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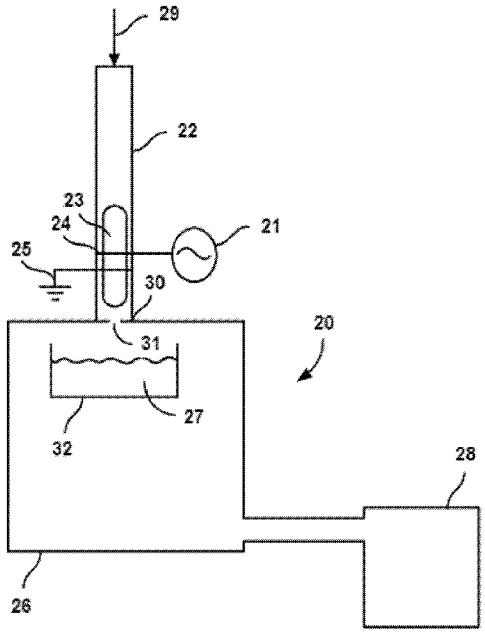
(54) Title: BIOCONJUGATED MOLECULE, METHOD OF PREPARING SAME, AND DIAGNOSTIC METHOD

(57) Abstract: A bioconjugated molecule comprises a quantum dot formed via a plasma process and a biomolecule. The quantum dot is bonded to a biomolecule via a divalent linking group. A method of preparing a bioconjugated molecule comprises providing a quantum dot formed via a plasma process, a biomolecule, and a cross-linking agent. The method further comprises bonding the cross-linking agent between the quantum dot and the biomolecule to prepare the bioconjugated molecule. A diagnostic method involving the bioconjugated molecule is also disclosed.

FIGURE 1



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BIOCONJUGATED MOLECULE, METHOD OF PREPARING SAME, AND DIAGNOSTIC METHOD

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The application claims priority to and all advantages of U.S. Provisional Patent Application No. 62/786,788 filed on 31 December 2018, the content of which is incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The disclosure relates to a bioconjugated molecule and, more specifically, to a bioconjugated molecule including a quantum dot and to a method of preparing the bioconjugated molecule.

DESCRIPTION OF THE RELATED ART

[0003] Nanoparticles are known in the art and can be prepared via various processes. For example, nanoparticles are often defined as particles having at least one dimension of less than 100 nanometers. Nanoparticles are produced either from a bulk material, which is initially larger than a nanoparticle, or from particles smaller than the nanoparticles, such as ions and/or atoms. Nanoparticles are particularly unique in that they may have significantly different properties than the bulk material or the smaller particles from which the nanoparticles are derived. For example, a bulk material that acts as an insulator or semiconductor can be, when in nanoparticle form, electrically conductive.

[0004] Quantum dots are nanoparticles that are characterized by certain properties, e.g. quantum mechanical properties. For example, conventional quantum dots comprise semiconductor crystals whose excitons are confined in all three spatial dimensions. Quantum dots may be formed via a variety of techniques, such as colloidal synthesis. Given the unique electronic properties of quantum dots, quantum dots are commonly utilized in various optical applications, e.g. in light-emitting devices. However, numerous opportunities remain for applications in which quantum dots may be utilized, particularly in view of different methods to synthesize quantum dots and the various materials that may be utilized to form quantum dots.

BRIEF SUMMARY OF THE INVENTION

[0005] Disclosed is a bioconjugated molecule. The bioconjugated molecule comprises a quantum dot formed via a plasma process. The quantum dot is bonded to a biomolecule via a divalent linking group.

[0006] A method of preparing a bioconjugated molecule is also disclosed. The method comprises providing a quantum dot formed via a plasma process, a biomolecule, and a cross-linking agent. The method further comprises bonding the cross-linking agent between

the quantum dot and the biomolecule to prepare the bioconjugated molecule. The bioconjugated molecule comprises a divalent linking group formed from the cross-linking agent bonded between the quantum dot and the biomolecule.

[0007] The present invention also provides a diagnostic method. The diagnostic method comprises administering the bioconjugated molecule to at least one biological sample.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] Other advantages and aspects of this invention may be described in the following detailed description when considered in connection with the accompanying drawings wherein:

[0009] Figure 1 is illustrates one embodiment of a low pressure high frequency pulsed plasma reactor for producing quantum dots;

[0010] Figure 2 illustrates an embodiment of a system including a low pressure pulsed plasma reactor to produce quantum dots and a diffusion pump to collect the quantum dots; and

[0011] Figure 3 illustrates a schematic view of one embodiment of a diffusion pump for collecting quantum dots produced via a reactor.

DETAILED DESCRIPTION OF THE INVENTION

[0012] The present invention provides a bioconjugated molecule, a method of preparing the bioconjugated molecule, and a diagnostic method involving the bioconjugated molecule. The bioconjugated molecule may be prepared based on a desired end use application thereof and may be utilized in diverse end use applications. For example, the bioconjugated molecule is particularly suited for diagnostic methods involving biological samples, such as cells or tissues. However, the bioconjugated molecule is not limited to such diagnostic methods.

[0013] The bioconjugated molecule comprises a quantum dot formed via a plasma process. The quantum dot is bonded to a biomolecule via a divalent linking group. The divalent linking group is formed from a cross-linking agent utilized to prepare the bioconjugated molecule. For example, the method to prepare the bioconjugated molecule comprises bonding the cross-linking agent between the quantum dot and the biomolecule to give the bioconjugated molecule. The cross-linking agent may be reacted first with the quantum dot or the biomolecule, as described below. The quantum dot, biomolecule, and divalent linking group are each described in detail below both with reference to the bioconjugated molecule and the method of its preparation.

[0014] The quantum dot of the bioconjugated molecule is formed via a plasma process. The bioconjugated molecule may comprise two or more quantum dots, which may be independently formed or selected, so long as at least one of the quantum dots of the

bioconjugated molecule is formed via the plasma process. Typically, if the bioconjugated molecule includes two or more quantum dots, the quantum dots are each formed via the plasma process. For clarity and consistency, "the quantum dot" encompasses embodiments where the bioconjugated molecule includes but one or two or more quantum dots.

[0015] Any plasma process may be utilized to form the quantum dot. The quantum dot may be obtained commercially in the event such quantum dots formed via a plasma process are available. However, given limitations for obtaining quantum dots formed via the plasma process, the quantum dot may be synthesized or prepared. For example, the method of preparing the bioconjugated molecule may further comprise preparing the quantum dot. In these embodiments, the quantum dot is first prepared and subsequently utilized to form the bioconjugated molecule. The typical plasma process utilized to prepare the quantum dot is described below with reference to the quantum dot and the method of preparing the bioconjugated molecule therewith, particularly when the method of preparing the bioconjugated molecule comprises preparing the quantum dot via the plasma process.

[0016] In various embodiments, the plasma process to prepare the quantum dot is carried out in a low pressure reactor. In various embodiments, the quantum dot comprises an independently selected Group IV element. As used herein, the group designations of the periodic table are generally from the CAS or old IUPAC nomenclature, although Group IV elements are referred to as Group 14 elements under the modern IUPAC system, as readily understood in the art. As used herein, the Group IV elements include C, Si, Ge, Sn, Pb, and Fl. Typically, the Group IV element(s) of the quantum dot is selected from Si, Ge, Sn, and combinations thereof.

[0017] In certain embodiments where the plasma process is carried out in the low pressure reactor, the plasma process comprises forming a nanoparticle aerosol in the low pressure reactor, wherein the aerosol comprises MX-functional quantum dots in a gas, with M being an independently selected Group IV element and X being a functional group independently selected from H and a halogen atom. The MX-functional quantum dots are generally MX-functional prior to forming the bioconjugated molecule. The MX-functional quantum dots are generally collected upon their formation. In certain embodiments as described below, the MX-functional quantum dots are collected by capturing the MX-functional quantum dots in a capture fluid, which is typically in fluid communication with the low pressure reactor. The MX-functional quantum dots may still have MX-functionality after forming the bioconjugated molecule depending upon a number of functional sites therein, as described below.

[0018] Regardless of the particular plasma system and process utilized to produce the quantum dot, the plasma system generally relies on a precursor gas. The precursor gas is generally selected based on the desired composition of the quantum dot. For example, as

introduced above, the nanoparticle aerosol comprises MX-functional quantum dots, where M is an independently selected Group IV element.

[0019] To this end, the precursor gas utilized generally comprises at least one compound comprising M, i.e., the precursor gas generally comprises at least one of silicon, germanium and tin (which are Group IV elements). For example, when the MX-functional quantum dots comprise SiX-functional quantum dots, the precursor gas generally comprises silicon. In this embodiment, the precursor gas may be selected from silanes, disilanes, halogen-substituted silanes, halogen-substituted disilanes, C_1-C_4 alkyl silanes, C_1-C_4 alkyldisilanes, and combinations thereof. In one form of the present disclosure, the precursor gas comprises silane, which accounts for 0.1 to 2% of the precursor gas, which may alternatively be referred to as the gas mixture or reactant gas mixture. Generally, the precursor gas designates the reactive gas utilized to nucleate the MX-functional quantum dots, and the precursor gas may be combined with other gasses, as described below, to form the gas mixture or reactant gas mixture including the precursor gas. The gas mixture may also comprise other percentages of silane. The precursor gas may additionally or alternatively comprise $SiCl_4$, $HSiCl_3$, and H_2SiCl_2. Alternatively, when the MX-functional quantum dots comprise GeH-functional quantum dots, the precursor gas generally comprises germanium. In this embodiment, the precursor gas may be selected from germane, digermanes, halogen-substituted germanes, halogen-substituted digermanes, C_1-C_4 alkyl germanes, C_1-C_4 alkyldigermanes, and mixtures thereof. The MX-functional quantum dots may comprise both silicon and germanium, with the precursor gas including combinations of the above precursor gasses.

[0020] Further, organometallic precursor molecules may also be used in or as the precursor gas. These molecules include a Group IV metal and independently selected organic groups. Organometallic Group IV precursors include, but are not limited to organosilicon, organogermanium and organotin compounds. Some examples of Group IV precursors include, but are not limited to, alkylgermaniums, alkylsilanes, alkylstannanes, chlorosilanes, chlorogermaniums, chlorostannanes, aromatic silanes, aromatic germaniums and aromatic stannanes. Other examples of silicon precursors include, but are not limited to, disilane (Si_2H_6), silicon tetrachloride ($SiCl_4$), trichlorosilane ($HSiCl_3$) and dichlorosilane (H_2SiCl_2). Still other suitable precursor molecules for use in forming silicon quantum dots include alkyl and aromatic silanes, such as dimethylsilane (H_3C-SiH_2-CH_3), tetraethyl silane ($(CH_3CH_2)_4Si$) and diphenylsilane (Ph-SiH_2-Ph). Particular examples of germanium precursor molecules that may be used to form germanium quantum dots include, but are not limited to, tetraethyl germane ($(CH_3CH_2)_4Ge$) and diphenylgermane (Ph-GeH_2-Ph).

[0021] In another form of the present disclosure, the quantum dots may undergo an additional doping step. For example, the quantum dots may undergo gas phase doping in the plasma, where a second precursor gas is dissociated and is incorporated in the quantum dots as they nucleate. The quantum dots may also or alternatively undergo doping in the gas phase downstream of the production of the quantum dots, but before the quantum dots are collected, e.g. by capturing in the capture fluid. Furthermore, when the capture fluid is utilized to collect the quantum dots, doped quantum dots may be produced in the capture fluid where the dopant is preloaded therein, in which case the quantum dots become doped in situ in the capture fluid. Doped quantum dots can be formed by contact with organosilicon gases or liquids, including, but not limited to trimethylsilane, disilane, and trisilane. Gas phase dopants may include, but are not limited to, BCl_3, B_2H_6, PH_3, GeH_4, or $GeCl_4$.

[0022] The precursor gas may be mixed with other gases, such as inert gases, in the gas mixture or reactant gas mixture. Examples of inert gases that may be included in the gas mixture include argon, xenon, neon, or a mixture of inert gases. When present in the gas mixture, the inert gas may be utilized in an amount of from 1 to 99 volume percent based on the total volume of the reactant gas mixture. The gas mixture may comprise the precursor gas in an amount of from 0.1 to 50, alternatively from 1 to 50, volume percent based on the total volume of the reactant gas mixture.

[0023] In certain embodiments, the reactant gas mixture further comprises a second precursor gas which itself can make up from 0.1 to 49.9 volume percent based on the total volume of the reactant gas mixture. The second precursor gas may comprise BCl_3, B_2H_6, PH_3, GeH_4, or $GeCl_4$. The second precursor gas may also comprise other gases that contain carbon, germanium, boron, phosphorous, and/or nitrogen. The combination of the first precursor gas and the second precursor gas together may make up from 0.1 to 50 volume percent based on the total volume of the reactant gas mixture.

[0024] In another form of the present disclosure, the reactant gas mixture further comprises hydrogen gas. Hydrogen gas can be present in an amount of from 1 to 50, alternatively 1 to 25, alternatively 1 to 10, volume percent based on the total volume of the reactant gas mixture. However, it is also contemplated that the reactant gas mixture may comprise other percentages of hydrogen gas.

[0025] In one form of the present disclosure, the quantum dots may comprise alloys of Group IV elements, e.g. silicon alloys. Silicon alloys that may be formed include, but are not limited to, silicon carbide, silicon germanium, silicon boron, silicon phosphorous, and silicon nitride. The silicon alloys may be formed by mixing at least one first precursor gas with the second precursor gas or using a precursor gas that contains the different elements. However, other methods of forming alloyed quantum dots are also contemplated.

[0026] As set forth above, X of the MX-functional quantum dots comprises a functional group independently selected from H and a halogen atom. The precursor gas (or gasses in the reactant gas mixture) is generally selected based on the desired functional group of the MX-functional quantum dots. For example, when X is H, the reactant gas mixture generally comprises hydrogen gas or a lesser concentration of halogenated species (e.g. $SiCl_4$, $HSiCl_3$, BCl_3, $GeCl_4$, etc.). In contrast, when X is the halogen atom, the precursor gas (or reactant gas mixture) comprises an increased concentration of halogenated species (e.g. $SiCl_4$, $HSiCl_3$, BCl_3, $GeCl_4$, etc.). Any of these chlorinated species may comprise halogen atoms other than chlorine, e.g. bromine, fluorine, or iodine. For example, $SiBr_4$ may be utilized in combination with or in lieu of $SiCl_4$ contingent on the desired functional group X. Further, in embodiments where the functional group X is a halogen atom, the reactant gas mixture may further comprise a halogen gas. For example, in embodiments where the functional group X is Cl, chlorine gas (Cl_2) may be utilized in the reactant gas mixture, either as a separate feed or along with the precursor gas. The relative amount of the halogen gas, if utilized, may be optimized on a variety of factors, such as the precursor gas selected, etc. For example, lesser amounts of the halogen gas may be utilized to prepare halogen-functional quantum dots when the precursor gas comprises halogenated species. In certain embodiments, the halogen gas may be utilized in an amount of from greater than 0 to 25, alternatively from 1 to 25, alternatively from 1 to 10, volume percent based on the total volume of the reactant gas mixture.

[0027] Specific embodiments of plasma reactor systems particularly suitable for the instant method are described below. It is to be appreciated that the specific embodiments described below are merely examples of exemplary plasma processes suitable for producing quantum dots, and particularly MX-functional quantum dots, may be utilized.

[0028] Referring to Figure 1, a plasma reactor system is shown at 20. In this embodiment, the plasma reactor system 20 comprises a plasma generating chamber 22 having a reactant gas inlet 29 and an outlet 30 having an aperture or orifice 31 defined thereby. A particle collection chamber 26 is configured to be in communication with the plasma generating chamber 22. The particle collection chamber 26 contains a capture fluid 27 in a container 32. The container 32 may be adapted to be agitated (not shown). For example, the container 32 may be positioned on a rotatable support (not shown) or may include a stirring mechanism. Typically, the capture fluid is a liquid at the temperatures of operation of the system. The plasma reactor system 5 also includes a vacuum source 28 in communication with the particle collection chamber 26 and plasma generating chamber 22.

[0029] The plasma generating chamber 22 comprises an electrode configuration 24 that is attached to a variable frequency RF amplifier 21. The plasma generating chamber 22 also comprises a second electrode configuration 25. The second electrode configuration 25 is either ground, DC biased, or operated in a push-pull manner relative to the electrode configuration 24. The electrodes 24, 25 are used to couple the high frequency (HF) or very high frequency (VHF) power to the reactant gas mixture to ignite and sustain a glow discharge of plasma within the area identified as 23. The first reactive precursor gas (or gases) is then dissociated in the plasma to provide charged atoms which nucleate to form MX-functional quantum dots. However, other discharge tube configurations are contemplated, and may be used in carrying out the method disclosed herein.

[0030] In the embodiment of Figure 1, the MX-functional quantum dots are collected in the particle collection chamber 26 in the capture fluid. To control the diameter of the MX-functional quantum dots which are formed, the distance between the aperture 31 in the outlet 30 of the plasma generating chamber 22 and the surface of the capture fluid ranges from 5 to 50 times a diameter of the aperture (i.e., from 5 to 50 aperture diameters). Positioning the surface of the capture fluid too close to the outlet of the plasma generating chamber may result in undesirable interactions of plasma with the capture fluid. Conversely, positioning the surface of the capture fluid too far from the aperture reduces particle collection efficiency. As collection distance is a function of the aperture diameter of the outlet and the pressure drop between the plasma generating chamber and the collection chamber, based on the operating condition described herein, an acceptable collection distance is from 1 to 20, alternatively from 5 to 10, cm. Said differently, an acceptable collection distance is from 5 to 50 aperture diameters.

[0031] The plasma generating chamber 22 also comprises a power supply. The power is supplied via a variable frequency radio frequency power amplifier 21 that is triggered by an arbitrary function generator to establish high frequency pulsed plasma in area 23. Typicallyably, the radiofrequency power is capacitively coupled into the plasma using a ring electrode, parallel plates, or an anode/cathode setup in the gas. Alternatively, the radiofrequency power may be inductively coupled mode into the plasma using an rf coil setup around the discharge tube.

[0032] The plasma generating chamber 11 may also comprise a dielectric discharge tube. Typicallyably, a reactant gas mixture enters the dielectric discharge tube where the plasma is generated. MX-functional quantum dots which form from the reactant gas mixture start to nucleate as the first reactive precursor gas molecules are dissociated in the plasma.

[0033] The vacuum source 28 may comprise a vacuum pump. Alternatively, the vacuum source 28 may comprise a mechanical, turbo molecular, or cryogenic pump.

[0034] In one embodiment, the electrodes 24, 25 for a plasma source inside the plasma generating chamber 22 comprise a flow-through showerhead design in which a VHF radio frequency biased upstream porous electrode plate 24 is separated from a downstream porous electrode plate 25, with the pores of the plates aligned with one another. The pores may be circular, rectangular, or any other desirable shape. Alternatively, the plasma generating chamber 22 may enclose an electrode 24 that is coupled to the VHF radio frequency power source and has a pointed tip that has a variable distance between the tip and a grounded ring inside the chamber 22.

[0035] In one embodiment, the HF or VHF radio frequency power source operates in a frequency range of 10 to 500 MHz. In an alternative embodiment, the pointed tip 13 can be positioned at a variable distance from a VHF radio frequency powered ring 14 operated in a push-pull mode (180° out of phase). In yet another alternative embodiment, the electrodes 24, 25 include an inductive coil coupled to the VHF radio frequency power source so that radio frequency power is delivered to the reactant gas mixture by an electric field formed by the inductive coil. Portions of the plasma generating chamber 22 can be evacuated to a vacuum level ranging between $1x10^{-7}$ to 500 Torr. However, other electrode coupling configurations are also contemplated for use with the method disclosed herein.

[0036] In the illustrated embodiment of Figure 1, the plasma in area 23 is initiated with a high frequency plasma via an RF power amplifier such as, for example, an AR Worldwide Model KAA2040, or an Electronics and Innovation Model 3200L, or an EM Power RF Systems, Inc. Model BBS2E3KUT. The amplifier can be driven (or pulsed) by an arbitrary function generator (e.g., a Tektronix AFG3252 function generator) that is capable of producing up to 200 watts of power from 0.15 to 150 MHz. In several embodiments, the arbitrary function may be able to drive the power amplifier with pulse trains, amplitude modulation, frequency modulation, or different waveforms. The power coupling between the amplifier and the reactant gas mixture typically increases as the frequency of the rf power increases. The ability to drive the power at a higher frequency may allow more efficient coupling between the power supply and discharge. At frequencies below 30 MHz, only 2 - 15% of the power is delivered to the discharge. This has the effect of producing high reflected power in the rf circuit that leads to increased heating and limited lifetime of the power supply. In contrast, higher frequencies allow more power to be delivered to the discharge, thereby reducing the amount of reflected power in the rf circuit.

[0037] In one embodiment, the power and frequency of the plasma system is preselected to create an optimal operating space for the formation of the MX-functional quantum dots. Typicallyably, tuning both the power and frequency creates an appropriate ion and electron

energy distribution in the discharge to help dissociate the molecules of the reactive precursor gas and nucleate the MX-functional quantum dots.

[0038] The plasma reactor system 20 illustrated in Figure 1 may be pulsed to enable an operator to directly manage the resident time for particle nucleation, and thereby control the particle size distribution and agglomeration kinetics in the plasma. The pulsing function of the system 20 allows for controlled tuning of the particle resident time in the plasma, which affects the size of the MX-functional quantum dots. By decreasing the "on" time of the plasma, the nucleating particles have less time to agglomerate, and therefore the size of the MX-functional quantum dots may be reduced on average (i.e., the quantum dot distribution may be shifted to smaller diameter particle sizes).

[0039] Advantageously, the operation of the plasma reactor system 20 at higher frequency ranges and pulsing the plasma provides the same conditions as in conventional constricted/filament discharge techniques that use a plasma instability to produce the high ion energies/densities, but with the additional advantage that users can control operating conditions to select and produce MX-functional quantum dots having various sizes, which impacts their characteristic physical properties, e.g. photoluminescence..

[0040] For a pulse injection, the synthesis of the MX-functional quantum dots can be done with a pulsed energy source, such as a pulsed very high frequency RF plasma, a high frequency RF plasma, or a pulsed laser for pyrolysis. Typicallyably, the VHF radiofrequency is pulsed at a frequency ranging from 1 to 50 kHz.

[0041] Another method to transfer the MX-functional quantum dots to the capture fluid is to pulse the input of the reactant gas mixture while the plasma is ignited. For example, one could ignite the plasma in which a first reactive precursor gas is present to synthesize the MX-functional quantum dots, with at least one other gas present to sustain the discharge, such as an inert gas. The synthesis of the MX-functional quantum dots is stopped when the flow of first reactive precursor gas is stopped with a mass flow controller. The synthesis of the MX-functional quantum dots continues when the flow of the first reactive precursor gas is started again. This produces a pulsed stream of MX-functional quantum dots. This technique can be used to increase the concentration of MX-functional quantum dots in the capture fluid if the flux of MX-functional quantum dots impinging on the capture fluid is greater than the absorption rate of the MX-functional quantum dots into the capture fluid.

[0042] In another embodiment, the nucleated MX-functional quantum dots are transferred from the plasma generating chamber 22 to particle collection chamber 26 containing capture fluid via the aperture or orifice 31 which creates a pressure differential. It is contemplated that the pressure differential between the plasma generating chamber 22 and the particle collection chamber 26 can be controlled through a variety of ways. In one configuration, the

discharge tube inside diameter of the plasma generating chamber 22 is much less than the inside diameter of the particle collection chamber 26, thus creating a pressure drop. In another configuration, a grounded physical aperture or orifice may be placed between the discharge tube and the collection chamber 26 that forces the plasma to reside partially inside the orifice, based on the Debye length of the plasma and the size of the chamber 22. Another configuration comprises using a varying electrostatic orifice in which a positive concentric charge is developed that forces the negatively charged plasma through the aperture 31.

[0043] As first introduced above, in the embodiment of Figure 1, upon the dissociation of the first reactive precursor gas in the plasma generation chamber 22, MX-functional quantum dots form and are entrained in the gas phase. The distance between the quantum dot synthesis location and the surface of capture fluid must be short enough so that no unwanted functionalization occurs while the MX-functional quantum dots are entrained. If the MX-functional quantum dots interact within the gas phase, agglomerations of numerous individual small MX-functional quantum dots will form and be captured in the capture fluid. If too much interaction takes place within the gas phase, the MX-functional quantum dots may sinter together and form MX-functional quantum dots having larger average diameters. The collection distance is defined as the distance from the outlet of the plasma generating chamber to the surface of the capture fluid. The capture fluid is described in detail below following the description of an alternative embodiment of a plasma reactor system.

[0044] Additional aspects relating to this particular embodiment in which the MX-functional quantum dots are produced via this plasma process are described in International (PCT) Publication No. WO 2011/109299 (PCT/US2011/026491), which is incorporated by reference herein in its entirety.

[0045] Referring to Figure 2, an alternative embodiment of a plasma reactor system is shown at 50. In this embodiment, the MX-functional quantum dots are prepared in a system having a reactor and a diffusion pump in fluid communication with the reactor for collecting the MX-functional quantum dots of the aerosol. For example, MX-functional quantum dots of various size distributions and properties can be prepared by introducing a nanoparticle aerosol produced in a reactor (e.g. a low-pressure plasma reactor) into a diffusion pump in fluid communication with the reactor, capturing the MX-functional quantum dots of the aerosol in a condensate from the capture fluid, and collecting the captured MX-functional quantum dots in a reservoir. In the embodiment of Figure 2, the capture fluid may alternatively be referred to as a diffusion pump fluid, although the capture fluid and diffusion pump fluid are generally referred to herein as “the capture fluid” and are described collectively below.

[0046] Example reactors are described in WO 2010/027959 and WO 2011/109229, with each of which being incorporated herein in its respective entirety. Such reactors can be, but

are not limited to, low pressure high frequency pulsed plasma reactors. For example, Figure 2 illustrates the plasma reactor of the embodiment of Figure 1, but includes the diffusion pump in fluid communication with the reactor. To this end, description relative to this particular plasma reactor is not repeated herein with respect to the embodiment of Figure 2.

[0047] In the embodiment of Figure 2, the plasma reactor system 50 includes a diffusion pump 120. As such, the MX-functional quantum dots can be collected by the diffusion pump 120. A particle collection chamber 26 may be in fluid communication with the plasma generating chamber 22. The diffusion pump 120 may be in fluid communication with the particle collection chamber 26 and the plasma generating chamber 22. In other forms of the present disclosure, the system 50 may not include the particle collection chamber 26. For example, the outlet 30 may be coupled to an inlet 103 of the diffusion pump 120, or the diffusion pump 120 may be in substantially direct fluid communication with the plasma generating chamber 22.

[0048] Figure 3 is a cross-sectional schematic of an example diffusion pump 120 suitable for the system 50 of the embodiment of Figure 2. The diffusion pump 120 can include a chamber 101 having an inlet 103 and an outlet 105. The inlet 103 may have a diameter of 2 to 55 inches, and the outlet may have a diameter of 0.5 to 8 inches. The inlet 103 of the chamber 101 is in fluid communication with the outlet 30 of the reactor 20. The diffusion pump 120 may have, for example, a pumping speed of 65 to 65,000 liters/second or greater than 65,000 liters/second.

[0049] The diffusion pump 120 includes a reservoir 107 in fluid communication with the chamber 101. The reservoir 107 supports or contains the capture fluid. The reservoir may have a volume of 30 cc to 15 liters. The volume of the capture fluid in the diffusion pump may be 30 cc to 15 liters.

[0050] The diffusion pump 120 can further include a heater 109 for vaporizing the capture fluid in the reservoir 107 to a vapor. The heater 109 heats up the capture fluid and vaporizes the capture fluid to form a vapor (e.g., liquid to gas phase transformation). For example, the capture fluid may be heated to 100 to 400 °C or 180 to 250 °C.

[0051] A jet assembly 111 can be in fluid communication with the reservoir 107 comprising a nozzle 113 for discharging the vaporized capture fluid into the chamber 101. The vaporized capture fluid flows and rises up though the jet assembly 111 and emitted out the nozzles 113. The flow of the vaporized capture fluid is illustrated in Figure 3 with arrows. The vaporized capture fluid condenses and flows back to the reservoir 107. For example, the nozzle 113 can discharge the vaporized capture fluid against a wall of the chamber 101. The walls of the chamber 101 may be cooled with a cooling system 113 such as a water cooled system. The cooled walls of the chamber 101 can cause the vaporized capture fluid to

condense. The condensed capture fluid can then flow along and down the walls of the chamber 101 and back to the reservoir 107. The capture fluid can be continuously cycled through diffusion pump 120. The flow of the capture fluid causes gas that enters the inlet 103 to diffuse from the inlet 103 to the outlet 105 of the chamber 101. A vacuum source 33 may be in fluid communication with the outlet 105 of the chamber 101 to assist removal of the gas from the outlet 105.

[0052] As the gas flows through the chamber 101, MX-functional quantum dots in the gas can be absorbed by the capture fluid, thereby collecting the MX-functional quantum dots from the gas. For example, a surface of the MX-functional quantum dots may be wetted by the vaporized and/or condensed capture fluid. Furthermore, the agitating of cycled capture fluid may further improve absorption rate of the MX-functional quantum dots compared to a static fluid. The pressure within the chamber 101 may be less than 1 mTorr.

[0053] The capture fluid with the MX-functional quantum dots can then be removed from the diffusion pump 120. For example, the capture fluid with the MX-functional quantum dots may be continuously removed and replaced with capture fluid that substantially does not include MX-functional quantum dots.

[0054] Advantageously, the diffusion pump 120 can be used not only for collecting MX-functional quantum dots but also evacuating the reactor 20 (and collection chamber 26). For example, the operating pressure in the reactor 20 can be a low pressure, e.g. less than atmospheric pressure, less than 760 Torr, or between 1 and 760 Torr. The collection chamber 26 can, for example, range from 1 to 5 milliTorr. Other operating pressures are also contemplated.

[0055] The system 50 may also include a vacuum pump or vacuum source 33 in fluid communication with the outlet 105 of the diffusion pump 120. The vacuum source 33 can be selected in order for the diffusion pump 120 to operate properly. In one form of the present disclosure, the vacuum source 33 comprises a vacuum pump (e.g., auxiliary pump). The vacuum source 33 may comprise a mechanical, turbo molecular, or cryogenic pump. However, other vacuum sources are also contemplated.

[0056] One method of producing MX-functional quantum dots with the system 50 of Figure 2 can include forming a nanoparticle aerosol in the reactor 20. The nanoparticle aerosol can comprise MX-functional quantum dots in a gas, and the method further includes introducing the nanoparticle aerosol into the diffusion pump 120 from the reactor 5. In this embodiment, the method also may include heating the capture fluid in a reservoir 107 to form a vapor, sending the vapor through a jet assembly 111, emitting the vapor through a nozzle 113 into a chamber 101 of the diffusion pump 120, condensing the vapor to form a condensate, and flowing the condensate back to the reservoir 107. Furthermore, the method can further

include capturing the MX-functional quantum dots of the aerosol in the condensate, which comprises the capture fluid, and collecting the captured MX-functional quantum dots in the reservoir 107. The step of capturing the MX-functional quantum dots of the aerosol in the condensation, which comprises the capture fluid, may be identical to the step of collecting the MX-functional quantum dots of the aerosol in the capture fluid. The method can further include removing the gas from the diffusion pump with a vacuum pump. In the embodiment described above and illustrated in Figure 1, the MX-functional quantum dots are collected directly in the capture fluid. However, in the embodiment described immediately above and illustrated in Figures 2 and 3, the capture fluid is vaporized and condensed in the diffusion pump, and the MX-functional quantum dots are ultimately capture or collected in the capture fluid (once condensed).

[0057] Additional aspects relating to this particular embodiment in which the MX-functional quantum dots are produced via this plasma process are described in U.S. Appln. Ser. No. 61/655,635, which is incorporated by reference herein in its entirety.

[0058] Independent of the particular low pressure reactor utilized to prepare the nanoparticle aerosol, the MX-functional quantum dots are collected, optionally in the capture fluid (or diffusion pump fluid, which may also serve as the capture fluid).

[0059] If utilized, the capture fluid may comprise any compounds, components, or fluids that may be suitable for capturing the MX-functional quantum dots. For example, conventional components utilized in conventional capture fluids may be utilized as the capture fluid. Specific examples of conventional capture fluids include silicone fluids, such as polydimethylsiloxane, phenylmethyl-dimethyl cyclosiloxane, tetramethyltetraphenyltrisiloxane, and/or pentaphenyltrimethyltrisiloxane; hydrocarbons; phenyl ethers; fluorinated polyphenyl ethers; sulfoxides (e.g., anhydrous methyl sulfoxide); and ionic fluids. Combinations of different components may be utilized in the capture fluid. The capture fluid may have a dynamic viscosity of 0.001 to 1 Pa·s, 0.005 to 0.5 Pa·s, alternatively 0.01 to 0.1 Pa·s, at 23 ± 3 °C. Furthermore, the capture fluid may have a vapor pressure of less than 1 x 10^{-4} Torr. In some embodiments, the capture fluid is at a temperature ranging from -20 °C to 150 °C and a pressure ranging from 1 to 5 milliTorr (0.133 Pa to 0.665 Pa). In some embodiments, the capture fluid has a vapor pressure less than the pressure in the particle collection chamber.

[0060] The quantum dots may have a largest dimension or average largest dimension less than 10, less than 9, less than 8, less than 7, less than 6, or less than 5, nm (while still being greater than 0 nm). Furthermore, the largest dimension or average largest dimension of the quantum dots may be between greater than 0 to 10, between 1 and 8, between 1.5 and 7, or between 2.2 and 4.7, nm. The largest dimension of the quantum dots can be measured

by a variety of methods, such as with a transmission electron microscope (TEM). For example, as understood in the art, particle size distributions are often calculated via TEM image analysis of hundreds of different quantum dots. Quantum dots have excitons confined in all three spatial dimensions and may comprise individual crystals, i.e., each quantum dot is a single crystal.

[0061] As introduced above, the bioconjugated molecule further comprises a biomolecule. As with the quantum dot, the bioconjugated molecule may comprise two or more biomolecules, which may be independently selected. For clarity and consistency, "the biomolecule" encompasses embodiments where the bioconjugated molecule includes but one or two or more biomolecules.

[0062] The biomolecule need not be derived from biological tissue or cells. Instead, the term "biomolecule," as used herein, designates any molecule that may serve a biological function or that has an affinity for or capacity to react or physically interact with a component(s) of a biological system. While many such biomolecules are derived from or included biological tissue or cells (e.g. proteins, amino acids, etc.), other biomolecules, such as active pharmaceutical ingredients, merely serve a biological function, but may be artificially synthesized. The biological function may be any biological function.

[0063] In certain embodiments, the biomolecule is selected from the group of proteins, antibodies, enzymes, peptides, hormones, amino acids, vitamins, lipids, carbohydrates, lectins, active pharmaceutical ingredients, biological response modifiers, genetic material, and combinations thereof. One of skill in the art readily understands how to select at least one of these biomolecules for preparing the bioconjugated molecule in view of the desired biological function thereof.

[0064] In certain embodiments, the biomolecule comprises a protein. The protein may be any protein, as understood in the art. In certain embodiments, the protein comprises a signaling protein molecule, such as tumor necrosis factor alpha, basic fibroblast growth factor, and vascular endothelial growth factor. Alternatively, the protein may comprise a bone morphogenic protein. Alternatively still, the protein may be derived from an infectious agent. Structural proteins may also be utilized as the biomolecule, such as collagen and/or laminin.

[0065] In certain embodiments, the biomolecule comprises an antibody or a fragment thereof. The antibody may be a primary or secondary antibody. Specific examples of suitable antiboies include those having as their antigens growth factors or cluster of differentiation (CD) molecules. For example, antibodies for CD4 and CD8 may be utilized, which are commonly employed to monitor HIV infections. Additional examples of antibodies include those for CD34, c-Kit, and Sca-1, which are commonly employed to identify stem cells.

Growth factors that may be targeted by the antibody include vascular endothelial growth factor (VEGF), basic fibroblast growth factor, and the bone morphogenic proteins (BMPs).

[0066] In certain embodiments, the biomolecule comprises an enzyme. The enzyme may be any enzyme, as understood in the art. When the biomolecule comprises the enzyme, the bioconjugated molecule may be utilized in enzymatically cleaved substrates. For example, the bioconjugated molecule may be utilized to detect or measure protease activity based on an amount of the substrate that is cleaved.

[0067] In certain embodiments, the biomolecule comprises a peptide. Peptides are known in the art and the peptide may be any peptide formed from any amino acids. The peptide is not limited to those derived from canonical amino acids, and non-amino acid groups can be bonded to or included in the peptide for added functionality. For example, p-propargyloxyphenylalanine or p-azidophenylalanine may be used to enable a 3+2 cycloaddition reaction to form the peptide.

[0068] In certain embodiments, the biomolecule comprises a hormone. The hormone is not limited and may be, for example, a phytohormone. Additional specific examples of suitable hormones include cortisol, lopoxins, arachidoic acid, insulin, and triiodothyronine.

[0069] In certain embodiments, the biomolecule comprises an amino acid. Specific examples of amino acids include arginine, lysine, aspartic acid, glutamic acid, glutamine, asparagine, histidine, serine, threonine, tyrosine, cysteine, methionine, tryptophan, alanine, isoleucine, leucine, phenylalanine, valine, proline, and glycine. Further, non-canonical amino acids may be utilized as the biomolecule, such as p-propargyloxyphenylalanine.

[0070] In certain embodiments, the biomolecule comprises a vitamin. As used herein, the term “vitamin” denotes any organic molecule that is required in relatively small quantities for the proper functioning of an organism’s metabolism, and that human cells frequently or always cannot synthesize in quantities adequate to sustain life. Specific examples of vitamins suitable for the biomolecule of the bioconjugated molecule include folic acid, biotin, and thiamine.

[0071] In certain embodiments, the biomolecule comprises a lipid. As used herein, the term “lipid” denotes any biologically derived molecule that is insoluble in water, but can be extracted by chloroform and other non-polar solvents. Specific examples of lipids suitable for the biomolecule include fatty acids, triglycerides, phospholipids, and steroids (e.g. cholesterol).

[0072] In certain embodiments, the biomolecule comprises a carbohydrate. Typically, the carbohydrate comprises a saccharide. Specific examples of suitable carbohydrates include polyhydroxy aldehydes, polyhydroxy ketones, or molecules that can form such molecules when hydrolyzed. Specific examples of saccharides suitable for the biomolecule range from

monosaccharides (ribose, deoxyribose, manose, etc.) to polysaccharides (dextran, chitin, glycosaminoglycan, etc.).

[0073] In certain embodiments, the biomolecule comprises a lectin. As used herein, the term "lectin" denotes proteins having a high bind affinity and specificity for saccharides. The lectin may be derived from a variety of sources, such as plants (e.g. Canavalia ensiformis), bacteria (e.g. Escherichia coli), and/or mammals (e.g. Homo sapiens). Non-limiting examples of a lectin include a cellulose-binding domain or a carbohydrate-binding module.

[0074] In certain embodiments, the biomolecule comprises an active pharmaceutical ingredient (API). As used herein, the API is any compound or molecule having a direct impact on disease prevention, diagnosis, treatment, and/or cure. One of skill in the art readily understands suitable APIs for such purposes, and the API is not limited. The API may be a prescription API or an over-the-counter (OTC) API.

[0075] In certain embodiments, the biomolecule comprises a biological response modifier. As understood in the art, biological response modifiers modify biological responses. Specific examples of biological response modifiers suitable for the biomolecule include interleukins, interferons, viruses, viral fragments, etc.

[0076] In certain embodiments, the biomolecule comprises genetic material. "Genetic material" may include certain species or types of other biomolecules described above. For example, viruses and other biological response modifiers may also constitute genetic material. Additional examples include plasmids, phages, cosmids, genes and gene fragments (e.g. exons and/or introns), nucleotides (e.g. oligonucleotides and substituted nucleic acid oligonucleotides), and nucleic acids. Specific examples of nucleic acids include deoxyribonucleic acid (DNA), which may be single or double-stranded, ribonucleic acid (RNA), which may be single or double-stranded, ribosomal RNA (rRNA), catalytic RNA (cRNA), small nuclear RNA (snRNA), messenger RNA (mRNA), transfer RNA (tRNA), DNA and/or RNA oligonucleotides, etc.

[0077] Additional examples of suitable biomolecules include radionuclides, toxins, antigens, pesticides, pheromones, and combinations thereof.

[0078] As introduced above, the bioconjugated molecule further comprises a divalent linking group bonded between the quantum dot and the biomolecule. In embodiments where the bioconjugated molecule includes two or more quantum dots and two or more biomolecules, the bioconjugated molecule may include more than one divalent linking group, which may be the same as or different from one another, e.g. in terms of atomic structure. Further, the quantum dot may include one or more monovalent groups, particularly when the quantum dot includes more than one functional group X for reacting with a cross-linking agent to form the divalent linking group, as described below. For example, the cross-linking agent may be

utilized to form the divalent linking group, while also resulting in one or more monovalent groups pending from the quantum dot. More specifically, the MX-functional quantum dots may have a plurality of functional sites of the functional group X, and different molecules of the cross-linking agent may bond to different functional sites on the same MX-functional quantum dot. Due to steric hindrance or molar ratios of the components, some of the molecules of the cross-linking agent may not also bond to the biomolecule, thus resulting in the monovalent groups pending from the quantum dots, which may also exist in the resulting bioconjugated molecule.

[0079] The divalent linking group may be formed from a variety of cross-linking agents, as described below, particularly in view of various functionalities suitable for the cross-linking agent. Alternatively, the divalent linking group may be formed from a substituent already present in, for example, the biomolecule. In these embodiments, the divalent linking group is not formed from a separate cross-linking agent. However, the cross-linking agent is typically utilized to form the divalent linking group of the bioconjugated molecule.

[0080] The divalent linking group may be organic or silicon-based. Organics are distinguished from silicones, which predominately comprise siloxane bonds (Si-O-Si), although carbon-carbon bonds may also be present in silicones. Typically, the divalent linking group is organic. When the divalent linking group is organic, the divalent linking group is generally free from siloxane bonds, alternatively free from silicon atoms. Even when classified as being organic, the divalent linking group may comprise one or more heteroatoms (e.g., O, S, N, etc.). In one embodiment, the divalent linking group is a hydrocarbon group, with is linear or branched, alternatively linear. In other embodiments, the divalent linking group may be substituted or unsubstituted, and may have pendent functional or nonfunctional groups (e.g. monovalent groups), as described below. By "substituted," it is meant that the organic compound may include at least one non-carbon based substituent or a carbon-based substituent substituted with atoms other than hydrogen. For example, the divalent linking group may be a substituted or unsubstituted hydrocarbylene, heterohydrocarbylene, or organoheterylene linking group. The divalent linking group and the cross-linking agent utilized to form the divalent linking group is described in greater detail below with reference to the method of preparing the bioconjugated molecule. The divalent group, which is between the biomolecule and the quantum dot, may alternatively be referred to as a spacer.

[0081] The bioconjugated molecule, the quantum dot, an exemplary plasma process to form the quantum dot, the biomolecule, and the divalent linking group are described above. A method of preparing the bioconjugated molecule is also disclosed. The method comprising bonding the cross-linking agent between the quantum dot and the biomolecule. In various

embodiments, the method further comprises preparing the quantum dot via the plasma process, e.g. any of the plasma processes described above.

[0082] The quantum dots include functional group X, and the biomolecule typically includes a functional group X'. In these embodiments, the cross-linking agent includes a functional group Y reactive with the functional group X of the quantum dot and a functional group Y' reactive with the functional group X' of the biomolecule. Y and Y' may be the same as or different from one another. Similarly, X and X' are the same as or different from one another. Typically, Y and Y' are different from one another, and X and X' are different from one another, which can prevent the formation of cross-linked quantum dots and/or cross-linked biomolecules rather than the inventive bioconjugated molecule. When Y and Y' are the same as each other, the cross-linking agent is referred to as a homobifunctional cross-linking agent. When Y and Y' are different from one another, the cross-linking agent is referred to as a heterobifunctional cross-linking agent. Although these designations recite "bifunctional," the cross-linking agent may have three or more functional groups, e.g. may be tri-functional, but the bifunctional designation relates to Y and Y'.

[0083] The selection of the functional group Y of the cross-linking agent is based on the functional group X of the MX-functional quantum dots. For example, certain functional groups are reactive with hydrogen but not halogen atoms, whereas other functional groups are reactive with halogen atoms but not hydrogen.

[0084] In certain embodiments, the functional group X of the MX-functional quantum dots is H, in which case the MX-functional quantum dots may be referred to as MH-functional quantum dots. In these embodiments, the cross-linking agent typically comprises an unsaturated organic compound, and the functional group Y of the cross-linking agent is an aliphatic carbon-carbon multiple bond. The aliphatic carbon-carbon multiple bond may be a double bond (C=C) or a triple bond (C≡C). Further, the cross-linking agent may have more than one carbon-carbon multiple bond, with each carbon-carbon multiple bond being independently selected from a double bond and a triple bond. The aliphatic carbon-carbon multiple bond may be within a backbone of the cross-linking agent, pendent from the cross-linking agent, or at a terminal location of the cross-linking agent. For example, the cross-linking agent may be linear, branched, or partly branched, and the aliphatic carbon-carbon multiple bond may be located at any location of the cross-linking agent. Typically, the cross-linking agent is aliphatic, although the cross-linking agent may have a cyclic and/or aromatic portion, so long as the carbon-carbon multiple bond is located in an aliphatic portion of the cross-linking agent, i.e., the carbon-carbon multiple bond of the cross-linking agent is not present in, for example, an aryl group. In certain embodiments, the aliphatic carbon-carbon multiple bond is present at a terminal location of the cross-linking agent, i.e., the alpha carbon

of the cross-linking agent is part of the carbon-carbon multiple bond. This embodiment generally reduces steric hindrance of the aliphatic carbon-carbon multiple bond during the step of bonding.

[0085] In these or other embodiments, the cross-linking agent includes at least 5, alternatively at least 10, alternatively at least 15, alternatively at least 20, alternatively at least 25, carbon atoms in its chain. However, as described above, at least one carbon atom may be substituted by an atom other than carbon, e.g. O, N, S, etc. In certain embodiments, it is desirable to minimize the number of carbon atoms or the molecular weight of the cross-linking agent so as to increase solubility of the bioconjugated molecule formed therefrom.

[0086] For example, in various embodiments, the cross-linking agent may comprise an ester having the carbon-carbon multiple bond. In this embodiment, at least one carbon atom of the chain of the cross-linking agent is replaced by an oxygen atom so as to form an ether linkage with a carbonyl group adjacent thereto, e.g. as in an ester. Specific examples of such esters suitable for the purposes of the cross-linking agent include, but are not limited to, allyl dodecanoate, dodecyl 3-butenoate, propyl 10-undecenoate, 10-undecenyl acetate, and dodecyl (meth)acrylate. It is to be appreciated that these compounds do not include any naming scheme or designation relative to the additional functional group Y' that is reactive with the X' functional group of the biomolecule, as described below. Said differently, these compounds and their functional groups relate to the bonding between X of the quantum dots and Y of the cross-linking agent.

[0087] In other embodiments, the functional group X of the MX-functional quantum dots is the independently selected halogen atom. In these embodiments, the functional group X is independently selected from fluorine (F), chlorine (Cl), bromine (Br), and iodine (I). Typically, X is Cl. In these embodiments, the functional group Y of the cross-linking agent is reactive with the functional group X of the MX-functional quantum dots , i.e., the functional group Y is reactive with a halogen atom.

[0088] In embodiments where the functional group X of the MX-functional quantum dots is the independently selected halogen atom, specific examples of Y for the cross-linking agent include, but are not limited to, an alcohol functional group, a thiol functional group; an amine functional group; a carboxylic acid functional group; a sulphide functional group; an amide functional group; a phosphine functional group; a metal halide functional group; a terminal alkyne functional group, an organolithium functional group, an aldehyde functional group, and a Grignard reagent functional group, e.g. an RMgBr group, where R is a portion of the cross-linking agent.

[0089] As noted above, the cross-linking agent typically further includes a functional group Y' reactive with a functional group X' of the biomolecule. The functional group X' of the

biomolecule may be an inherently present functional group based on a particular structure of the biomolecule, or the biomolecule may be modified so as to introduce the functional group X' to the biomolecule. The functional group X' may be pendent or terminal in the biomolecule so long as the functional group X' does not inhibit the function of the biomolecule.

[0090] The functional group Y' of the cross-linking agent is chosen based on the particular biomolecule and its functional group X'. One of skill in the art readily understands reactivity between functional groups and how to select the functional group Y' based on the functional group X' of the biomolecule. For example, as understood in the art, when the biomolecule is a protein molecule, the protein molecule may include an amine functional group (typically a primary amine functional group), a carboxyl functional group, a sulfhydryl functional group, a hydroxyl group, and/or a carbonyl functional group (e.g. as a ketone or aldehyde). Such groups may be present in the protein molecule, or a group the protein molecule may be modified (e.g. reduced or oxidized) to form such a functional group.

[0091] Specific examples of the functional group Y' that is reactive with the exemplary examples of the functional group X' above are known in the art. For example, when X' is the hydroxyl functional group, Y' may be an isocyanate group, an epoxide group, an oxirane group, etc. When X' is the sulfhydryl functional group, Y' may be a haloacetyl group, a thiosulfonate group, a vinylsulfone group, a maleimide group, a pyridyldithiol group, an aziridine group, a sulfhydryl group, etc. When X' is the primary amine functional group, Y' may be an N-hydroxysuccinimide group, an N-hydroxysulfosuccinimide group, an O-acylisourea group, an aldehyde group, an acid anhydride group, etc. When X' is the carboxylic acid functional group, Y' may be a diazoalkane group, a diazoacetyl group, a N,N'-carbonyl diimidazole group, etc.

[0092] If desired, the cross-linking agent may have additional functionality (i.e., functionality other than and in addition to the functional groups Y and Y'). For example, in certain embodiments, the cross-linking agent further comprises at least one functional group Z in addition to the functional groups Y and Y', with the functional group Z being convertible to a hydrophilic functional group. The functional group Z may, in certain embodiments, be selected from some of the functional groups set forth above suitable for the functional groups Y and/or Y', although in such embodiments, the functional group Z is separate from and in addition to the functional groups Y and Y' in the cross-linking agent.

[0093] Specific examples of hydrophilic functional groups include carboxylic acid functional groups, alcohol functional groups, hydroxy functional groups, azide functional groups, silyl ether functional groups, ether functional groups, phosphonate functional groups, sulfonate functional groups, thiol functional groups, amine functional groups, and combinations

thereof. The amine functional group may be primary, secondary, tertiary, or cyclic. Such hydrophilic functional groups may be bonded directly to the chain of the cross-linking agent, e.g. to a carbon atom of the chain, or may be bonded via a heteroatom or divalent linking group.

[0094] In certain embodiments, the cross-linking agent may include the hydrophilic functional group, such as any of the hydrophilic functional groups set forth above. Alternatively, the cross-linking agent may include the at least one functional group Z convertible to a hydrophilic functional group such that the cross-linking agent does not include a hydrophilic functional group until the at least one functional group Z is converted thereto.

[0095] Specific examples of the at least one functional group Z convertible to a hydrophilic functional group include, but are not limited to: ester functional groups (RCO_2R^1), including those of oxo acids, such as esters of carboxylic acid, sulfuric acid, phosphoric acid, nitric acid, and boric acid; acid halide functional groups (RCOX); amide functional groups ($RCONH_2$); nitrile functional groups (RCN); epoxide functional groups; silyl ether functional groups; ethylenically unsaturated groups in addition to the aliphatic carbon-carbon multiple bond; oxazoline functional groups (RC_3H_5NO); and anhydride functional groups, where R represents the cross-linking agent, R^1 is a hydrocarbyl group, and X is a halogen atom. The esters of oxo acids may be derived from the condensation of any alcohol with the particular oxo acid. For example, the alcohol may be aliphatic or aromatic. The at least one functional group Z may be a substituent of the cross-linking agent or a moiety within the cross-linking agent. For example, when the cross-linking agent includes an ester functional group, the ester functional group is generally a moiety within the cross-linking agent, as opposed to a substituent bonded thereto.

[0096] The at least one functional group Z of the cross-linking agent is generally selected based on the functional group X of the MX-functional quantum dots, the functional groups Y and Y' of the cross-linking agent, and the functional group X' of the biomolecule. For example, when X is H, reacting X and Y results in Si-C bonds. In contrast, when X is the independently selected halogen atom, reacting X with Y may result in SiC bonds, Si-O-C bonds, and/or Si-N-C bonds. Because Si-O-C bonds and/or Si-N-C bonds may hydrolyze, further reaction to form the hydrophilic functional group is generally not carried out in an aqueous medium. In these embodiments, the cross-linking agent may further comprise a butoxycarbonyl group.

[0097] Generally, when the cross-linking agent includes the functional group Z, the functional group Z is a protected group from reacting with the biomolecule and the quantum dot. Further, the functional group Z is desirable for increasing hydrophobicity, as an aqueous

medium is generally desirable for the biomolecule and the bioconjugated molecule. One specific example of a suitable cross-linking agent is an ester compound including functional groups Y and Y'. Specific examples of ester compounds include polycaprolactones, polylactic acid, poly(lactic-co-glyolic acid), etc., although such compounds include functional groups Y and Y', which may not be specifically accounted for in the species above, but one of skill in the art readily understands how to introduce or modify functionalities of such compounds so as to include functional groups Y and Y'. Another specific example of a suitable cross-linking agent is a polyalkylene glycol including functional groups Y and Y'. Typically, the polyalkylene glycol is a polyethylene glycol (PEG). Other examples of suitable cross-linking agents include peptides and peptide-based materials such as gelatin, polyhydroxyethylmethacrylate, chitosan, poly(N-isopropylacrylamide, hyaluronan, alginic acid, agarose, and polyvinylalcohol, although such compounds include functional groups Y and Y', which may not be specifically accounted for in the species above, but one of skill in the art readily understands how to introduce or modify functionalities of such compounds so as to include functional groups Y and Y'. The bioconjugated molecule may be formed at any stage after formation of the quantum dot. For example, the cross-linking agent may be present in the capture fluid such that the cross-linking agent and the quantum dot are combined upon the formation and collection of the quantum dot. Alternatively, the quantum dots may be collected and optionally stored, removed from the capture fluid, isolated, and/or treated prior to forming the bioconjugated molecule. In other embodiments, the cross-linking agent may be first combined and optionally reacted with the biomolecule. Alternatively still, the quantum dot, the cross-linking agent, and the biomolecule may be combined simultaneously.

[0098] In certain embodiments, the cross-linking agent is present in the capture fluid at the time of collecting the quantum dots. In these embodiments, the cross-linking agent may react with the MX-functional quantum dots in the capture fluid to form a reaction intermediate by bonding the cross-linking agent to the MX-functional quantum dots. The cross-linking agent becomes a monovalent group (or ligand) on the quantum dots upon this bonding, and the reaction intermediate may then react with the biomolecule to form the bioconjugated molecule. In embodiments where the reaction intermediate comprises the reaction product of the cross-linking agent and the quantum dot, the reaction intermediate is referred to as a quantum dot ligand. However, as introduced above, the reaction intermediate may instead comprise the biomolecule in lieu of the quantum dot when the cross-linking agent is not first reacted with the MX-functional quantum dot. Further, the cross-linking agent may not inherently react with the cross-linking agent absent a curing condition, e.g. irradiation. In these embodiments, the cross-linking agent and the MX-functional quantum dots may remain

separate in the capture fluid. The biomolecule may be disposed in the capture fluid to form the bioconjugated molecule. Typically, the cross-linking agent and the quantum dot are first reacted to form the reaction intermediary, which still includes functional group Y' on the monovalent group (or ligand) formed from the cross-linking agent, which subsequently reacts with functional group X' of the biomolecule to form the bioconjugated molecule (and the monovalent group or ligand becomes the divalent linking group).

[0099] When the capture fluid comprises the cross-linking agent at the time of collecting the MX-functional quantum dots, the capture fluid generally comprises the cross-linking agent in an amount sufficient to provide a molar ratio of the functional group Y to MX bonds in the MX-functional quantum dots of at least 1:1, alternatively at least 1.2:1, alternatively at least 1.4:1. Molar ratios much higher than 1.4:1 may advantageously be utilized. Typically, the cross-linking agent is utilized in a molar excess to ensure bonding to the MX-functional quantum dots.

[00100] In various embodiments, the capture fluid comprises the cross-linking agent in an amount of from greater than 0 to 100, alternatively from greater than 0 to 50, alternatively from 1 to 40, alternatively from 2 to 30, alternatively from 5 to 15, percent by weight based on the total weight of the capture fluid. The balance of the capture fluid may comprise any of the conventional components or conventional capture fluids set forth above, although the balance of the capture fluid comprises hydrocarbons for miscibility with the cross-linking agent. The capture fluid may comprise the cross-linking agent, consist essentially of the cross-linking agent, or consist of the cross-linking agent.

[00101] Typically, it is desirable to form the bioconjugated molecule in an aqueous medium. The aqueous medium may comprise, in addition to water, other carrier vehicles or solvents, such as dipolar aprotic solvents. Notably, the capture fluid is generally not an aqueous medium. Thus, in various embodiments, the cross-linking agent is present in the capture fluid and the cross-linking agent and the quantum dot first react to form the quantum dot ligand. In embodiments where the cross-linking agent includes the functional group Z, the method may further comprise converting the functional group Z to the hydrophilic group. Typically, when the cross-linking agent includes the functional group Z, the functional group Z is present in the quantum dot ligand. The functional group Z may be converted to the hydrophilic group at any stage of the method to prepare the bioconjugated molecule. Generally, however, the functional group Z is converted to the hydrophilic group after forming the quantum dot ligand. Conversion after formation of the quantum dot ligand is believed to increase stability of the quantum dot and provide additional benefits.

[00102] For example, conversion of the functional group Z to the hydrophilic group increases hydrophilicity of the quantum dot ligand. The method may further comprise isolating the

quantum dot ligand from the capture fluid. Alternatively, the method may further comprise isolating the MX-functional quantum dot from the capture fluid when the quantum dot ligand is not formed therein. In the former embodiments, the quantum dot ligand may be disposed in the aqueous medium for preparing the bioconjugated molecule by bonding the biomolecule to the quantum dot ligand. Embodiments relating to the reaction to form the quantum dot ligand are described below.

[00103] The MX-functional quantum dots and the cross-linking agent may be reacted via known methods. When X is H, this reaction is generally referred to as an addition reaction. For example, in hydrosilylation, i.e., when the MH-functional quantum dots comprise SiH-functional quantum dots, the carbon-carbon multiple bond of the cross-linking agent undergoes an addition reaction with the SiH-functional quantum dots. For SiH-functional quantum dots, this addition reaction is referred to as hydrosilylation; for GeH-functional quantum dots, this addition reaction is referred to as hydrogermylation; for SnH-functional quantum dots, this addition reaction is referred to as hydrostannylation. Alternatively, when X is the halogen atom, the reaction between the MX-functional quantum dots and the cross-linking agent is generally classified based on the selection of the cross-linking agent.

[00104] In certain embodiments, particularly X is H and Y is the carbon-carbon multiple bond, reacting the MX-functional quantum dots and the cross-linking agent comprises irradiating a suspension of the MH-functional quantum dots and the cross-linking agent in the capture fluid with UV radiation. For example, reacting the MH-functional quantum dots with the cross-linking agent may be photoinitiated. When reacting the MH-functional quantum dots and the cross-linking agent comprises irradiating the suspension of the MH-functional quantum dots and the cross-linking agent in the capture fluid with radiation, the radiation typically has a wavelength of from 10 to 400, alternatively 280 to 320, nm.

[00105] Alternatively or in addition to radiation, reacting the MX-functional quantum dots and the cross-linking agent may comprise heating a suspension of the MX-functional quantum dots and the cross-linking agent in the capture fluid to or at a first temperature for a first period of time. When heat is utilized to react the MX-functional quantum dots and the cross-linking agent, the first temperature is typically from 50 to 250 °C and the first period of time is from 5 to 500 minutes.

[00106] Alternatively still, the MX-functional quantum dots may inherently react with the cross-linking agent once the MX-functional quantum dots are collected in the capture fluid including the cross-linking agent such that no reaction condition (e.g. irradiation or heat) is utilized or applied. However, utilizing heat or irradiation generally

improves the reaction between the MX-functional quantum dots and the cross-linking agent, which may improve physical properties of the quantum dot ligand and ultimately the bioconjugated molecule, including photoluminescence and photoluminescent intensity.

[00107] If desired, a catalyst or photocatalyst may be utilized during the step of reacting the MX-functional quantum dots and the cross-linking agent. Such catalysts are well known in the art based on the desired reaction mechanism, e.g. when X is H, any catalysts suitable for addition (e.g. hydrosilylation) may be utilized, which are typically based on precious metals, e.g. platinum. However, catalysts or photocatalysts are not required for the step of reacting the MX-functional quantum dots and the cross-linking agent.

[00108] The functional group Z may be converted to a hydrophilic functional group via known methods. In various embodiments, converting the functional group Z comprises hydrolyzing the functional group Z. Thus, when functional group Z is present in the quantum dot ligand, the functional group Z is hydrolyzed as a moiety in the quantum dot ligand. The functional group Z may be converted to a hydrophilic functional group while the quantum dot ligand is present in the capture fluid, or the quantum dot ligand may be isolated or separated therefrom prior to converting the functional group Z.

[00109] For example, the functional group Z may be converted to a hydrophilic functional group by acidic or basic treatment. In these embodiments, the acid or base utilized is generally selected such that the acid or base is miscible with the capture fluid. Further, the acid is typically selected such that it can be removed from the capture fluid, e.g. by vacuum or washing with solvent. To this end, the acid may be selected from trifluoroacetic acid, hydrofluoric acid, and combinations thereof. The acid may be utilized in various concentrations in an aqueous form.

[00110] Independent of whether the MX-functional quantum dots are utilized to form the quantum dot ligand in the capture fluid, the method may further comprise separating the MX-functional quantum dots and/or the quantum dot ligands from the capture fluid to form separated quantum dots and/or separated quantum dot ligands. For example, the MX-functional quantum dots and/or the quantum dot ligands may be separated from the capture fluid by centrifuging and/or decanting. The separated MX-functional quantum dots and/or the separated quantum dot ligands may be further washed by suspension in a solvent, e.g. toluene, followed by repeated separation from the solvent by centrifuging and/or decanting. The separated MX-functional quantum dots and/or the separated quantum dot ligands may ultimately be dried, e.g. in vacuo, to form a dried solid. In this embodiment, the separated

MX-functional quantum dots and/or the separated quantum dot ligands are free-standing and not in solution or suspension.

[00111] Further, when the cross-linking agent includes the functional group Z convertible to a hydrophilic functional group, and when the method further comprises converting the functional group to a hydrophilic functional group, the quantum dot ligand may advantageously be suspended in a polar solvent, which offers significant advantages. For example, in this embodiment, the method may further comprise suspending the separated quantum dot ligand in a polar solvent, such as an aqueous solution, optionally along with ions, e.g. from disassociated sodium bicarbonate. The polar solvent may be selected from water and a dipolar aprotic organic solvent. Typically, bonding the quantum dot ligand and the biomolecule to form the bioconjugated molecule is carried out in an aqueous medium. To this end, the biomolecule may be provided in the aqueous medium, or the biomolecule may be disposed in an aqueous medium including the quantum dot ligand. Alternatively, the biomolecule and the quantum dot ligand may be disposed in the aqueous medium simultaneously or in any order.

[00112] The reaction between the functional group Y' and the functional group X' of the biomolecule may be carried out via a variety of techniques based on the selections of Y' and X'. One of skill in the art readily understands conditions, including any cure conditions, for such a reaction based on these selections.

[00113] Due to the unique and excellent properties of the quantum dot of the bioconjugated molecule, the bioconjugated molecule is suitable for numerous end uses and applications. The present invention also provides a diagnostic method involving the bioconjugated molecule. The diagnostic method comprises administering the bioconjugated molecule to at least one biological sample.

[00114] The biological sample may be any biological sample and may be derived from various cells, tissues, or portions/derivatives thereof. Thus, the term "biological sample" as used herein encompasses animal tissue cultures, cell cultures, immortalized cell lines, unicellular organisms, *ex situ* reactions comprising various components derived from biological systems (e.g., metabolic reactions comprising enzymes, metabolites, and substrates). The biological sample may be derived from any of various biological systems encompassing all domains of life as well as both unicellular and multicellular organisms. The particular type of biological sample is generally contingent on the type of biomolecule of the bioconjugated molecule and the specific diagnosis desired. The bioconjugated molecule is particularly suited for color/light-based assay diagnosis techniques, such as optical microscopy, cytometry, competitive immunoassays, sandwich immunoassays, DNA sequencing, fluorescence in-situ hybridization, magnetic resonance aging, fluorescence

reflectance imaging, and fluorescence-mediated tomography. Such diagnosis techniques may be utilized, for example, for locating tumors, identifying infectious agents, etc. in biological samples, e.g. humans or other mammals, or cells or tissue samples thereof. Such diagnostic methods are known in the art and one of skill in the art would readily understand how to utilize the instant bioconjugated molecule in any of these techniques.

[00115] The bioconjugated molecule may be used for any number of methods useful in characterizing or analyzing a biological sample. For example, the bioconjugated molecule may be used for *in vivo* or *ex vivo* imaging of a biological sample or the bioconjugated molecule may be used for monitoring, quantifying, or visualizing metabolic or cellular activities within a biological sample. As the term is used in the present disclosure, "diagnostic" is to be understood to encompass the methods just referenced as well as those methods specific to determining the presence or absence of disease. Thus, as used herein, "diagnostic method" is used to describe a method intended to provide evidential insight into the nature of a biological system and, in some instances, to determine a medical state of a biological system. The bioconjugated molecule, therefore, may be used in any number of methods finding utility in basic or applied research as well as in medical contexts.

[00116] The step of administering may be carried out via any technique for contacting the bioconjugated molecule with the biological sample. For example, the diagnostic method may be utilized in assay techniques. Alternatively, the bioconjugated molecule may be introduced into a living organism, such as a mammal or human, e.g. intravenously, orally, transdermally, etc.

[00117] The quantum dot of the bioconjugated molecule may exhibit a number of unique electronic, magnetic, catalytic, physical, optoelectronic and optical properties due to quantum confinement effects. For example, many semiconductor nanoparticles exhibit photoluminescence effects that are significantly greater than the photoluminescence effects of macroscopic materials having the same composition.

[00118] In various embodiments, the quantum dot (and bioconjugated molecule including the same) may be photoluminescent when excited by exposure to UV light. Depending on the average diameter of the quantum dot, the quantum dot may photoluminescence in any of the wavelengths in the visible spectrum and may visually appear to be red, orange, green, blue, violet, or any other color in the visible spectrum. For example, when the quantum dot of the bioconjugated molecule has an average diameter less than 5 nm, visible photoluminescence may be observed, and when the quantum dot has an average diameter less than 10 nm, near infrared (IR) luminescence may be observed. In one form of the present disclosure, the quantum dot has a photoluminescent intensity of at least 1×10^6 at an excitation wavelength of 365 nm. The photoluminescent intensity may be measured with

a Fluorolog3 spectrofluorometer (commercially available from Horiba of Edison, NJ) with a 450 W Xe excitation source, excitation monochromator, sample holder, edge band filter (400 nm), emission monochromator, and a silicon detector photomultiplier tube. To measure photoluminescent intensity, the excitation and emission slit width are set to 2 nm and the integration time is set to 0.1s. In these or other embodiments, the quantum dot may have a quantum efficiency of at least 4% at an excitation wavelength of 395 nm as measured on an HR400 spectrophotometer (commercially available from Ocean Optics of Dunedin, Florida) via a 1000 micron optical fiber coupled to an integrating sphere and the spectrophotometer with an absorption of >10% of the incident photons. Quantum efficiency was calculated by placing a sample into the integrating sphere and exciting the sample via a 395 nm LED driven by an Ocean Optics LED driver. The system is calibrated with a known lamp source to measure absolute irradiance from the integrating sphere. The quantum efficiency is then calculated by the ratio of total photons emitted by the quantum dots to the total photons absorbed by the quantum dots. Further, in these or other embodiments, the quantum dot may have a full width at half maximum emission of from 20 to 250 at an excitation wavelength of 270-500 nm.

[00119] Furthermore, both the photoluminescent intensity and luminescent quantum efficiency may continue to increase over time, particularly when the quantum dot (optionally as a part of the bioconjugated molecule) is exposed to air. In another form of the present disclosure, the maximum emission wavelength of the quantum dot shifts to shorter wavelengths over time when exposed to oxygen. The luminescent quantum efficiency of the quantum dot may be increased by 200% to 2500% upon exposure to oxygen. However, other increases in the luminescent quantum efficiency are also contemplated. The photoluminescent intensity may increase from 400 to 4500% depending on the time exposure to oxygen and the concentration of the quantum dots in the fluid. However, other increases in the photoluminescent intensity are also contemplated. The wavelength emitted from the direct capture composition also experiences a blue shift of the emission spectrum. In one form of the present disclosure, the maximum emission wavelength shifts 100 nm, based on a 1 nm decrease in quantum dot core size, depending on the time exposed to oxygen. However, other maximum emission wavelength shifts are also contemplated herein.

[00120] The following examples, illustrating the compositions and methods of this disclosure, are intended to illustrate and not to limit the disclosure.

[00121] Example 1:

[00122] Hydroxyl-terminated silicon nanoparticles (4.3 mg) are added to a solution consisting of 25.6 mg of carbonyldiimidazole (CDI) and 500 µL of anhydrous methyl sulfoxide

in a 1.5 mL centrifuge tube. The mixture is sonicated to disperse the nanoparticles particles into solution. The solution is allowed to react for two hours at room temperature.

[00123] The solution is centrifuged at 21,100 g for two minutes to settle the nanoparticles out of the solution, and liquid is decanted to remove excess CDI and reaction byproducts. Then, 500 µL of fresh anhydrous methyl sulfoxide is added to the tube and the nanoparticles are dispersed using a sonic mixer. The solution is centrifuged at 21,100 g for two minutes, and liquid is decanted from the tube. The washing process is repeated two additional times, resulting in a pellet of CDI-activated nanoparticles.

[00124] Next, 6.1 mg of folic acid is added to a solution containing 300 µL of anhydrous methyl sulfoxide and 300 µL of 50 mM borate buffer at pH 9.3 to give a mixture. The mixture is added to the tube containing the CDI-activated nanoparticles. The tube is placed in a sonic bath to disperse the CDI-activated nanoparticles into solution, and placed on a tube rotator at room temperature for 16 hours.

[00125] The tube is centrifuged at 21,100 g for 20 minutes to settle the nanoparticles out of solution. The liquid is decanted and a pellet of nanoparticles is washed three times with 800 µL of anhydrous methyl sulfoxide by suspending the nanoparticles, followed by centrifugation. The nanoparticles are re-suspended in 600 µL of acetone, centrifuged, and the acetone is decanted from a pellet of the nanoparticles. The pellet is dried under a stream of dry nitrogen. The weight of the nanoparticles is measured to have increased by 0.2 mg based on this exemplified process.

[00126] It is to be understood that the appended claims are not limited to express and particular compounds, compositions, or methods described in the detailed description, which may vary between particular embodiments which fall within the scope of the appended claims. With respect to any Markush groups relied upon herein for describing particular features or aspects of various embodiments, different, special, and/or unexpected results may be obtained from each member of the respective Markush group independent from all other Markush members. Each member of a Markush group may be relied upon individually and or in combination and provides adequate support for specific embodiments within the scope of the appended claims.

[00127] Further, any ranges and subranges relied upon in describing various embodiments of the present invention independently and collectively fall within the scope of the appended claims, and are understood to describe and contemplate all ranges including whole and/or fractional values therein, even if such values are not expressly written herein. One of skill in the art readily recognizes that the enumerated ranges and subranges sufficiently describe and enable various embodiments of the present invention, and such ranges and subranges may be further delineated into relevant halves, thirds, quarters, fifths, and so on. As just one

example, a range “of from 0.1 to 0.9” may be further delineated into a lower third, i.e., from 0.1 to 0.3, a middle third, i.e., from 0.4 to 0.6, and an upper third, i.e., from 0.7 to 0.9, which individually and collectively are within the scope of the appended claims, and may be relied upon individually and/or collectively and provide adequate support for specific embodiments within the scope of the appended claims. In addition, with respect to the language which defines or modifies a range, such as “at least,” “greater than,” “less than,” “no more than,” and the like, it is to be understood that such language includes subranges and/or an upper or lower limit. As another example, a range of “at least 10” inherently includes a subrange of from at least 10 to 35, a subrange of from at least 10 to 25, a subrange of from 25 to 35, and so on, and each subrange may be relied upon individually and/or collectively and provides adequate support for specific embodiments within the scope of the appended claims. Finally, an individual number within a disclosed range may be relied upon and provides adequate support for specific embodiments within the scope of the appended claims. For example, a range “of from 1 to 9” includes various individual integers, such as 3, as well as individual numbers including a decimal point (or fraction), such as 4.1, which may be relied upon and provide adequate support for specific embodiments within the scope of the appended claims.

CLAIMS

What is claimed is:

1. A bioconjugated molecule, comprising:

a quantum dot formed via a plasma process, and

a biomolecule bonded to the quantum dot via a divalent linking group.

2. The bioconjugated molecule of claim 1 wherein said quantum dot comprises a Group IV element.

3. The bioconjugated molecule of claim 1 or 2 wherein said biomolecule is selected from the group of proteins, antibodies, enzymes, peptides, hormones, amino acids, vitamins, lipids, carbohydrates, lectins, active pharmaceutical ingredients, biological response modifiers, genetic material, and combinations thereof.

4. The bioconjugated molecule any one of claims 1-3 wherein said divalent linking group is organic.

5. A method of preparing a bioconjugated molecule, said method comprising:

providing a quantum dot formed via a plasma process, a biomolecule, and a cross-linking agent; and

bonding the cross-linking agent between the quantum dot and the biomolecule to prepare the bioconjugated molecule;

wherein the bioconjugated molecule comprises a divalent linking group formed from the cross-linking agent bonded between the quantum dot and the biomolecule.

6. The method of claim 5 wherein the quantum dot is further defined as an MX-functional quantum dot, where M is an independently selected Group IV element and X is independently selected from H and a halogen atom; and wherein the cross-linking agent includes a functional group Y reactive with X of the MX-functional quantum dot.

7. The method of claim 5 or 6 wherein the biomolecule includes a functional group X' and the cross-linking agent includes a functional group Y' reactive with the functional group X' of the biomolecule.

8. The method of claim 5 wherein the quantum dot is further defined as an MX-functional quantum dot, where M is an independently selected Group IV element and X is independently selected from H and a halogen atom; wherein the biomolecule includes a

functional group X'; and wherein the cross-linking agent includes a functional group Y reactive with the functional group X of the quantum dot and a functional group Y' reactive with the functional group X' of the biomolecule; wherein Y and Y' are the same as or different from one another, and wherein X and X' are the same as or different from one another.

9. The method any one of claims 5-8 further comprising preparing the quantum dot via the plasma process, which is carried out in a low pressure reactor.

10. The method of claim 9, wherein the plasma process to produce the quantum dot comprises:

forming a nanoparticle aerosol in the low pressure reactor, wherein the nanoparticle aerosol comprises MX-functional quantum dots in a gas, wherein M is an independently selected Group IV element and X is a functional group independently selected from H and a halogen atom; and

collecting the MX-functional quantum dots.

11. The method of claim 10, wherein the plasma process to produce the quantum dots further comprises:

applying a preselected VHF radio frequency having a continuous frequency ranging from 10 to 500 MHz and a coupled power ranging from 5 to 1000 W to a reactant gas mixture in the low pressure reactor having a reactant gas inlet and an outlet having an aperture defined thereby, to generate a plasma for a time sufficient to form the MX-functional quantum dots, and

collecting the MX-functional quantum dots.

12. The method of claim 11, wherein the MX-functional quantum dots are collected in a capture fluid in fluid communication with the low pressure reactor.

13. The method of claim 12 further comprising:

introducing the nanoparticle aerosol into a diffusion pump from the low pressure reactor;

heating the capture fluid in a reservoir to form a vapor and sending the vapor through a jet assembly;

emitting the vapor through a nozzle into a chamber of the diffusion pump and condensing the vapor to form a condensate comprising the capture fluid;

flowing the condensate back into the reservoir; and

capturing the MX-functional quantum dots of the nanoparticle aerosol in the condensate comprising the capture fluid.

14. The method of claim 12 or 13, wherein the cross-linking agent is present in the capture fluid when collecting the MX-functional quantum dots therein.

15. The method of claim 14, wherein bonding comprises reacting the cross-linking agent and the quantum dot in the capture fluid to form a quantum dot ligand and reacting the quantum dot ligand with the biomolecule to form the bioconjugated molecule.

16. The method of any one of claims 5-15, wherein the step of bonding is carried out in an aqueous medium.

17. The method of any one of claims 5-16, wherein (i) the cross-linking agent includes at least one functional group Z convertible to a hydrophilic functional group and wherein the method further comprises converting the functional group Z to a hydrophilic functional group; or (ii) the cross-linking agent includes at least one hydrophilic functional group.

18. A diagnostic method, comprising administering the bioconjugated molecule of claim 1 to at least one biological sample.

19. The diagnostic method of claim 18, wherein the at least one biological sample is further defined as animal tissue.

20. A method, comprising administering the bioconjugated molecule of claim 1 to at least one biological sample.

FIGURE 1

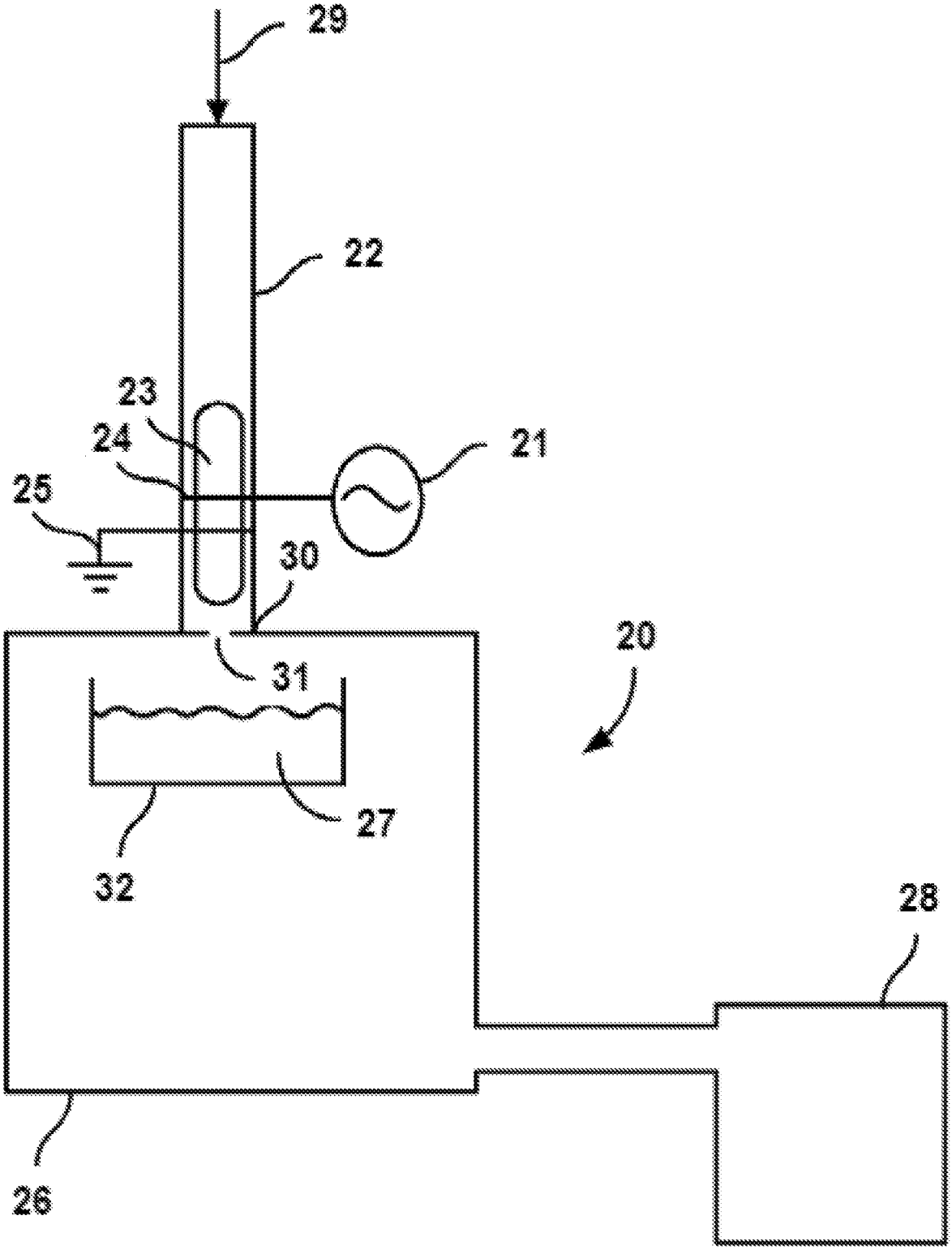


FIGURE 2

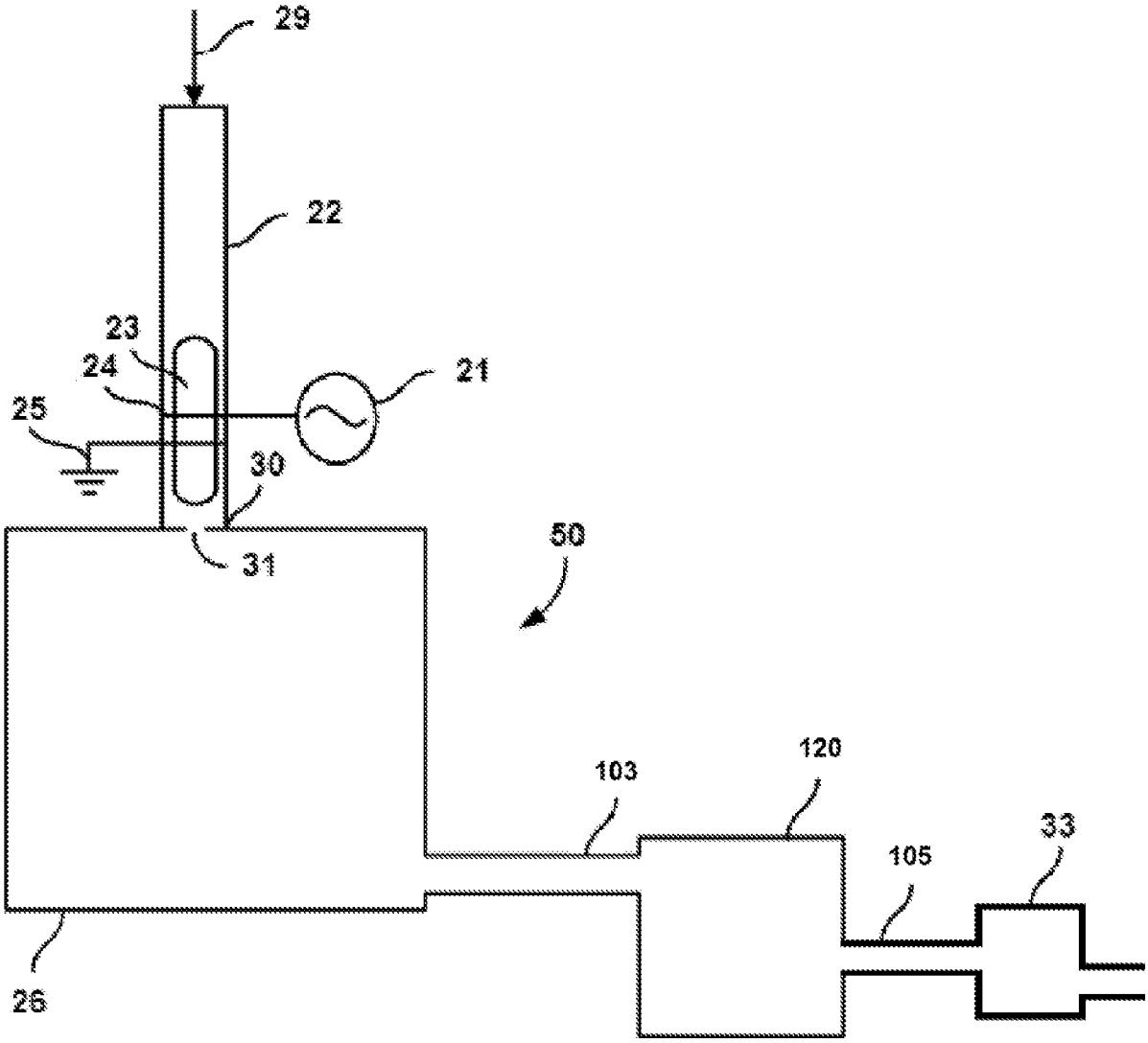
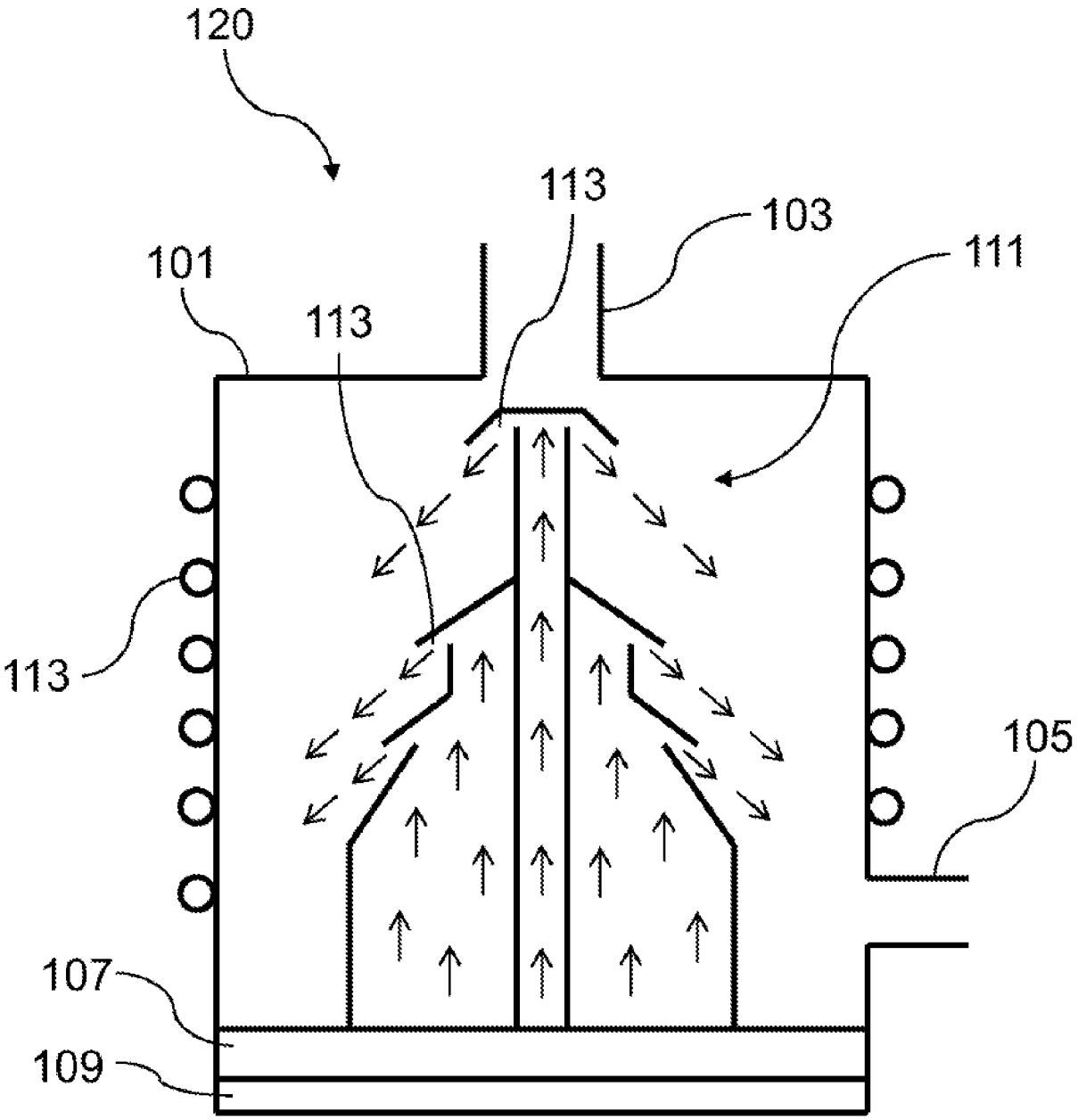


FIGURE 3



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/068022

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/543
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
G01N

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	JP 2016 136104 A (KONICA MINOLTA INC) 28 July 2016 (2016-07-28) paragraphs [0036], [0033], [0043] - [0044]	1-20
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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 April 2020	17/04/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Lunter, Pim

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INTERNATIONAL SEARCH REPORT

International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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