

# WATER-SOLUBLE FLUORESCENT MATERIAL WITH BALANCED HYDROPHILICITY AND HYDROPHOBICITY

## FIELD OF THE INVENTION

**[0001]** The present invention relates to fluorescent materials, particularly water-soluble fluorescent materials and method of forming them.

## BACKGROUND OF THE INVENTION

**[0002]** Fluorescent particles are useful in various applications. For instance, fluorophores are useful as probes, labels or tags, e.g., in many biochemical fields, such as drug and gene research, cell/microorganism imaging, disease diagnosis, analyte detection, and the like. As the aqueous environment is a common environment, it is desirable that the fluorescent particles are soluble in water. However, many fluorophores are not soluble in water. Further, when the fluorophores are exposed to the environment, their performance may be affected by the environment and may be unstable. It is thus desirable to encapsulate the fluorophores with a water-soluble outer layer, which renders the resulting particles soluble in an aqueous solution and insulates the inner fluorophore from the environment.

**[0003]** Some conventional fluorescent particles have hydrophobic fluorescent segments and hydrophilic or amphiphilic segments that encapsulate the hydrophobic segments. However, a drawback of these particles is that in an aqueous environment these particles tend to either aggregate/precipitate or disintegrate, and are unstable. As a result, performance will decay over time. It is thus desirable to improve the stability of fluorescent particles formed from amphiphilic fluorescent molecules. It is known that the stability of particles formed from amphiphilic molecules can be affected by the balance between their hydrophilicity and hydrophobicity. However, it is difficult to predict when the hydrophilicity and hydrophobicity of a particular type of amphiphilic fluorescent polymers is sufficiently balanced for forming stable particles.

## SUMMARY OF THE INVENTION

**[0004]** Amphiphilic molecules are provided for forming fluorescent particles that can remain stable in water. Each molecule comprises a backbone and side chains grafted to the backbone. At least three backbone units are hydrophobic and fluorescent and at least one side chain unit is hydrophilic. The weight ratio within the molecule of backbone and side chain units that are hydrophilic to those that are hydrophobic is from about 1:4 to about 4:1. To form fluorescent particles, a solution comprising water, an organic solvent and the amphiphilic molecule dissolved in the organic solvent is provided. The concentration of the molecule in the solution is from about 1 to about 1000 CAC, such as from about 10 to about 100 CAC, where CAC is the critical aggregation concentration of the amphiphilic molecule. The organic solvent is removed from the solution, thus allowing the amphiphilic molecule to form particles that have a peripheral size from about 10 nm to about 10 microns. The particles can remain stable in water for more than six months.

**[0005]** Accordingly, in a first aspect of the present invention, there is provided a method of forming fluorescent particles. The method comprises providing a solution comprising water, an organic solvent and an amphiphilic molecule dissolved in the organic solvent; and removing the organic solvent from the solution, thus allowing the amphiphilic molecule to form the fluorescent particles having a peripheral size from about 10 nm to about 10 microns. The amphiphilic molecule comprises a plurality of backbone units forming a molecular backbone and a plurality of side chains grafted to the molecular backbone, with each one of the side chains formed as at least one side chain unit. At least one of the side chain units within the molecule is hydrophilic and at least three of the backbone units are hydrophobic and fluorescent. The weight ratio within the molecule of backbone units and side chain units that are hydrophilic to those that are hydrophobic is from about 1:4 to about 4:1, such as from about 3:7 to about 7:3. The concentration of the amphiphilic molecule in the solution is from about 1 to about 1000 CAC, such as from about 10 to about 100 CAC, wherein the CAC is the critical aggregation concentration of the amphiphilic molecule in the solution. The solution may be prepared by mixing water with a precursor solution that comprises the organic solvent and the amphiphilic molecule. The organic solvent may be removed from

the solution by evaporation. The solution may have a pH of about 2 to about 12, and may be at a temperature from about 0 to about 80 °C, such as from about 4 to about 70 °C.

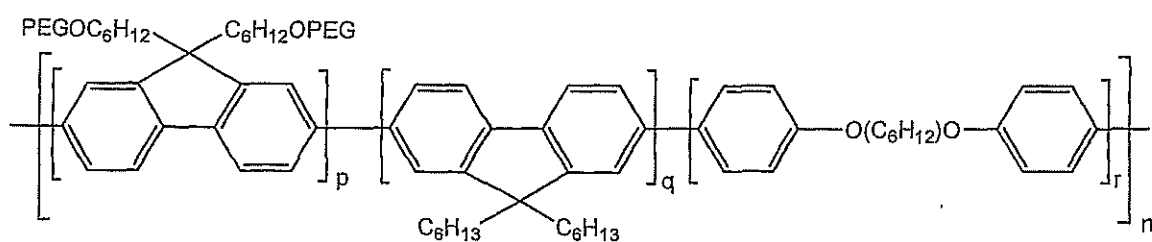
**[0006]** In accordance with another aspect of the present invention, there is provided a water-soluble fluorescent particle formed according to the above method. The particle may comprise a ligand having a specific affinity to a selected target. The ligand may be selected from avidin, biotin, antibody, antigen, and DNA, and the target may be selected from a target molecule, a cell and an organism.

**[0007]** In accordance with a further aspect of the present invention, there is provided a molecule comprising a plurality of backbone units forming a molecular backbone and a plurality of side chains grafted to the molecular backbone, with each one of the side chains formed as at least one side chain unit. At least one of the side chain units within the molecule is hydrophilic and at least three of the backbone units are hydrophobic and fluorescent. The weight ratio within the molecule of backbone units and side chain units that are hydrophilic to those that are hydrophobic is from about 1:4 to about 4:1, such as from about 3:7 to about 7:3.

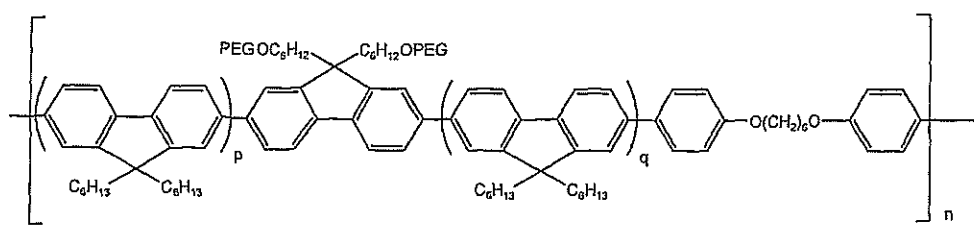
**[0008]** In accordance with another aspect of the present invention, there is provided a water-soluble fluorescent particle comprising the molecule described in the preceding paragraph. The particle may comprise a ligand having a specific affinity to a selected target. The ligand may be selected from avidin, biotin, antibody, antigen, and DNA, and the target may be selected from a target molecule, a cell and an organism.

**[0009]** For the amphiphilic molecule described in the four preceding paragraphs, the backbone units may comprise fluorene units and the side chains may comprise polyethyleneglycol. The backbone units may comprise an arylene, heteroarylene, arylene vinylene, heteroarylene vinylene, arylene ethylene, or heteroarylene ethylene unit, or a derivative thereof. The backbone units may comprise a unit substituted with an alkyl, alkoxy, alkenyl, alkynyl, alkylsilyl, arylsilylaryl, heteroaryl, aryloxy, heteroaryloxy, alkylthio, alkylamino, dialkylamino, arylamino, diarylamino, aryl ether, heteroaryl ether, aryl thioether, heteroaryl thioether, halogen, cyano,

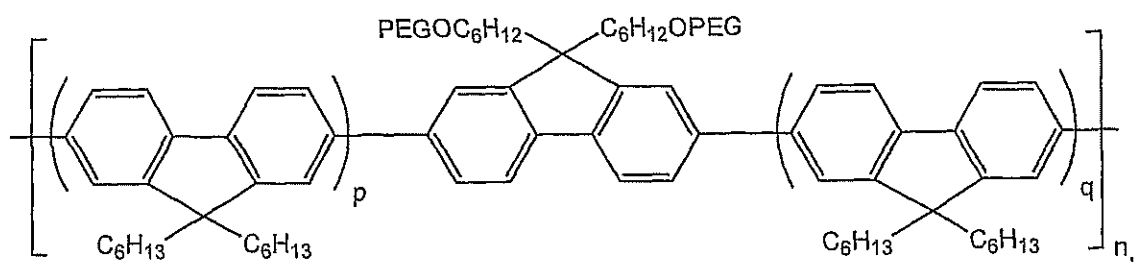
nitro, carbonyl, thionyl, sulphonyl, or perfluoroalkyl group, or an amino group comprising a heteroaryl group. The backbone units may comprise a phenylene, thienylene, spirobifluorenylene, indenofluorenylene, pyridylene, bipyridylene, carbazoylene, indenocarbazoylene, benzothiazoylene, or oxadiazoylene unit, or a derivative thereof. At least one of the backbone units may be linked to a vinylene group or an ethylene group. At least two of the backbone units may be linked with each other through a single carbon bond, a methylene group, or an atom selected from O, S, N, Si, and P. The backbone units may comprise a flexible group connecting two hydrophobic and fluorescent backbone units. The flexible group may be hydrophilic or hydrophobic. The side chains may comprise side chain units. The side chain units may comprise polyethyleneglycol, polyethyleneimine, polyamide, polyvinylpyrrolidone, polyacrylic acid, polyvinyl alcohol, polylysine, or derivatives thereof. At least one of the side chains may be bonded to the backbone through a single carbon bond, single phosphor bond, ether group, thioether group, amino group, imino group, silyl group, ester group, thioester group, amide group or an imide group. The amphiphilic molecule may have the formula,



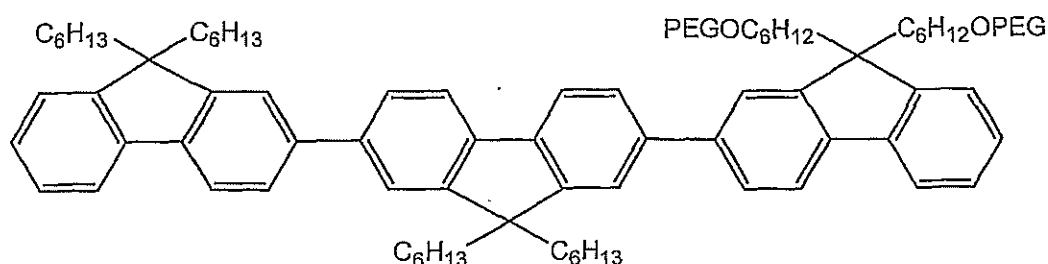
where p, q, r, and n are integers, p is from 1 to 10, q is from 1 to 200, r is from 0 to 20 such as 0, and n is from 1 to 20. The amphiphilic molecule may have the formula,



where p, q, and n are integers, n is from 1 to 20, and p+q is from 2 to 200 such as 4 or 10. The amphiphilic molecule may have the formula,



where  $p$ ,  $q$  and  $n$  are integers,  $n$  is from 1 to 20, and  $p+q$  is from 2 to 200. For example, in an exemplary embodiment,  $n=1$ , and  $p=q=1$ . In another embodiment,  $n=1$ , and  $p=q=2$ . The amphiphilic molecule may have the formula,



**[0010]** The above amphiphilic molecule may be formed by forming precursors for the backbone units, the precursors comprising hydrophobic fluorescent groups; grafting hydrophilic groups to the precursors, forming grafted precursors; and linking the grafted precursors, thus forming the amphiphilic molecule. The linking may comprise linking through a coupling reaction. The linking may comprise linking through Suzuki coupling reaction, Grignard coupling reaction, Stille coupling reaction, Heck coupling reaction, Sologashira coupling reaction, oxidation polymerization reaction, reduction polymerization reaction, or polycondensation reaction.

**[0011]** Other aspects and features of the present invention will become apparent to those of ordinary skill in the art upon review of the following description of specific embodiments of the invention in conjunction with the accompanying figures.

#### BRIEF DESCRIPTION OF THE DRAWINGS

**[0012]** In the figures, which illustrate, by way of example only, embodiments of the present invention,

**[0013]** FIGS. 1 to 15 are schematic chemical reaction diagrams showing the synthesis routes for respective specific compounds, exemplary of embodiments of the present invention;

**[0014]** FIG.16 is a schematic chemical reaction diagram showing a generalized synthesis route of a polymer, exemplary of embodiments of the present invention;

**[0015]** FIG.17 is a schematic chemical reaction diagram showing a generalized synthesis route of a polymer with a flexible segment in the backbone, exemplary of embodiments of the present invention;

**[0016]** FIGS. 18 to 20 are bar graphs showing measured particles sizes formed of different compounds;

**[0017]** FIG. 21 is a line graph of measured cell numbers;

**[0018]** FIG. 22 is a transmission electron microscopy (TEM) image of fluorescent particles formed from a sample compound exemplary of an embodiment of the present invention; and

**[0019]** FIG. 23 is a transmission electron microscopy (TEM) image of fluorescent particles formed from another sample compound exemplary of an embodiment of the present invention.

#### DETAILED DESCRIPTION

**[0020]** It has been discovered that a graft amphiphilic fluorescent molecule can be used to form stable micellar particles with sufficiently balanced hydrophilicity and hydrophobicity when the weight ratio of the hydrophilic units to the hydrophobic units within the molecule is from about 1:4 to about 4:1, such as from about 3:7 to about 7:3. A graft molecule has a molecular backbone and side chains grafted to the backbone and each of said backbone and side chains is formed of at least one backbone unit or side chain unit, respectively. As would be understood, unit as used herein refers to a building block of a particular molecule, for example, where the molecule is formed by polymerizing monomers, the unit is monomeric unit. In an exemplary embodiment of the present invention, the backbone includes at least three hydrophobic fluorescent backbone units, and at least one side chain includes

at least one hydrophilic side chain unit. In one embodiment, the backbone may include only hydrophobic backbone units. In a different embodiment, the backbone may also include one or more hydrophilic backbone units. To form fluorescent particles, a solution comprising water, an organic solvent and the amphiphilic molecule dissolved in the organic solvent is provided. The concentration of the molecule in the solution is from about 1 to about 1000 CAC, such as from about 10 to about 100 CAC, where CAC is the critical aggregation concentration of the amphiphilic molecule. The organic solvent is removed from the solution, thus allowing the amphiphilic molecule to form micellar particles that have a peripheral size from about 10 nm to about 10 microns. The particles can remain stable in water for more than six months.

**[0021]** Without being limited to any particular theory, it is believed that sufficient balance between hydrophilicity and hydrophobicity in the molecules is achieved when the weight ratio is in the range as described above. As can be understood, the balance between hydrophilicity and hydrophobicity affects the stability of the particles in water. When the hydrophilicity of the molecules is too strong, the particles tend to disintegrate in water. When the hydrophobicity of the molecules is too strong, the particles tend to aggregate or precipitate in water. In either case, the particles are unstable in water. When the weight ratio of the hydrophilic and hydrophobic units within the molecule is within the range given above, the interactions between the various segments in the particles and the aqueous environment are at a dynamic equilibrium. Thus, the particles can remain stable in water or an aqueous environment. Particles are considered stable when their sizes remain substantially the same over an extended period of time, such as over a week or up to six months. The particle sizes are considered substantially the same when the variation in size is permissible for the particular application in which the particles are used. For different applications, the permissible size variation may be different.

**[0022]** Stability of the particles in an aqueous solution may be determined using any suitable technique. For example, the solution may be inspected visually over time to see if it stays clear. If the particles precipitate or aggregate, precipitation or unclear solution may be observed. If the solution stays clear, the particle sizes in

the solution may be measured or monitored using a suitable optical technique such as a light scattering technique. Exemplary methods of particle size measurement are described in, e.g., Z. Yang et al., *Langmuir*, 2003, vol.19, p.943; and W. Brown et al., *J. Phys. Chem.*, 1991, vol. 95, p.1850; the contents of each one of which are incorporated herein by reference.

**[0023]** In exemplary embodiments, the graft molecule may be a conjugated polymer or conjugated oligomer. The backbone is formed of backbone units, which include at least 3 units that are each hydrophobic and fluorescent. The hydrophobic and fluorescent units may be repeating units. The side chains include hydrophilic side chain units. The weight ratio of the hydrophilic side chain units to the hydrophobic repeating units in the molecule may be from about 1:4 to about 4:1, if no other hydrophobic or hydrophilic segments are present in the molecule. In some embodiments, the weight ratio may be from about 3:7 to about 7:3.

**[0024]** The backbone may include an arylene, heteroarylene, arylene vinylene, heteroarylene vinylene, arylene ethylene, or heteroarylene ethylene unit, or a derivative thereof. The term "or" in the preceding sentence or in other similar context indicates that each of the listed items is itself a possible alternative and that any combination of any two or more of the listed items is also a possible alternative. A backbone unit may also be phenylene, thienylene, spirobifluorenylene, indenofluorenylene, pyridylene, bipyridylene, carbazoylene, indenocarbazoylene, benzothiazoylene, oxadiazoylene, or a derivative thereof. In one embodiment of the present invention, the repeating units in the backbone may include fluorene units. In one embodiment, a backbone unit may be linked to a vinylene group or an ethylene group. A backbone unit may also be linked to a single or two C-C triple bonds. In another embodiment, the backbone units may be linked with each other through a single carbon bond, a methylene group, or an atom selected from O, S, N, Si, and P.

**[0025]** In different embodiments, the backbone units may include a unit substituted with an organic group. The organic group may be an alkyl, alkoxy, alkenyl, alkynyl, alkylsilyl, arylsilylaryl, heteroaryl, aryloxy, heteroaryloxy, alkylthio, alkylamino, dialkylamino, arylamino, diarylamino, aryl ether, heteroaryl ether, aryl thioether, heteroaryl thioether, halogen, cyano, nitro, carbony, thionyl, sulphonyl, or

perfluoroalkyl group, or an amino group that includes a heteroaryl group. The substituted group may be selected to improve the solubility of the result molecule in a selected solvent. The solvent may be an organic solvent, such as tetrahydrofuran (THF), chloroform, dichloromethane, toluene, or the like. For example, the molecule may include hexyl substituted fluorene blocks.

**[0026]** The backbone may further include a flexible group connecting the fluorescent units in the backbone. The flexible group may be hydrophilic or hydrophobic. For example, the flexible group may be an alkyl group, substituted or unsubstituted alkylene, alkenylene, alkynylene, ether group, thioether group, amino group, imino group, ester group, thioester group, amide group, imide group, silyl group, or the like. The substituents can include any one of the above listed group, any substituted or unsubstituted aryl or heteroaryl group, any hetero atoms, or any combination thereof.

**[0027]** The side chains may include side chain units, which in turn include hydrophilic units. Hydrophilic units, either in the side chains or in the backbone, may include a polyethyleneglycol (PEG), polyethyleneimine, polyamide, polyvinylpyrrolidone, polyacrylic acid, polyvinyl alcohol, or polylysine unit, or a derivative thereof. The hydrophilic units in the side chains may be bonded to the backbone through a single carbon or phosphor bond, ether group, thioether group, amino group, imino group, silyl group, ester group, thioester group, amide group or an imide group. The hydrophilic side chains may be linked to any position of the backbone, either at the end or in the middle of the backbone.

**[0028]** Given the chemical structure or formula of a particular molecule, the hydrophilic and hydrophobic units (segments) in the molecule may be readily identified by persons skilled in the art. The weight ratio of these two types of units (segments) within the molecule may then be calculated based on the atomic weights of the elements present in the respective units (segments). The total weight of all hydrophilic segments and the total weight of all hydrophobic segments in the molecule may be respectively calculated and used to calculate their ratio. Alternatively, for a polymer or oligomer formed of repeating monomer units, the weight ratio of the two types of segments in each monomer unit of the polymer may be calculated and used to determine the weight ratio of the entire polymer. The

weight ratio may also be determined in another suitable manner known to skilled person in the art.

**[0029]** In one embodiment, the graft molecule may be formed as follows. The precursors for the backbone units are prepared or obtained from available commercial sources, such as Sigma-Aldrich™, Fluka™, Merck™, TCL™, Alfa Aesar™. The precursors may be prepared using any suitable techniques known to skilled person in the art. At least some of the precursors include hydrophobic fluorescent groups. Side chains including hydrophilic side chain groups are grafted to respective precursors, forming grafted precursors. The grafted precursors may be same or different molecules. The grafted precursors are then linked to form the final graft molecule. The ratio of the side chains to the precursors are selected so that the weight ratio of the hydrophilic segments to the hydrophobic segments is from about 1:4 to about 4:1, such as from about 3:7 to about 7:3. Each grafted precursor may be a monomer, an oligomer, or a polymer. The stoichiometric ratio of different grafted precursors in the graft molecule may vary and may be selected depending on the desired backbone size, molecular weight, solubility, bio-compatibility, optical property, or other relevant characteristics. The grafted precursors may be linked together through a coupling reaction. The coupling reaction may be a Suzuki coupling reaction, a Grignard coupling reaction, or a Stille coupling reaction. The grafted precursors may also be linked to form the grafted molecules through oxidation polymerization reaction, reduction polymerization reaction, polycondensation reaction, Heck reaction, Sologashira reaction, or the like. The grafted precursors may be directly linked, or indirectly linked by other backbone units, as can be understood by those skilled in the art. A linking backbone unit that links grafted precursors may have no side chain attached to it or have one or more side chains attached to it. For example, the linking backbone unit may be flexible group.

**[0030]** In a different embodiment, the graft molecules may be formed using other procedures. For example, a polymer backbone may be first prepared and the side chains are then grafted to the backbone in postpolymerization reactions. In one example, PEG with OH end groups or amino groups may be grafted to polymer backbones that include alkylbromo groups, acid chloride groups, or anhydride

groups. In another example, side chains with acid chloride groups or anhydride groups may be grafted to backbones that include OH groups or amino groups. Exemplary postpolymerization techniques are disclosed in Cuihua Xue *et al.*, "Facile, versatile prepolymerization and postpolymerization functionalization approaches for well-defined fluorescent conjugated fluorene-based glycopolymers", *Macromolecules*, 2006, vol. 39, no. 17, pp. 5747-5752; K. Buga *et al.*, "Postpolymerization grafting of aniline tetramer on polythiophene chain: Structural organization of the product and its electrochemical and spectroelectrochemical properties", *Chemistry Of Materials*, 2005, vol. 17, no. 23, pp. 5754-5762; K. Buga *et al.*, "Poly(alkylthiophene) with pendant dianiline groups via postpolymerization functionalization: preparation, spectroscopic, and spectroelectrochemical characterization", *Macromolecules*, 2004, vol. 37, no. 3, pp. 769-777; and J.S. Liu and R.D. McCullough, "End group modification of regioregular polythiophene through postpolymerization functionalization", *Macromolecules*, 2002, vol. 35, no. 27, pp. 9882-9889, the contents of each one of which are incorporated herein by reference.

**[0031]** Some exemplary embodiments of the present invention relate to water-soluble fluorescent particles formed from the graft molecules described above. The particles may have a peripheral size from 10 nm to 10 microns. The peripheral size is the particle diameter when the particle is spherical. When the particles have irregular shapes or non-uniform sizes, the peripheral size refers to the effective or average diameter for the particles. An effective diameter of a non-spherical particle is the diameter of a spherical particle that has the same volume as the non-spherical diameter. The size of the particles may be measured using any suitable technique including optical or electronic imaging techniques. For example, the sizes of the particles may be measured using a light scattering technique.

**[0032]** The particles may include a ligand having a specific affinity to a selected target. For instance, the ligand may be avidin, biotin, antibody, antigen, or DNA, and the target may be a molecule, a cell or an organism.

**[0033]** In an exemplary embodiment, the particles may be formed as follows.

**[0034]** A first solution containing the graft molecules dissolved in an organic

solvent is obtained. The organic solvent may be THF, chloroform, dichloromethane, toluene, or the like. The first solution may be a product solution from the formation process for the graft molecule. The product solution may be subject to further optional or necessary treatment depending on the particular case. The first solution may also be separately prepared by dissolving the graft molecules in the organic solvent. The first solution is mixed with water, thus forming a second solution. To form the second solution, water or an aqueous solution may be added to the first solution, or the first solution may be added to water or the aqueous solution. The aqueous solution includes water, and may also include a PH buffer solution. The concentration of the graft molecules in the second solution should be controlled. Assuming the particular graft molecule has a critical aggregation concentration, CAC, in the second solution, the concentration of the graft molecules in the second solution should be from about 1 to about 1000 CAC in one embodiment, and from about 10 to about 100 CAC in another embodiment. The CAC for a given graft molecule in a given solution may be determined by monitoring intensity of light emitted from the solution while increasing the concentration of the graft molecule. When the concentration of the graft molecule in the solution is at or near the CAC value, the emission light intensity exhibits a sharp increase. CAC may be measured according to exemplary methods disclosed in, e.g., K. Holmberg et al., *Handbook of applied surface and colloid chemistry*, 2002, vol. 2, Chapter 13, the contents of which are incorporated herein by reference.

**[0035]** The organic solvent is next removed from the second solution, thus allowing the graft molecules to form the particles, such as through self-assembly. The organic solvent may be removed by evaporation, or another technique. For example, a rotary evaporation technique may be used. The evaporation of the organic solvent may be facilitated by exposing the second solution to vacuum, by heating, or by both. In some embodiments, at least 90% of the organic solvent should be removed. The self-assembly process is driven by the hydrophilic and hydrophobic interactions among the different segments in the graft molecules and the water. Generally, hydrophobic segments and water molecules repel each other, while hydrophilic segments and water molecules attract each other. To facilitate the self-assembly process, the second solution may have a pH of about 2 to about 12, depending on the application. During evaporation and particle

formation, the second solution may be maintained at a temperature from about 0 °C to about 80 °C, such as from about 4 °C to about 70 °C.

**[0036]** The resulting solution and the formed particles therein may be subject to any suitable post-formation treatment such as extraction, purification, drying, or the like, depending on the application.

**[0037]** In different embodiments, the particles may be formed using different procedures. For example, the particles may be formed by dissolving amphiphilic block copolymers into a mixture of water and an organic solvent to form a mixture solution, and the organic solvent may be extracted from the mixture solution in different processes. In an exemplary process (dialysis process), the mixture solution may be transferred into a dialysis tube, which is then immersed into a water bath. Due to concentration differential, the organic solvent will diffuse from the tube into the water bath. The water bath may be refreshed from time to time, such as every two (2) hours. The refreshing process may be repeated until the organic solvent is substantially removed from the solution in the tube. Typically, this process may take two to three days to complete. The remaining solution in the tube will contain the particles formed. The dialysis procedure may be used even when the organic solvent is not very volatile. A further alternative solvent removal technique is the sonication-injection process, which may be used when the organic solvent is volatile, such as THF or acetone. In this technique, the mixture solution may be slowly injected into a volume of (pure) water under sonication. The resulting solution may be sonicated for a period of time, such as about 10 minutes. The sonicated solution may be stirred at room temperature for e.g. two days to remove the organic solvent by evaporation.

**[0038]** It is expected that the resulting fluorescent particles prepared in the exemplary processes described above are soluble in water and can exhibit stable fluorescent emission over a relatively long period of time such as up to 6 months in an aqueous environment such as in water (see e.g. Example 13 below). In comparison, when nanosized particles are formed using amphiphilic molecules that have similar constituents but the weight ratio of the hydrophilic segments to the hydrophobic segments in the particles are outside the range of 1:4 to 4:1, the particles may be unstable in water and their performance as a fluorescent probe is

expected to deteriorate over a relatively short period of time, such as within a day to one week. For example, amphiphilic graft polymers 2,7-dibromo-9,9(6'-polyethyleneglycol-hexyl)fluorene and 2-bromo-9,9(6'-polyethyleneglycol-hexyl)fluorene have been found to be unstable in water. The weight ratio of the hydrophilic segments and hydrophobic segments in these two compounds is about 10:1. In contrast, test results showed that when the weight ratio was in the range from about 1:4 to about 4:1, and the concentration of the graft molecules in the second solution was from about 1 to about 1000 CAC, such as from about 10 to about 100 CAC, the tested sample graft molecules formed fluorescent particles that were observed to be stable in water over an extended period of time, such as more than six months.

**[0039]** In one particular embodiment, the backbones of the graft molecules include oligofluorene or polyfluorene and the side chains include PEG. THE PEG units may have any suitable size or weight.

**[0040]** The grafted precursors for the graft molecules may be linked through a Suzuki coupling reaction. The weight ratio of PEG to oligo- or polyfluorene in the molecules is from about 1:4 to 4:1, or from about 3:7 to about 7:3. In the first solution, the organic solvent may be toluene or dichloromethane. The first solution may be added to water drop-wise as the mixed solution is stirred. The organic solvent may be removed by evaporation. The resulting particles may have peripheral sizes from a few nanometers to several micrometers. Both the first solution and the resulting particles when dispersed in water will exhibit intense fluorescence emission. The fluorescence can be measured using a fluorescence spectrometer or a confocal spectrometer.

**[0041]** The intense fluorescence exhibited by these particles may be utilized in imaging or detection applications, such as in bio-applications. For example, the particles may be attached to a specific ligand. The ligand may be a nucleotide, single-stranded DNA, double-stranded DNA, single-stranded RNA, double-stranded RNA, a peptide, a protein, a hormone, an antibody, a receptor, an antigen, an epitope, a nucleic acid binding protein, a molecule, an enzyme substrate or an analogue thereof, avidin, streptavidin, biotin, a monosaccharide, a polysaccharide, or the like. The particles with the specific ligand may be used to target a specific

cell or an organism. When the particle size is generally in the nanometer range, it can be easily up-taken by cells or organisms. The particles can also be prepared so that they are compatible with the selected environment such as the surrounding cells. For example, the components of the particles may be selected so that the presence of the particles would not bring about undesired effects, such as being toxic to the cells.

**[0042]** Examples:

**[0043]** Example 1 (Synthesis of Compound 1)

**[0044]** Compound 1 was 2,7-dibromo-(9,9-dihexyl) fluorene, prepared according to the reaction shown in **FIG. 1**. 2,7-dibromofluorene (9.72g, 30mmol, obtained from Sigma-Aldrich) was added to a mixture of aqueous sodium hydroxide (54.3ml, 50%), tetrabutylammonium bromide (1.82g, 5.63mmol), and 1-bromohexane (25.36ml, 180mmol) at 80°C. After having been stirred for 5 hours, the mixture was cooled down to room temperature. The mixture was mixed with dichloromethane to extract the reaction product. The organic layers were sequentially washed with water, aqueous HCl, water, and brine. The washed layers were dried using anhydrous MgSO<sub>4</sub>. Remaining solvent and excess 1-bromohexane were removed. The residue was purified using silica gel column chromatography and hexane as the eluent. The purified product contained a white solid, which weighed 14.55g corresponding to a yield of 98.6% by weight. The measured spectra of the white solid were: <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400MHz) δ[ppm]: 7.513 (m, 6H), 1.908 (m, 4H), 1.119 (m, 12H), 0.782 (t, 6H), 0.583 (m, 4H), confirming that the product contained Compound 1, 2,7-dibromo-(9,9-dihexyl) fluorene.

**[0045]** Example 2 (Synthesis of Compound 2)

**[0046]** Compound 2 was 2,7-Bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)9,9-dihexylfluorene, prepared according to the reaction shown in **FIG. 2**, using Compound 1.

**[0047]** 2.46g (5mmol) of Compound 1 was added to THF (50ml) to form a solution. The solution was cooled to a temperature of -78°C. 15ml (18mmol) of n-butyllithium (1.2M in hexane, obtained from Aldrich™) was added to the solution to

form a mixture. The mixture was stirred for 1 hour with the solution temperature maintained at -78°C. 2-Isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (2.7ml, 12.98mmol) was next added rapidly to the mixture. The resulting mixture was warmed to room temperature and stirred overnight. The mixture was poured into water. The reaction product in mixture was extracted using ether. The extraction process was repeated 3 times. The extracted organic layers were combined and washed with brine and dried over anhydrous magnesium sulfate. The remaining solvent was removed by rotary evaporation. The residue was purified by silicon column chromatography (ethyl-acetate:hexane=1:15). The purified product contained 1.823g of a white solid (giving a yield of 62% by weight). The measured spectra of the white solid were <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400MHz) δ[ppm]: 7.793 (d, 2H, J=7.6Hz), 7.742 (s, 2H), 7.725 (d, 2H, J=7.6Hz), 1.995 (m, 4H), 1.386 (s, 24H), 1.064 (m, 12H), 0.740 (t, 6H), 0.546 (m, 4H), confirming that the product was Compound 2, 2,7-Bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)9,9-dihexylfluorene.

**[0048]** Example 3 (Synthesis of Compound 3)

**[0049]** Compound 3 was 2-bromo-(9,9-dihexyl)fluorene, synthesized according the reaction shown in **FIG. 3**. 2-bromofluorene (12.25g, 50mmol) was added to a mixture of aqueous sodium hydroxide (91ml, 50%), tetrabutylammonium bromide (3.03g, 9.38mmol), and 1-bromohexane (42.3ml, 300mmol) at 80°C. After having been stirred for 4 hours, the mixture was cooled down to room temperature. After extraction with dichloromethane, the combined organic layers were sequentially washed with water, aqueous HCl, water, and brine, and then dried over anhydrous MgSO<sub>4</sub>. After removal of remaining solvent and excess 1-bromohexane from the dried layers, the residue was purified by silica gel column chromatography using hexane as the eluent. The resulting product contained 20.03g (with 97wt% yield) of a light-yellow liquid product, with the measure spectra of <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400MHz) δ[ppm]: 7.681 (m, 1H), 7.57 (d, J=7.7Hz, 1H), 7.45 (m, 2H), 7.35 (m, 3H), 1.95 (m,4H), 1.09 (m, 12H), 0.79 (t, J=7.1Hz, 6H), 0.62 (m, 4H). This product was Compound 3, 2-bromo-(9,9-dihexyl)fluorene.

**[0050]** Example 4 (Synthesis of Compound 4)

**[0051]** Compound 4 was 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-dihexylfluorene, prepared according to the reaction shown in **FIG. 4**, using compound 3. 6.2g (15mmol) of Compound 3 was mixed with 100 ml anhydrous THF to form a solution. 22.5ml of n-BuLi (27mmol) was added to the solution, which was at -78°C. The solution was stirred for one hour before adding 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (3.9ml, 18.75mmol) thereto. The resulting mixture was stirred overnight. Water was then added to quench further reaction. The reaction product in the mixture was extracted with dichloromethane (100ml) for 3 times. The extracted organic layer was washed with brine, dried over anhydrous MgSO<sub>4</sub>, and then concentrated in vacuo. The concentrated layer was subject to column chromatography (silica gel, ethyl acetate: hexane = 1: 20), producing a product weighing 5.83g (with 84.5wt% yield). The measured spectra of the product were <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400MHz) δ[ppm]: 7.812 (m, 1H), 7.747 (m, 3H), 7.322 (m, 3H), 7.322 (m, 3H), 1.990 (m, 4H), 1.39 (s, 12H), 1.023 (m, 12H), 0.752 (m, 6H), 0.597 (m, 4H), confirming that the product was Compound 4, 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-dihexylfluorene.

**[0052]** Example 5 (synthesis of Compound 5)

**[0053]** Compound 5 was 2,7-dibromo-9,9-bis(6'-bromohexyl)fluorene, prepared according to the reaction shown in **FIG. 5**. 2,7-dibromofluorene (0.972g, 3mmol) was added to a mixture of aqueous potassium hydroxide (60ml, 50%), tetrabutylammonium bromide (0.198g, 0.6mmol), and 1,6-dibromohexane (7.32g, 30mmol) at 75°C. After having been stirred for 15 minutes, the mixture was cooled down to room temperature. After extraction with dichloromethane, the combined organic layers were sequentially washed with water, aqueous HCl, water, and brine, and then dried over anhydrous MgSO<sub>4</sub>. After removal of remaining solvent and excess 1, 6-dibromohexane, the residue was purified by silica gel column chromatography using hexane:chloroform (v/v: 9:1) as the eluent. The resulting product contained 1.47g (75wt% yield) of a white solid, with measured spectra of <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400MHz) δ (ppm): 7.433-7.535 (m, 6H), 3.294 (t, 4H), 1.923 (m, 4H), 1.652-1.687 (m, 4H), 1.203 (m, 4H), 1.083 (m, 4H), 0.587 (m, 4H), confirming that the product was Compound 5, 2,7-dibromo-9,9-bis(6'-bromohexyl)fluorene.

**[0054]** Example 6 (synthesis of Compound 6)

**[0055]** Compound 6 was 2,7-dibromo-9,9-(6'-polyethyleneglycol-hexyl)fluorene, prepared according the reaction shown in **FIG. 6**, using compound 5. Sodium hydride (0.96g, 40mmol) and anhydrous THF (30ml) were placed in a three-necked 150ml flask under argon atmosphere. PEG (8g, 4mmol) in THF (50ml) was then added drop-wise at room temperature. As shown in **FIG. 6**, the molecular weight of the PEG used was 2000 Dalton, denoted as PEG2000 or PEG<sub>2000</sub>. However, it should be understood that PEG2000 was used for testing purposes and in different embodiments of the present invention the weight of the PEG may vary.

**[0056]** The resulting mixture was stirred for 4 hours. 0.65g (1mmol) of Compound 5 was next added to the flask. The mixture in the flask was stirred at room temperature for about one week. Water was next added drop-wise to terminate further reaction. The organic solvent in the mixture was evaporated off and the residue was dissolved in 20ml of dichloromethane and 4ml of methanol. The solution is mixed with 500ml of ether to form a precipitate. The precipitate was collected by centrifuging off the solvent. The precipitate was further purified by repeating the above process for 3 times and then dried in vacuum. The resulting product was Compound 6.

**[0057]** Example 7 (Synthesis of Compound 7)

**[0058]** Compound 7 was 2-bromo-9,9-bis(6'-bromohexyl)fluorene, prepared according to the reaction shown in **FIG. 7**. 2-bromofluorene (4.9g, 20mmol) was added to a mixture of aqueous potassium hydroxide (400ml, 50%), tetrabutylammonium bromide (1.32g, 4mmol), and 1,6-dibromohexane (48.8g, 200mmol) at 75°C. After having been stirred for 15 minutes, the mixture was cooled down to room temperature. After extraction with dichloromethane, the combined organic layers was sequentially washed with water, aqueous HCl, water, and brine, and then dried over anhydrous MgSO<sub>4</sub>. After removal of remaining solvent and excess 1, 6-dibromohexane, the residue was purified by silica gel column chromatography using hexane/chloroform (v:v = 9:1) as the eluent. The product was a light-yellow liquid, weighing 8.6g (75wt% yield), with measured spectra of <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400MHz)  $\delta$  (ppm): 7.669 (m, 1H), 7.548 (m, 1H), 7.453 (m, 2H), 7.322 (m, 3H), 3.280 (t, 4H), 1.943 (m, 4H), 1.657 (m, 4H), 1.193 (m, 4H), 1.073 (m, 4H), 0.604 (m, 4H), confirming that the product was Compound 7, 2-

bromo-9,9-bis(6'-bromohexyl)fluorene.

**[0059]** Example 8 (Synthesis of Compound 8)

**[0060]** Compound 8 was 2-bromo-9,9(6'-polyethyleneglycol-hexyl)fluorene was synthesized according to the reaction shown in **FIG. 8**, using compound 7.

**[0061]** Sodium hydride (0.96g, 40mmol) and anhydrous THF (30ml) were placed in a three-necked 150ml flask under argon atmosphere. PEG (8g, 4mmol) in THF(50ml) was added drop-wise at room temperature. The mixture was stirred for 4 hours. 0.57g (1mmol) of Compound 7 was next added to the mixture. The mixture was stirred further at room temperature for about one week. Water was then added drop-wise to the flask to terminate further reaction. The solution was allowed to evaporate to remove THF. The remaining mixture was dissolved in 20ml dichloromethane and 4ml methanol, and then mixed with 500ml ether to form a precipitate. The solvents in the mixture were removed by centrifuge. The above precipitation process was repeated 3 times. The resulting precipitate was dried in vacuum. The resulting product was Compound 8.

**[0062]** Example 9 (Synthesis of Compound 9)

**[0063]** Compound 9 was prepared according the reaction shown in **FIG. 9**.

**[0064]** A mixture of p-bromophenol 2.63g (15mmol), dibromohexane 1.22g (5mmol) and anhydrous potassium carbonate 1.38g (10mmol) was dissolved in acetone and heated to reflux. After 8 hours of refluxing, water was added to terminate further reaction. The mixture was mixed with 50ml of ether (3 times) to extract an organic precipitate. The precipitate was sequentially washed with 2M sodium hydroxide solution, brine, and then dried over anhydrous MgSO<sub>4</sub>. After removal of remaining solvent, the crude product was re-crystallized in ethanol, forming Compound 9 weighing 1.15g (46wt% yield) and with measured spectra of <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400MHz) δ[ppm]: 7.369-7.347(d, 4H, J=8.8Hz), 7.776-7.754(d, 4H, J=8.8Hz), 3.929(t, 4H), 1.803(m, 4H), 1.533(m, 4H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 400MHz) δ (ppm):158.429, 132.409, 116.548, 112.885, 68.299, 29.290, 25.982.

**[0065]** Example 10 (Synthesis of Compound 10)

**[0066]** Compound 10 was denoted 3F1-PEG2000, prepared according to the reaction shown in **FIG. 10**, using Compounds 2, 3 and 4. A mixture of Compound 2 (0.15g, 0.25mmol), Compound 4 (1.103g, 0.25mmol), tetrakis(triphenylphosphine)palladium (50mg, 0.04mmol), aqueous sodium carbonate (2M, 1.24ml), and toluene (10.16ml) was deoxygenated and then heated to reflux under nitrogen. The mixture was stirred for 4 hours. 0.103g (0.18mmol) of Compound 3 dissolved in 1 ml of toluene was next added to the mixture. The mixture was further stirred for about 2 days and then cooled to the room temperature. The organic solvent in the mixture was allowed to evaporate. The residue was dissolved in 4 ml dichloromethane and mixed with 100ml ether to form a precipitate. The solvents were removed by centrifuge. The precipitation process was repeated 3 times. The crude product was dissolved in dichloromethane, and subject to dialysis for about one week using an 8k dialysis tube. The solution was then freeze-dried, forming a pale powder product, which was Compound 10. The yield of the synthesis process was 24wt%.

**[0067]** In Compound 10, the PEG segments are hydrophilic and the other segments are hydrophobic. The weight ratio of hydrophilic to hydrophobic units in a Compound 10 molecule is about 4:1. The CAC of Compound 10 in water is 0.05 mg/mL.

**[0068]** To prepare stable particles from Compound 10, 10 mg of Compound 10 was dissolved in 10 mL of water (equivalent to 20 CAC). The resulting solution was stirred for 3 days. Micellar particles were formed in the solution, which stayed stable for 6 months while in storage.

**[0069]** Example 11 (Synthesis of Compound 11)

**[0070]** Compound 11 was 3F2-PEG2000, prepared according to the reaction shown in **FIG. 11**, using Compounds 4 and 6. A mixture of Compound 4 (0.46g, 1mmol), Compound 6 (1.123g, 0.25mmol), tetrakis(triphenylphosphine)palladium (50mg, 0.04mmol), aqueous sodium carbonate (2M, 1.24ml), and toluene (10.16ml) was deoxygenated and then heated to reflux under nitrogen. The mixture was stirred for 2 days and then cooled down to room temperature. The organic solvent in the mixture was allowed to evaporate. The residue was dissolved in 20ml of

dichloromethane, and mixed with 800ml of ether to form a precipitate. The solvents were removed by centrifuge. The precipitation process was repeated 3 times. The crude product was dissolved in dichloromethane and subject to dialysis using an 8k dialysis tube for about one week. The solution was freeze-dried to form a pale powder product, which was Compound 11. The yield of the process was 50%. As in Compound 10, the weight ratio of hydrophilic to hydrophobic segments in Compound 11 is about 4:1.

**[0071]** The weight average molecular weight ( $M_w$ ) and number-average molecular weight ( $M_n$ ) of Compound 11 were determined based on gel permeation chromatography (GPC) analysis, with PEG being used as the standard for calibration, and were found to be 4123 and 3969 respectively. The GPC result indicated that each Compound 11 molecule contained only one block in which PEG side chains were attached to a fluorenyl group.

**[0072]** The CAC of Compound 11 was measured using a dynamic light scattering (DLS) technique at room temperature in aqueous solution, and was found to be 0.1 mg/mL. The CAC of Compound 11 in water is 0.10 mg/mL.

**[0073]** To prepare stable particles from Compound 11, 10 mg of Compound 11 was dissolved in 10 mL of water (equivalent to 10 CAC). The resulting solution was stirred for 3 days. Micellar particles were formed in the solution, which stayed stable for 6 months while in storage.

**[0074]** Example 12 (Synthesis of Compound 12)

**[0075]** Compound 12 was 5F3-PEG2000, prepared according to the reaction shown in **FIG. 12**, using Compounds 2, 3 and 6. A mixture of Compound 2 (0.23g, 0.4mmol), Compound 3 (0.25g (0.6mmol), Compound 6 (0.45g, 0.1mmol), tetrakis(triphenylphosphine)palladium ( 32mg, 0.028mmol), aqueous sodium carbonate (2M, 0.88ml), and toluene (2.6ml) was deoxygenated and then heated to reflux under nitrogen. The mixture was under reflux for 2 days and then cooled to room temperature. The organic solvent in the mixture was allowed to evaporate and the residue was dissolved in 20ml of dichloromethane. The solution was mixed with 800ml of ether to form a precipitate. The solvents were removed by centrifuge.

The precipitation process was repeated 3 times. The crude product was dissolved in dichloromethane and subject to dialysis using an 8k dialysis tube for about one week. The solution was freeze-dried to form a pale powder product, which was Compound 12. The yield was 46% in this example. The weight ratio of hydrophilic to hydrophobic units in Compound 12 is about 2.4:1.

**[0076]** The weight average molecular weight ( $M_w$ ) and number-average molecular weight ( $M_n$ ) of Compound 12 were determined based on GPC analysis, with PEG being used as the standard for calibration, and were found to be 4409 and 3919 respectively. The GPC result indicated that each Compound 12 molecule contained only one block in which PEG side chains were attached to a fluorenyl group.

**[0077]** The CAC of Compound 12 was measured using the DLS technique at room temperature in an aqueous solution, and was found to be 0.08 mg/mL.

**[0078]** To prepare stable particles from Compound 12, 10 mg of Compound 12 was dissolved in 10 mL of water (equivalent to 12.5 CAC). The resulting solution was stirred for 3 days. Micellar particles were formed in the solution, which stayed stable for 6 months while in storage.

**[0079]** Example 13 (Synthesis of Compound 13)

**[0080]** Compound 13 (PF1-PEG2000) was prepared according to the reaction shown in **FIG. 13**, using Compounds 1, 2, and 6. The symbols p and q used herein represent integers. As shown in **FIG. 13**,  $p+q=19$ . A mixture of compound 1 (0.089g, 0.18mmol), Compound 2 (0.117g, 0.2mmol), Compound 6 (0.0898g, 0.02mmol), tetrakis(triphenylphosphine)palladium (2mg, 0.002mmol), aqueous sodium carbonate (2M, 0.284ml), and toluene (1.15ml) was deoxygenated and then heated to reflux under nitrogen. The mixture was under reflux for 2 days and then cooled to room temperature. The organic solvent in the mixture was allowed to evaporate and the residue was dissolved in 4ml dichloromethane. The solution was mixed with 100ml of ether to form a precipitate. The solvents were removed by centrifuge. The precipitation process was repeated 3 times. The crude product was dissolved in dichloromethane and subject to dialysis using a 10k dialysis tube

for about one week. The solution was then freeze-dried, forming a light yellow powder product, Compound 13. The resulting product had a particle size of about 85 nm. The yield was 14% in this Example. The measure spectra of the product were  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400MHz)  $\delta$ [ppm]: 7.854(m, 30H), 7.674(m, 90H), 3.643(m, 360H), 2.129(m, 80H), 1.414(m, 240H), 0.795(m, 200H). The weight ratio of hydrophilic to hydrophobic units in Compound 13 is about 1:1.6. The CAC of Compound 13 was found to be 0.008 mg/mL.

**[0081]** The fluorescence of Compound 13 and its stability were tested. For this purpose, BV-2 cells were cultured for 2 days. An aqueous solution containing 0.3 mg/g of nanoparticles formed from Compound 13 (PF1-PEG2000) was prepared as follows. 3 mg of Compound 13 was dissolved in 1.5 mL of THF to form an initial solution. The initial solution was further diluted with THF until the total volume of the resulting solution was 2 mL. 8 mL of deionized water was slowly added to the resulting solution over 3 hours. The solution was contained in a bottle. The bottle was covered with a piece of Kimwipes™ tissue paper, to allow THF to evaporate over a 3-day period. After the 3-day period, water was added to the bottle to adjust the concentration of the final solution which had a total volume of 10 mL. The final solution contained about 0.3 mg/g of Compound 13 (equivalent to about 40 CAC).

**[0082]** The solution was added to the culture media with a ratio of 1:10 (v:v). The cells were kept in the culture media for 6 hours. Next, the cells were fixed with 4% paraformaldehyde for 2 hours and then washed with PBS for 3 times. The cells were observed under a confocal microscope. Two batches of Compound 13 were used in the test. The first batch of Compound 13 was freshly prepared. The second batch of Compound 13 was stored in an aqueous solution at room temperature for at least 6 months. In both cases, the fixed cells showed similar intense fluorescence.

**[0083]** In another test, the cultured BV-cells were allowed to settle down after 2 days of culturing. Next, the aqueous solution containing particles formed from Compound 13 described above was added to the culture media. Different tests were conducted with different batches where the concentrations of the aqueous solution in the culture media was 1%, 2%, 5%, 10% or 20% (v/v) respectively. For each batch, the cell viability of BV2 cells was measured four times at 12 hour

intervals (i.e. at 12, 24, 48, and 72 hours after addition). Representative measured results are shown in **FIG. 21**. For batches where the concentration of the particle solution was in the range from 1% to 5%, the measured number of cells in the culture media increased significantly with time. The increase was less pronounced for batches of higher concentrations (10% and 20%). This was expected as the solvent for the particle solution was water. The significant increase of the cell numbers indicated that the fluorescent particles of Compound 13 was non-toxic or only of very low toxicity to the cells.

**[0084]** It was observed that the cells could take up more fluorescent particles formed from compound 13 after the cells were activated. This suggests that the particles could be used as a carrier to deliver drugs or other substance to selectively activated cells/organisms.

**[0085]** Example 14 (Synthesis of Compound 14)

**[0086]** Compound 14 was PF2-PEG2000, prepared according to the reaction shown in **FIG. 14**, using Compounds 1, 2, 6, and 9. Like symbols "p" and "q", symbol "n" used herein also represent an integer. In **FIG. 14**, the value of n may be from 1 to 100 such as from 1 to 20, and  $p+q=10$ . A mixture of Compound 1 (0.16g, 0.32mmol), Compound 2 (0.28g, 0.48mmol), Compound 6 (0.36g, 0.08mmol), Compound 9 (40mg, 0.08mmol), tetrakis(triphenylphosphine)palladium (78mg, 0.07mmol), aqueous sodium carbonate (2M, 0.93ml), and toluene (3.72ml) was deoxygenated and then heated to reflux under nitrogen. The mixture was under reflux for 2 days and then cooled to room temperature. The organic solvent was allowed to evaporate and the residue was dissolved in 10ml dichloromethane. The solution was mixed with 400ml of ether to form a precipitate. The solvents were removed by centrifuge. The precipitation process was repeated 3 times. The crude product was dissolved in dichloromethane and subject to dialysis using a 10k dialysis tube for about one week. The solution was freeze-dried, forming a light yellow powder product, Compound 14. The product particles had a size of about 154 nm. The yield was 37wt%. The weight ratio of hydrophilic to hydrophobic units in Compound 14 is about 1:0.9. The CAC of Compound 14 was found to be 0.00032 mg/mL.

**[0087]** An aqueous solution containing particles formed from Compound 14 was prepared using the same procedure as that described above for Compound 13 by replacing Compound 13 with Compound 14. The final aqueous solution contained about 0.3 mg/mL of Compound 14 (equivalent of about 940 CAC). The micellar particles in the final solution remained stable for 6 months while in storage.

**[0088]** Example 15 (Synthesis of Compound 15)

**[0089]** Compound 15 was PF3-PEG2000, prepared according to the reaction shown in **FIG. 15**, using Compounds 1, 2, 6, and 9. In **FIG. 15**,  $n$  may be from 1 to 100 such as from 1 to 20, and  $p+q=4$ . A mixture of Compound 1 (0.16g, 0.32mmol), Compound 2 (0.28g, 0.48mmol), Compound 6 (0.36g, 0.08mmol), Compound 9 (40mg, 0.08mmol), tetrakis(triphenylphosphine)palladium (64.1mg, 0.055mmol), aqueous sodium carbonate (2M, 0.88ml), and toluene (3.5ml) was prepared. The mixture was processed as described in Example 14. The resulting product was a pale powder product, Compound 15. The product particles had a size of about 178 nm. The yield was 21 wt%. The weight ratio of hydrophilic to hydrophobic units in Compound 15 is about 5:2. The CAC of Compound 15 was found to be 0.0004 mg/mL.

**[0090]** An aqueous solution containing particles formed from Compound 15 was prepared using the same procedure as that described above for Compound 13 by replacing Compound 13 with Compound 15. The final aqueous solution contained about 0.3 mg/mL of Compound 15 (equivalent of about 750 CAC). The micellar particles in the final solution remained stable for 6 months while in storage.

**[0091]** Example 16

**[0092]** BV-2 cells were cultured for 2 days and then activated for 24 hours by adding different concentration of a simulating agent, lipopolysaccharide. An aqueous solution containing fluorescent particles formed from Compound 13 was prepared as described in Example 13. The solution was added to the cell culture media. The volume ratio of the solution to the culture media was 1:100. The fluorescence from the media was monitored using a confocal imaging technique. It was observed that when the stimulation intensity was increased (after adding the

simulation agent), the fluorescent emission from the cells became stronger, indicating that more fluorescent particles had become engulfed by the activated cells.

**[0093]** As can be appreciated, the above example synthesis routes may be modified or generalized to produce other graft molecules according aspects of the present invention. For example, water-soluble fluorescent polymers may be formed generally according to the reaction shown in **FIG. 16**, and polymers with flexible segments in the backbone may be prepared according to the reaction shown in **FIG. 17**.

**[0094]** For the reaction shown in **FIG. 16**, the values of  $x$ ,  $y$ ,  $z$ ,  $p$ ,  $q$ , and  $n$ , which represent integers, may vary. The sum,  $p+q$  ( $=x+y+z$ ) may also vary. As can be understood, to form Compound 13,  $x=9$ ,  $y=10$ ,  $z = 1$ ; and in Compound 13,  $p=1$ ,  $q=19$ , and  $n=1$ . In different embodiments, the value of  $p+q$  may vary such as from 2 to 200. Molecules with different sum of  $p+q$  may be prepared by adjusting the relative molar or weight ratio of the reactants present in the reaction mixture.

**[0095]** Generally, a fluorescent polymer that can form stable nanosized particles may be formed as follows. Appropriate amounts of Compounds 1, 2, and 6 are mixed with tetrakis(triphenylphosphine)palladium, aqueous sodium carbonate, and toluene, to form a mixture. The concentrations of the reactants in the mixture may vary. In one embodiment, the concentration of total reactants may be from 0.1 M to 1.0 M. The mixture may be deoxygenated and then heated to reflux under nitrogen. The mixture may be under reflux and stirred for an extended period such as 2 days to 1 week and then cooled to a lower temperature such as the room temperature. The organic solvent in the mixture is allowed to evaporate. The residue may be dissolved in a minimal amount of dichloromethane or another suitable solvent. The new solution is mixed with a suitable amount of ether to form a precipitate. The volume ratio of the new solution to ether may be from 1:100 to 1:500. The solvents may be removed such as by centrifuge. The precipitation process may be repeated, such as 3 times. The crude product obtained may be dissolved in dichloromethane or another suitable low boiling point solvent, and may be subject to dialysis, such as using a 10k dialysis tube, for an extended period such as from about 3 days to one week. The solution may be freeze-dried to form

the final product which contains particles formed from the desired graft molecules. The sizes of the particles may be controlled by changing the molecular structure, molecular weight, or solution concentration.

**[0096]** A sample compound was prepared according to the reaction in **FIG. 16** and the above procedure, where the reagent concentrations were selected so that  $p=1$  and the total number of backbone units was 18. The CAC of the resulting compound was measured using the DLS technique at room temperature in an aqueous solution, and was found to be 0.008 mg/mL. The weight average molecular weight ( $M_w$ ) and number-average molecular weight ( $M_n$ ) of this sample compound were determined based on GPC analysis, with PEG being used as the standard for calibration, and were found to be 19585 and 9886 respectively. The value of  $M_n$  was also calculated based on NMR measurement, and was found to be 9976. The GPC result indicated that the sample compound molecule contained only one block in which PEG side chains were attached to a fluorenyl group.

**[0097]** For preparing a compound according to the reaction shown in **FIG. 17**, a similar procedure may be followed with the exception that an additional compound, Compound 9, is included in the initial mixture. Symbols  $a$ ,  $b$ ,  $c$ ,  $d$ , and  $r$  in **FIG. 17** also represent integers. The molar ratio between Compound 9 and all other constituents may be from 1:100 to 1:2, such as from 1:50 to 1:2. Generally,  $p$  may be from 1 to 10,  $q$  may be from 1 to 200,  $r$  ( $=d$ ) may be from 1 to 20, and  $n$  may be from 1 to 100 such as 1 to 20. The values of  $a$ ,  $b$ ,  $c$  and  $d$  may be selected according to the desired end products. As can be appreciated, when  $r=0$ , the compound in **FIG. 17** is the same as the compound shown in **FIG. 16**. In different embodiments, the sum of  $p+q$  may vary such as from 2 to 200.

**[0098]** The particles formed from the compounds prepared according to the reaction routes shown **FIGS. 10** or **17** can also have desired particle size distribution. For example, as shown in **FIGS. 18**, **19** and **20**, the particle sizes (effective diameters) of Compound 11, 12 or 13 in aqueous solutions were from about 10 to about 300 nm. The particle sizes were measured based on transmission electron microscopy (TEM) images of the particles. **FIGS. 22** and **23** show exemplary TEM images of fluorescent particles formed from Compound 10 and Compound 11, respectively. The samples for TEM imaging were prepared as

follows. A 90  $\mu\text{L}$  of particle aqueous solution was mixed with 10  $\mu\text{L}$  1% PTA aqueous solution by vortex. A drop of the mixture was then put onto a 400-mesh carbon-coated copper grid and air-dried overnight. The particles shown in **FIG. 22** had effective diameters ranging from 100 to 200 nm, and the particles shown in **FIG. 23** had effective diameters ranging from 10 to 20 nm and from 50 to 80 nm.

**[0099]** As discussed earlier, the particles prepared according to embodiments of the present invention can exhibit good stability in an aqueous environment. For example, the particles can remain stable for more than 6 months in water.

**[00100]** Some of the terms used herein can be understood with reference to "Glossary of basic terms in polymer science (IUPAC Recommendations 1996)", published in *Pure Appl. Chem.*, 1996, vol. 68, No.12, pp. 2287-2311, the entire contents of which are incorporated herein by reference.

**[00101]** As can be appreciated, embodiments of the present invention have various applications in different fields and industries. For example, the graft molecules and particles described above may be used as fluorescent probes, labels or tags in many fields such as biochemical fields. They are useful in drug and gene research, cell/microorganism imaging, disease diagnosis, analyte detection, and the like.

**[00102]** Other features, benefits and advantages of the embodiments described herein not expressly mentioned above can be understood from this description and the drawings by those skilled in the art.

**[00103]** Of course, the above described embodiments are intended to be illustrative only and in no way limiting. The described embodiments are susceptible to many modifications of form, arrangement of parts, details and order of operation. The invention, rather, is intended to encompass all such modification within its scope, as defined by the claims.

## WHAT IS CLAIMED IS:

1. A method of forming fluorescent particles, comprising:

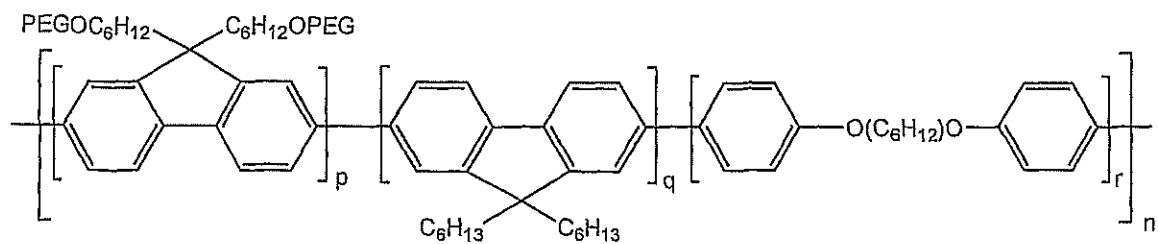
providing a solution comprising water, an organic solvent and an amphiphilic molecule dissolved in said organic solvent, said amphiphilic molecule comprising a plurality of backbone units forming a molecular backbone and a plurality of side chains grafted to said molecular backbone, with each one of said side chains formed as at least one side chain unit, at least one of said side chain units within said molecule being hydrophilic and at least three of said backbone units being hydrophobic and fluorescent, the weight ratio within said molecule of backbone units and side chain units that are hydrophilic to those that are hydrophobic being from about 1:4 to about 4:1, the concentration of said amphiphilic molecule in said solution being from about 1 to about 1000 CAC, wherein said CAC is the critical aggregation concentration of said amphiphilic molecule in said solution, and

removing said organic solvent from said solution, thus allowing said amphiphilic molecule to form said particles having a peripheral size from about 10 nm to about 10 microns.

2. The method of claim 1, wherein said concentration of said amphiphilic molecule in said solution is from about 10 to about 100 CAC.
3. The method of claim 1 or claim 2, wherein said solution is prepared by mixing water with a precursor solution that comprises said organic solvent and said amphiphilic molecule.
4. The method of any one of claims 1 to 3, wherein said removing comprises removing said organic solvent from said solution by evaporation.
5. The method of any one of claims 1 to 4, wherein said solution has a pH of about 2 to about 12, and is at a temperature from about 0 to about 80 °C.
6. The method of any one of claims 1 to 5, wherein said weight ratio is from about 3:7 to about 7:3.

7. The method of any one of claims 1 to 6, wherein said backbone units comprise fluorene units and said side chains comprise polyethyleneglycol.
8. The method of any one of claims 1 to 6, wherein said backbone units comprise an arylene, heteroarylene, arylene vinylene, heteroarylene vinylene, arylene ethylene, or heteroarylene ethylene unit, or a derivative thereof.
9. The method of claim 8, wherein said backbone units comprise a unit substituted with an alkyl, alkoxy, alkenyl, alkynyl, alkylsilyl, arylsilylaryl, heteroaryl, aryloxy, heteroaryloxy, alkylthio, alkylamino, dialkylamino, arylamino, diarylamino, aryl ether, heteroaryl ether, aryl thioether, heteroaryl thioether, halogen, cyano, nitro, carbonyl, thionyl, sulphonyl, or perfluoroalkyl group, or an amino group comprising a heteroaryl group.
10. The method of any one of claims 1 to 6, wherein said backbone units comprise a phenylene, thienylene, spirobifluorenylene, indenofluorenylene, pyridylene, bipyridylene, carbazoylene, indenocarbazoylene, benzothiazoylene, or oxadiazoylene unit, or a derivative thereof.
11. The method of any one of claims 1 to 10, wherein (1) at least one of said backbone units is linked to a vinylene group or an ethylene group, or (2) wherein at least two of said backbone units are linked with each other through a single carbon bond, a methylene group, or an atom selected from O, S, N, Si, and P.
12. The method of any one of claims 1 to 11, wherein said backbone units comprise a flexible group connecting two hydrophobic and fluorescent backbone units, said flexible group being hydrophilic or hydrophobic.
13. The method of any one of claims 1 to 12, wherein said side chain units comprise polyethyleneglycol, polyethyleneimine, polyamide, polyvinylpyrrolidone, polyacrylic acid, polyvinyl alcohol, polylysine, or derivatives thereof.
14. The method of any one of claims 1 to 13, wherein at least one of said side chains is bonded to said backbone through a single carbon bond, single phosphor bond, ether group, thioether group, amino group, imino group, silyl group, ester group, thioester group, amide group or an imide group.

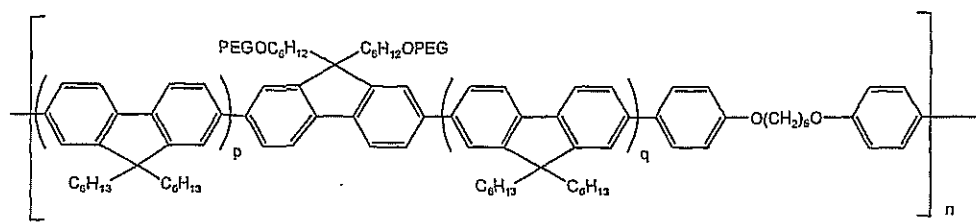
15. The method of any one of claims 1 to 5, wherein said amphiphilic molecule has the formula,



where  $p$ ,  $q$ ,  $r$ , and  $n$  are integers,  $p$  is from 1 to 10,  $q$  is from 1 to 200,  $r$  is from 0 to 20, and  $n$  is from 1 to 20.

16. The method of claim 15, wherein  $r = 0$ .

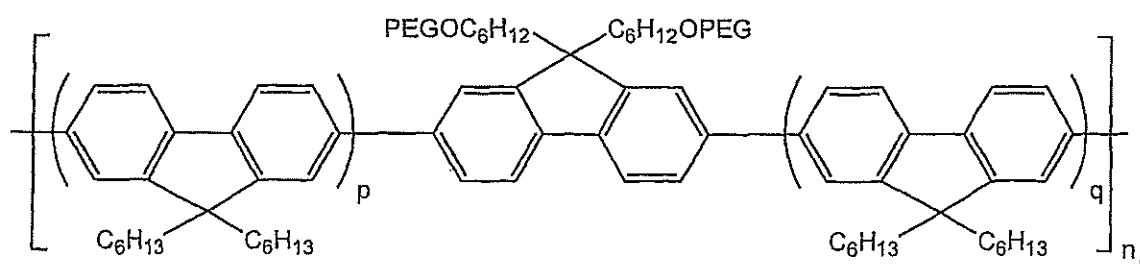
17. The method of any one of claims 1 to 5, wherein said amphiphilic molecule has the formula,



where  $p$ ,  $q$ , and  $n$  are integers,  $n$  is from 1 to 20, and  $p+q$  is from 2 to 200.

18. The method of claim 17, wherein  $p+q = 4$  or 10.

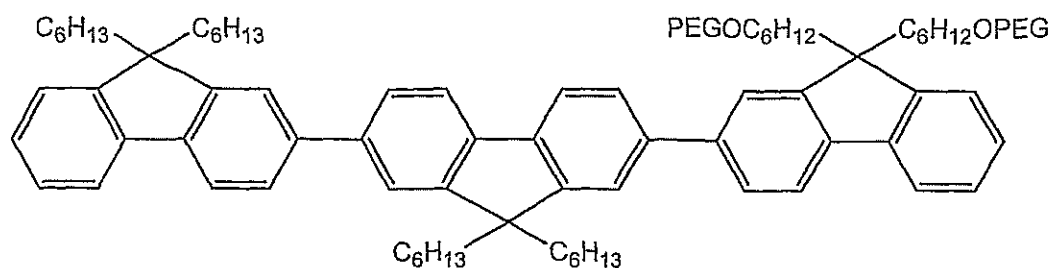
19. The method of any one of claims 1 to 5, wherein said amphiphilic molecule has the formula,



where  $p$ ,  $q$  and  $n$  are integers,  $n$  is from 1 to 20, and  $p+q$  is from 2 to 200.

20. The method of claim 19, wherein  $n = 1$ , and  $p = q = 1$  or  $p = q = 2$ .

21. The method of any one of claims 1 to 5, wherein said amphiphilic molecule has the formula,



22. The method of any one of claims 1 to 21, wherein said amphiphilic molecule is formed by:

forming precursors for said backbone units, said precursors comprising hydrophobic fluorescent groups;

grafting hydrophilic groups to said precursors, forming grafted precursors; and

linking said grafted precursors, thus forming said amphiphilic molecule.

23. The method of claim 22, wherein said linking comprises linking through a coupling reaction.

24. The method of claim 22, wherein said linking comprises linking through Suzuki coupling reaction, Grignard coupling reaction, Stille coupling reaction, Heck coupling reaction, Sologashira coupling reaction, oxidation polymerization reaction, reduction polymerization reaction, or polycondensation reaction.

25. A water-soluble fluorescent particle comprising the molecule formed according to the method of any one of claims 1 to 24.

26. A molecule comprising:

a plurality of backbone units forming a molecular backbone; and

a plurality of side chains grafted to said molecular backbone, with each one of said side chains formed as at least one side chain unit,

wherein at least one of said side chain units within said molecule is hydrophilic and at least three of said backbone units are hydrophobic and fluorescent, and

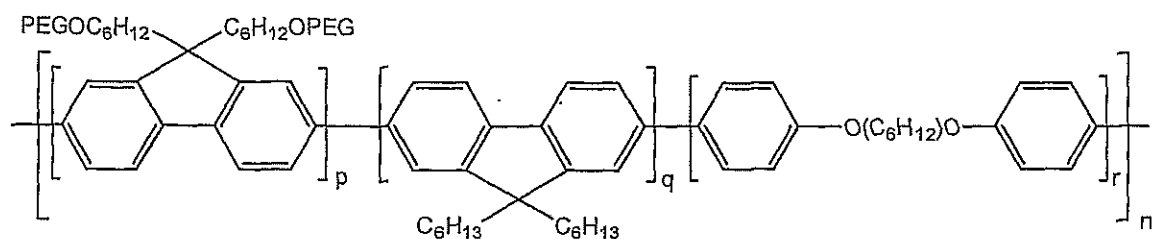
wherein the weight ratio within said molecule of backbone units and side chain units that are hydrophilic to those that are hydrophobic is from about 1:4 to about 4:1.

27. The molecule of claim 26, wherein said weight ratio is from about 3:7 to about 7:3.
28. The molecule of claim 26 or claim 27, wherein said backbone units comprise fluorene units and said side chains comprise polyethyleneglycol.
29. The molecule of claim 26 or claim 27, wherein said backbone units comprise an arylene, heteroarylene, arylene vinylene, heteroarylene vinylene, arylene ethylene, or heteroarylene ethylene unit, or a derivative thereof.
30. The molecule of claim 29, wherein said backbone units comprise a unit substituted with an alkyl, alkoxy, alkenyl, alkynyl, alkylsilyl, arylsilylaryl, heteroaryl, aryloxy, heteroaryloxy, alkylthio, alkylamino, dialkylamino, arylamino, diarylamino, aryl ether, heteroaryl ether, aryl thioether, heteroaryl thioether, halogen, cyano, nitro, carbony, thionyl, sulphonyl, or perfluoroalkyl group, or an amino group comprising a heteroaryl group.
31. The molecule of claim 26 or claim 27, wherein said backbone units comprise a phenylene, thienylene, spirobifluorenylene, indenofluorenylene, pyridylene, bipyridylene, carbazoylene, indenocarbazoylene, benzothiazoylene, or oxadiazoylene unit, or a derivative thereof.
32. The molecule of any one of claims 26 to 31, wherein (1) at least one of said backbone units is linked to a vinylene group or an ethylene group, or (2) wherein at least two of said backbone units are linked with each other through a single carbon bond, a methylene group, or an atom selected from O, S, N, Si, and P.
33. The molecule of any one of claims 26 to 32, wherein said backbone units comprise a flexible group connecting two hydrophobic and fluorescent backbone units, said flexible group being hydrophilic or hydrophobic.
34. The molecule of any one of claims 26 to 33, wherein said side chain units comprise polyethyleneglycol, polyethyleneimine, polyamide,

polyvinylpyrrolidone, polyacrylic acid, polyvinyl alcohol, polylysine, or derivatives thereof.

35. The molecule of any one of claims 26 to 34, wherein at least one of said side chains is bonded to said backbone through a single carbon bond, single phosphor bond, ether group, thioether group, amino group, imino group, silyl group, ester group, thioester group, amide group or an imide group.

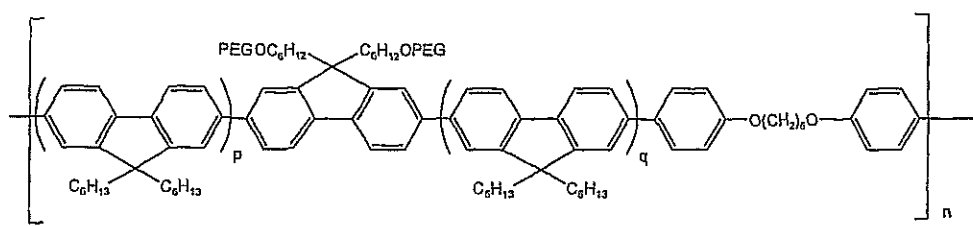
36. The molecule of claim 26, having the formula,



where p, q, r, and n are integers, p is from 1 to 10, q is from 1 to 200, r is from 0 to 20, and n is from 1 to 20.

37. The molecule of claim 36, wherein  $r = 0$ .

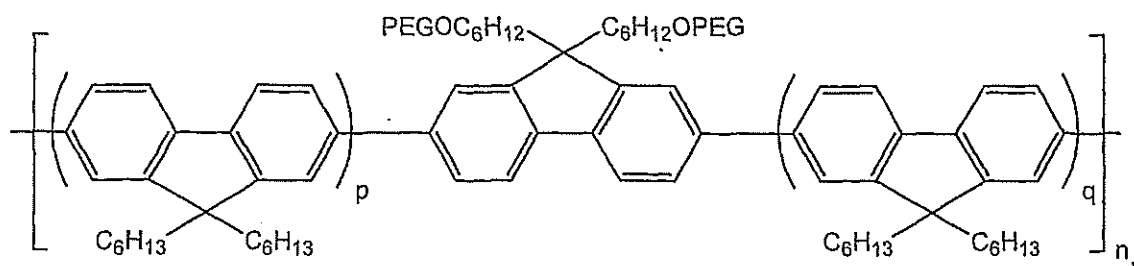
38. The molecule of claim 26, having the formula,



where p, q, and n are integers, n is from 1 to 20, and  $p+q$  is from 2 to 200.

39. The molecule of claim 38, where  $p+q = 4$  or 10.

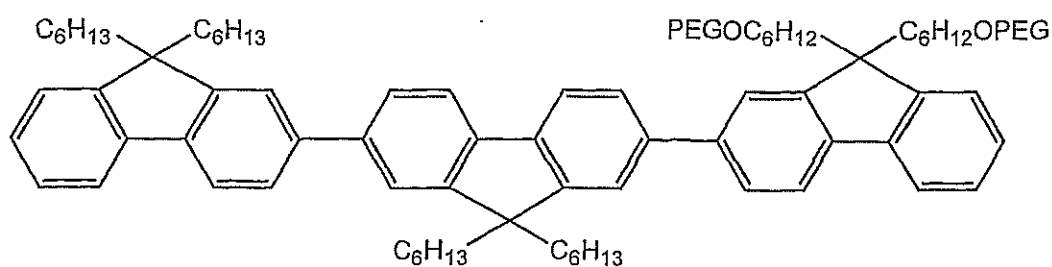
40. The molecule of claim 26, having the formula,



where  $p$ ,  $q$  and  $n$  are integers,  $n$  is from 1 to 20, and  $p+q$  is from 2 to 200.

41. The molecule of claim 40, wherein  $n = 1$ , and  $p = q = 1$  or  $p = q = 2$ .

42. The molecule of claim 26, having the formula,



43. A water-soluble fluorescent particle comprising the molecule of any one of claims 26 to 42.

44. The particle of claim 25 or claim 43, comprising a ligand having a specific affinity to a selected target.

45. The particle of claim 44, wherein said ligand is selected from avidin, biotin, antibody, antigen, and DNA, and wherein said target is selected from a target molecule, a cell and an organism.