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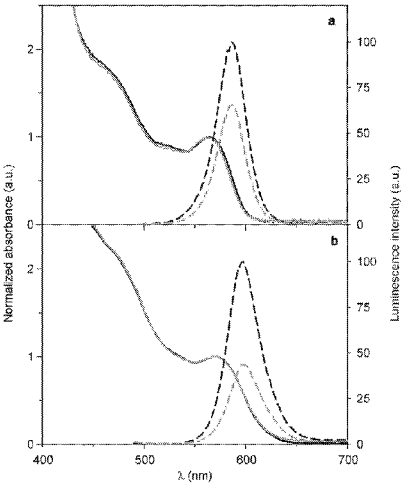

FIGURE 1

(57) Abstract: The present invention refers to the field of luminescent semiconductor nanocrystals (quantum dots). In particular, the present invention relates to quantum dots (QD) functionalized with ligands bearing a dithiolane group and an acid group salified with countercations, said QDs being able to solubilize in water and other polar solvents. The invention also relates to a method for the manufacturing of said quantum dots and to their possible uses and applications in biological, medical and other technical fields.

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METHOD FOR CONTROLLING SOLUBILITY OF QUANTUM DOTS

DESCRIPTION

TECHNICAL FIELD

The present invention refers to the field of luminescent semiconductor nanocrystals (quantum dots).

In particular, the present invention relates to the functionalization of quantum dots (QD) surface by exchanging the native ligands with other ligands capable of adjusting the solubility of said nanoparticle, in particular in water and other polar solvents.

BACKGROUND OF THE INVENTION

Quantum dots (QDs) are semiconductor nanocrystals endowed with unique optical and electronic properties, such that they are emerging as substitutes for molecular fluorophores in a variety of technological applications. QDs of, e.g., CdSe exhibit a high light absorption and an intense luminescence in the UV-visible region, whose wavelength can in both cases be modulated by adjusting the diameter of the particle. QDs are chemically and photochemically very stable, and are excellent two-photon absorbers. For these peculiar properties, QDs are employed in several applications related to the use of luminescence: (bio)chemical analysis, diagnostic imaging, medical therapy, photovoltaic solar cells, and LED devices for lighting and displays. In the past 5-10 years most fine chemicals companies (e.g., Sigma-Aldrich, Strem Chemicals) have introduced various kinds of QDs in their catalogues, and several small-medium enterprises have started with the mission of developing QDs tailored for specific applications in the above mentioned fields.

Synthetic methods that enable the preparation of QDs with accurate control of their properties afford nanocrystals whose surface is coated with a layer of highly hydrophobic molecular ligands. These QDs are therefore (moderately) soluble only in apolar organic solvents such as toluene, hexane or chloroform. Several applications of QDs, however - e.g., biological imaging or medical therapy - require water soluble nanocrystals; in general, the control of the solubility of QDs in common solvents is a crucial requirement for a viable processing of these nanomaterials. This objective can be reached with the functionalization of the nanocrystals' surface by appropriate molecular ligands (I. L. Medintz, H. T. Uyeda, E. R. Goldman, H. Mattoussi, "Quantum dot bioconjugates for imaging, labeling and sensing", *Nat. Mater.,* **2005,** 4, 435-446).

Another reason for modifying the capping layer of QDs is to link functional molecular units (e.g., receptors, fluorophores, switches, sensitizers, biomolecules) to the nanocrystal, with the aim of developing hybrid nanomaterials with predetermined properties.

A frequently used methodology for the above purposes involves the exchange of the hydrophobic native ligands with new functional (e.g., hydrophilic) ligands. Unfortunately, the optical properties and the solution stability of the nanoparticles are often dramatically worsened upon ligand exchange.

For applications that require hydrophilic nanocrystals, post-synthetic surface modification is a strict requirement. The surface can be made water-compatible following two main strategies: 1) exchange of the native ligands with capping agents that combine an anchoring group for the metal surface and a hydrophilic part; 2) encapsulation of the hydrophilic nanocrystals using amphiphilic molecules or polymers.

The first strategy (ligand exchange) allows for the preparation of hydrophilic nanocrystals with a compact shell layer (i.e. with small overall size, suitable for crossing biological barriers) but with reduced luminescence quantum yield (because of the non-optimal surface passivation obtained after ligand exchange).

The surface ligands should have two main functional domains: a hydrophilic moiety that allows the solubilization of nanocrystals in polar media, and an anchoring group which can bind the surface of the quantum dot. Thiols are widely employed anchoring groups. Examples of the first strategy include the substitution of the hydrophobic ligand with carboxy-terminated thiols, such as mercaptopropionic and mercaptoacetic acid and thiol-containing zwitterionic molecules, such as cysteine. The surface functionalization using monothiolated ligands, while it is a simple approach, affords nanocrystals with lower emission quantum yield (this is true in general for all the hydrophilic nanocrystals obtained following the first strategy) and poor long-term stability, due to the desorption of the ligands from the surface. Bis-thiol derivatives such as dihydrolipoic acid, dihydrolipoic acid coupled with poly(ethylene)glycol, multiple-thiols ligand greatly enhance the stability over a wide range of biological conditions while maintaining a small hydrodynamic radius. Ligands based on lipoic acid and related compounds, which contain the 1,2-dithiolane anchoring unit, have become increasingly popular, owing to their ability to form robust capping monolayers on the surface of metal and semiconductor nanoparticles. The high stability of the layer arises from the presence of two efficient surface binding sites in each anchoring moiety, generated by the rupture of the S-S bond of the dithiolane moiety. The cleavage of the disulfide bond occurs spontaneously in the presence of a noble metal surface, while it has to be activated in the case of semiconductor surfaces. Specifically, the dithiolane group is chemically reduced to bis-thiol. Therefore, all these types of ligands exploit dihydrolipoic acid (DHLA) as the anchoring group for the nanocrystals surface. DHLA is commonly synthesized from lipoic acid by reduction of the 1,2-dithiolane moiety.

The lipoic acid reduction is commonly carried out using a solution of $NaBH_4$ (A.F. Wagner, E. Walton, G. E. Boxer, M. P. Pruss, F. W. Holly, K. Folkers, "Properties and Derivatives of a-Lipoic Acid", *J. Am. Chem. Soc,* **1956,** 78, 5079-5081 ; C. Gunsalus, L. S. Barton, W. Gruber, "Biosynthesis and Structure of Lipoic Acid Derivatives", *J. Am. Chem. Soc,* **1956,** *78,* 1763-1766). The protocols currently available for the reduction of lipoic acid involve the breaking of S-S bond through reaction with $NaBH_4$ at low temperatures for several hours. The obtained product is then acidified and purified through extraction with water/toluene or water/chloroform (H. Mattoussi, J. M. Mauro, E.R. Goldman, G.P. Anderson, V.C. Sundar, F.V. Mikulec, M.G. Bawendi, "Self-Assembly of CdSe-ZnS Quantum Dot Bioconjugates Using an Engineered Recombinant Protein", *J. Am. Chem. Soc,* **2000,** *122,* 12142-121 50; H. T. Uyeda, I. L. Medintz, J. K. Jaiswal, S. M. Simon, H. Mattoussi, "Synthesis of Compact Multidentate Ligands to Prepare Stable Hydrophilic Quantum Dot Fluorophores", *J. Am. Chem. Soc,* **2005,** *127,* 3870-3878; A. R. Clapp, E. R. Goldman, H. Mattoussi, "Capping of CdSe-ZnS quantum dots with DHLA and subsequent conjugation with proteins", *Nat. Protoc,* **2006,** 1, 1258-1266; B. C Mei, K. Susumu, I. L Medintz, H. Mattoussi, "Polyethylene glycol-based bidentate ligands to enhance quantum dot and gold nanoparticle stability in biological media", *Nat. Protoc,* **2009,** 4,412-423). The reduced bis-thiol ligand needs to be stored in a refrigerator under inert atmosphere to prevent re-oxidation.

In some cases, the use of $NaBH_4$ as a reducing agent is not possible; for example, with functional ligands with metal ion receptors, which are therefore sensitive to metal ions.

An alternative, recently published method relies on the use of UV light to cleave the S-S bond of lipoic acid, thus generating the bis-thiol function (G. Palui, T. Avellini , N. Zhan, F. Pan, D. Gray, I. Alabugin, H. Mattoussi, "Photo-Induced Phase Transfer of Luminescent Quantum Dots to Polar and Aqueous Media", *J. Am. Chem. Soc,* **2012,** *134,* 16370-16378). This method avoids the use of borohydride chemicals and allows the ligand activation and QD functionalization in a single step, with a considerable advantage in terms of time. However, it requires a specific equipment (device for UV irradiation) and, more importantly, it cannot be used with photosensitive ligands.

Another type of ligand exchange involves the use of thiolated silanes. These ligands are directly absorbed on the QD surface after displacement of the native ligands (M. Bruchez Jr., M. Moronne, P. Gin, S. Weiss, A. P. Alivisatos, *Science,* **1998,** *281,* 2013-2016; D. Gerion, F. Pinaud, S. C. Williams, W. J. Parak, D. Zanchet, S. Weiss, A. P. Alivisatos, *J. Phys. Chem. B,* **2001,** *105,* 8861-8871). The addition of a base triggers the hydrolysis of the silanol groups with the formation of a silica-siloxane shell. Further addition of the siloxane precursor produces a much thicker shell with the aim of a better water compatibility.

Ligands for quantum dots bearing a dithiolane group and functionalized with PEG are known. See for example the following literature.

I. Yildiz, S. Ray, T. Benelli, F. M. Raymo, "Dithiolane ligands for semiconductor quantum dots", *J. Mater. Chem.,* **2008,** *18,* 3940-3947, discloses a quantum dot functionalized with ligands bearing a dithiolane group as an anchor and a PEG (polyethylene glycol) chain.

H. T. Uyeda, I. L. Medintz, H. Mattoussi, "Design of water-soluble quantum dots with novel surface ligands for biological applications", *Mater. Res. Soc. Symp. P.,* **2004** *789,* 111-116, discloses water-soluble quantum dots with oligo- and polyethylene glycol (PEG) based surface capping ligand.

H. T. Uyeda, I. L. Medintz, J. K. Jaiswal, S. M. Simon, H. Mattoussi, "Synthesis of Compact Multidentate Ligands to Prepare Stable Hydrophilic Quantum Dot Fluorophores", *J. Am. Chem. Soc,* **2005,** *127,* 3870-3878, discloses multidentate ligands for preparing stable hydrophilic quantum dots obtained by modification of thioctic acid with various lengths of PEG.

S. Mignani, J. Aszodi, D. Babin, M. Liutkus, O. Bedel, "Synthesis of new macromolecular, functionalized carboxylic-acid-PEG-DHLA surface ligands", *Tetrahedron Lett.,* **2010,** *51,* 5364-5367, discloses the synthesis of surface ligands for quantum dots functionalization containing a dihydrolipoic acid unit connected to a mono- or a diacid terminal function by a PEG chain.

Ligands with a dithiolane group which can be used for quantum dots are also disclosed in FR2925492 and in WO2013025347.

US6426513 discloses the superficial functionalization of semiconductor nanocrystals using ligand containing mono-thiols. As explained above, the use of mono-thiol ligands has some disadvantages in terms of stability of the nanocrystal, in particular in aqueous solution.

US6369098 discloses methods for synthetizing dithiolane derivatives, which are ligands for certain cell receptors and are useful in the treatment of a series of disorders.

US6649138 discloses a method for the water solubilization of semiconductor nanocrystals using amphiphilic polymers having a hydrophobic moiety able to interact with the nanocrystal through non-covalent bindings and a hydrophilic moiety able to promote the solubilization of the nanocrystals in a water environment. Said method of functionalization differs therefore from the method of ligand exchange, in particular since the interaction ligand-nanocrystal is of non-covalent type. Furthermore, the disclosed ligands are all polymers.

US6319426 discloses a water-soluble semiconductor nanocrystal including a semiconductor nanocrystal core, a shell-layer overcoating the core comprising a semiconductor material, and an outer layer comprising a molecule having at least one linking group for attachment of the molecule to the overcoating shell-layer and at least one hydrophilic group, optionally spaced apart from the linking group by a hydrophobic region sufficient to prevent electron charge transfer across the hydrophobic region. Different types of ligands for the solubilization in water of the nanocrystals are reported, in particular ligands having thiols groups for the anchoring to the nanocrystals surface. Also lipoic acid is disclosed and it is reduced using $NaBH_4$ according to a method known in the art (C. Gunsalus, L. S. Barton, W. Gruber, "Biosynthesis and Structure of Lipoic Acid Derivatives", *J. Am. Chem. Soc,* **1956,** *78,* 1763-1766).

The inventors of the present invention have found a method for the production of functionalized QDs, which involves the quick reduction of ligands based on dithiolane group, in particular lipoic acid, and their subsequent use in the functionalization of QDs through exchange with the native surface ligands. Said method is based on the use of a borohydride ion-exchange resin (N.M. Yoon, H.J. Lee, J.H. Ahn, J. Choi, "Selective reduction of alkyl halides with borohydride exchange resin-nickel acetate in methanol", *J. Org. Chem.,* **1994,** *59,* 4687-4688) for the reduction of the dithiolane unit.

US6888019 discloses a method of preparing a rhenium complex by use of a borohydride exchange resin, where the resin is used as a reducing agent for breaking the S-S bond of disulphide, thus converted into sulphide, which is then combined with rhenium. The obtained rhenium complexes are useful as radioactive pharmaceuticals. The compounds obtained by the disclosed method and their use are completely different from the compounds obtained in the present invention and their field of application.

The advantages in the use of the resin-supported reducing agent in the reduction of ligands for the functionalization of QDs are the followings:

1. Removal of the supported reactive by decantation or filtration; no additional extraction required;
2. Possibility to carry out the reaction at room temperature and in aerated solvents;
3. Quick reaction time (ca. 1 h compared to ca. 5 h for currently known procedures);
4. Possibility to quickly prepare just the required amount of reduced ligand for the immediate cap exchange, thus avoiding the storage of stock solutions of reduced ligand under inert atmosphere.

Furthermore, since the resin does not contain any metal cation, the strategy reported here is particularly appropriate for the activation of ligands that are sensitive to metal ions (e.g., because they contain metal ion receptors).

The inventors of the present invention have found that the use of the method of the invention for the reduction of ligands allows for the production of QDs covered with a reduced ligand in the form of a carboxylate ion and a layer of countercations non-covalently bound to the carboxylate ion.

Nag et al. ("Effect of metal ions on photoluminescence, charge transport, magnetic and catalytic properties of all-inorganic colloidal nanocrystals and nanocrystal solids", *J. Am. Chem. Soc.*, **2012,** *134,* 13604-13615) disclose colloidal semiconductor nanocrystals (NC) with anionic inorganic ligands covalently bound to their surface; the cationic parts of inorganic ligands are used to engineer the nanocrystal properties and impart additional functionalities. The authors show that said cationic species can be employed for engineering almost every property of all-inorganic NCs, in particular photoluminescence efficiency, electron mobility, doping, magnetic susceptibility and electrocatalytic performance. However, this document refers only to all-inorganic NC, i.e. only NC with anionic inorganic ligands. The problem of solubility is not addressed not even mentioned in the paper.

NCs with a coating of cationic ions are also disclosed in the work of Kovalenko et al. ("Nanocrystal superlattices with thermally degradable hybrid inorganic-organic capping ligands", *J. Am. Chem. Soc,* **2010,** *132,* 15124-1 5126). NCs functionalized with metal chalcogenide complexes (MCCs) are turned from highly hydrophilic to nonpolar and lipophilic using cationic surfactant molecules which bind to the negatively charged surfaces forming dense hydrophobic monolayers. To form the hydrophobic coating, Na^+ or NH_4^+ counterions are partially replaced with different tertiary alkylammonium ions; other long-chain cationic surfactants can be used. Said cationic surfactants are therefore used to make the MCC-capped NCs compatible with common nonpolar molecules, therefore soluble in nonpolar solvents. The problem of solubility is therefore mentioned in this paper only with regard to non-polar solvents and the cationic molecules are used for making the NCs surface hydrophobic. Also in this document, the disclosed ligands are all inorganic.

Different approaches have been tried for modulating solubility of nanocrystals. For example, oligo(ethyleneglycol)-based molecular or polymeric ligands afford NCs that are soluble in various solvents depending on chain length (H. T. Uyeda, I. L. Medintz, J. K. Jaiswal, S. M. Simon, H. Mattoussi, "Synthesis of Compact Multidentate Ligands to Prepare Stable Hydrophilic Quantum Dot Fluorophores", *J. Am. Chem. Soc,* **2005,** *127,* 3870-3878; I. Yildiz, B. McCaughan, S. F. Cruickshank, J. F. Callan, F. M. Raymo, "Biocompatible CdSe-ZnS Core-Shell Quantum Dots Coated with Hydrophilic Polythiols", *Langmuir,* **2009,** *25,* 7090-7096). Modulation of solubility by this approach, however, requires a large synthetic effort because, for each targeted solvent, a specific ligand type has to be prepared and successively used for the NCs functionalization. Moreover, since these ligands often have a high molecular weight, they increase substantially the hydrodynamic radius of resulting NCs, often posing problems in biological applications.

In view of the above, there is still the need of a method for controlling and efficiently modulating the solubility of quantum dots, in particular in polar solvents; more in particular, a method for easily obtaining quantum dots soluble in different solvents without substantially impair their properties is still needed.

It has surprisingly been found that the functionalization of QDs with different countercations, in particular using the method of the present invention, allows for the modulation and control of the solubility of the QDs in aqueous solution and in other polar solvents. In fact, the functionalized QD has different solubility properties in different polar solvents depending on the type of countercations with which it is functionalized.

SUMMARY OF THE INVENTION

It is an object of the present invention a quantum dot functionalized with ligands bearing a dithiolane group and an acid group salified with countercations, wherein said countercations are selected from the group consisting of Na^+, Li^+, K^+, Zn^{++}, Fe^{++}, Cu^{++}, **C-i-Cs** tetralkylammonium.

When the countercation is a C_1-C_8 tetralkylammonium, the alkyl chains can be the same or different, linear or branched. Preferably, the tetralkylammonium is selected from the group consisting of: tetramethylammonium (TMA^+), tetrabutylammonium (TBA^+), tetraethylammonium (TEA^+) and tetraoctylammonium (TOA^+).

Said ligand comprises a moiety with a dithiolane group, binding the quantum dot, and a moiety with a salified acid group.

Said salified acid group can be a carboxylate, a sulfonate or a phosphate group. Preferably, it is a carboxylate group.

Preferably, said ligands are ligands based on lipoic acid.

A method for manufacturing said quantum dot is also an object of the present invention. Said method comprises the following steps:

a. adding a resin loaded with BH_4^- to a solution of a ligand bearing a dithiolane group and a salifiable acid group in a first solvent, said solvent dissolving said ligand and not dissolving said resin;
b. removing the solvent in order to obtain the resin with the attached ligand;
c. adding a solution of a base or a salt thereof to said resin, wherein the cation of said base or salt is selected from the group consisting of Na^+, Li^+, K^+, Zn^{++}, Fe^{++}, Cu^{++}, **C-i-Cs** tetralkylammonium, and stirring to extract the reduced ligand from the resin;
d. washing the resin with said first solvent to obtain a solution containing said reduced ligand;
e. adding quantum dots covered with hydrophobic ligands, said quantum dots being in a solid form or dissolved in a second solvent not miscible with said first solvent, to the solution of step d), thus obtaining a biphasic mixture;
f. shaking said mixture to allow the transfer of said quantum dots to said solution

to allow exchange of native hydrophobic ligands with the reduced, hydrophilic ones;

g. isolating said quantum dots.

In an embodiment of the invention, the quantum dots added in step e) are dissolved in a second solvent not miscible with said first solvent; a biphasic mixture is thus obtained. In this embodiment, when said mixture is shaken in step f) the quantum dots are transferred from said second solvent to the solution of step d), typically forming a suspension. The second solvent is then removed and the suspension is washed with said second solvent. After separation of the solvents, the first solvent can be removed and the QDs isolated (step g).

In a different embodiment, said quantum dots added in step e) are in a solid form. In said embodiment, shaking of the mixture (step f) allows the transfer of the solid quantum dots to the solution containing the reduced, hydrophilic ligands, typically forming a suspension. Said suspension can then be washed with a second solvent not miscible with the first solvent of said solution. After separation of the solvents, the first solvent can be removed and QDs isolated (step g).

Said method allows for the preparation of quantum dots covered with the desired hydrophilic ligands and countercations, which can be then solubilized in water or other polar solvent.

Examples of polar solvents in which said QDs can be solubilized are water, methanol, acetone, acetonitrile.

Depending on the cation of said base or salt used in step c) of the method of the invention, quantum dots with different characteristics of compatibility with polar solvents can be obtained.

The QDs obtained with the method of the present invention maintain largely their optical properties and are stable in solution for a long time.

The use of the quantum dots of the invention as luminescent probes/labels in biology and medical diagnostics, as components of photosensitizers for photodynamic therapy, in light absorbing materials for solar cells and in light emitting materials for lighting and display technologies is also within the scope of the present invention.

The present invention will be now disclosed in detail also by means of examples.

DESCRIPTION OF THE INVENTION

Definitions

Within the context of the present invention, the term "quantum dot" means a semiconductor nanocrystal with size-dependent optical and electronic properties. The term "nanocrystal" is used in the present invention as a synonym of "quantum dot". Within the context of the present invention, "ligand" means a molecule able to bind to the surface of a quantum dot.

Figures

Figure 1. a) Absorption (full line) and emission (λ_{exc} = 485 nm; dashed line) spectra of TOP/TOPO CdSe-3ZnS QDs (core diameter 3.4 nm) in $CHCl_3$ (black) and $DHLA^-Na^+$ capped in H_2O (grey). b) Absorption (full line) and emission (λ_{exc} = 480 nm; dashed line) spectra of TOP/TOPO CdSe-5ZnS QDs (core diameter 3.6 nm) in $CHCl_3$ (black) and $DHLA^-Na^+$ capped in H_2O (grey).

Figure 2. Absorption (full lines) and emission (A_{exc} = 480 nm; dashed lines) spectra of CdSe-5ZnS QDs (core diameter 3.6 nm, 130 nM) capped with $DHLA^-Na^+$ in water, freshly prepared (black) and after 21 days of storage at 5°C (grey). The inset shows the evolution of the luminescence quantum yield over time.

Figure 3. Absorption (full lines) and emission (dashed lines) spectra of CdSe-3ZnS QDs (core diameter 2.9 nm) TOP/TOPO capped in $CHCl_3$ (black lines), DHLA7Na $^+$ capped in H_2O (light gray lines) and DHLA7TMA $^+$ capped in H_2O (dark gray lines). Aexc = 420 nm.

Figure 4: Photographs of 0.5 μM CdSe-5ZnS QDs capped with $DHLA^-TBA^+$ in different solvents under ambient light (top) and UV light (A_{exc} = 365 nm, bottom).

Figure 5. Absorption changes observed upon addition of BH_4^- resin (2 equivalents) to a methanol solution of lipoic acid $1.6x10^{-2}$ M (total stirring time, 20 min).

Figure 6. a) Absorption spectra of a lipoic acid/DHLA methanol solution before (full line) and after (dashed line) addition of TMAOH. Part (b) shows a magnification of the region of the S-S absorption band peaking at 330 nm.

Figure 7. a) Absorption spectrum of a lipoic acid/DHLA solution soon after the addition of the base TMAOH (dashed line), and changes observed on stirring for up to 20 min (full line). b) Absorption spectra of lipoic acid before reduction (full line), and after reduction and base extraction (dashed line).

Figure 8. Absorption spectrum of $1.6x10^{-2}$ M lipoic acid in methanol before (a) and after the addition of BH_4^- resin (2 equivalents) and 30 min stirring (b). Curve (c) is the spectrum obtained upon treating the mixture in (b) with NaOH (2 equivalents with respect to lipoic acid) and 30 min stirring.

Detailed description of the invention

A quantum dot includes a "core" of one or more first semiconductor materials, which can be surrounded by a "shell" of a second semiconductor material. A semiconductor nanocrystal core surrounded by a semiconductor shell is referred to as a "core/shell" semiconductor nanocrystal. The core and/or the shell can be a semiconductor material including, but not limited to, those of the group II-VI (e.g., ZnS, ZnSe, ZnTe, CdS, CdSe, CdTe, HgS, HgSe, HgTe, MgTe and the like) and III-V (e.g., GaN, GaP, GaAs, GaSb, InN, InP, InAs, InSb, AIAs, AIP, AlSb, AIS, and the like) and IV (e.g., Ge, Si, Pb and the like) materials, and an alloy thereof, or a mixture, including ternary and quaternary mixtures, thereof.

The core can be synthesized using the published procedure developed by Peng and coworkers (Z. A. Peng, X. Peng, "Formation of High-Quality CdTe, CdSe, and CdS Nanocrystals Using CdO as Precursor", *J. Am. Chem. Soc,* **2001 ,** *123,* 183-1 84). The shell overcoating reaction can be carried out using either the successive ion layer adsorption and reaction (SILAR) (J. J. Li, Y. A. Wang, W. Guo, J. C. Keay., T. D. Mishima, M.B. Johnson, X. Peng, "Large-Scale Synthesis of Nearly Monodisperse CdSe/CdS Core/Shell Nanocrystals Using Air-Stable Reagents via Successive Ion Layer Adsorption and Reaction", *J. Am. Chem. Soc,* **2003,** *125,* 12567-12575) or the one-time-precursors-injection approach (M. A. Hines, P. Guyot-Sionnest, "Synthesis and Characterization of Strongly Luminescing ZnS-Capped CdSe Nanocrystals", *J. Phys. Chem,* **1996,** *100,* 468-471 ; B. O. Dabbousi, J. Rodriguez-Viejo, F. V. Mikulec, J. R. Heine, H. Mattoussi, R. Ober, K. F. Jensen, M. G. Bawendi, "(CdSe)ZnS Core-Shell Quantum Dots: Synthesis and Optical and Structural Characterization of a Size Series of Highly Luminescence Materials", *J. Phys. Chem.* B, **1997,** *101,* 9463-9475). Core/shell semiconductor nanocrystals can be prepared in high-boiling point non-coordinating organic solvents using a two step approach in which a relatively thick shell is grown onto core nanocrystals synthesized in the first step. The resulting nanocrystals are covered with hydrophobic ligands, for example TOPO (tris-octylphosphineoxide), TOP (trioctylphosphine), OIA (oleic acid), ODA (octadecylamine) and/or HDA (hexadecylamine); they act as passivating hydrophobic surface agents, preventing particle aggregation.

In particular, CdSe, CdTe and CdS are quantum dot cores more suitable for the present invention.

ZnS, CdS and ZnSe are quantum dot shells particularly suitable for the present invention.

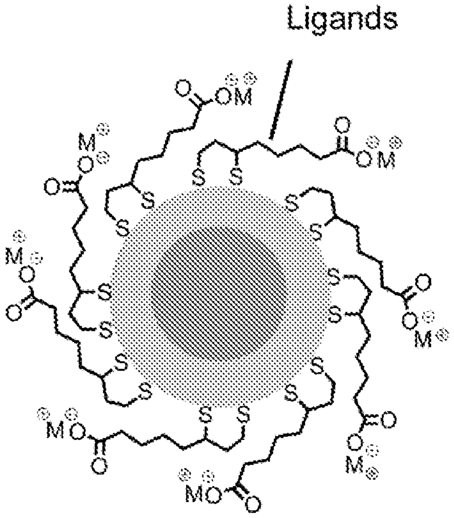
Preferably, the QD core is CdSe and the shell is ZnS.

The QD can vary in size and shell thickness depending on the number of shell monolayers. Preferably, the number of shell monolayers is 3 or 5.

In a preferred embodiment, the QDs are prepared in high-boiling point non-coordinating organic solvents using a two step approach in which a relatively thick ZnS shell is grown onto CdSe core nanocrystals synthesized in the first step. The resulting nanocrystals are covered with TOPO (tris-octylphosphineoxide) and TOP (trioctylphosphine), OIA (oleic acid), ODA (octadecylamine) and/or HDA (hexadecylamine). Said QDs are then used in step e) of the method of the invention to obtain the quantum dots of the invention.

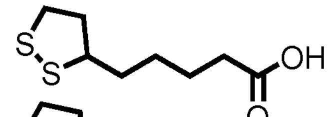
The quantum dot of the present invention is covered with hydrophilic ligands bearing a dithiolane group and a salified acid group and countercations.

A schematic example of a quantum dot of the invention is the following (M^+ = countercation):

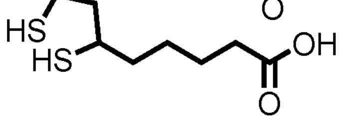


Preferably, the ligands are ligands based on lipoic acid.

More preferably, the ligand is lipoic acid, which is reduced to di-hydro lipoic acid (DHLA) in order to bind to the quantum dot.



Lipoic acid



Di-hydro lipoic acid (DHLA)

The QD of the present invention is characterized in that it is soluble in polar solvent and, in particular, in that its solubility can be modulated by the type of surface functionalization. In fact, according to the countercation with which the QD surface is functionalized, the QD is endowed with specific characteristics of compatibility with different polar solvents.

More in detail, it is possible to tune the solubility of the QDs by changing the type of salt or base used in the extraction step (step c) of the method of the invention, in particular by changing the countercation of said salt or base.

With respect to known methods, the method of the invention has in fact the further advantage to allow the modulation of the solubility of the QDs in different polar solvents by simply changing the kind of base (and thus of countercation) to be used in the method. This avoids the effort of synthesizing different QDs with different ligands according to the solvent, as done in the prior art. Importantly, the use of the method of the invention does not substantially change or alter the properties of the functionalized quantum dots.

The quantum dots of the present invention are obtained by the method above described, comprising the steps a)-g), which is also an object of the present invention. Said method will be now described more in detail.

In step a) of said method, a resin loaded with BH_4^- is added to a solution of the ligand bearing a dithiolane group and a salifiable acid group in a first solvent.

Preferably, said first solvent is selected from the group consisting of methanol, ethanol or water.

The molar ratio between the ligand and BH_4^- in step a) is preferably of 1:2. The obtained mixture is stirred for a time sufficient for the ligand to go inside the resin, react and stick to the resin. Said stirring time is at least 30 min, preferably higher than 30 min.

The resin loaded with BH_4^- is commercially available and can be purchased or otherwise prepared with methods known in the art. For example, it can be prepared starting from a commercially available anion-exchange resin loaded with anions CP, where said anions are subsequently exchanged with anions BH_4^- using an aqueous solution of $NaBH_4$ (N.M. Yoon, H.J. Lee, J.H. Ahn, J. Choi, "Selective reduction of alkyl halides with borohydride exchange resin-nickel acetate in methanol", *J. Org. Chem.,* **1994,** 59, 4678-4688). This second option is economically more advantageous.

Said resin is preferably in the form of beads.

In step b), the solvent is removed and the resin with the attached ligand is obtained. Said resin can be optionally washed with the solvent, to remove the unreacted ligand

and the hydrolyzed borohydride products.

In step c), a base or a salt thereof is added to the resin, preferably in a ratio ranging from 1.2 to 4 equimolar with respect to the BH_4^- content of the resin. The mixture is stirred for a suitable time to extract the reduced ligand from the resin; preferably said time is 30 minutes.

When a base is used, it is a Bronsted base. Preferably, it is a hydroxide.

When a salt is used, it is preferably a triflate ($CF3SO3^-$), a bromide (Br^-) or a perchlorate (ClO_4^-) salt.

The cation of said base or salt thereof is selected from the group consisting of Na^+, Li^+, K^+, Zn^{++}, Fe^{++}, Cu^{++}, C_1-C_8 tetralkylammonium.

When the cation is a C_1-Cs tetraalkylammonium cation, it is preferably selected from the group consisting of tetramethylammonium (TMA^+), tetraethylammonium (TEA^+), tetra(n-butyl)ammonium (TBA^+) and tetra(n-octyl)ammonium (TOA^+). A more preferred cation is TBA^+.

The resin is then washed with the solvent (step d). Optionally, said washing with the solvent can be repeated in order to recover as much reduced ligand as possible; all the solvent fractions are then joined together and the residual solid are discarded.

In step e), the quantum dots covered with hydrophobic ligands, obtained as above described, are added to the solution of step d) containing the reduced ligand.

The amount of the added QD in step e) may vary between 1/20000 and 1/30000 QD/ligand ratio, depending on the QD size.

The quantum dots covered with hydrophobic ligands can be added to the solution of step d) in a solid form or dissolved in a second solvent not miscible with said first solvent.

A biphasic mixture is thus obtained.

Said mixture is shaken to allow the transfer of the QDs to the solution containing the reduced ligand to allow exchange of the native hydrophobic ligands with the hydrophilic, reduced ligands (step f). It can be further stirred for a suitable time, preferably overnight, to allow a complete ligands exchange.

The obtained QDs are then isolated (step g).

Isolation of QDs can be done according to the general knowledge of the skilled person.

For example, in case the QDs are added in solid form, once they are transferred to the solution containing the reduced, hydrophilic ligands, typically a suspension is formed. The suspension can be washed with a second solvent not miscible with the first solvent of said solution in order to remove unreacted QDs and native hydrophobic ligands. After separation of the solvents, the first solvent can be removed, preferably under reduced pressure, and QDs isolated.

In another exemplary embodiment, in case QDs are added dissolved in a second solvent, not miscible with the first solvent, the QDs, once transferred to the solution containing reduced, hydrophilic ligands, typically form a suspension. The non-miscible solvents are separated and the suspension can be treated as described above.

Preferably, said second solvent is hexane.

Quantum dots covered with hydrophilic ligands and countercations according to the present invention are thus obtained.

Said QDs can then be dissolved in water or in other polar solvent thus obtaining a solution of QDs.

Said solution of QDs can be filtered to remove possible large aggregates. For example, a syringe filter may be employed. Said solution can be further purified by removing the reduced ligand in excess with cycles of dilution/concentration. Preferably, the number of said cycles is 3 and a centrifugal filter is preferably employed.

A concentrated solution of quantum dots covered with hydrophilic ligands and countercations is thus obtained.

The method of the invention above described is preferably carried out at a temperature ranging from 20 to 60°C.

The concentration of the ligand in step a) is preferably lower than 50 mM.

The QD/ligand ratio is preferably comprised between 1/20000 and 1/30000.

In a preferred embodiment, the QD is functionalized with dihydrolipoic acid (DHLA), as the reduced ligand, and Na^+ as the countercation.

In a more preferred embodiment, the QD is a DHLA7Na^+-coated core-shell CdSe-ZnS QD.

The present invention also provides a method for controlling solubility of quantum dots characterized in that when the solvent is water the acid group of the ligand according to the invention is salified with a countercation selected from the group consisting of Na^+, Li^+, K^+, TMA^+, TEA^+ and TBA^+.

The present invention also provides a method for controlling solubility of quantum dots characterized in that when the solvent is dimethyl sulfoxide (DMSO) the acid group of the ligand according to the invention is salified with TBA^+.

The present invention also provides a method for controlling solubility of quantum dots characterized in that when the solvent is methanol the acid group of the ligand according to the invention is salified with a countercation selected from the group consisting of K^+, TMA^+, TEA_4^-, TBA^+ and TOA^+.

The present invention also provides a method for controlling solubility of quantum dots characterized in that when the solvent is acetonitrile the acid group of the ligand according to the invention is salified with a countercation selected from the group consisting of TEA_4^- and TBA^+.

The present invention also provides a method for controlling solubility of quantum dots characterized in that when the solvent is acetone the acid group of the ligand according to the invention is salified with TBA^+.

The quantum dot of the present invention can be used as luminescent probe/label for *in vitro* biological applications; for example, for biochemical analysis.

In another embodiment, the quantum dots of the invention can be used as luminescent probes/labels in medical diagnostics and/or for medical imaging.

In a further embodiment of the invention, the quantum dot can be used as a component of photosensitizers for photodynamic therapy. Photodynamic therapy is a form of phototherapy used for treating a variety of medical conditions, wherein light-sensitive compounds are exposed selectively to light.

A photosensitizer comprising the quantum dot of the invention is also within the scope of the present invention.

The quantum dots of the invention can also be included in light absorbing materials; said materials can be used, for example, for the manufacturing of solar cells.

A light absorbing material comprising the quantum dot of the invention is also an object of the present invention.

In a further embodiment, the quantum dots are included in light emitting material, which can be used for lighting apparatus and displays. For this application of the quantum dots, reference can be made to Y. Shirasaki, G. J. Supran, M. G. Bawendi, V. Bulovic, "Emergence of colloidal quantum-dot light-emitting technologies", *Nat. Photonics,* **2013,** 7, 13-23, and references therein.

A light emitting material comprising the quantum dot of the invention is also an object of the present invention.

The following examples will further illustrate the invention.

EXAMPLES

Example 1

Reduction of lipoic acid and quantum dot cap exchange

The preparation of the quantum dots was carried out using the following procedure:

1) The reduction of lipoic acid is achieved by stirring 5.5 mg of lipoic acid (2.66x10^{-5} mol) with 19 mg of BH_4^- resin (2.7 mmol BH_4^- per g) in 500 µL of methanol at 400 rpm for at least 30 min. A longer stirring time afforded a better reduction. During this time, the lipoic acid goes inside the resin, reacts and sticks into it.

2) The methanol layer was removed and the beads were washed 3 times with 500 µL of methanol. The purpose of this operation is to remove unreacted lipoic acid and hydrolyzed borohydride products.

3) Methanol (500 µL) and NaOH (from 1.2 to 4 eq with respect to the BH_4^- content) were added to the beads. The mixture was stirred for 30 min in order to extract the reduced lipoic acid from the resin.

4) The beads were washed 2 times with 200-300 µL of fresh methanol, in order to recover as much reduced ligand as possible. All the methanol fractions were joined together, and the residual solid was discarded.

5) The desired amount of QDs (from 1/20000 to 1/30000 QD/lipoic acid ratio, depending on the QD size) was dissolved in 1000 µL of hexane. The QD solution was added to the vial containing the reduced ligand in methanol, thus forming a biphasic mixture.

6) Shaking of the biphasic system results in the fast transfer of the QDs from the hexane to the methanol phase. The resulting methanol suspension appeared turbid. The mixture was further stirred overnight to allow complete exchange of the native hydrophobic ligands with the new hydrophilic ones.

7) The hexane layer (turned colorless) was removed, and the methanol suspension was washed 5 times with hexane (2 mL) in order to remove unreacted nanocrystals and native hydrophobic ligands.

8) The methanol solvent was removed under reduced pressure and resulting dried QDs were dissolved in water.

9) The mixture was first passed through a syringe filter (0.46 µm pore size) to remove possible large aggregates, and was successively purified with 3 cycles of

dilution/concentration with a centrifugal filter (Millipore, 30 kDa, 7000 rpm, 12 minutes for each cycle) to eliminate the excess of free dihydrolipoic acid (B. C Mei, K. Susumu, I. L. Medintz, H. Mattoussi, "Polyethylene glycol-based bidentate ligands to enhance quantum dot and gold nanoparticle stability in biological media", *Nat. Protoc,* **2009,** 4, 412-423). A relatively concentrated water solution of QDs (from 5 to 10 μM) was eventually obtained.

The borohydride-loaded resin was prepared following the protocols reported in literature (N. M. Yoon, H. J. Lee, J. H. Ahn, J. Choi, "Selective reduction of alkyl halides with borohydride exchange resin-nickel acetate in methanol", *J. Org. Chem.,* **1994,** 59, 4687-4688). The amount of BH_4^- loaded, estimated by acid titration, is in agreement with that reported for a commercially available borohydride-loaded resin (Sigma-Aldrich, Borohydride on Amberlite® IRA-400, Catalog Number: 328642). In all cases, we used a resin loaded with 2.7 mmol of BH_4^- per gram of resin.

The functionalization with DHLA7Na$^+$ using the methodology reported above enabled us to dissolve in water different types of core and core-shell semiconductor nanocrystals. DHLA7Na$^+$-coated core-shell CdSe-ZnS QDs of various size and different shell thickness (3 monolayers and 5 monolayers) yielded clear water solutions which resulted to be stable for at least 3 months. Only a very minor shift in the absorption and emission peak wavelengths was observed with respect to the starting hydrophobic QDs, indicating that no aggregation takes place and the spectroscopic properties of the final products are preserved. The luminescence efficiency of the final QDs in aqueous solution is 30%-50% of that of the starting nanoparticles in organic solvent, as widely reported in literature (A. R. Clapp, E. R. Goldman, H. Mattoussi, "Capping of CdSe-ZnS quantum dots with DHLA and subsequent conjugation with proteins", *Nat. Protoc,* **2006,** 1, 1258-1266). We found that CdSe QD cores are not emissive after phase transfer, again in line with literature reports. The time required for complete transfer from the hexane to the methanol phase, the solubility and stability in water of the hydrophilic QDs, and the photophysical properties of the aqueous solutions are reported in Table 1.

Table 1

	Phase transfer time (min)	Solubility in H_2O	Stability in H_2O	Absorption peak shift (nm)	Emission peak shift (nm)
CdSe-3ZnS green	< *1*	Yes	≈ *3 months*^{b,c}	0 nm	1 nm
CdSe-5ZnS orange	< *1*	Yes	≈ *3 months*^{b,c}	0 nm	1-2 nm
CdSe-3ZnS red(1)	< *1*	Yes	≈ *3 months*^{b,c}	1 nm	0 nm
CdSe-3ZnS red(2)	< *1*	Yes	≈ *3 months*^{b,c}	2 nm	1 nm

Table 1. Photophysical properties of different semiconductor nanocrystals capped with DHLA7Na$^+$ in water. CdSe-ZnS red (1) and (2) were synthesized from two different reactions. Particle diameter: CdSe-3ZnS green, 4.6 nm; CdSe-5ZnS orange, 6.6 nm; CdSe-3ZnS red(1), 5.9 nm; CdSe-3ZnS red(2), 5.4 nm. [b] A red solid crashes out from the solution with time; this is most likely due to protonation of the lipoic acid on the QD surface, as confirmed by the prompt dissolution of the precipitate upon addition of NaOH. [c] The solid showed luminescence under the UV lamp.

The obtained QDs are compared with CdSe-5ZnS orange TOP/TOPO capped, which are the QDs covered with the native hydrophobic ligands. Figure 1 shows the absorption and emission spectra of CdSe-3ZnS and CdSe-5ZnS orange capped with DHLA7Na$^+$ in water, compared with the same nanocrystals TOP/TOPO capped in CHCl3. As it can be seen in figure 1, the absorption spectrum of the QDs capped with DHLA7Na$^+$ is unchanged with respect to the TOP/TOPO capped QDs, thus confirming that the functionalized QDs are intact and their absorption properties are not modified. The emission intensity of the DHLA7Na$^+$ QDs is 30-50% lower than that of the TOP/TOPO capped QDs, as expected and already known in literature for QDs covered with hydrophilic ligands.

The long term stability of DHLA7Na$^+$ capped QDs prepared as above described was evaluated. For example, a dilute solution (130 nM) of the nanocrystals of Figure 1b (CdSe-5ZnS QDs DHLA7Na$^+$ capped) in deionized water was stored in a refrigerator at 5°C, and the absorption and luminescence spectra were monitored over 3 weeks (Figure 2). No precipitation was observed, although the emission quantum yield decreased from 0.081 to 0.05 during the first two weeks, in line with literature reports for DHLA-capped QDs (D. Liu, P. T. Snee, "Water-Soluble Semiconductor Nanocrystals Cap Exchanged with Metalated Ligands", *ACS Nano,* **2011,** 5, 546-550).

Example 2

Effect of the anion of the salt used to extract DHLA from the resin

We explored the influence of the nature of the counteranions of the sodium salt used for the extraction of reduced lipoic acid (DHLA) from the resin (step 3 of the protocol of example 1). The procedure for the production of the QDs was the same as that reported in example 1. Different sodium salts were used in the place of sodium hydroxide mentioned in step 3 of the protocol of example 1. CdSe-ZnS core-shell nanocrystals were used in these experiments. The results are gathered in Table 2.

Table 2

Sodium salt	**Phase transfer**	**Solubility in H_2O**	**Absorption peak shift (nm)**	**Emission peak shift (nm)**
NaCl	No	No	/	/
$NaBH_4$	No	No	/	/
NaBr	Yes	Yes	1 nm	2 nm
Na_2CO_3	Yes	Yes	1 nm	2 nm
CH_3COONa	Yes	Yes	2 nm	2 nm
$NaCF_3SO_3$	Yes	Yes	2 nm	1 nm
$NaClO_4$	Yes	Yes	0 nm	1 nm
NaOH	Yes	Yes	0 nm	2 nm

Table 2. Photophysical properties of CdSe-5ZnS QDs in water capped with $DHLA^-/Na^+$, extracted from the resin using different sodium salts.

When NaCl or $NaBH_4$ was employed in the extraction, no phase transfer was observed. Sodium carbonate and acetate afford an incomplete extraction of the DHLA ligand and consequently a partial cap exchange. Triflate, bromide and perchlorate salts allow a complete extraction and consequently afford cap-exchanged nanocrystals endowed with stability in water and photophysical properties similar to those obtained using sodium hydroxide. Sodium bromide leads to a complete cap exchange but longer stirring time is required.

Example 3

Effect of the cation of the base or salt used to extract DHLA from the resin: adjusting the solubility

Different types of salts and hydroxides have been used in the extraction (step 3) with different results. We performed several cap exchange experiments following the protocol reported in example 1. Different alkali metal ions and tetraalkylammonium ions were successfully employed as the cations for the salts and hydroxides. The results of these experiments are summarized in Table 3.

Table 3

	Phase transfer	**Solubility in H_2O**	**Absorption peak shift (nm)**	**Emission peak shift (nm)**
LiOH	Yes	Yes	0 nm	1 nm
NaOH	Yes	Yes	0 nm	2 nm
KOH	Yes	Yes	4 nm	3 nm
TMAOH	Yes	Yes	0 nm	0 nm
$TEANO_3$	Yes	Yes	2 nm	2 nm
TBAOH[a]	Yes	Yes[b]	0 nm	5 nm

Table 3. Photophysical properties of CdSe-ZnS QDs capped using $DHLA^-$ with different countercations in water. TMA^+ = tetramethylammonium, TEA^+ = tetraethyl-ammonium, TBA^+ = tetra(n-butyl)ammonium. [a]$TBAPF_6$ gives the same results. [b] Clear water suspension but background was detected in the absorption spectra.

Figure 3 shows the absorption and emission spectra of QDs capped with $DHLA^-$ and either Na^+ or TMA^+ as the countercations. As for example 1, absorption spectra remain unchanged, thus confirming the integrity of the QDs, while the emission spectra of the functionalized QDs are lowered.

QDs capped with the same anchoring group ($DHLA^-$) and different countercations (e.g. Na^+, TMA^+, etc.) show different solubility features. The solubility data are reported in Table 4 in comparison with those of the native TOP/TOPO-capped hydrophobic QDs.

Table 4

	$^{a}Li^{+}$	$^{a}Na^{+}$	$^{a}K^{+}$	$^{a}TMA^{+}$	$^{b,c}TEA^{+}$	$^{a}TBA^{+}$	$^{a}TOA^{+}$	TOPO QD
Hexane	No	No	No	No	No	No	No	Yes
Toluene	Yes	No	No	No	No	No	No	Yes
$CHCl_3$	Yes	Yes	No	No	No	Yes	No	Yes
THF	No	Yes	No	No	No	Yes	No	Yes
Acetone	No	No	No	No	No	Yes	No	No
Acetonitrile	No	No	No	No	Yes	Yes	No	No
Methanol	No	No	Yes	Yes	Yes	Yes	Yes	No
DMSO	No	No	No	Yes	No	Yes	No	No
Water	Yes	Yes	Yes	Yes	Yes	Yes	No	No

Table 4. Solubility properties of CdSe-3ZnS (5.7 nm) QDs capped with $DHLA^{-}$ and different countercations in various solvents. TMA^{+} = tetramethylammonium, TEA^{+} = tetraethylammonium, TBA^{+} = tetra(n-butyl)ammonium, TOA^{+} = tetra(n-octyl)ammonium. ^{a}From hydroxide; ^{b}from perchlorate; ^{c}from nitrate. DMSO=Dimethyl sulfoxide. THF= tetrahydrofuran.

The results reported in Table 4 show that using the same cap-exchange procedure and the same capping agent (DHLA) it is possible to tune the solubility of the QDs by changing the type of salt/base used in the extraction step. Alkali cations Li^{+}, Na^{+} and K^{+} allow the solubilization of the QDs mainly in water or methanol. QD-$DHLA^{-}$ bearing ammonium cations with short alkyl chains, such as TMA^{+}, are soluble in methanol and water. Tetraalkylammonium cations with longer alkyl chains afford QDs which are more compatible with organic solvents. For example, TEA^{+} enables the solubilization of the QDs in acetonitrile as well as in water and methanol. TBA^{+} offers solubility in a wide range of solvents but in this case the QDs are less soluble in water, owing to the fact that the nanocrystals' surface bear long hydrophobic alkyl chains. TOA^{+}-covered QDs are soluble only in methanol.

The spectroscopic properties of the QDs are maintained in all the final dispersions, with absorption and emission peak shifts not exceeding 5 nm in comparison with the native QDs. As an example, Figure 4 shows photographs of QDs capped with $DHLA^{-}$ TBA^{+} in different solvents.

Example 4

Lipoic acid reduction: spectophotometric measurements

The reduction of the lipoic acid was studied by following the change of the S-S absorption band at 330 nm. 2.5 ml_ of a methanol solution of lipoic acid (1.6x10^{-2} M) was placed in a spectrophotometric cell together with a certain amount of borohydride resin (2 equivalents of BH_4^-). The solution was stirred. The absorption changes are depicted in Figure 5.

The absorption changes shown in Figure 5, and particularly the decrease of the 330 nm band, can be taken as an indication that lipoic acid is reduced by the resin. Moreover, it can be noticed that also the band at 230 nm, which is initially out of scale, decreases. Since this band is present also in the reduced form of lipoic acid (G. Bucher, C.Lu, W. Sander, "The Photochemistry of Lipoic Acid: Photoionization and Observation of a Triplet Excited State of a Disulfide", *ChemPhysChem,* **2005,** 6, 2607-2618) its decrease suggest that DHLA is chemisorbed inside the resin, most likely because of the interaction between the carboxylate residue of lipoic acid and the ammonium moieties of the resin.

This hypothesis is supported by the fact that after addition of base (tetramethylammonium hydroxide, TMAOH) the band at 230 nm is immediately restored, while the band at 330 nm is recovered only slightly (Figure 6). These observations indicate that TMAOH causes the release of reduced lipoic acid ($DHLA^-$) from the resin.

After stirring, the absorbance at 330 nm increased again, reaching a maximum after 20 min, because of the release of unreacted lipoic acid from the resin (Figure 7a). By comparing the absorption intensity at 330 nm after 20 min with the initial intensity (Figure 7b), it can be estimated that ca. 90% of lipoic acid has disappeared (i.e. it has been reduced to DHLA or has remained adsorbed within the resin).

The actual yield of the reduction of lipoic acid to DHLA afforded with the above described protocol was evaluated using NaOH to extract the carboxylate products from the resin. The addition of NaOH to the resin suspension caused a substantial absorption increase between 220 and 250 nm (DHLA band), whereas the signal at 330 nm (lipoic acid band) was only slightly recovered (Figure 8). As discussed above for figures 6 and 7 in which TMAOH was employed, these observations are consistent with the release of $DHLA^-$ from the resin, together with a minor amount of unreacted lipoic acid.

The increase of the absorption intensity at 330 nm was used to estimate the amount of unreacted lipoic acid released in the solution, whereas the amount of released $DHLA^-$ was determined with Ellman's reagent (G. L. Ellman, *Arch. Biochem.*

Biophys., **1959,** 82, 70-77). Under the adopted experimental conditions (room temperature; 30 min stirring with the BH_4^- resin, addition of 2 equivalents of NaOH and 30 min stirring), the $DHLA^-$ yield in the methanol solution was 29%; 7% of unreacted LA was also extracted.

CLAIMS

1. A quantum dot functionalized with ligands bearing a dithiolane group and an acid group salified with countercations, wherein said countercations are selected from the group consisting of Na^+, Li^+, K^+, Zn^{++}, Fe^{++}, Cu^{++}, C-i-Cs tetralkylammonium.
2. The quantum dot according to claim 1, wherein said countercation is a C-i-Cs tetralkylammonium selected from the group consisting of tetramethylammonium (TMA^+), tetrabutylammonium (TBA^+), tetraethylammonium (TEA^+) and tetraoctylammonium (TOA^+).
3. The quantum dot according to anyone of claims 1-2 wherein said salified acid group is selected from the group consisting of a carboxylate, a sulfonate and a phosphate group, preferably it is a carboxylate group.
4. The quantum dot according to anyone of claims 1-3 wherein said ligands are ligands based on lipoic acid.
5. The quantum dot according to anyone of claims 1-4, which is a core-shell CdSe-ZnS quantum dot.
6. The quantum dot according to anyone of claims 1-5, wherein said ligand is DHLA and said countercation is Na^+.
7. The quantum dot according to claim 1, which is a DHLA7Na^+-coated core-shell CdSe-ZnS quantum dot.
8. A method for manufacturing the quantum dot of anyone of claims 1-7, comprising the following steps:
   a. adding a resin loaded with BH_4^- to a solution of a ligand bearing a dithiolane group and a salifiable acid group in a first solvent, said solvent dissolving said ligand and not dissolving said resin;
   b. removing said solvent in order to obtain the resin with the attached ligand;
   c. adding a solution of a base or a salt thereof to said resin, wherein the cation of said base or salt is selected from the group consisting of Na^+, Li^+, K^+, Zn^{++}, Fe^{++}, Cu^{++}, Ci-C_8 tetralkylammonium, and stirring to extract the reduced ligand from the resin;
   d. washing the resin with said first solvent to obtain a solution containing said reduced ligand;
   e. adding quantum dots covered with hydrophobic ligands, said quantum

dots being in a solid form or dissolved in a second solvent not miscible with said first solvent, to the solution of step d), thus obtaining a biphasic mixture;

f. shaking said mixture to allow the transfer of said quantum dots to said solution to allow exchange of native hydrophobic ligands with the reduced, hydrophilic ones;

g. isolating said quantum dots.

9. The method according to claim 8 further comprising the step of solubilizing said obtained quantum dots in a polar solvent, preferably water.

10. The method according to anyone of claims 8-9 wherein said first solvent is methanol, ethanol or water.

11. The method according to anyone of claims 8-10, wherein said base of step c) is a Bronsted base, preferably a hydroxide.

12. The method according to anyone of claims 8-1 1, wherein said salt of step c) is a triflate ($CF_3S0_3^-$), a bromide (Br^-) or a perchlorate ($CI0_4^-$) salt.

13. The method according to anyone of claims 8-12, wherein said cation of said base or salt of step c) is a C_1-Cs tetraalkylammonium cation and it is selected from the group consisting of tetramethylammonium (TMA^+), tetraethylammonium (TEA^+), tetra(n-butyl)ammonium (TBA^+) and tetra(n-octyl)ammonium (TOA^+).

14. The method according to anyone of claims 8-13, wherein said second solvent is hexane.

15. Use of the quantum dot of anyone of claims 1-7 as luminescent probe/label for *in vitro* biological applications.

16. The quantum dot according to anyone of claims 1-7 for use as luminescent probe/label in medical diagnostics and in medical imaging.

17. The quantum dot according to anyone of claims 1-7 for use as a component of photosensitizers for photodynamic therapy.

18. Photosensitizer comprising the quantum dot according to anyone of claims 1-7.

19. Light absorbing material comprising the quantum dot according to anyone of claims 1-7 for the manufacturing of solar cells.

20. Light emitting material comprising the quantum dot according to anyone of claims 1-7 for lighting apparatus and display.

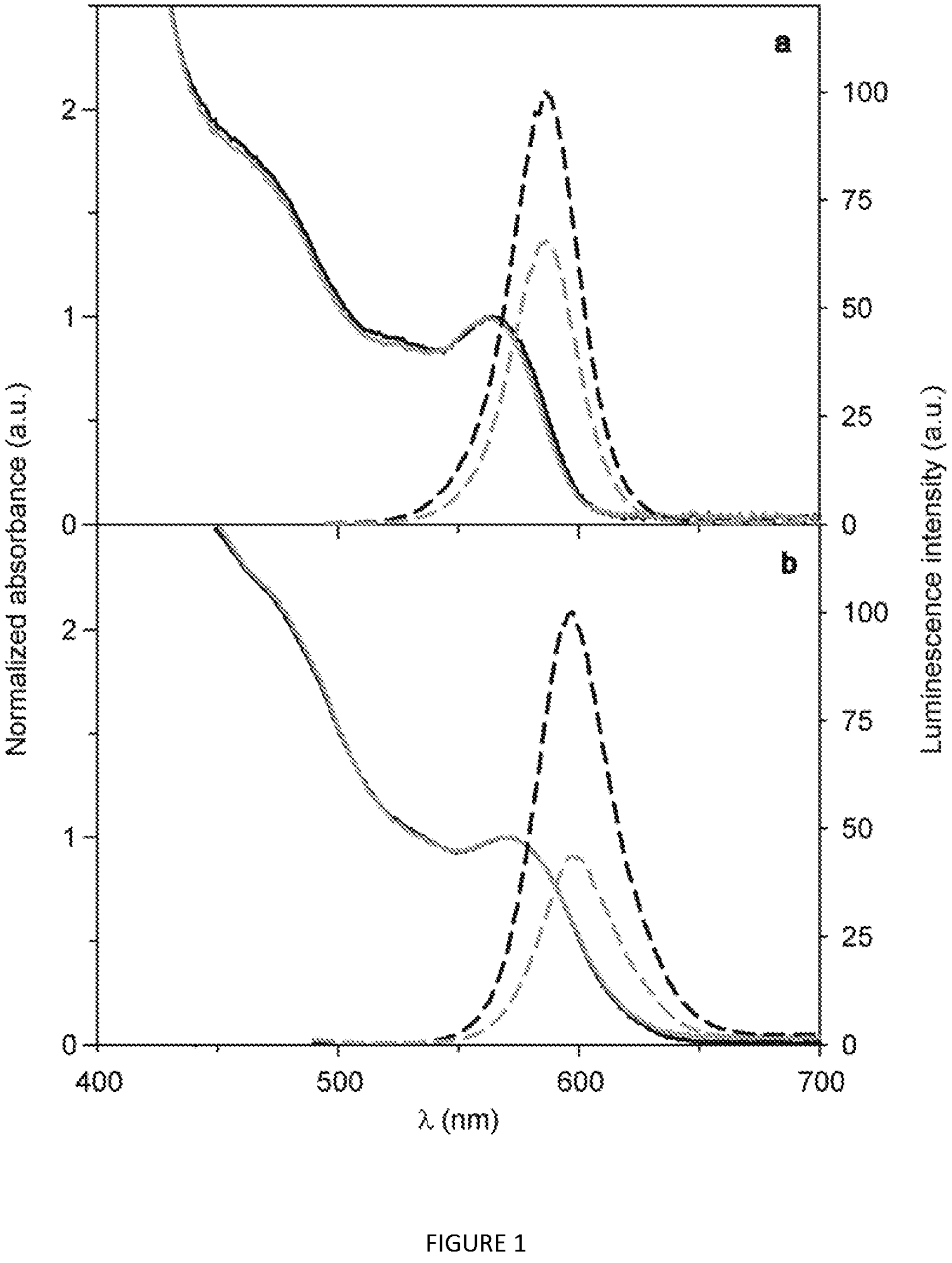

a
b
Normalized absorbance (a.u.)
Luminescence intensity (a.u.)
0
1
2
0
1
2
0
25
50
75
100
0
25
50
75
100
400
500
600
700
λ (nm)

FIGURE 1

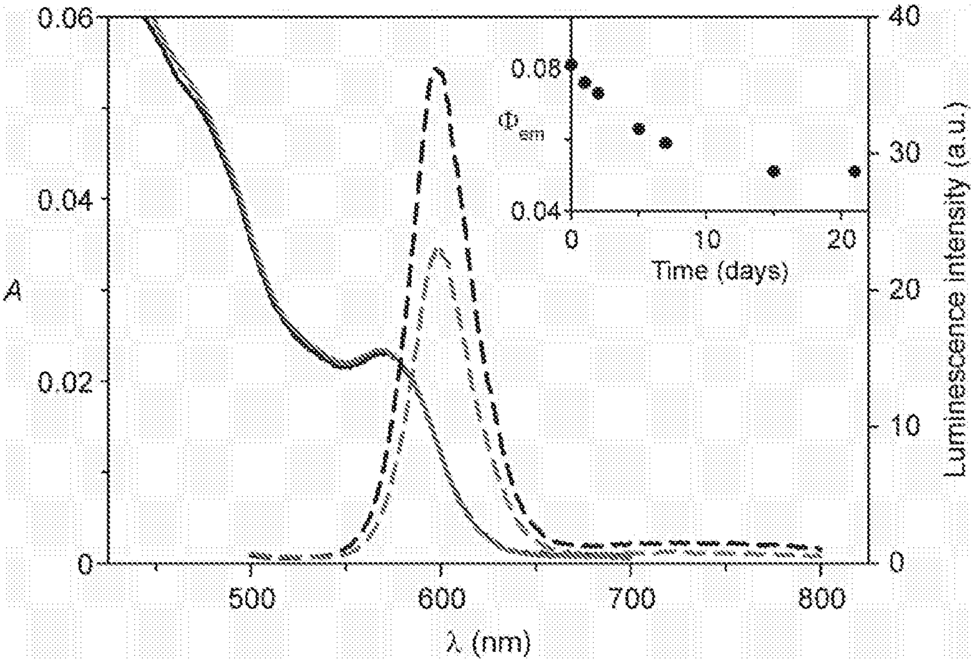


FIGURE 2

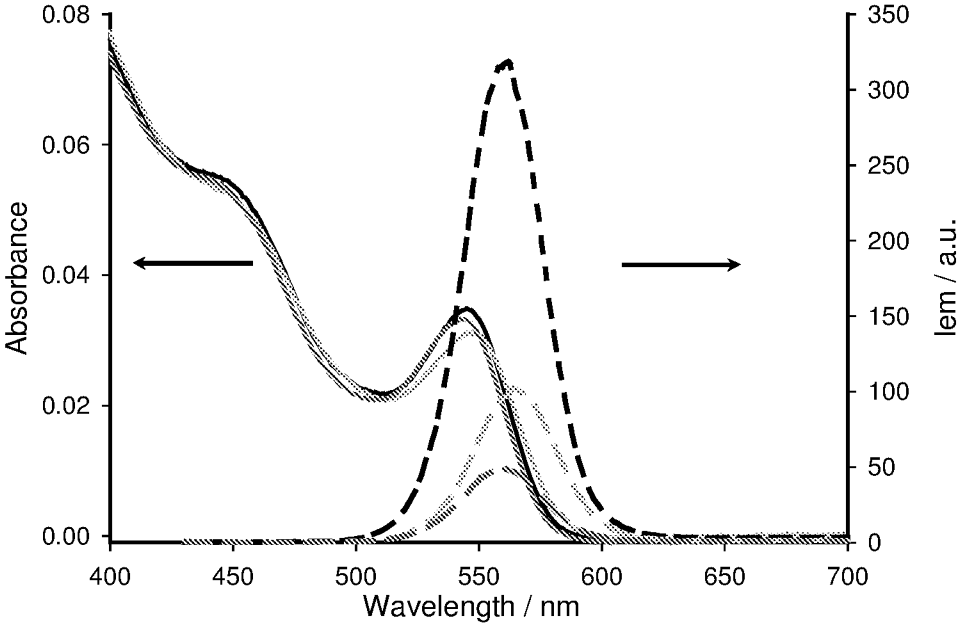


FIGURE 3

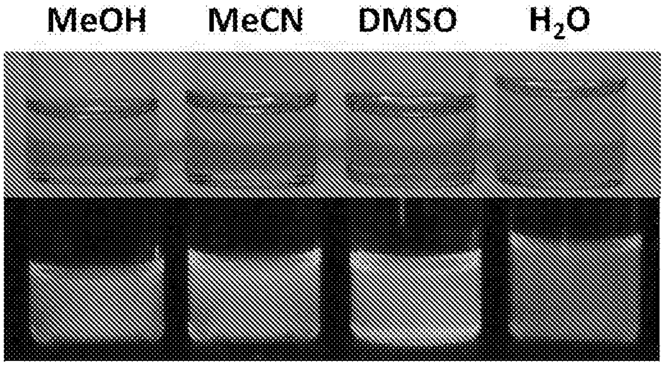


FIGURE 4

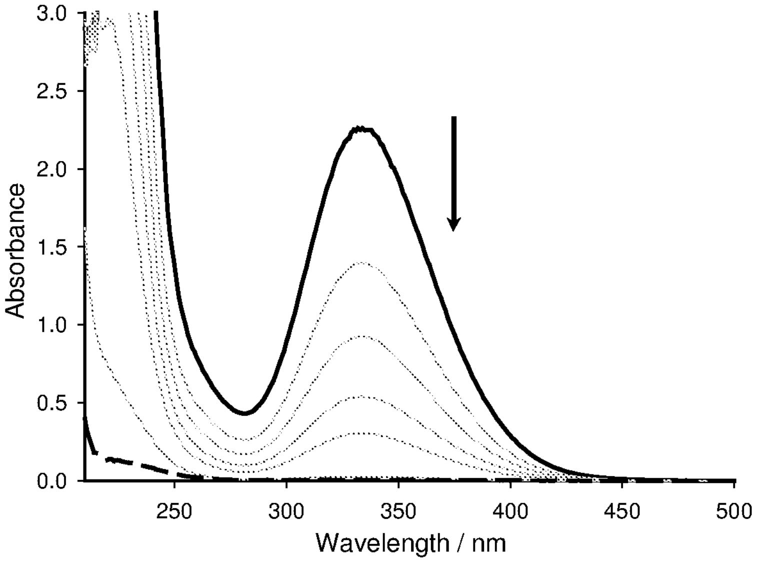


FIGURE 5

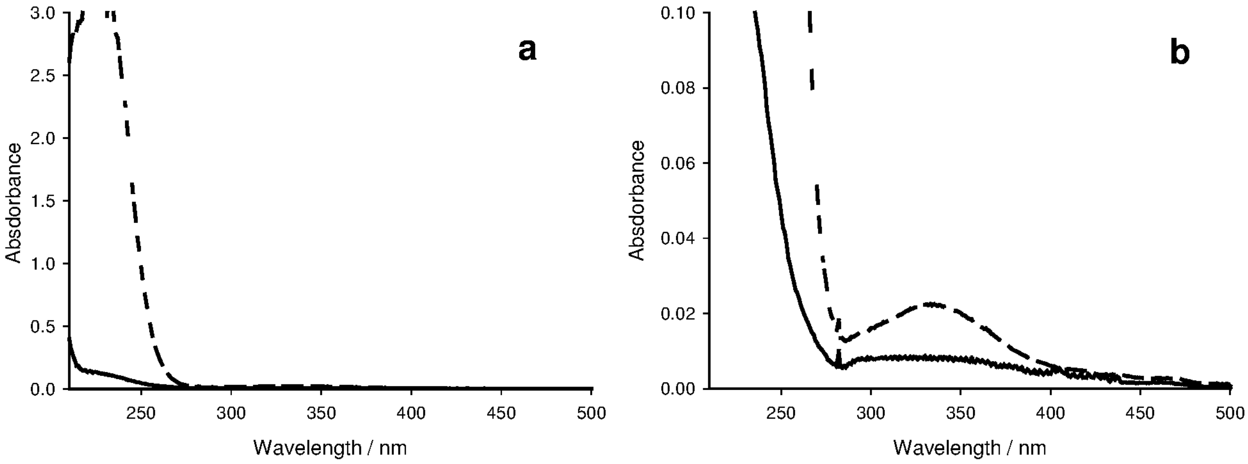


FIGURE 6

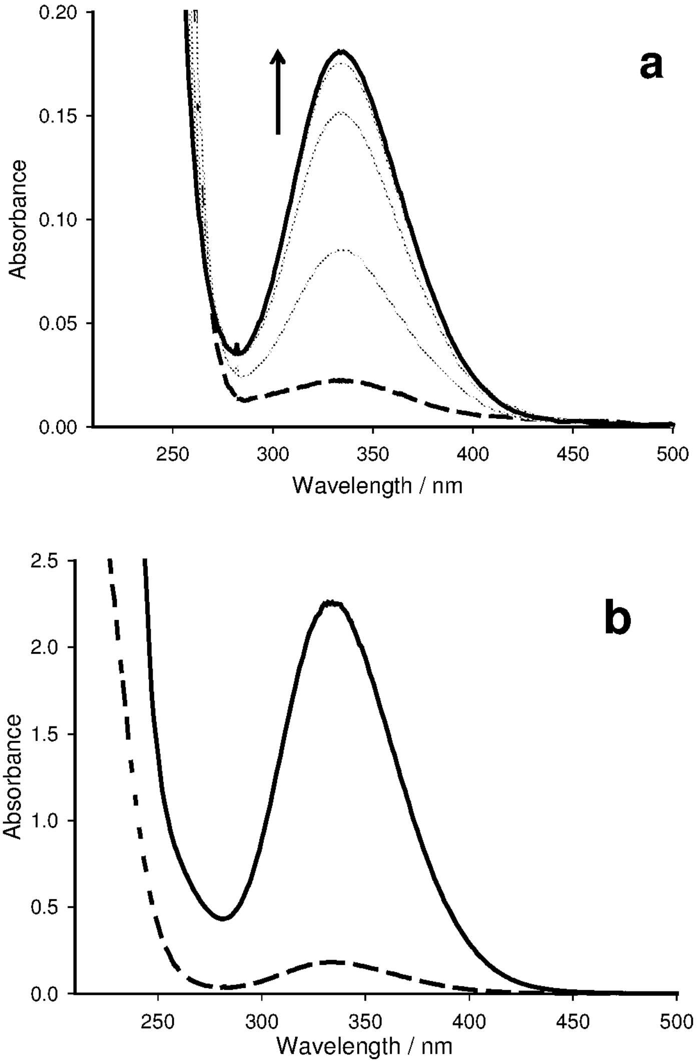


FIGURE 7

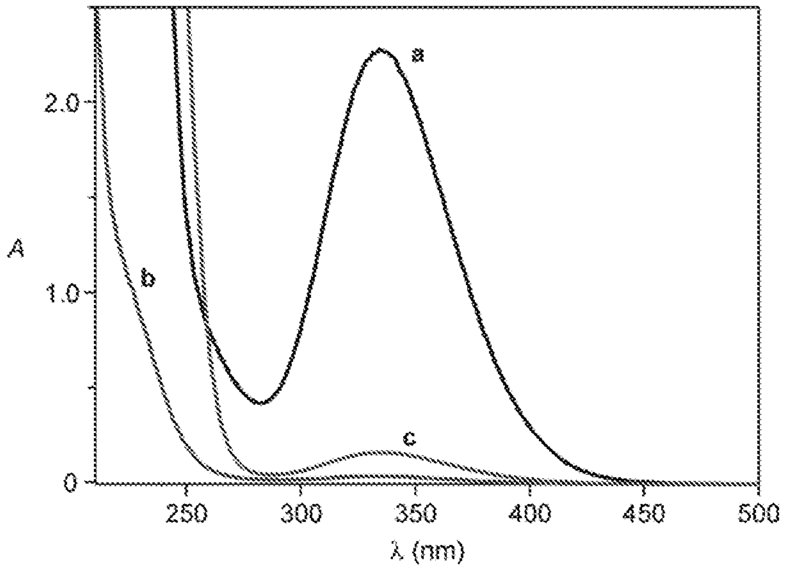


FIGURE 8

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2014/06123O

A. CLASSIFICATION OF SUBJECT MATTER

INV. C09K11/02 C09K11/88 C09K11/56

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)

C09K

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 , INSPEC, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	IBRAHIM YILDIZ ET AL: "Dithiolane ligands for semiconductor quantum dots", JOURNAL OF MATERIALS CHEMISTRY, vol. 18, no. 33, 1 January 2Q08 (2008-01-01), page 3940, XP055042181, ISSN: 0959-9428, D0I: 10.1039/b806247a page 3942, last paragraph -----	1-20
A	FR 2 925 492 AI (SAN0FI AVENTIS SA [FR]) 26 June 2009 (2009-06-26) claims 1-13 -----	1-20
A	W0 2013/025347 AI (US GOVERNMENT [US]; SUSUMU KIMIHI R0 [US]; MEDINTZ IGOR L [US]; STEWART) 21 February 2013 (2013-02-21) claims 1-20 ----- -/--	1-20

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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Date of the actual completion of the international search

28 July 2014

Date of mailing of the international search report

04/08/2014

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Authorized officer

Kovecs, Monika

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2014/06123O

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	UYEDA H T ET AL: "Design of water-soluble quantum dots with novel surface ligands for biological applications", MATERIALS RESEARCH SOCIETY SYMPOSIUM PROCEEDINGS; [MATERIALS RESEARCH SOCIETY SYMPOSIUM PROCEEDINGS], MATERIALS RESEARCH SOCIETY, PITTSBURG, PA; US, vol. 789, 1 January 2004 (2004-01-01), pages N5.8. 1-N5.8. 6, XP002996402, ISBN: 978-1-55899-828-5 page N5.8.4 -----	1-20
A	H. TETSU0 UYEDA ET AL: "Synthesis of Compact Multidentate Ligands to Prepare Stable Hydrophilic Quantum Dot Fluorophores", JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 127, no. 11, 1 March 2005 (2005-03-01), pages 3870-3878, XP055013251, ISSN: 0002-7863, D0I: 10. 1021/ja044031w the whole document -----	1-20
A	SERGE MIGNANI ET AL: "Synthesis of new macromolecular, functionalized carboxylic-acid PEGDHLA surface ligands", TETRAHEDRON LETTERS, PERGAM0N, GB, vol. 51, no. 41, 21 July 2010 (2010-07-21), pages 5364-5367, XP028239939, ISSN: 0040-4039, D0I: 10. 1016/J.TETLET.2010.07.118 [retrieved on 2010-08-01] the whole document -----	1-20

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2014/061230

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO 2013025347 A1	21-02-2013	US 2013045499 A1	21-02-2013
		US 2013323777 A1	05-12-2013
		WO 2013025347 A1	21-02-2013

Form PCT/ISA/210 (patent family annex) (April 2005)