1, further comprising a third molecule having a third detectable functionality, wherein the third molecule is attached to the nanospecies.
6. The nanostructure of claim 5, wherein the first detectable functionality, the second detectable functionality, and the third detectable functionality are each selected from the following: an optical functionality, a magnetic functionality, an x-ray functionality, and a radioactive functionality; and wherein the first detectable functionality, the second detectable functionality, and the third detectable functionality are different.

7. The nanostructure of claim 1, wherein the nanospecies is a quantum dot and the second molecule is ^{67}Ga (gallium).
8. The nanostructure of claim 1, wherein the nanospecies is a quantum dot and the second molecule is selected from ^{111}In (indium), yttrium (^{90}Y), $^{99\text{m}}\text{Tc}$ (technetium), ^{201}Tl (thallium), ^{67}Ga (gallium), ^{123}I (iodine), ^{131}I (iodine), ^{32}P (phosphorus), ^{153}Sm (samarium), ^{186}Re (rhenium), ^{188}Re , ^{165}Dy (dysprosium), ^{166}Ho (holmium), and combinations thereof.
9. The nanostructure of claim 1, wherein the nanospecies is a quantum dot and the second molecule is selected from Cr, Mn, Fe, Cu, Eu, Gd, Dy, and combinations thereof.
10. The nanostructure of claim 5, wherein the nanospecies is a quantum dot and the second molecule is selected from ^{111}In (indium), yttrium (^{90}Y), $^{99\text{m}}\text{Tc}$ (technetium), ^{201}Tl (thallium), ^{67}Ga (gallium), ^{123}I (iodine), ^{131}I (iodine), ^{32}P (phosphorus), ^{153}Sm (samarium), ^{186}Re (rhenium), ^{188}Re , ^{165}Dy (dysprosium), ^{166}Ho (holmium), and combinations thereof; and the third molecule is selected from Cr, Mn, Fe, Cu, Eu, Gd, Dy, and combinations thereof, wherein the second molecule and third molecule are different.
11. The nanostructure of claim 1, wherein the nanospecies is a magnetic nanoparticle and the second molecule is selected from an organic dye, quantum dot, ^{111}In (indium), yttrium (^{90}Y), $^{99\text{m}}\text{Tc}$ (technetium), ^{201}Tl (thallium), ^{67}Ga (gallium), ^{123}I (iodine), ^{131}I (iodine), ^{32}P (phosphorus), ^{153}Sm (samarium), ^{186}Re (rhenium), ^{188}Re , ^{165}Dy (dysprosium), ^{166}Ho (holmium), and combinations thereof.

12. The nanostructure of claim 5, wherein the nanospecies is a magnetic nanoparticle; the second molecule is selected from an organic dye and a quantum dot; and the third molecule is selected from, ^{111}In (indium), yttrium (^{90}Y), $^{99\text{m}}\text{Tc}$ (technetium), ^{201}Tl (thallium), ^{67}Ga (gallium), ^{123}I (iodine), ^{131}I (iodine), ^{32}P (phosphorus), ^{153}Sm (samarium), ^{186}Re (rhenium), ^{188}Re , ^{165}Dy (dysprosium), ^{166}Ho (holmium), and combinations thereof; wherein the second molecule and third molecule are different.
13. The nanostructure of claim 12, wherein the magnetic nanoparticle is an iron oxide nanoparticle.
14. The nanostructure of claim 1, further comprising:
 - a hydrophobic protection structure including at least one compound selected from a capping ligand, an amphiphilic copolymer, and combinations thereof, wherein the hydrophobic protection structure encapsulates the nanospecies; and
 - a bio-compatibility compound disposed substantially on the surface of the hydrophobic protection structure;
 - wherein the second molecule is attached to the hydrophobic protection structure.
15. The nanostructure of claim 14, further comprising:
 - a third molecule having a third detectable functionality, wherein the third molecule is attached to the hydrophobic protection structure.
16. The nanostructure of claim 1, further comprising:
 - a polymer layer disposed substantially on the surface of the nanospecies; and
 - a bio-compatibility compound disposed substantially on the surface of the hydrophobic protection structure;
 - wherein the second molecule is attached to the polymer layer.
17. The nanostructure of claim 16, further comprising:

a third molecule having a third detectable functionality, wherein the third molecule is attached to the polymer layer.

18. A method of preparing a multimodality nanostructure, comprising the steps of:
providing a nanospecies having a first detectable functionality; and
disposing a second molecule to the nanospecies, wherein the second molecule has a second detectable functionality, and wherein the first detectable functionality and the second detectable functionality are different.
19. The method of claim 18, wherein the first detectable functionality and the second detectable functionality are each selected from the following: an optical functionality, a magnetic functionality, an x-ray functionality, and a radioactive functionality.
20. The method of claim 18, wherein the nanospecies is a quantum dot and the second molecule is selected from ^{111}In (indium), yttrium (^{90}Y), $^{99\text{m}}\text{Tc}$ (technetium), ^{201}Tl (thallium), ^{67}Ga (gallium), ^{123}I (iodine), ^{131}I (iodine), ^{32}P (phosphorus), ^{153}Sm (samarium), ^{186}Re (rhenium), ^{188}Re , ^{165}Dy (dysprosium), ^{166}Ho (holmium), and combinations thereof.
21. The method of claim 18, further comprising:
linking a third molecule to the nanospecies, wherein the third molecule has a third detectable functionality.

22. The method of claim 21, wherein the first detectable functionality, the second detectable functionality, and the third detectable functionality are each selected from the following: an optical functionality, a magnetic functionality, an x-ray functionality, and a radioactive functionality; and wherein the first detectable functionality, the second detectable functionality, and the third detectable functionality are different.
23. The method of claim 21, wherein the nanospecies is a quantum dot and the second molecule is selected from ^{111}In (indium), yttrium (^{90}Y), $^{99\text{m}}\text{Tc}$ (technetium), ^{201}Tl (thallium), ^{67}Ga (gallium), ^{123}I (iodine), ^{131}I (iodine), ^{32}P (phosphorus), ^{153}Sm (samarium), ^{186}Re (rhenium), ^{188}Re , ^{165}Dy (dysprosium), ^{166}Ho (holmium), and combinations thereof; and the third molecule is selected from Cr, Mn, Fe, Cu, Eu, Gd, Dy, and combinations thereof, wherein the second molecule and third molecule are different.
24. The method of claim 18, further comprising:
linking a bio-compatibility compound to the nanospecies.
25. A method of detecting a target in a subject, comprising:
providing a nanostructure having:
at least one nanospecies having a first detectable functionality,
a second molecule having a second detectable functionality,
wherein the second molecule is attached to the nanospecies, wherein the first detectable functionality and the second detectable functionality are different, and
at least one probe attached to the nanospecies, wherein a first probe has an affinity for the target;
introducing the nanostructure to a subject; and
determining the presence of the target in the subject that corresponds to the probe by detecting the first detectable functionality of the nanospecies and the second detectable functionality of the second molecule.
26. The method of claim 25, wherein nanostructure includes a third molecule having a third functionality, wherein the first detectable functionality, the

second detectable functionality, and the third detectable functionality are different, and further comprising:

determining the presence of the target in the subject corresponding to the probe by detecting the first detectable functionality of the nanospecies, the second detectable functionality of the second molecule, and the second detectable functionality of the second molecule.

27. The method of claim 26, wherein the target is a cancerous disease.
28. The method of claim 27, wherein the determination is made *in vivo*.
29. The method of claim 25, wherein the first probe is selected from a polynucleotide, a polypeptide, an antibody, an antigen, and combinations thereof.
30. The method of claim 25, wherein the introduction is performed by a method selected from at least one of the following: a subcutaneous injection and a systemic injection.
31. The method of claim 25, wherein the nanostructure further comprises a bio-compatibility compound attached to the nanospecies.

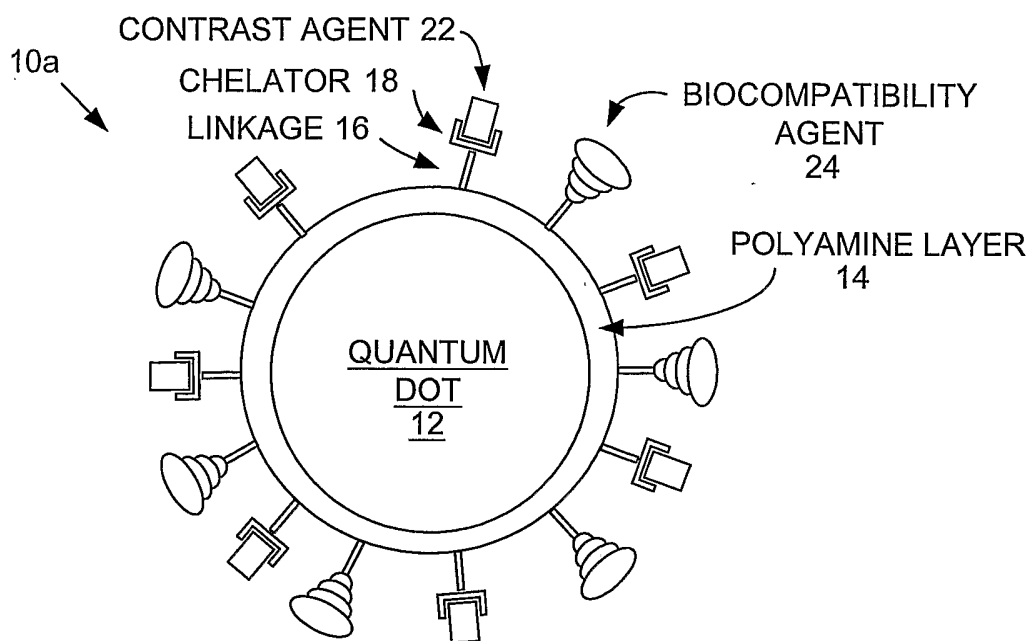


FIG. 1

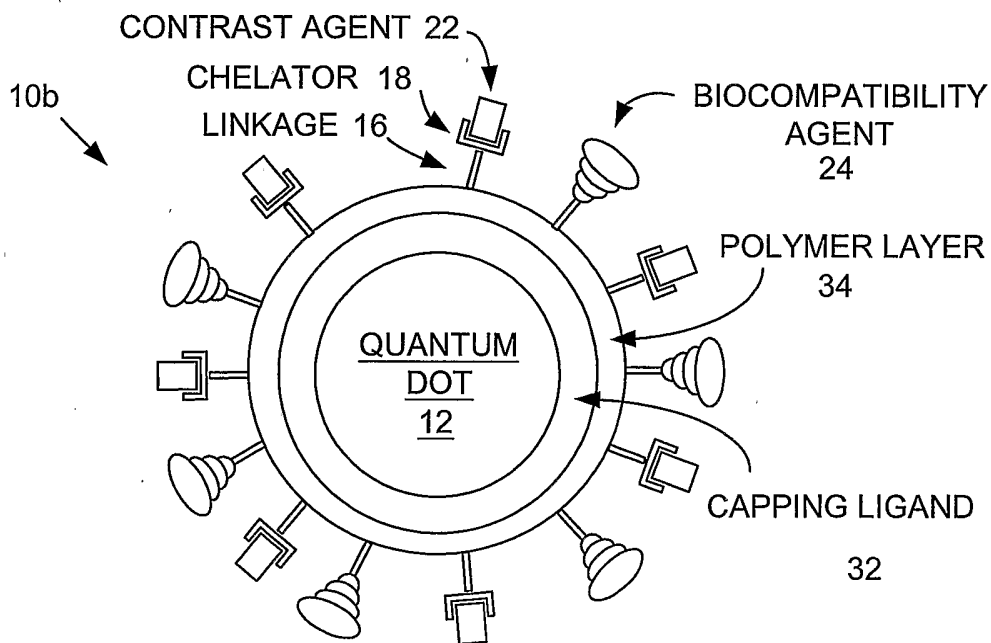


FIG. 2

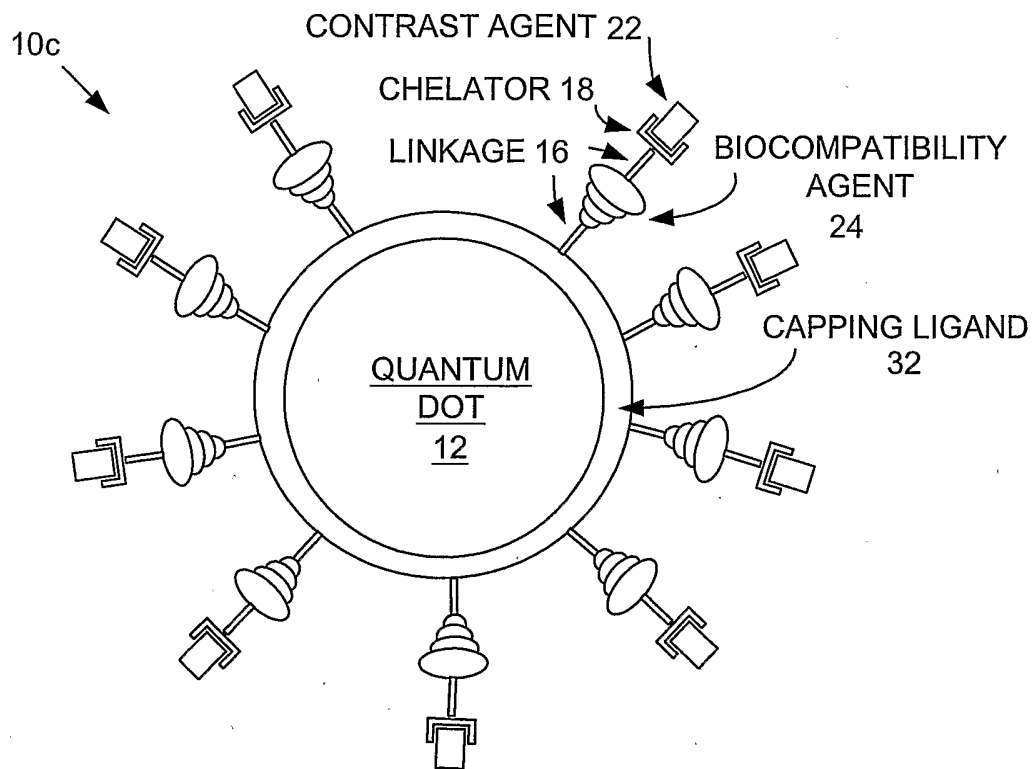


FIG. 3

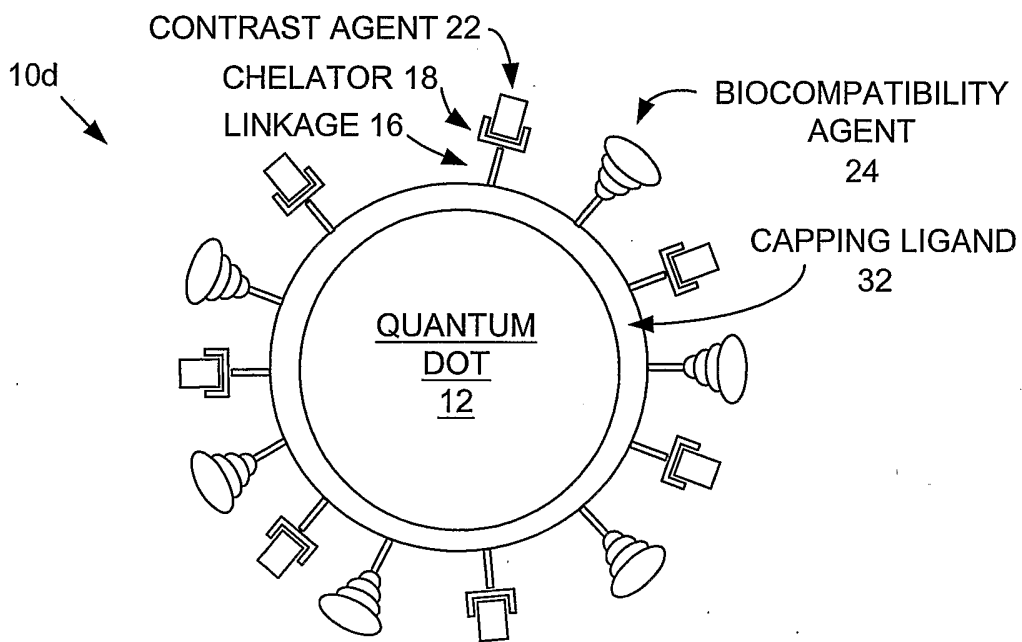


FIG. 4

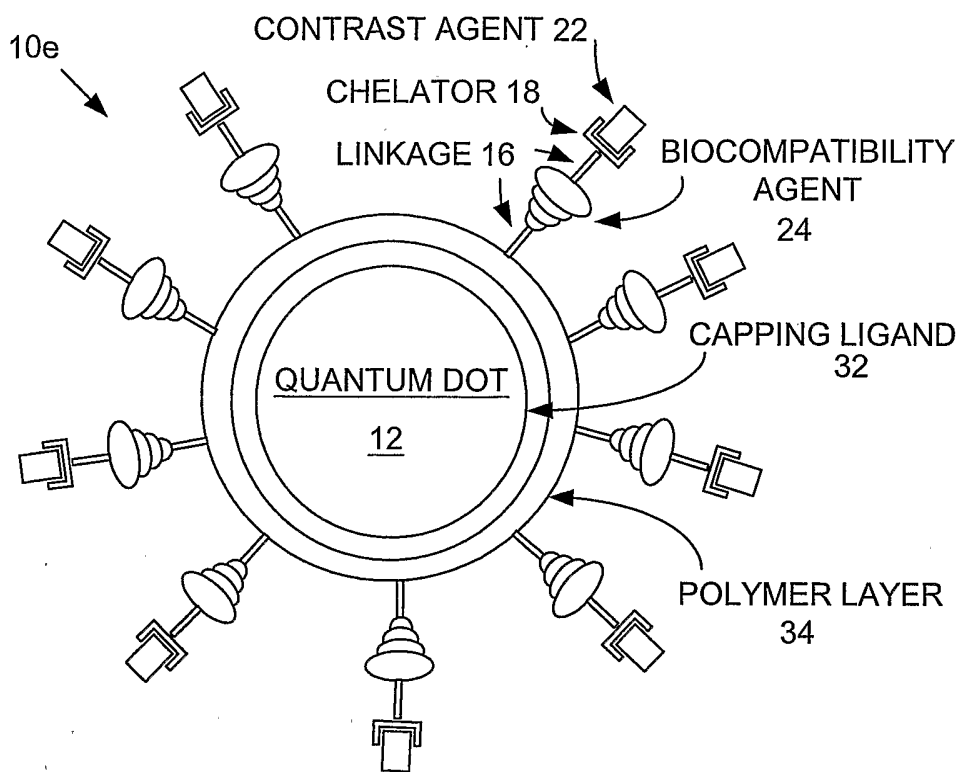


FIG. 5

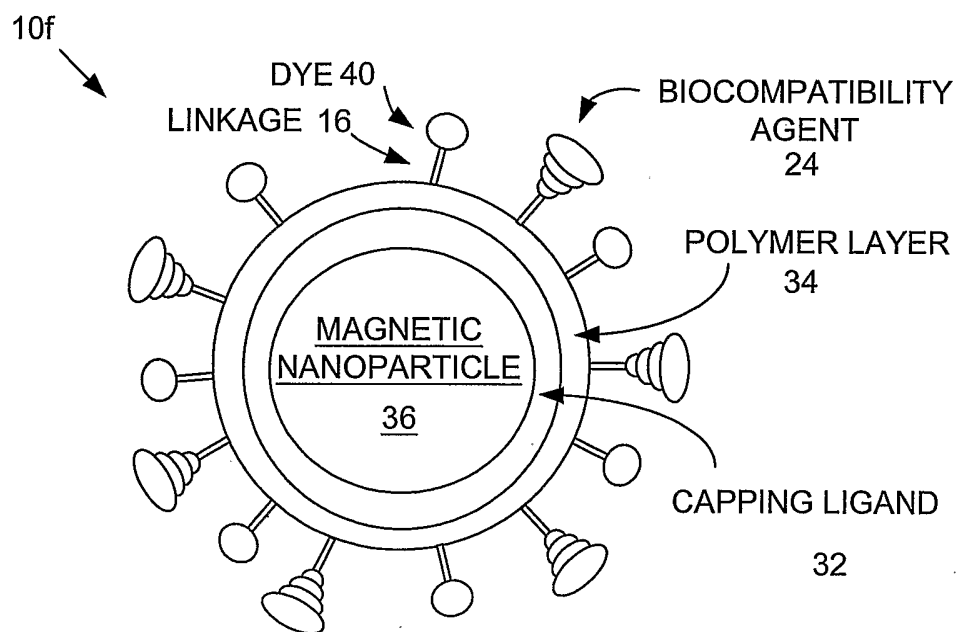


FIG. 6

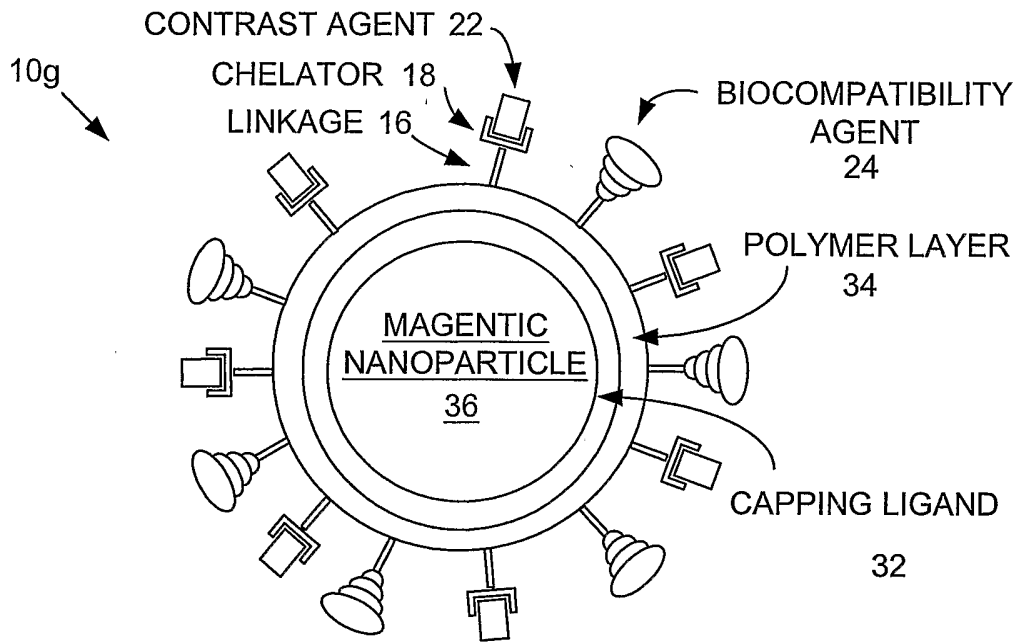


FIG. 7

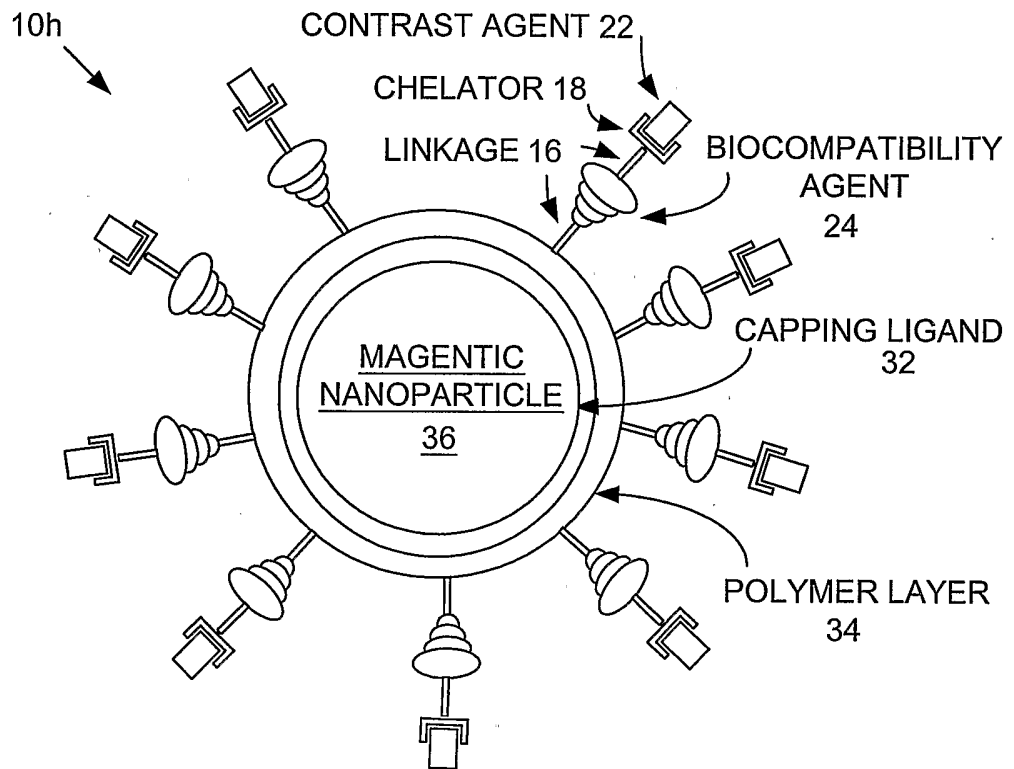


FIG. 8

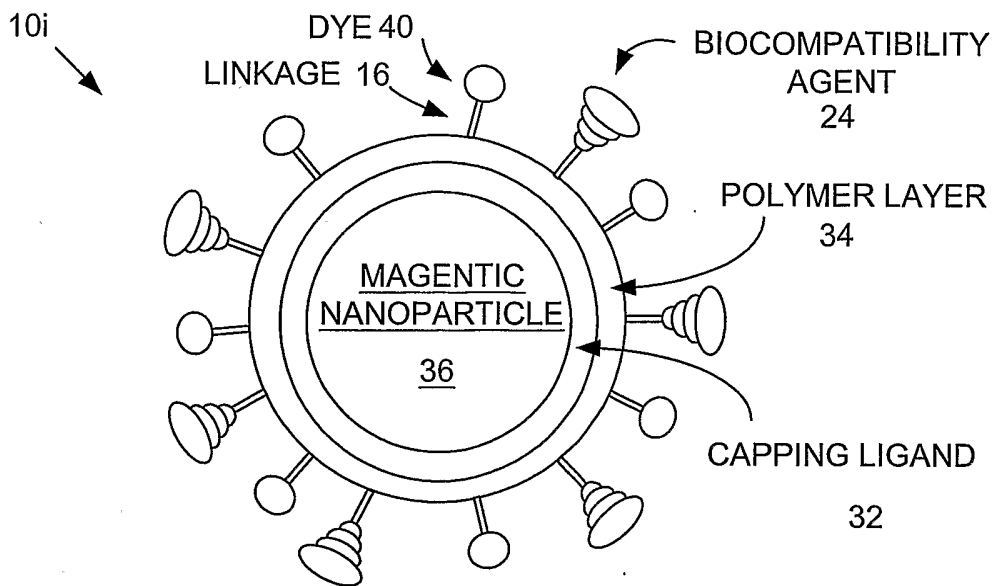


FIG. 9

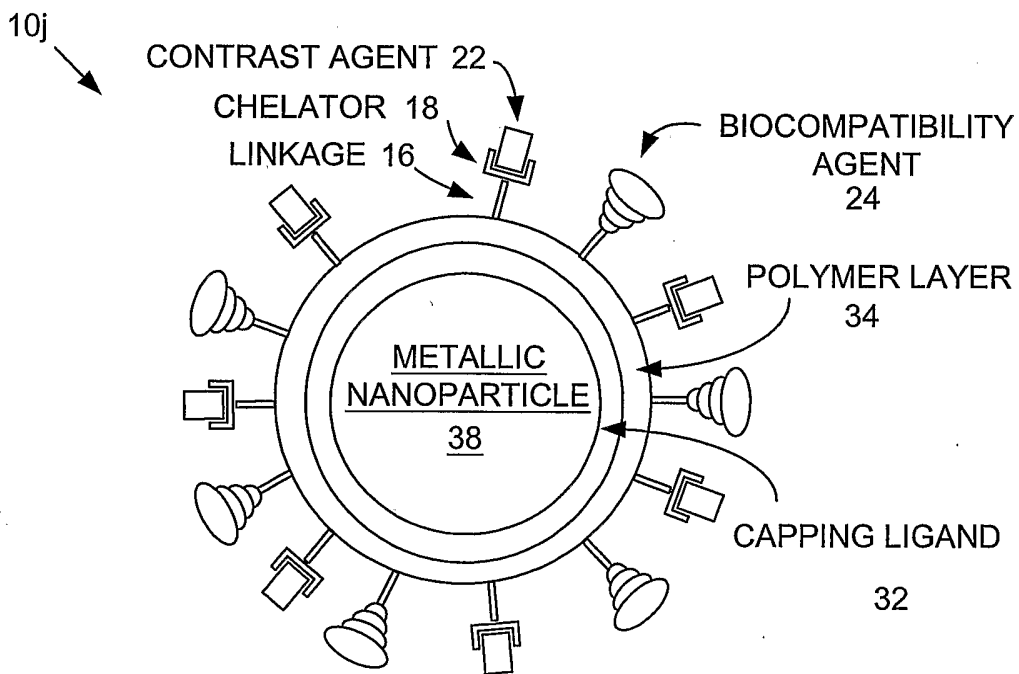


FIG. 10

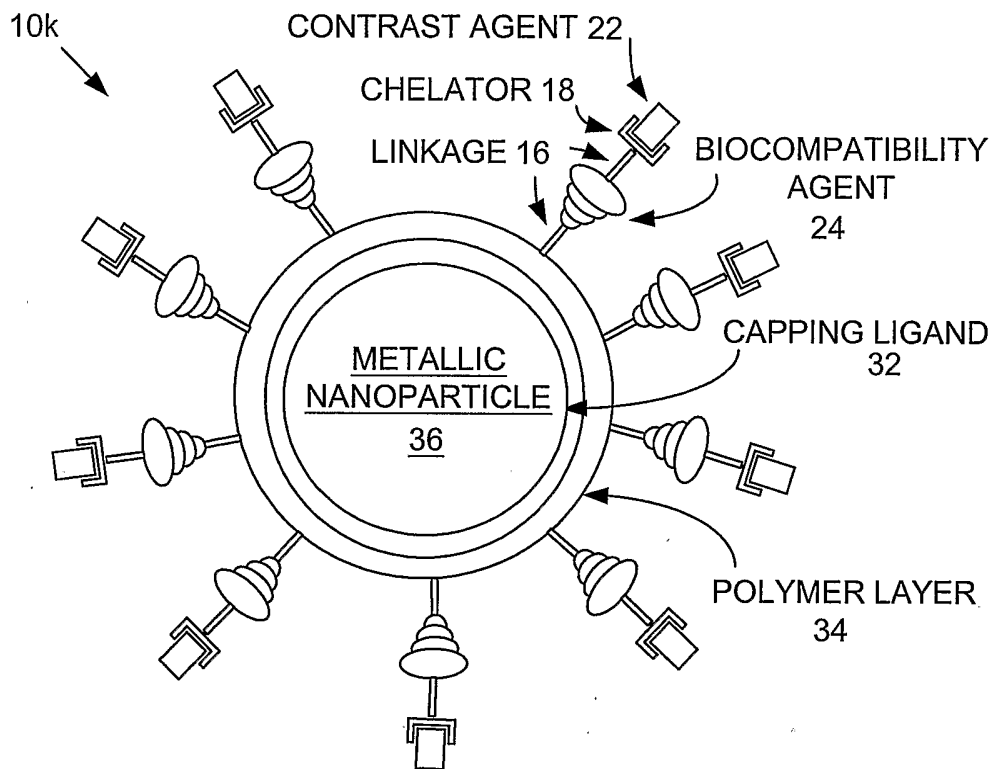


FIG. 11

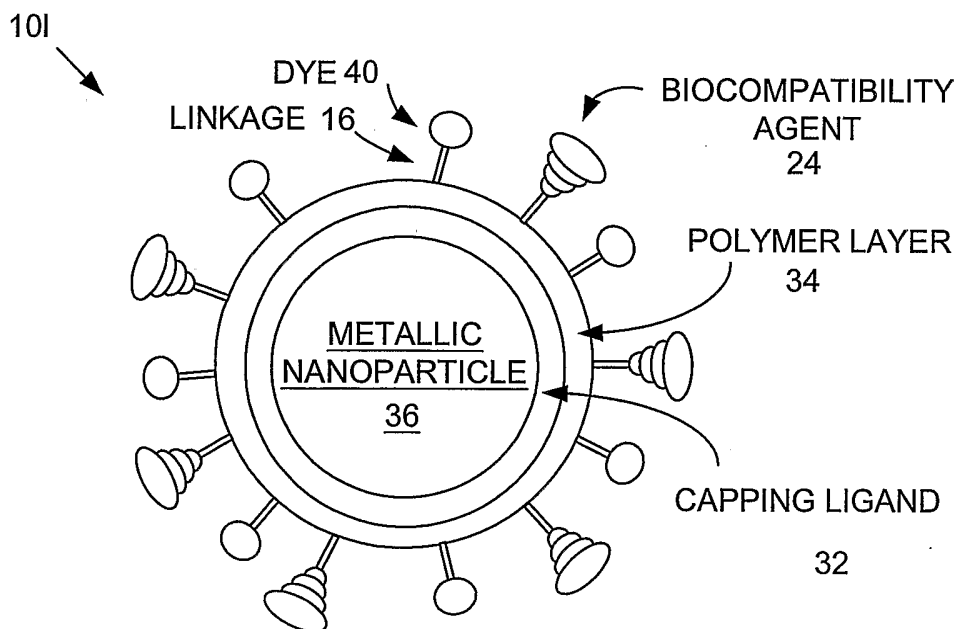


FIG. 12

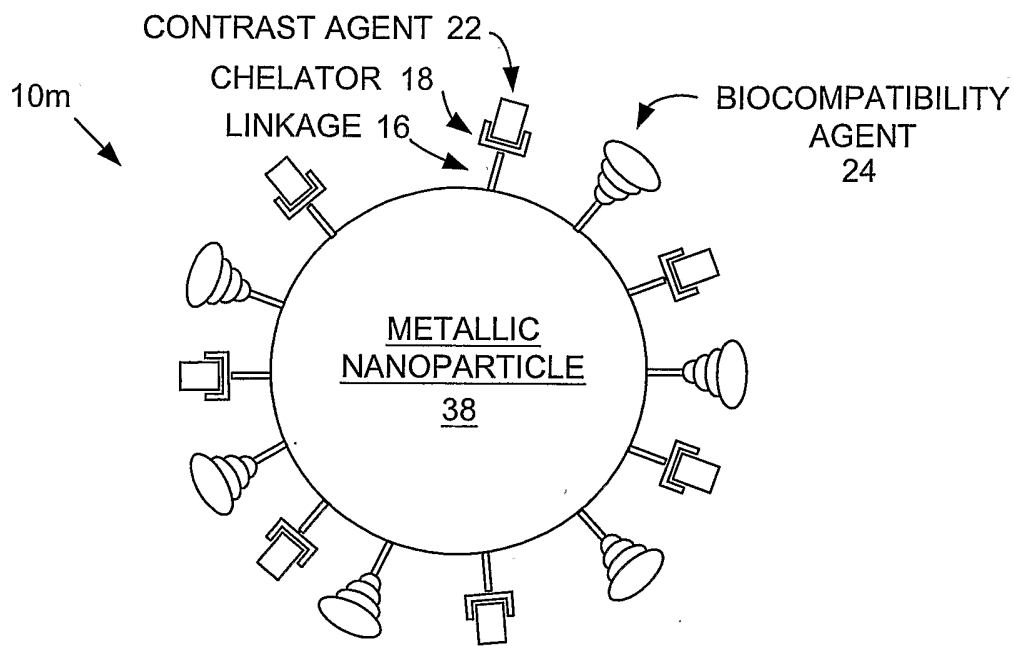


FIG. 13

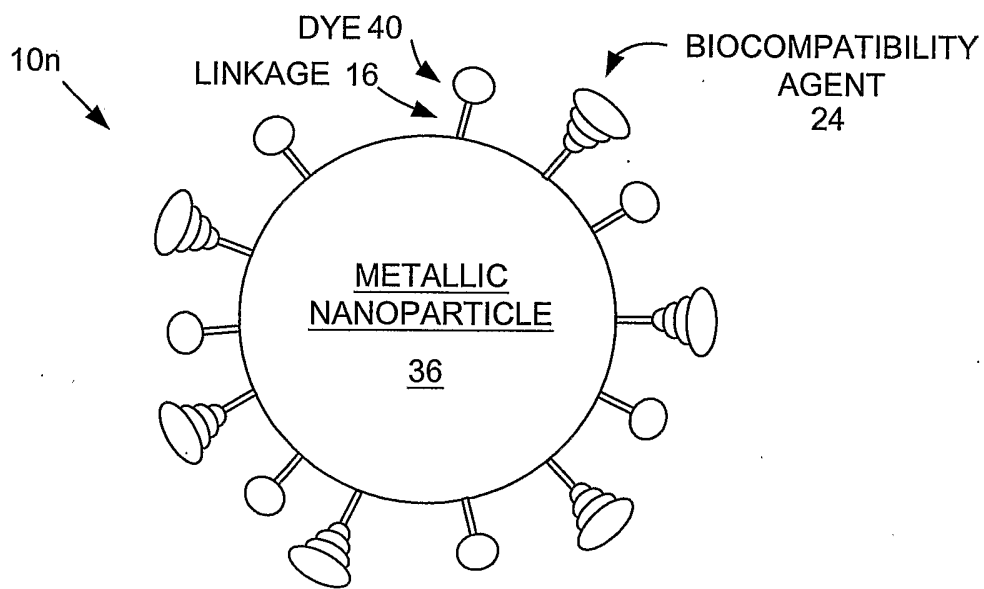


FIG. 14

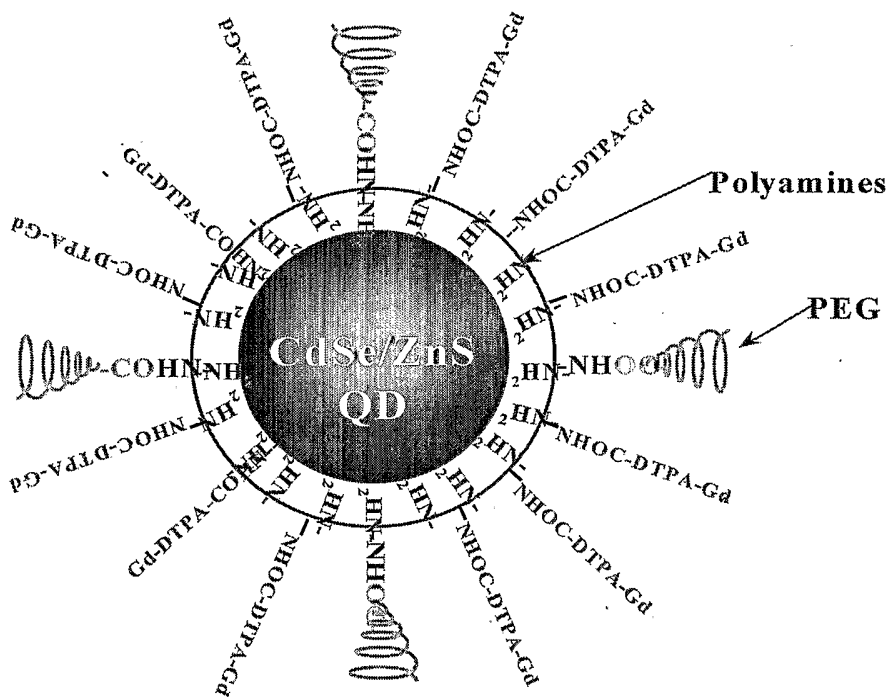


FIG. 15

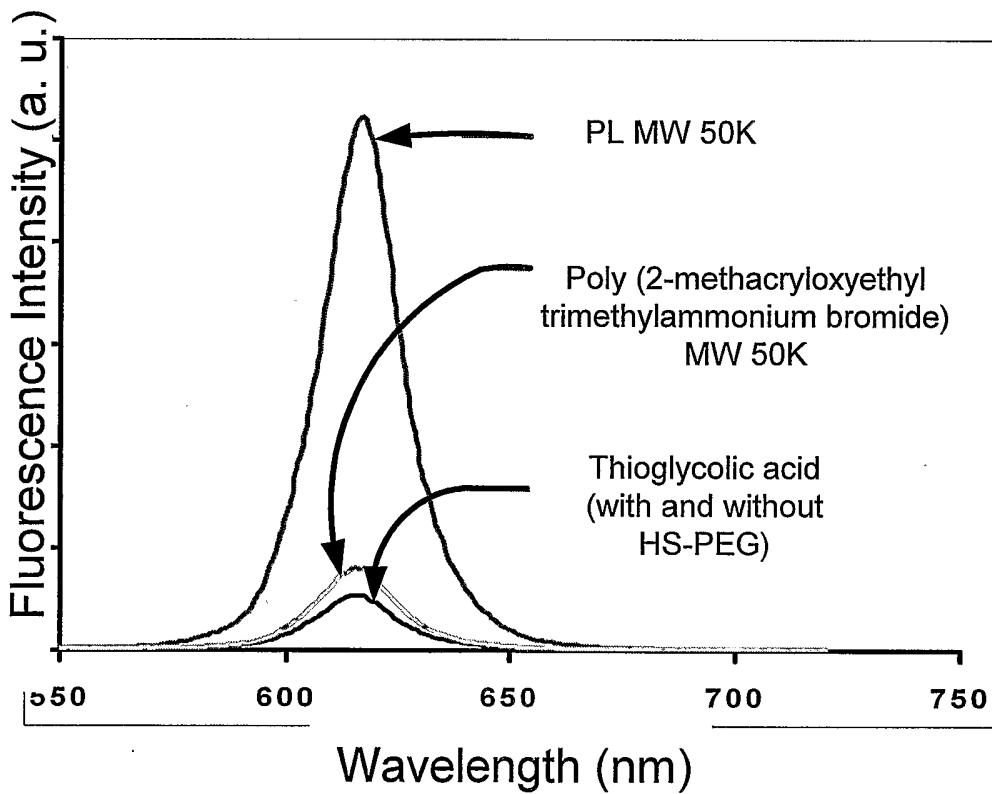


FIG. 16

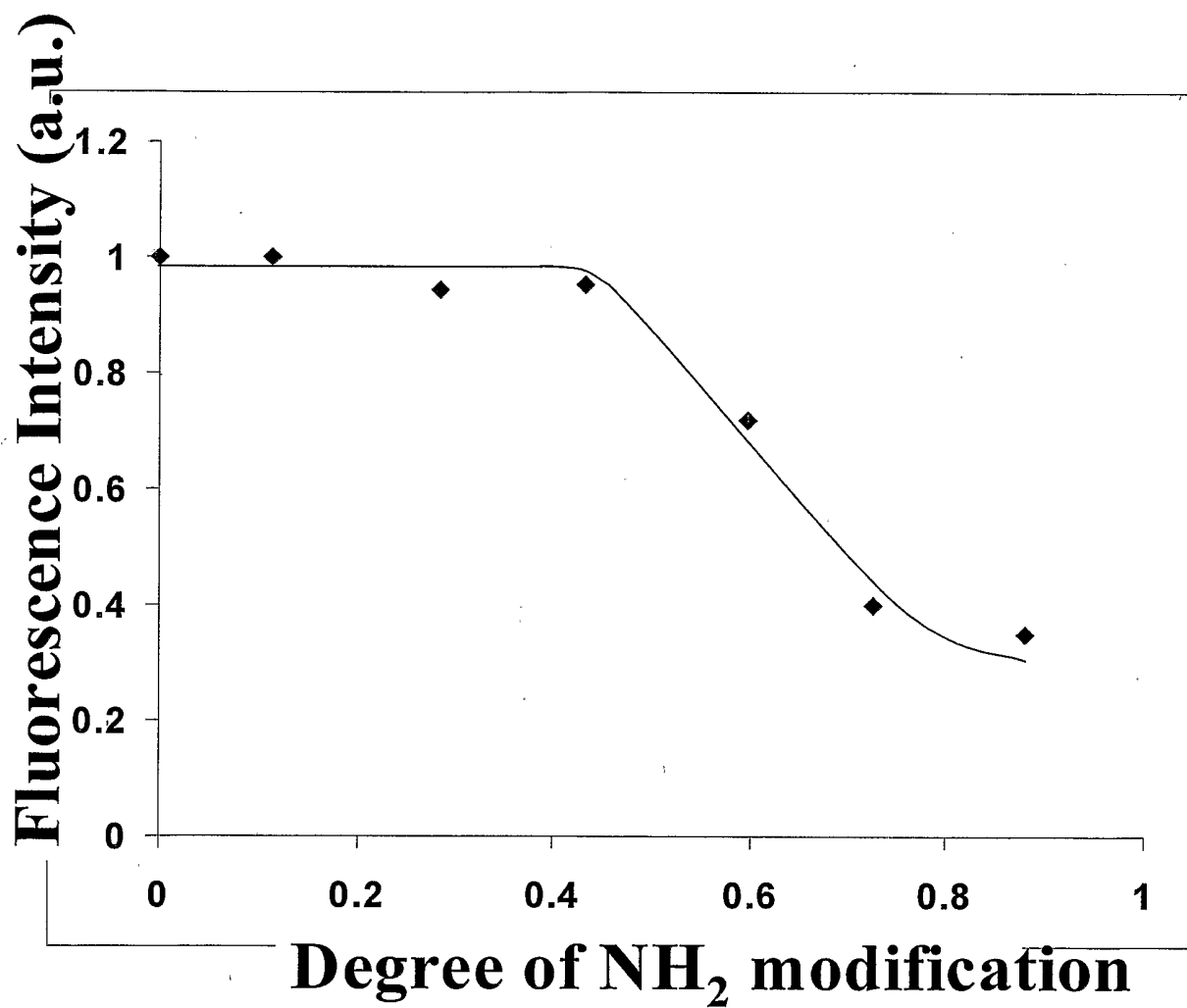


FIG. 17

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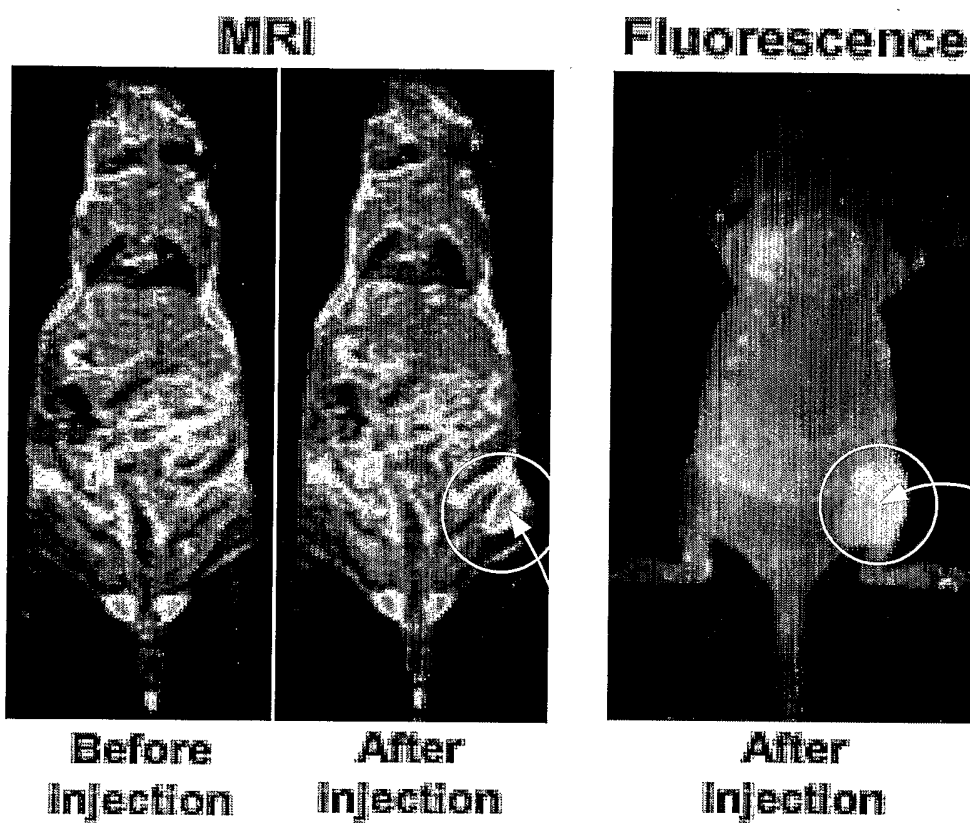


FIG. 18