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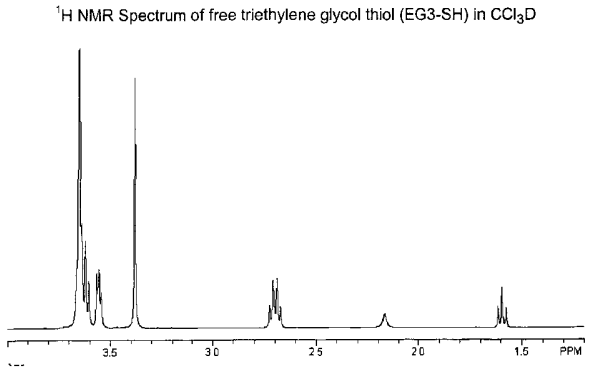

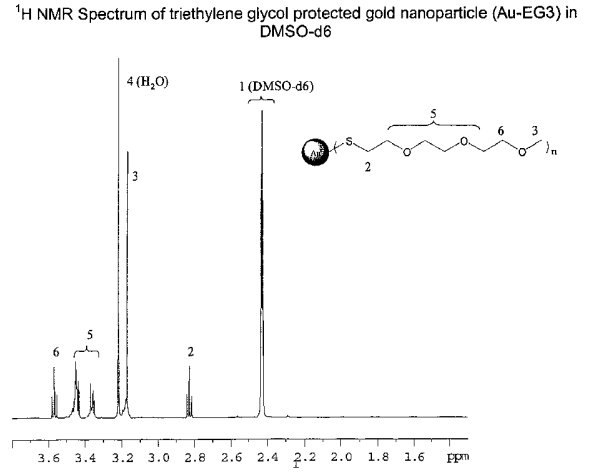

(57) Abstract: Metallic nanoparticles coated with a mixed monolayer having a defined ratio of shielding component to capture component are isolated for the specific capture of a ligand of interest.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TITLE

NANOPARTICLES COMPRISING A MIXED MONOLAYER FOR SPECIFIC LIGAND BINDING

This Application claims the benefit of United States Provisional Application 60/400,144, filed August 1, 2002.

FIELD OF INVENTION

The invention relates to the preparation of metal nanoparticles coated with a mixed monolayer optimized for maximal specific binding of a ligand of interest. Accordingly, the method of the present invention provides a controlled number of specific binding sites for biological entities (such as proteins, DNA, and cells) on a single nanoparticle.

BACKGROUND

Nanomaterials and their hybrids with biological molecules are recognized to have potential applications in electronic, optics, genomics, proteomics, and biomedical and bioanalytical areas. The usefulness of the hybrid materials largely depends on how well can one achieve rational design based on specific binding between inorganic nanomaterials and biological molecules. It is generally recognized that the surfaces of many nanoparticles, such as Au, Ag, Pt and Cu are charged, which cause non-specific binding with biological molecules via electrostatic interactions. Alkyl thiol protected nanoparticles reduce the electrostatic interactions but induce hydrophobic interactions, another type of non-specific interaction. In addition, alkyl thiol protected nanoparticles are not soluble in water, which makes them non-compatible with biomolecules that require aqueous environment for activity. Prime et al. (*Science* (1991), 252(5009), 1164-7) and Lahiri et al. (*Analytical Chemistry*, 1999 Feb 15, 71(4) 777-90) both describe a method to prepare a model system for the adsorption of proteins on surfaces by preparing self-assembled monolayers of alkanethiols on gold films. Singh et al. describes a method for using gold nanorods to extract groups of biomolecules, using derivatized alkanethiols (U.S. Patent Appl. 20020034827).

Nanoparticles coated with tiopronin (N-2-mercaptopropionyl-glycine, TP) have the advantage of being water-soluble, but are subject to non-specific binding of biomolecule ligands. Templeton et al. (JACS, 1999, 121, pg. 7081) prepared gold clusters coated with single monolayers of tiopronin and acidic, water soluble ligands. Gold and silicon surfaces have been coated with ethylene glycol derivatives and have been

shown to resist protein binding on a flat gold surface. Additionally gold nanoparticles coated with polyethylene glycol have been shown to be water soluble. However, the neutral glycols con not react easily with biomolecules, and the monolayer thickness is not well defined, particularly with the large polymer chains.

Foos et.al (*Chem. Mater.* (2002), 14, pg. 2401-2408) have prepared a gold nanocluster coated with short chain ethylene glycol oligomers in a monolayer. However, the authors used a two-phase organic solvent system (as opposed to an aqueous/organic mixed solvent) for preparation and not all of the coated particles were found to be water-soluble.

Applicants have previously constructed a water soluble, metallic nanoparticle with a mixed monolayer, comprising a capture component and a shielding component with well defined thickness or length using a single phase organic/aqueous solvent system (U. S. Provisional Application 60/400,144). The shielding component, thiolated ethylene glycol short chain oligomers with well defined thickness or length, functioned to minimize the non-specific interaction between nanoparticles and biological molecules, whereas the capture coating component, (e.g. tiopronin), served as ligands to engage biological molecules specifically. Applicants have improved upon this system by developing a method to determine the critical ratio of the capture component to the shielding component, optimizing the ability to be inert to non-specific binding with biological molecules while still maintaining specific binding to biological molecules.

SUMMARY OF THE INVENTION

The invention provides a method for the preparation of a nanoparticle coated with a mixed monolayer specifically optimized for the specific binding a ligand of interest. The monolayer contains a shielding component and a capture component. The shielding component is designed to prevent non-specific binding of ligands and particularly biomolecules, to the monolayer and is typically an ethylene glycol derivative. The capture component is designed to comprise a binding site for the ligand of interest (typically a biomolecule such as a peptide of nucleic acid). Applicants have discovered that by varying the ratio of capture component to shielding component the nanoparticle may be engineered to specifically bind the ligand of interest while excluding all other ligands or materials. The invention provides methods of determining

the specific critical ratio of the two monolayer components for the specific ligand to be bound. Accordingly the invention provides a process for the isolation of a population of water soluble metallic nanoparticle having a specific affinity for a ligand of interest comprising:

a) providing a multiplicity of soluble metallic nanoparticles each coated with a mixed monolayer, the monolayer comprising:

i) an ethylene glycol component; and

ii) a capture coating component having an affinity for a ligand of interest;

wherein the ratio of ethylene glycol component to capture coating component on each nanoparticle is different;

b) contacting each metallic nanoparticle of (a) with a mixture of standard diverse ligands for a time and under conditions where non-specific ligands binding occurs on some nanoparticles and no ligand binding occurs on other nanoparticles;

c) identifying a first subpopulation of nanoparticles of (b) where no ligand binding occurs;

d) contacting the first subpopulation of nanoparticle of (c) with a ligand of interest for a time and under conditions whereby binding of the ligand to the nanoparticle occurs; and

e) isolating a second subpopulation of nanoparticles of (d) whereby ligand binding to the nanoparticle is specific.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1(a) is a ^1H NMR spectrum of free triethylene glycol thiol in CCl_3D

Figure 1(b) is a ^1H NMR spectrum of triethylene glycol protected gold nanoparticle in DMSO-d6.

Figure 2 is a ^1H NMR spectrum of triethylene glycol and tiopronin mixed monolayer protected gold nanoparticle in DMSO-d6. The molar feeding ratio of EG3-SH and Tp for synthesis of the nanoparticle is 4/1.

Figure 3 is a graph plotting the reactivity of EG3-SH vs. tiopronin in the formation of a mixed monolayer protected nanoparticle.

Figure 4(a) is a gel electrophoresis image illustrating the change and migration of EG3/Tp mixed monolayer protected nanoparticle controlled by the percentage of Tp on the nanoparticle surface.

Figure 4(b) is a gel electrophoresis image of the nanoparticles from Figure 4(b) bonded with lysozyme.

Figure 5(a) is a gel electrophoresis image illustrating the change and migration of EG3/GSH mixed monolayer protected nanoparticles controlled by the percentage of GSH on the nanoparticle surface.

Figure 5(b) is a gel electrophoresis image of the nanoparticles from Figure 5(a) bonded with lysozyme.

Figure 6 is a gel electrophoresis image illustrating specific binding of a nanoparticle with a mixed monolayer of EG3 and GSH at feeding ratio of 19 ([EG3-SH]/[GSH]) with GST protein.

Figure 7 is a gel electrophoresis image illustrating specific binding of a nanoparticle with a mixed monolayer of EG3 and GSH-biotin with streptavidin protein.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to nanoparticles coated with a mixed monolayer that has been designed to bind a specific ligand while excluding other ligands. The coated nanoparticles of the present invention are useful in the fabrication of nanodevices where uniform water soluble particles are needed as binding agents for the immobilization of, and attachment to, proteins and nucleic acids and other materials in a specific fashion. Additionally the coated nanoparticles of the invention may be used in of nanoscale electronic devices, multifunctional catalysts, chemical sensors, and many biological applications such as biosensors, biological assays.

The following definitions and abbreviations will be used herein for the interpretation of the claims and the specification:

"EG2" is the abbreviation for diethylene glycol

"EG3" is the abbreviation for triethylene glycol

"EG4" is the abbreviation for tetraethylene glycol

"GSH" is the abbreviation for glutathione

"GST" is the abbreviation for glutathione s-transferase

"TBE" is the abbreviation for tris-borate-EDTA

"DMSO" is the abbreviation for dimethyl sulfoxide

"EDC" is the abbreviation for 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.

"GSH" is the abbreviation for glutathione.

"MES" is the abbreviation for morpholinoethane sulfonic acid.

"TP" is the abbreviation for tiopronin

" V/V" is the abbreviation for volume/volume.

"Nanoparticles" are herein defined as metallic particles with an average particle diameter of between 1 and 100 nm. Preferably, the average particle diameter of the particles is between about 1 and 40 nm. As used herein, "particle size" and "particle diameter" have the same meaning. The metallic nanoparticles include, but are not limited to, particles of gold, silver, platinum, palladium, iridium, rhodium, osmium, iron, copper, cobalt, and alloys composed of these metals. An "alloy" is herein defined as a homogeneous mixture of two or more metals.

A "monolayer" refers to a layer of material coated on a nanoparticle that is the thickness of single molecule.

A "mixed monolayer" refers to a monolayer having at least two different molecular components.

A "ligand" refers to a substance that binds specifically to a capture coating component. Ligands may be any substance and typically are biopolymers such as peptides, proteins, cell and nucleic acid fragments. A "ligand of interest" is a particular ligand that is the focus of any particular investigation.

The term "standard diverse ligands" means a group of ligands that do not contain a ligand of interest. Typically the standard diverse ligands comprise biomolecules that are found in the same environment where the ligand of intents is found.

The term "binding pair" refers to chemical or biopolymer based couples that bind specifically to each other. Common examples of binding pairs are immune-type binding pairs, such as antigen/antibody or hapten/anti-hapten systems.

A "capture coating component" as used herein refers to a material capable of forming a monolayer on a nanoparticle that has an affinity for the ligand.

A "shielding coating component" refers to a material capable of forming a monolayer on a nanoparticle that has the ability to prevent non-specific binding of substances that are not ligands of interest. Shielding coating components may be comprised of a variety of materials where ethylene glycol is particularly suitable.

The term "entanglement molecular weight" as used in reference to the shielding component of the mixed monolayer means the minimum molecular weight beyond which the polymer molecules used as shielding component show entanglement. Methods of determining the entanglement

molecular weight of a polymer are known, see for example Friedrich et al., Progress and Trends in Rheology V, Proceedings of the European Rheology Conference, 5th, Portoroz, Slovenia, Sept. 6-11, 1998 (1998), 387. Editor(s): Emri, I. Publisher: Steinkopff, Darmstadt, Germany.

The term "nano-structure" means tubes, rods, cylinders, bundles, wafers, disks, sheets, plates, planes, cones, slivers, granules, ellipsoids, wedges, polymeric fibers, natural fibers, and other such objects which have at least one characteristic dimension less than about 100 microns.

The terms "nano-rod" means a variety of nano-structures which may be either hollow or solid and may or may not have a circular crossectional shape. Nano-rods of the invention may include nanotubes, nanofibers, polymeric nanofibers, bundles and multiwalled structures.

The term "nanotube" refers to a hollow article having a narrow dimension (diameter) of about 1-200 nm and a long dimension (length), where the ratio of the long dimension to the narrow dimension, i.e., the aspect ratio, is at least 5. In general, the aspect ratio is between 10 and 2000.

By "nanoplanes" is meant surfaces that have one characteristic dimension less than 500 nanometer, for example a single or a dual layer of graphite or graphene sheets.

By "nanofibers" is meant natural or polymeric filaments that have a small dimension of less than 1000 nanometer.

The term "metal binding functionality" refers to a chemical group that effects attachment of a molecule to a metal surface. One non-limiting example of a metal binding functionality is the sulfhydral (-SH) functional group that effects the binding of materials to gold and other metals.

The term "mixed solvent" refers to a solvent comprising both an organic component and an aqueous component, wherein the organic component is substantially water miscible.

A "substantially water miscible organic solvent" is herein defined as an organic solvent that dissolves completely in water up to a concentration of at least 80% by volume.

The term "Ligand-replacement method" refers to a method of coating a nanoparticle with a mixed monolayer where a first component of the mixed monolayer is coated on the nanoparticle and then elements of the first component are subsequently replaced with elements of a second component until a mixed monolayer is achieved.

The term "direct-synthesis method" refers to a method of coating a nanoparticle with a mixed monolayer where both components of the mixed monolayer are reacted in a single solution in the presence of the metal nanoparticle where a mixed monolayer is formed on the nanoparticle.

Coated Nanoparticles

The invention provides a water soluble metal nanoparticle coated with a mixed monolayer having a shielding coating component capture component in such a ratio as to provide for specific binding of a ligand of interest while excluding the binding of other ligands.

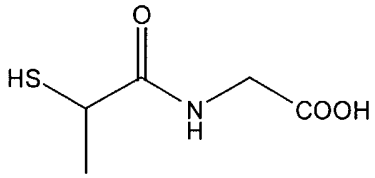
Nanoparticles of the invention may be comprised of a variety of metals, including but not limited to gold, silver, platinum, palladium, iridium, rhodium, osmium, iron, copper, cobalt, and alloys composed of these metals. Preferred for use herein are nanoparticles comprised of gold. Typically un-coated nanoparticles of the invention range in diameter from bout 1nm to about 100 nm, where from about 1nm to about 40 nm is preferred. Methods of preparing metal nanoparticles are well known in the art (see for example Templeton, A.C.*et al.*, *Acc. Chem. Res.* 2000, 33, 27-36) and are additionally available commercially from sources such as Sigma Chemical Company.

The nanoparticles of the invention are coated with a mixed monolayer having a capture component and a shielding component as part of the same monolayer. The capture component may range from about 10% to about 90% of the monolayer however more typically the capture component comprises less than about 50% of the mixed monolayer where about 20%-40% is preferred. Conversely the shielding component typically forms the major component of the monolayer attaining from about 10% to about 90% of the monolayer where at least about 50% of the monolayer is suitable, and where about 60% to about 90% is preferred.

The capture component functions to bind various materials to the coated nanoparticle. It is preferred if the capture component has a specific affinity for a single ligand. Typical ligands of the invention include for example, biopolymers such as peptides, proteins, nucleic acid fragments, and collagen, as well as nanomaterials useful in the assembly and synthesis of nanodevices such as for example various nano-structures (nano-rods, nano-tubes, nano-planes and nano-fibers as defined herein). Ligands may also comprise one member of a binding pair. Chemical and

protein based binding pairs are well known in the art and include, but are not limited to combinations such as, Glutathione-S-transferase/glutathione, 6X Histidine Tag/Ni-NTA, Streptavidin/biotin, S-protein/S-peptide, Cutinase/phosphonate inhibitor. Additionally binding pairs will include any of the class of immune-type binding pairs, such as antigen/antibody or hapten/anti-hapten systems; and also any of the class of nonimmune-type binding pairs, such as biotin/avidin; biotin/streptavidin; folic acid/folate binding protein; complementary nucleic acid segments, including peptide nucleic acid sequences; protein A or G/immunoglobulins; and binding pairs, which form covalent bonds, such as sulfhydryl reactive groups including maleimides and haloacetyl derivatives, and amine reactive groups such as isotriocyanates, succinimidyl esters and sulfonyl halides.

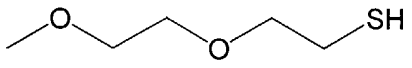
The capture component may be functionalized with various chemical groups that allow for binding to a ligand of interest. Non-limiting examples of such chemical reactive groups include those selected from the group consisting of: $-NH_2$, -COOH, -CHO-, -OH, -X (Cl, Br, I), succinimide, and epoxy groups. Preferred examples of suitable capture components are tiopronin and GSH. Tiopronin (abbreviated TP), is N-2-mercaptopropionyl-glycine is particularly suitable as a capture component because of the presence of exposed carboxy groups (as illustrated below) which serves as a convenient binding site for ligands.



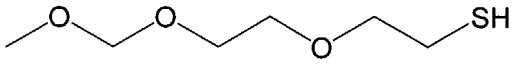
The shielding component of the mixed monolayer serves to block the binding of ligands that are not of interest to the coated nanoparticle and permits the nanoparticle to be used to bind, isolate or immobilize specific ligands of interest. The principal requirement of the shielding component is that it not bind the ligand, and typically will be an uncharged, water soluble molecule of well defined length. Polymers of excessive length may have the effect of blocking the binding sites on the capture

component and thus polymer length must be controlled. Suitable shielding components will include but are not limited to short chain ethylene glycol oligomers, ethylene glycol methacrylate, sugars, crown ethers, and acrylamide, where the short chain ethylene glycol oligomers are preferred.

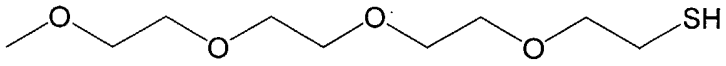
The shielding component may be functionalized to bind to the surface of the metal nanoparticle. A common method of conveying metal binding functionality to a material is the addition of a sufhydral group as illustrated below for two of the preferred short chain ethylene glycol oligomers:



EG2-SH



EG3-SH



EG4-SH

Although any short chain ethylene glycol oligomers are suitable it is generally preferred if they are limited in size such that they are less than the entanglement molecular weight of the oligomer. While not meaning to suggest a mechanism for this preference, it is believed that short chain shielding components are more suitable as opposed to long polymer chains to avoid blocking the binding sites of the capture component. It is reasonable to expect that short chain shielding components will allow the ligand binding sites on the capture component to be accessible to the ligand. Longer chain major components may block the ligand binding functionality, preventing any binding from occurring.

Synthesis of Coated Nanoparticles

The present invention provides nanoparticles coated with a mixed monolayer having a rationally designed ratio of coating component to shielding component. Preferred herein are two methods for the synthesis of such nanoparticles referred to as the ligand-replacement method and the direct synthesis method. The two methods differ principally in the

order of the addition of components, and will be described in greater detail below.

Ligand-Replacement

The ligand-replacement method proceeds with the coating of a metal nanoparticle with a first component of the mixed monolayer, followed by exposure of the coated particle to a second component. Under the appropriate reaction conditions, elements of the first component are replaced by elements of the second component such that a mixed monolayer is formed. The ligand-replacement method comprises the following process steps:

a) providing a water soluble metallic nanoparticle coated with monolayer of a capture coating component having an affinity for a ligand of interest; and

b) mixing the coated nanoparticle of (a) with a shielding coating component in a mixed solvent, the shielding component having metal binding functionality; the mixed solvent comprising at least one substantially water miscible organic solvent and at least one aqueous solvent wherein the mixed solvent is at a pH of less than 7.0; wherein exchange occurs between the shielding coating component and the capture coating component to form a nanoparticle coated with a mixed monolayer.

Optionally the mixed monolayer coated metal nanoparticle of may be isolated from solution by standard methods such as filtration, centrifugation, or distillation.

Of particular importance in the ligand-replacement method for the preparation of the mixed monolayer is the use of a mixed solvent at an acidic pH. The mixed solvent comprises an aqueous portion and an organic portion where the organic portion is substantially miscible in water. Suitable organic solvents include, but are not limited to, C1-C6 alkanols (e.g. methanol, ethanol, isopropanol), dimethyl sulfoxide, tetrahydrofuran, dimethylformamide, Dioxane, and acetone. Suitable solvents also include mixtures of organic solvents that are completely miscible with each other and that result in a mixture which is a substantially water-miscible organic solvent. Examples of multi-component organic solvents include, but are not limited to, ethyl acetate and methanol; ethyl acetate and ethanol; ethyl acetate and isopropanol; ethyl acetate and acetone; ethyl acetate, dimethylformamide and dimethyl sulfoxide; and ethyl acetate,

tetrahydrofuran, and dioxane. The preferred organic solvent is methanol or ethanol.

The aqueous portion of the mixed solvent may simply be water, however it is generally an acid such as acetic acid since it is preferable if the reaction take place in an acid environment, generally at a pH of less than about 7.0 where a pH of less than about 5.5 is preferred.

The ligand replacement method relies on providing a metallic nanoparticle coated with a monolayer. Methods of synthesizing a nanoparticle coated with a single monolayer are known. For example, Templeton et al. (*Langmuir* 15:66-76 (1999)), herein incorporated by reference, describe a method for the preparation of, charged, water-soluble gold nanoparticles protected by tiopronin or coenzyme A monolayers. To prepare the tiopronin-protected gold nanoparticles, tetrachloroauric acid and N-(2-mercaptopropionyl)glycine (tiopronin) were codissolved in a mixture of methanol and acetic acid. Sodium borohydride was added with rapid stirring. The coenzyme A protected gold nanoparticles were prepared in a similar manner by substituting coenzyme A for tiopronin in the reaction. In addition to gold, other metals may be used. For example Heath et al. in US Patent No. 6,103,868, describe the coating of gold, silver, platinum, palladium, cobalt and nickel with a solution of an organic surface passivant that had a functional group such as a thiol, phosphine, disulfide, amine, oxide, or amide. Chen et al. (*Colloids and Surfaces A* 169:107-116 (2000)), herein incorporated by reference describes and alternate method involving the preparation of nanoparticles in an ethanol-water mixture by the reduction of chloroplatinic acid by ethanol in the presence of poly(N-vinylisobutyramide). Other methods are also described, see for example Hagemeyer et al. in US Patent 6,074,979; Wuelfing et al. (*J. Am. Chem. Soc.* 120:12696-12697 (1998)); Chan et al. (*Science* 281:2016-2018 (1998)); Mitchell et al. (*J. Am. Chem. Soc.* 121:8122-8123 (1999)); and Napper (*J. Colloid. Interface. Sci* 58:390-407 (1977)).

Accordingly a preferred method of synthesis of a nanoparticle coated with the capture component for use in the present invention involves the following process steps:

a) mixing a metal salt with a capture coating component having an affinity for a ligand of interest, the capture coating component comprising a metal binding functionality to form a

first reaction mixture, wherein the first reaction mixture is at a pH of less than 7.0; and

b) adding a suitable reducing agent to the first reaction mixture of (a) to form a second reaction mixture comprising metal nanoparticles coated with said capture coating component.

When using the above, or similar method for the preparation of the nanoparticle coated with a single monolayer it is useful to begin with a metal salt. Suitable metal salts include, but are not limited to, any salts of the metals, gold, silver, platinum, palladium, iridium, rhodium, osmium, iron, copper, cobalt, and alloys composed of these metals. Particularly suitable salts include $HAuCl_4$, $AgNO_3$, $Cu(CH3CO2)_2$, $Cu(NO_3)_2$, $HPtCl_6$, and K_2PdCl_4.

A reducing agent is needed for the bonding of the metal binding functionalized capture or shielding component to the surface of the metal nanoparticle. Metal binding functionality is generally conveyed to the capture or shielding coating component by the addition of various reactive groups that will bind metal surfaces after reduction. A typical reactive group of this sort is the sulfhydral (-SH) group which can be used to derivativize many suitable capture coating components. Typical reducing agents are those that interact with the metal-binding functional group on the capture component. Suitable reducing agents where the metal binding functional group is –SH are $NaBH_4$, lithium triethylborohydride and hydrogen peroxide.

Direct-Synthesis

In contrast to the two step ligand-replacement method, it is also possible to synthesize the mixed monolayer coated nanoparticles of the invention by a single step, direct synthesis method. This method is useful where the shielding component is comprised of ethylene glycol. The direct synthesis method proceeds by the following process steps:

a) providing;
   i) a metallic nanoparticle
   ii) an ethylene glycol component having metal binding functionality;
   iii) a capture coating component having metal binding functionality and having an affinity for a ligand of interest;
   iv) a suitable reducing agent; and

v) a mixed solvent comprising at least one substantially water miscible organic solvent and at least one aqueous solvent wherein the mixed solvent is at a pH of less than 7.0; and

b) mixing elements (i) – (iv) in the mixed solvent of (v) to form a reaction mixture, wherein the final concentration of water in the reaction mixture is from about 9% to about 18% V/V, and wherein a mixed monolayer forms on the metallic nanoparticle.

Optionally the coated nanoparticles may be isolated according to any means known in the art.

Alternatively the direct synthesis method may employ a series of steps that initially separate the components of the mixed solvent. Good results have been achieved when the metal salt, shielding component, capture component are dissolved in an organic solvent and then mixed with a reducing agent dissolved in water. The final solution should be at pH of less than 7.0, and it is preferred if the final concentration of water in the mixture is from about 9% to about 18% V/V. Accordingly the invention provides a process for the synthesis of a water soluble metallic nanoparticle coated with a mixed monolayer comprising:

a) providing a first reaction mixture comprising:
   (i) a metal salt
   (ii) a shielding component having metal binding functionality;
   (iii) a capture coating component having metal binding functionality and having an affinity for a ligand of interest;
   (iv) an organic solvent;

b) providing a second reaction mixture comprising a suitable reducing agent in an aqueous solvent; wherein the second reaction mixture is at a pH of less than 7.0; and

c) mixing the first and second reaction mixtures wherein the final concentration of water in the mixture is from about 9% to about 18% V/V, and wherein a water soluble metallic nanoparticle coated with a mixed monolayer is formed.

The direct synthesis method offers the advantage of single reaction step, however it is essential that the amount of water in the reaction mixture be controlled. For example, final water content of the reaction should be from about 5% to about 20% (V/V) where from about 9% to about 18% (V/V) is preferred. In the direct synthesis method the solubility

of the nanoparticles is sensitive to the concentration of water and fluctuations in the water concentration may result in the nanoparticles precipitating out of solution.

The average size of the resulting nanoparticles synthesized by either method may be controlled by changing the molar ratio of the metal salt and the capture coating component, as described in "Water-Soluble, Isolable Gold Clusters Protected by Tiopronin and Coenzyme A Monolayers", A. C. Templeton, S. Chen, S. M. Gross, and R. W. Murray, Langmuir, 1999, 15, pp 66 - 76.

Either of the above described processes may be performed at room temperature, although higher or lower temperatures can also be utilized.

Isolation and Separation of A Nanoparticle Binding a Specific Ligand

The method of the invention is used to isolate monolayer coated nanoparticles that will bind a specific ligand of interest while excluding materials and ligands that bind nonspecifically. An essential feature of the invention is that the ratio of the shielding component to the capture component of the monolayer is varied to allow for optimal binding of the ligand of interest and maximal exclusion of all other ligands or materials. To accomplish this is it necessary to provide a multiplicity of nanoparticles, each with a different ratio of shielding : capture components. This may be effected during the preparation process by altering the feed ratios of the shielding and capture components in any of the methods described above.

Once a pool of nanoparticles are prepared with monolayers of different compositions, the nanoparticles may then be contacted with a mixture of standard diverse ligands. These ligands generally do not contain the ligand of interest and comprise materials, (generally other biomoleclues) that are found in the environment of the ligand of interest to be detected. Particularly suitable standard diverse ligand mixtures will include, but will not be limited to lysozyme, bovine serum albumin and cytochrome. The ligand standards will typically bind nonspecifically to many of the nanoparticles, however some will be completely resistant. The sub-population of nanoparticles that successfully resist the non-specific binding of the diverse ligands are then selected out. This sub-population is then exposed to the ligand of interest and those nanoparticles showing high binding affinities for the ligand of interest are selected as a second sub-population. The nanoparticles within this second

sub-population have the optimal ratio of shielding component to capture component in the monolayer to effect specific binding of the ligand of interest. Nanoparticles with this specific monolayer composition may then be used in assays to detect or isolate ligands of interest. Alternatively, the ligands of interest may be used to immobilize or assemble the nanoparticles into nanowires or other components of nano-electric devices.

Accordingly it is an object of the invention to provide a process for the isolation of a population of water soluble metallic nanoparticles having a specific affinity for a ligand of interest comprising:

a) providing a multiplicity of soluble metallic nanoparticles each coated with a mixed monolayer, the monolayer comprising:
   i) a shielding component consisting of ethylene glycol; and
   ii) a capture coating component having an affinity for a ligand of interest;

wherein the ratio of shielding component to capture coating component on each nanoparticle is different;

b) contacting each metallic nanoparticle of (a) with a mixture of standard diverse ligands for a time and under conditions where non-specific ligand binding occurs on some nanoparticles and no ligand binding occurs on other nanoparticles;

c) identifying a first subpopulation of nanoparticles of (b) where no ligand binding occurs;

d) contacting the first subpopulation of nanoparticle of (c) with a ligand of interest for a time and under conditions whereby binding of the ligand to the nanoparticle occurs; and

e) isolating a second subpopulation of nanoparticles of (d) whereby ligand binding to the nanoparticle is specific.

EXAMPLES

The present invention is further defined in the following Examples. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

Materials and Methods

Unless otherwise specified, all the reagents were purchased from Aldrich Chemicals (Milwaukee, WI) and used without further purification.

The meaning of abbreviations is as follows: "h" means hour(s), "min" means minute(s), "sec" means second(s), "d" means day(s), "mL" means milliliters, "L" means liters.

EXAMPLE 1

Synthesis of diethylene glycol thiol (EG2-SH)

1-Bromo-2-(2-methoxyethoxy)ethane (90%, 10.0 g)and urea (99%, 8.3 g) were added into a 250 mL dried round bottom flask. Then 80 mL of ethanol (99.9%) was added into the flask. The mixture was refluxed for 6 hours. After the mixture was cooled down to room temperature, EtOH was removed by rotary evaporation. Then 150 g of 20% NaOH was added and refluxed for 3 hours. The mixture was cooled down to room temperature and poured to a 500 mL beaker. 15% HCl (prepared from the concentrated HCl) was slowly added into the mixture with stirring until the pH reached 2. The mixture was extracted 4 times with 200 mL of ether. Liquid in ether phase was collected in a 1000 mL beaker and the ether phase was extracted with 200 mL D.I. water to further remove the salt and other impurities. The ether was removed by rotary evaporation and the crude product was distilled at 41-42°C under 1.2 mm Hg pressure. The final product was colorless with a typical thiol odor, and weighed 6.0 g, giving a yield of 57%. The structure was confirmed by nmR measurements. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.60-1.65 (t, 1H), 2.70-2.80 (m, 2H), 3.45 (s, 3H), 3.55-3.75 (m, 6H).

EXAMPLE 2

Synthesis of triethylene glycol thiol (EG3-SH)

Phosphorous tribromide (99%, 22.0 g, 0.081 mole) in 50 mL CH_2Cl_2 was slowly added to a stirred mixture of 20.0 g (0.122 mole) of tri(ethylene glycol) monomethyl ether (98%, Alfa Aesar), 7.0 mL of pyridine and 30 mL CH_2Cl_2 at 0°C. The resulting mixture was stirred at room temperature for 16 hours. Then 10 mL of D.I. water was added. The mixture was rinsed successively with 160 mL of 10% sodium carbonate aqueous solution, 160 mL of saturated NaOH, 160 mL of 5% sulfuric acid, and 160 mL of D.I. water, and further dried over anhydrous magnesium sulfate. The solvent was removed by rotary evaporation. The crude product, $CH_3O(CH_2CH_2O)_2CH_2CH_2Br$ (7.8 g, 0.034 mole), urea (99%, 5.6 g,

0.073 mole) and 65 mL of ethanol (99.9%) were mixed and refluxed for 6 hours. After the mixture was cooled down to room temperature, EtOH was removed by rotary evaporation. Then 150 g of 20% NaOH was added and refluxed for 3 hours. The mixture was cooled down to the room temperature, and acidified with15% HCl until the pH reached 2.0. The mixture was extracted 4 times with 200 mL of ether for each. The ether phase was extracted with 200 mL D.I. water to further remove the salt and other impurities. The ether was removed by rotary evaporation and the crude product was distilled to give the final product (EG_3-SH) with a yield of 70%. ^1H NMR (500 MHz, $CDCl_3$) δ: 1.50-1.55(t, 1H), 2.62-2.67(dt, 2H), 3.33(s, 3H), 3.49-3.51(dt, 2H) and 3.55-3.62 (m, 8H).

EXAMPLE 3

Synthesis of tetraethylene glycol thiol (EG4-SH)

Phosphorous tribromide (99%, 22.0 g, 0.081 mole) was slowly added to a stirred mixture of 10.0 g (0.048mole) of tetra(ethylene glycol) monomethyl ether (98%, Alfa Aesar) and 2.0 g of pyridine at 0°C. The resulting mixture was stirred at room temperature for 16 hours. Then 10 mL of D.I. water was added. The mixture was extracted 3 times with 40 mL of carbon tetrachloride for each. The combined organic extracts were rinsed successively with 25 mL of 10% sodium carbonate aqueous solution, 5% sulfuric acid, and D.I. water, and further dried over anhydrous magnesium sulfate. The solvent was removed by rotary evaporation. The crude product, $CH_3O(CH_2CH_2O)_3CH_2CH_2Br$ (90%, 10.0 g), urea (99%, 8.3 g)and 80 mL of ethanol (99.9%) were mixed and refluxed for 6 hours. After the mixture was cooled down to room temperature, EtOH was removed by rotary evaporation. Then 150 g of 20% NaOH was added and refluxed for 3 hours. The mixture was cooled down to the room temperature, and acidified with15% HCl until the pH reached 2.0. The mixture was extracted 4 times with 200 mL of ether for each. The ether phase was extracted with 200 mL D.I. water to further remove the salt and other impurities. The ether was removed by rotary evaporation and the crude product was distilled to give the final product (EG_4-SH) with a yield of 22%. ^1H NMR (500 MHz, $CDCl_3$) δ: 1.50-1.55(t, 1H), 2.60-2.65(dt, 2H), 3.31(s, 3H), 3.45-3.50(dt, 2H) and 3.53-3.62 (m, 14H).

EXAMPLE 4

Synthesis of Tiopronin Monolayer Protected Gold Nanoparticles [Au-$(TP)_n$] with Average Diameter of 3.5 nm

In a typical reaction, 60 mL MeOH (HPLC grade) and 10 mL acetic acid (HPLC grade) were mixed in a 250 mL Erlenmeyer flask by stirring for 2-5 minutes. Then, 0.37 g (0.94 mmol) tetrachloroauric acid ($HAuCl_4 \cdot xH_2O$) (99.99%) and 16.32 mg (0.1 mmol) N-(2-mercaptopropionyl)glycine (tiopronin, 99%) were added to the above mixed solvents and dissolved by stirring for 5 minutes, which gave a clear yellow solution. Next, 0.6 g (16 mmol) sodium borohydride ($NaBH_4$, 99%) was dissolved in 30 g Nanopure® water. The $NaBH_4$ solution was drop-wise added into the above solution with rapid stirring. With the first drop of $NaBH_4$ was added, the $HAuCl_4$ solution was immediately turned to dark brown from yellow. It was noticed that this reaction was exothermic. The heat generated in the reaction made the solution warm for ~ 15 min. During the reaction, the pH of the solution changed from 1.2 to ~ 5.0. Rapid stirring was kept for two hours. The tiopronin protected gold nanoparticles were soluble in water. When diluted, it became purple and clear. The particle solution was transferred into a filter tube (50K MW cut-off, Millipore) and purified by centrifuging at 3,500 rpm and washing with Nanopure water 4 times, then dried in the lypholizer for 3 days.

The average size of the nanoparticles could be tuned by changing the molar ratio of [$HAuCl_4$] and [tiopronin]. With the increasing ratio, the average particle size also increases.

EXAMPLE 5

Preparation of Glutathione Monolayer Protected Gold Nanoparticles

In a typical reaction, 60 mL of methanol (HPLC grade) and 10 mL of acetic acid (HPLC grade) were mixed in an Erlenmeyer flask by stirring for 2-5 min. Tetrachloroauric acid ($HAuCl_4 \cdot xH_2O$, 99.99%) (0.37 g)and 61.4 mg of glutathione (GSH) (99% minimum, obtained from Sigma, St. Louis, MO) were added to the above mixed solvents and dissolved by stirring for 5 min, resulting in a clear, yellow solution. A sodium borohydride solution was prepared by dissolving 0.6 g of $NaBH_4$ (99%) in 30 g of Nanopure® water. The $NaBH_4$ solution was added dropwise into the above solution with rapid stirring. When the first drop of $NaBH_4$ solution was added, the $HAuCl_4$ solution immediately turned dark brown from yellow. This reaction was exothermic, warming the solution for

approximately 15 min. During the reaction, the pH of the solution changed from 1.2 to about 5.0. The reaction solution was stirred rapidly for 2 h. The glutathione monolayer-protected gold nanoparticles were soluble in water and when diluted, the solution became clear purple. The particle solution was transferred into a filter tube (50K MW cut-off, Millipore) and purified by centrifuging at 3,500 rpm and washing with Nanopure® water 4 times, then dried in the lypholizer for 3 days.

EXAMPLE 6

Synthesis of Triethylene Glycol Monolayer-Protected Gold Nanoparticles (Au-EG3) with Average Diameter of 3.0 nm

In a typical reaction, 30 mL MeOH (HPLC grade) and 5.0 mL acetic acid (HPLC grade) were mixed in a 150 mL Erlenmeyer flask by stirring for 2-5 minutes. Then, 0.175 g (0.44 mmol) tetrachloroauric acid ($HAuCl_4 \cdot xH_2O$) (99.99%) and 40.0 mg (0.22 mmol) triethylene glycol thiol (EG3-SH) were added to the above mixed solvents and dissolved by stirring for 5 minutes, which gave a clear and yellow solution. Next 0.15 g (4.0 mmol) sodium borohydride ($NaBH_4$, 99%) was dissolved in 5.0 mL Nanopure® water. The $NaBH_4$ solution was drop-wise added into the above solution with rapid stirring. With the first drop of $NaBH_4$ was added, the $HAuCl_4$ solution immediately turned to dark brown from yellow. It was noticed that this reaction was exothermic. The heat generated in the reaction made the solution warm for ~15 min. During the reaction, the pH of the solution changed from 1.2 to ~5.0. Rapid stirring was continued for two hours. The diethylene glycol protected gold nanoparticles are soluble in water. When diluted, it became red purple and clear. The particle solution was transferred into a filter tube (50K MW cut-off, Millipore) and purified by centrifuging at 3,500 rpm and washing with Nanopure water 4 times, then dried in the lypholizer for 3 days.

EXAMPLE 7

Synthesis of Tetraethylene Glycol Monolayer-Protected Gold Nanoparticles (Au-EG4) with Average Diameter of 3.0 nm

In a typical reaction, 30 mL MeOH (HPLC grade) and 5.0 mL acetic acid (HPLC grade) were mixed in a 150 mL Erlenmeyer flask by stirring for 2-5 minutes. Then, 0.0885 g (0.23 mmol) tetrachloroauric acid ($HAuCl_4 \cdot xH_2O$) (99.99%) and 22.4 mg (0.1 mmol) tetraethylene glycol thiol (EG4-SH) added to the above mixed solvents and dissolved by stirring for 5 minutes, which gave a clear and yellow solution. Next 0.075 g

(2.0 mmol) sodium borohydride ($NaBH_4$, 99%) was dissolved in 5.0 mL Nanopure® water. The $NaBH_4$ solution was drop-wise added into the above solution with rapid stirring. When the first drop of $NaBH_4$ was added, the $HAuCl_4$ solution immediately turned to dark brown from yellow. It was noticed that this reaction was exothermic. The heat generated in the reaction would make the solution warm for ~15 min. During the reaction, the pH of the solution changed from 1.2 to ~5.0. Rapid stirring was continued for two hours. The tetraethylene glycol protected gold nanoparticles are soluble in water. When diluted, it became red purple and clear. The particle solution was transferred into a filter tube (50K MW cut-off, Millipore) and purified by centrifuging at 3,500 rpm and washing with Nanopure water 4 times, then dried in the lypholizer for 3 days.

EXAMPLE 8

1H NMR spectra of Free EG3-SH, Au-EG3 and mixed monolayer protected gold nanoparticles

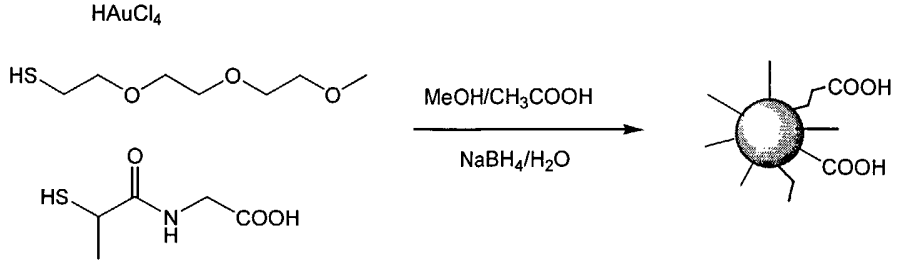
The ^1H NMR sample for free EG3-SH was prepared by mixing 3 mg pure material and 2 mL CCl_3D in a NMR tube. ^1H NMR spectrum of free EG3-SH was recorded with a Bruker 500 MHz at room temperature in CCl_3D, as shown in Figure 1(a). Nanoparticle samples were prepared by dissolving 15.0 mg dry nanoparticle in 2 mL DMSO-d6 solvent. The ^1H NMR spectrum of Au-EG3 monolayer protected nanoparticles was recorded with a Varian Inova 400 MHz at room temperature in DMSO-d6, as shown in Figure 1(b). ^1H NMR spectra of mixed monolayer (Au-EG3/Tp) protected nanoparticles were recorded with a Varian Inova 400 MHz at room temperature in DMSO-d6, as shown in Figure 2. The samples were run with 1 pulse experiment using 90° pulse with 20 seconds delay. The baseline was flattened by using a spline baseline corrector.

The ^1H NMR spectra of the mixed monolayer protected nanoparticles clearly showed that the doublet peak at 1.32-1.36 (δ) is attributed to the –CH3 group from tiopronin and the triplet peak at 2.87-2.92 (δ) is attributed to the –SCH2 group. The ratio of these two peaks' integral determines the ratio of triethylene glycol and tiopronin on the surface of gold nanoparticle.

EXAMPLE 9

Synthesis of Triethylene Glycol and Tiopronin Mixed Monolayer Protected Gold Nanoparticles with Average Diameter of 3.0 nm

In a typical reaction of feeding molar ratio of triethylene glycol and tiopronin at 1:1, 45 mL MeOH (HPLC grade) and 7.5 mL acetic acid



(HPLC grade) were mixed in a 150 mL Erlenmeyer flask by stirring for 2-5 minutes. Then, 0.236 g (0.6 mmol) tetrachloroauric acid ($HAuCl_4 \cdot xH_2O$) (99.99%), 27.0 mg (0.15 mmol) tetraethylene glycol thiol (EG4-SH), and 24.5 mg of tiopronin (0.15 mmol) were added to the above mixed solvents and dissolved by stirring for 5 minutes, which gave a clear and yellow solution. Next 0.225 g (6.0 mmol) sodium borohydride ($NaBH_4$, 99%) was dissolved in 7.5 mL Nanopure® water. The $NaBH_4$ solution was drop-wise added into the above solution with rapid stirring. With the first drop of $NaBH_4$ was added, the $HAuCl_4$ solution immediately turned to dark brown from yellow. It was noticed that this reaction was exothermic. The heat generated in the reaction made the solution warm for ~15 min. During the reaction, the pH of the solution changed from 1.2 to ~ 5.0. Rapid stirring was continued for two hours. The triethylene glycol/tiopronin mixed monolayer protected gold nanoparticles were soluble in water. When diluted, it became red purple and clear. The particle solution was transferred into a filter tube (50K MW cut-off, Millipore) and purified by centrifuging at 3,500 rpm and washing with Nanopure water 4 times, then dried in the lypholizer for 3 days.

The synthesis of different molar ratio of triethylene glycol and tiopronin mixed monolayer protected gold nanoparticles followed the same protocol as the above with varying weights of each reagent, as listed below in Table 1.

Table 1.
Synthesis of different molar ratio of triethylene glycol (EG3-SH) and tiopronin (Tp) mixed monolayer protected gold nanoparticles

Reaction agents	Feeding molar ratio [EG3-SH]/[Tp]			
	4	12.3	14	19
MeOH CH_3COOH $HAuCl_4$	45 mL 7.5 mL 0.236g (0.6mmol)	45 mL 7.5 mL 0.236g (0.6mmol)	45 mL 7.5 mL 0.236g (0.6mmol)	45 mL 7.5 mL 0.236g (0.6mmol)
EG3-SH	43.2 mg (0.24 mmol)	50.0 mg (0.278 mmol)	50.4 mg (0.28 mmol)	51.3 mg (0.285 mmol)
Tp	9.78 mg (0.06 mmol)	3.67 mg (0.023 mmol)	3.26 mg (0.02 mmol)	2.45 mg (0.015 mmol)
$NaBH_4$ H_2O	0.225g (6 mmol) 7.5 mL	0.225g (6 mmol) 7.5 mL	0.225g (6 mmol) 7.5 mL	0.225g (6 mmol) 7.5 mL

After synthesis of these above mixed monolayer protected gold nanoparticles, the quantitative surface components of triethylene glycol and tiopronin were determined from ^1H NMR spectra, as described in Example 8. The results are shown in Table 2. The surface ratio is plotted against the feeding ratio, as shown in Figure 3. The linear relationship of Figure 3 with the slope of 3.2 means the reactivity of EG3-SH is roughly 3 times higher than Tp in the synthesis of a mixed monolayer protected gold nanoparticle.

Table 2.
Molar ratio of ethylene glycol and tiopronin on gold nanoparticle vs. the feeding molar ratio in the synthesis.

Feeding molar ratio in synthesis [EG3-SH]/[Tp]	Molar ratio on gold nanoparticle surface [EG3-S-]/[Tp]
0.5	1.7
1	3.7
4	12.6
9	29.3

EXAMPLE 10

Synthesis of Triethylene Glycol (EG3-SH) and Glutathione (GSH) Mixed Monolayer Protected Gold Nanoparticles

In a typical reaction of feeding molar ratio of triethylene glycol and glutathione at 1:1, 45 mL MeOH (HPLC grade) and 7.5 mL acetic acid (HPLC grade) were mixed in a 150 mL Erlenmeyer flask by stirring for 2-5 minutes. Then, 0.175 g (0.6 mmol) tetrachloroauric acid ($HAuCl_4 \cdot xH_2O$) (99.99%), 27.0 mg (0.15 mmol) triethylene glycol thiol (EG3-SH), and 46.1 mg of glutathione (0.15 mmol) were added to the above mixed solvents and dissolved by stirring for 5 minutes, which gave a clear and yellow solution. Next 0.225 g (6.0 mmol) sodium borohydride ($NaBH_4$, 99%) was dissolved in 5.0 mL Nanopure® water. The $NaBH_4$ solution was drop-wise added into the above solution with rapid stirring. With the first drop of $NaBH_4$ was added, the $HAuCl_4$ solution was immediately turned to dark brown from yellow. It was noticed that this reaction is exothermic. The heat generated in the reaction would make the solution warm for ~15 min. During the reaction, the pH of the solution changed from 1.2 to ~ 5.0. Rapid stirring was continued for two hours. The EG3-SH/GSH protected gold nanoparticles were soluble in water. When diluted, it became red purple and clear. The particle solution was transferred into a filter tube (50K MW cut-off, Millipore) and purified by centrifuging at 3,500 rpm and washing with Nanopure water 4 times, then dried in the lypholizer for 3 days.

The synthesis of different molar ratio of EG3-SH and GSH mixed monolayer protected gold nanoparticles followed the same protocol as above with varying weights of each reagent, as listed in Table 3.

Table 3.

Recipes for synthesis of different molar ratio of triethylene glycol (EG3-SH) and glutathione (GSH) mixed monolayer protected gold nanoparticles

Reaction agents	Feeding molar ratio [EG3-SH]/[GSH]			
	4	12.3	14	19
MeOH CH_3COOH $HAuCl_4$	45 mL 7.5 mL 0.236g (0.6mmol)	45 mL 7.5 mL 0.236g (0.6mmol)	45 mL 7.5 mL 0.236g (0.6mmol)	45 mL 7.5 mL 0.236g (0.6mmol)
EG3-SH	43.2 mg (0.24 mmol)	50.0 mg (0.278 mmol)	50.4 mg (0.28 mmol)	51.3 mg (0.285 mmol)

Reaction agents	Feeding molar ratio [EG3-SH]/[GSH]			
	4	12.3	14	19
GSH	18.4 mg (0.06 mmol)	6.9 mg (0.023 mmol)	6.2 mg (0.02 mmol)	4.6 mg (0.015 mmol)
$NaBH_4$	0.225g (6 mmol)	0.225g (6 mmol)	0.225g (6 mmol)	0.225g (6 mmol)
H_2O	7.5 mL	7.5 mL	7.5 mL	7.5 mL

EXAMPLE 11

Synthesis of Biotinated Triethylene Glycol/Glutathione (EG3/GSH) Mixed Monolayer Protected Gold Nanoparticles

This example shows that the functional group or ligand can be introduced by a simple reaction with a mixed monolayer protected nanoparticle. In this example, a biotin ligand was introduced to the nanoparticle by the reaction with the surface carboxylic acid group. A typical reaction scheme is given below.

7.7 mg EG3/Tp mixed monolayer protected Au nanoparticle [Au(EG3)4Tp] (prepared in Example 10 with the feeding ratio of EG3-SH and GSH at 4:1) was mixed with 1.5 mL of 0.1 M N-morpholinoethane sulfonic acid (MES) at pH 5.5 in a 5 mL round bottom flask, then 7.3 mg of EZ-Link® 5-(biotinamido)pentylamine (MW 328.48 from Pierce, Rockford, IL) and 50 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (EDC) (from Pierce, Rockford, IL) were added. The mixture was stirred overnight at the room temperature. The reaction mixture was then transferred into a filter tube (50K MW cut-off, Millipore) and purified by centrifuging at 3,500 rpm and washing with Nanopure® water 4 times.

EXAMPLE 12

Gel electrophoresis Analysis of EG3/Tp Mixed Monolayer Protected Nanoparticle - Protein Binding

Protein binding to Au nanoparticles can be conveniently monitored by gel electrophoresis, since protein-nanoparticle complex are expected to migrate differently than the free Au particles. Gel Electrophoresis Experiment. Gel electrophoresis was run in 1X TBE buffer (Tris-borate-EDTA) at 90V constant voltage for 20 min. Gel pictures were taken by directly scanning the gel on a HP ScanJet 6300C. Protein binding reactions were done by mixing 1 μL of ~1uM Au particles with certain amount of protein in 10 μL of sodium phosphate buffer (50 mm, pH 7.3).

After incubation at room temperature for 10 min, the entire reaction mixture was loaded on 1% agarose gel.

Results for EG3/Tp mixed monolayer protected nanoparticles are shown in Figure 4. In Figure 4a, Lane 1 is 7 μL of Au-(EG3)3Tp (representing a surface EG3/Tp ratio on the Au nanoparticle surface at 3:1) at ~ 0.1 mM in 10 μL of sodium phosphate buffer (50 mM, pH 7.3). Lane 2, 3, 4, 5 and 6 are the same amount of Au particles with EG3 and Tp ratio at 12:1, 27:1, 42:1, 57/1, and pure Au-EG3, respectively. The migration speed of EG3/Tp mixed monolayer protected nanoparticles decreased with decreasing percentage of tiopronin on the nanoparticle surface. In Figure 4b, Lane 1, 3, 5 and 7 are 7 μL of EG3/Tp mixed monolayer protected nanoparticles with ratio of 4, 12, 27, and 42, respectively at ~ 0.1 mM in 10 μL of sodium phosphate buffer (50 mM, pH 7.3). Lane 2, 4, 6, and 8 are identical to lane 1,3 5,and 7, respectively, except that particles were, mixed with 7 μg of lysozme in10 μL of sodium phosphate buffer (50 mM, pH 7.3). When Tp% (molar percentage) is less than 7.7%, EG3/Tp mixed monolayer protected nanoparticles have negligible binding with lysozyme.

EXAMPLE 13

Gel electrophoresis Analysis of EG3/GSH Mixed Monolayer Protected Nanoparticle - Protein Binding

The protocols for gel migration and protein binding experiments are the same as that described in Example 12. Results for EG3/GSH mixed monolayer protected nanoparticles are shown in Figure 5. In Figure 5a, Lane 1 is 7 μL of EG3/GSH mixed monolayer protected Au nanoparticle with EG3-SH/GSH feeding ratio at 3:1) at ~ 0.1 mM in 10 μL of sodium phosphate buffer (50 mM, pH 7.3). Lane 2, 3, 4, 5 and 6 are the same amount of Au particles with EG3/GSH feeding ratio at 4, 14, 19, and pure Au-EG3, respectively. The migration speed of EG3/GSH mixed monolayer protected nanoparticles decreased with decreasing percentage of GSH on the nanoparticle surface. In Figure 5b, Lane 1, 3, 5 and 7 are 7 μL of EG3/GSH mixed monolayer protected nanoparticles with feeding ratio at 1, 4, 9, and 14, respectively at ~ 0.1 mM in 10 μL of sodium phosphate buffer (50 mM, pH 7.3). Lane 2, 4, 6, and 8 are EG3/GSH mixed monolayer protected Au nanoparticles with the feeding ratio at 1, 4, 9, and 14, respectively, mixed with 7 μg of lysozme in10 μL of sodium phosphate buffer (50 mM, pH 7.3). In this example, the ratio of EG3/GSH on the

nanoparticle surface was not measured. When GSH% (feeding molar percentage) is less than 20%, EG3/GSH mixed monolayer protected nanoparticles have negligible binding with lysozyme.

EXAMPLE 14

Nanoparticle with Specific Binding with Proteins

The protocols for gel migration and protein binding experiments were the same as that described in Example 11. Example 12 showed that the EG3/GSH mixed monolayer protected nanoparticle has negligible binding with lysozyme when the feeding ratio of [EG3-SH]/[GSH] is higher than 14. For the feeding ratio at 19, the nanoparticle still had substantial migration toward the cathode, indicating this nanoparticle has GSH molecules on the surface. Such a nanoparticle suggests two functions: eliminating non-specific binding with proteins like lysozyme, and enabling specific binding. Figure 6 shows that this nanoparticle has a specific binding with GST protein through SGH-GST interaction. Lane 1 is GSH monolayer protected nanoparticles at ~ 0.1 mM in 10 μL of sodium phosphate buffer (50 mM, pH 7.3); lane 2 and 3 are the same as Lane 1, except that 10 μg of lysozyme and 5 μg of glutathione S-transferase were added, respectively. Lane 4, 5, and 6 are similar to Lane 1,2 and 3, except that [EG3-SH]19/[GSH] Au nanoparticles at a concentration of ~ 0.01 mM were used.

EXAMPLE 15

Biotinated Nanoparticle with Specific Binding with Streptavidin

The protocols for gel migration and protein binding experiments are the same as that described in Example 12. A biotinated Au nanoparticle (from Example 11) has two functions: eliminating non-specific binding with proteins like lysozyme, and enabling specific binding. Figure 7 shows that this nanoparticle has a specific binding with streptavidin protein through biotin-streptavidin interaction. Lane 1 is the biotinated nanoparticles at ~ 0.1 mM in 10 μL of sodium phosphate buffer (50 mM, pH 7.3); lanes 2, 3 and 4 are the same as Lane 1, except that 10 μg of BSA, 10 μg of lysozyme and 10 μg of streptavidin were added, respectively. The gel experiment showed that the biotinated Au nanoparticle did not have non-specific binding with BSA, a negatively charged protein and lysozyme, a positively charged protein, however strong binding with the target protein, streptavidin.

CLAIMS

What is claimed is:

1. A process for the isolation of a population of water soluble metallic nanoparticle having a specific affinity for a ligand of interest comprising:

a) providing a multiplicity of soluble metallic nanoparticles each coated with a mixed monolayer, the monolayer comprising:

i) a shielding component comprising ethylene glycol; and

ii) a capture coating component having an affinity for a ligand of interest;

wherein the ratio of shielding component to capture coating component on each nanoparticle is different;

b) contacting each metallic nanoparticle of (a) with a mixture of standard diverse ligands for a time and under conditions where non-specific ligand binding occurs on some nanoparticles and no ligand binding occurs on other nanoparticles;

c) identifying a first subpopulation of nanoparticles of (b) where no ligand binding occurs;

d) contacting the first subpopulation of nanoparticle of (c) with a ligand of interest for a time and under conditions whereby binding of the ligand to the nanoparticle occurs; and

e) isolating a second subpopulation of nanoparticles of (d) whereby ligand binding to the nanoparticle is specific.

2. A method according to Claim 1 wherein the amount of shielding component in the monolayer from about 90% to about 10%.

3. A method according to Claim 1 wherein the amount of capture coating component monolayer is from about 90% to about 10%.

4. A method according to Claim 1 wherein the mixture of diverse standard ligands contains ligands selected from the group consisting of nucleic acids, proteins, and peptides.

5 A method according to Claim 4 wherein the proteins are selected from the group consisting of lysozyme, bovine serum albumin, and cytochrome C.

6. A process according to Claim 1 wherein the ligand of interest is selected from the group consisting of: peptides, proteins, nucleic acid fragments, collagen, nano-rods, nano-tubes, nano-planes and nano-fibers.

7. A method according to Claim 1 wherein the ligand is one member of a binding pair.

8. A method according to Claim 7 wherein the binding pair is selected from the group consisting of glutathione-S-transferase/glutathione, 6X Histidine Tag/Ni-NTA, streptavidin/biotin, biotin/avidin, S-protein/S-peptide, cutinase/phosphonate inhibitor, antigen/antibody, hapten/anti-hapten, folic acid/folate binding protein, and protein A or G/immunoglobulins.

9. A process according to Claim 1 wherein the metal of the metallic nanoparticle is selected from the group consisting of; gold, silver, platinum, palladium, iridium, rhodium, osmium, iron, copper, cobalt, and alloys composed of these metals.

10. A process according to Claim 1 wherein said capture coating component is selected from the group consisting of:

a) molecules having reactive groups selected from the group consisting of: $-NH_2$, -COOH, -CHO-, -OH, -X (Cl, Br, I), succinimide, and epoxy groups; and

b) ligands selected from the group consisting of: peptides; tiopronin and GSH.

11. A process according to Claim 1 wherein said shielding component is a short chain ethylene glycol oligomer.

12. A process according to Claim 11 wherein the short chain ethylene glycol oligomer has a molecular weight less than the entanglement molecular weight.

13. A process according to Claim 12 wherein the short chain ethylene glycol oligomer is selected from the group consisting of tetraethylene glycol thiol, triethylene glycol thiol and diethylene glycol thiol.

14.A process according to claim 1 wherein the water soluble metallic nanoparticle is coated with a mixed monolayer by a process comprising the steps of:

a) providing a water soluble metallic nanoparticle coated with monolayer of a capture coating component having an affinity for a ligand of interest;

b) mixing the coated nanoparticle of (a) with a shielding coating component in a mixed solvent, the shielding component having metal binding functionality, the mixed solvent comprising at least one substantially water miscible organic solvent and at least one aqueous solvent wherein the mixed solvent is at a pH of less than 7.0; wherein exchange occurs between the shielding coating component and the capture coating component to form a nanoparticle coated with a mixed monolayer; and

c) optionally isolating the mixed monolayer coated metal nanoparticle of (b).

15. A process according to claim 1 wherein the water soluble metallic nanoparticle is coated with a mixed monolayer by a process comprising the steps of:

a) providing;

i) a metal salt

ii) a shielding component having metal binding functionality;

iii) a capture coating component having metal binding functionality and having an affinity for a ligand of interest;

iv) a suitable reducing agent; and

v) a mixed solvent comprising at least one substantially water miscible organic solvent and at least one aqueous solvent wherein the mixed solvent is at a pH of less than 7.0;

b) mixing elements (i) – (iv) in the mixed solvent of (v) to form a reaction mixture, wherein the final concentration of water in the reaction mixture is from about 9% to about 18% V/V, and wherein a mixed monolayer forms on the metallic nanoparticle; and

c) optionally isolating the mixed monolayer coated metal nanoparticle of (b).

16. A process according to claim 1 wherein the water soluble metallic nanoparticle is coated with a mixed monolayer by a process comprising the steps of:

a) providing a first reaction mixture comprising:

(i) a metal salt

(ii) a shielding component having metal binding functionality;

(iii) a capture coating component having metal binding functionality and having an affinity for a ligand of interest;

(iv) an organic solvent;

b) providing a second reaction mixture comprising a suitable reducing agent in an aqueous solvent; wherein the second reaction mixture is at a pH of less than 7.0; and

c) mixing the first and second reaction mixtures wherein the final concentration of water in the mixture is from about 9% to about 18% V/V, and wherein a water soluble metallic nanoparticle coated with a mixed monolayer is formed.

Figure 1(a)
^{1}H NMR Spectrum of free triethylene glycol thiol (EG3-SH) in CCl_3D

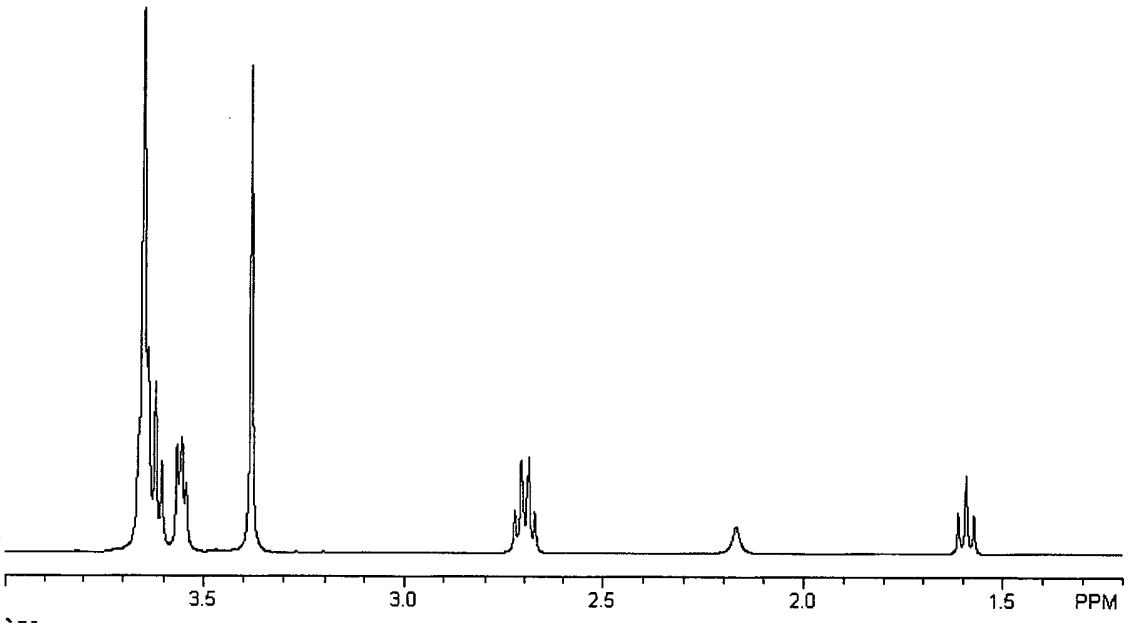


Figure 1(b)
^{1}H NMR Spectrum of triethylene glycol protected gold nanoparticle (Au-EG3) in DMSO-d6

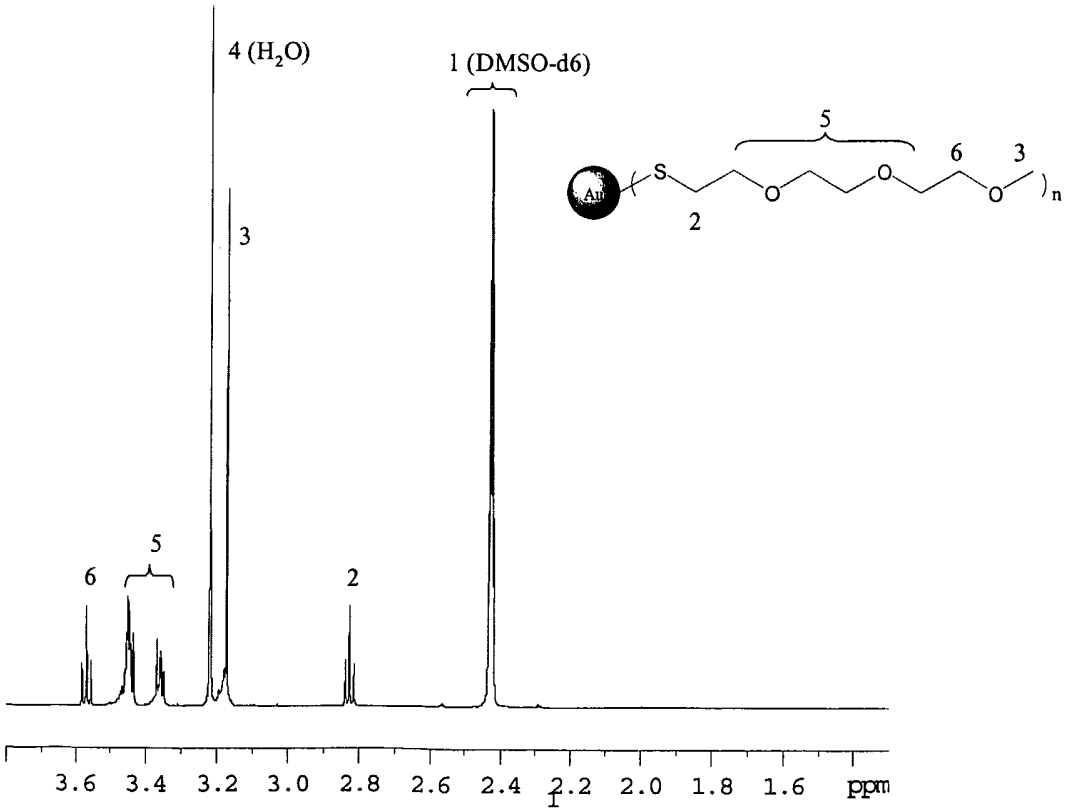


Figure 2
^{1}H NMR Spectrum of triethylene glycol and tiopronin mixed monolayer protected gold nanoparticle (Au-EG3/Tp) in DMSO-d6

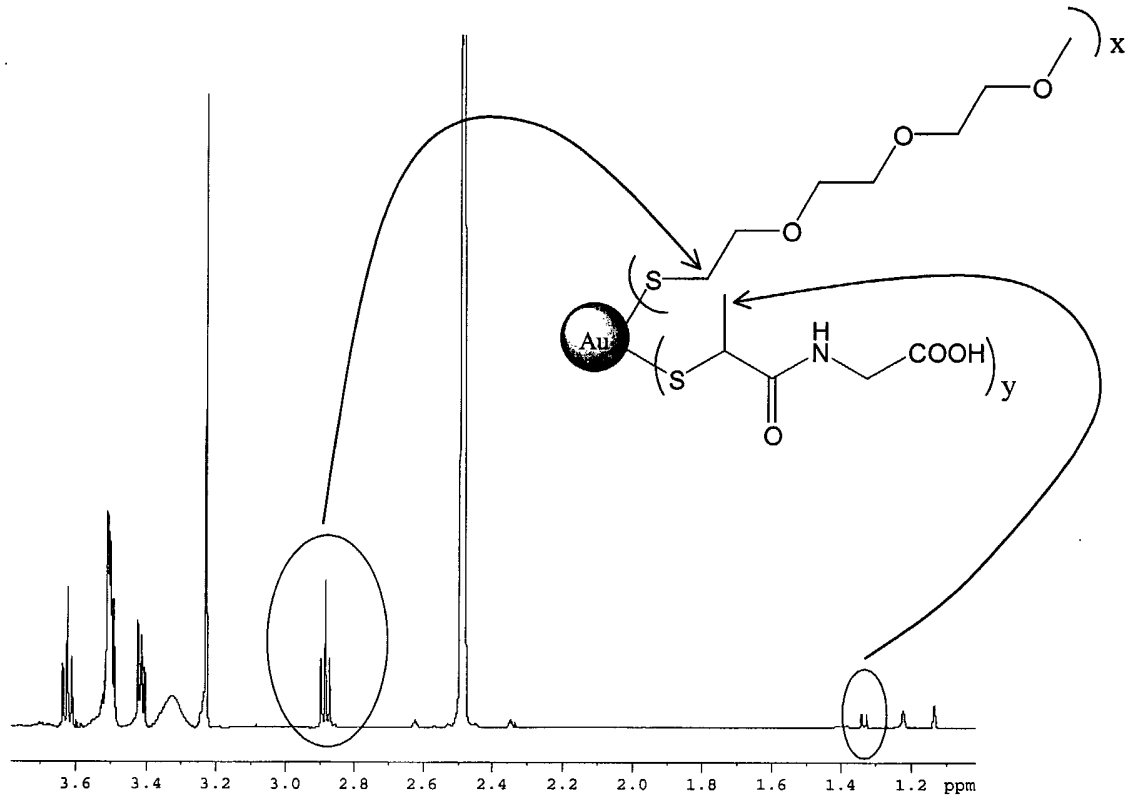


Figure 3
Reactivity of EG3-SH vs. tiopronin in the formation of a mixed monolayer protected nanoparticle

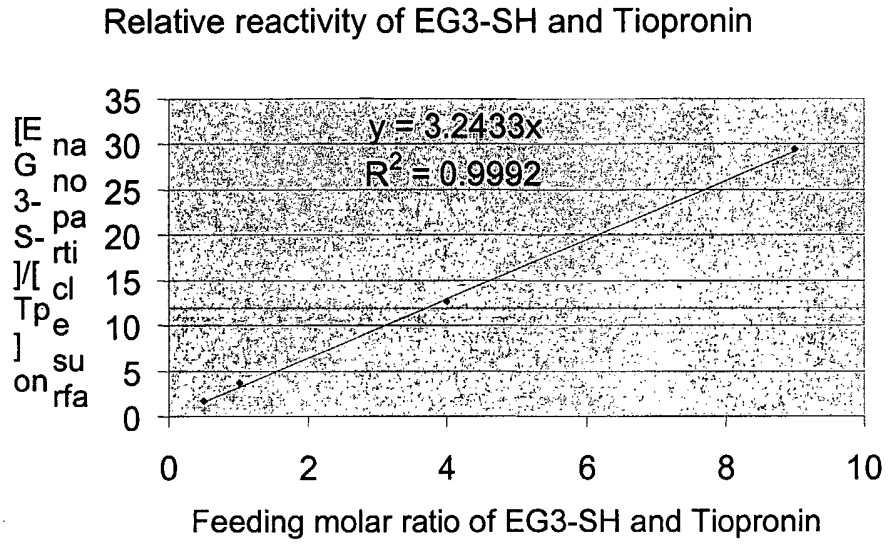
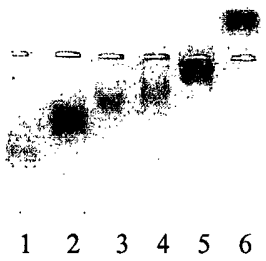


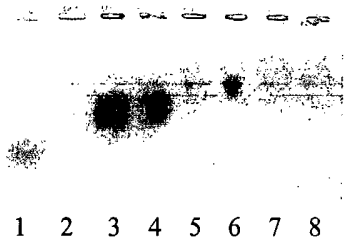
Figure 4(a)
Gel electrophoresis image of EG3/Tp mixed monolayer protected nanoparticle controlled by the percentage of Tp on the nanoparticle surface.



	Feeding ratio [EG3-SH]/[TP]	Surface ratio [EG3-S-]/[Tp]
1	1/1	3/1
2	4/1	12/1
3	9/1	27/1
4	14/1	42/1
5	19/1	57/1
6	Pure EG3-SH	

1% TBE gel, 90V, 40 min

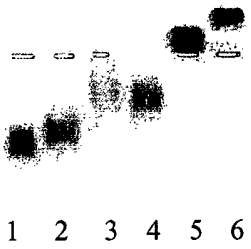
4(b)
Gel electrophoreis of the nanoparticles from Figure 4(b) bonded with lysozyme



	Feeding ratio [EG3-SH]/[TP]	Tp Surface Coverage (%)
1	1/1	25
3	4/1	7.7
5	9/1	3.6
7	14/1	2.3

1, 3, 5, 7: nanoparticle samples
2, 4, 6, 8: nanoparticle + Lysozyme at pH 7.2

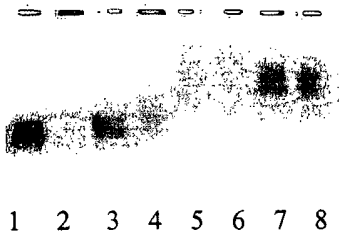
Figure 5(a)
Gel electrophoresis of EG3/GSH mixed monolayer protected nanoparticles controlled by the percentage of GSH on the nanoparticle surface



	Feeding ratio [EG3-SH]/[GSH]
1	1/1
2	4/1
3	9/1
4	14/1
5	19/1
6	Pure EG3-SH

4% TBE gel, 90V, 40 min

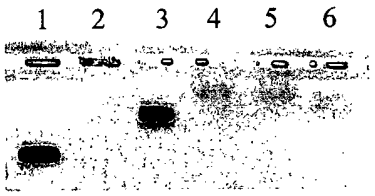
Figure 5(b)
Gel electrophoreis of the nanoparticles from Figure 5(a) bonded with lysozyme



	Feeding ratio [EG3-SH]/[GSH]
1	1/1
3	4/1
5	9/1
7	14/1

1, 3, 5, 7: nanoparticle samples
2, 4, 6, 8: nanoparticle + Lysozyme at pH 7.2

Figure 6
Gel electrophoresis image of a nanoparticle with a mixed monolayer of EG3 and GSH at feeding ratio of 19 ([EG3-SH]/[GSH]) with GST protein



1% gel, 90 v, 20 min

1. Au-GSH
2. 1 + Lysozyme
3. 1+ GST
4. Au-(EG3)19/GSH
5. 4 + Lysozyme
6. 4+ GST

Figure 7
Gel electrophoresis image illustrating specific binding of a nanoparticle with a mixed monolayer of EG3 and GSH-biotin with streptavidine protein.