

PLANTS COLOURED WITH NANOCRYSTALS

FIELD OF THE INVENTION

[0001] The present invention refers to a plant comprising at least one type of nanocrystal through which the plant emits a specific colour or several colours when exposed to light of a certain wavelength. In another aspect the present invention refers to a method of coloring the outer surface of a plant with at least one type of a nanocrystal. The present invention also refers to a method of coloring the tissue of a plant with at least one type of a nanocrystal.

BACKGROUND

[0002] Semiconductor nanocrystals (quantum dots) have been receiving great fundamental and technical interest for their use in a variety of technologies, such as light-emitting devices (Colvin et al, Nature 370, 354-357, 1994; Tessler et al, Science 295, 1506-1508, 2002), lasers (Klimov et al, Science 290, 314-317, 2000), solar cells (Huynh et al, Science 295, 2425-2427, 2002) or as fluorescent biological labels in biochemical research areas such as cell biology. For example, see Bruchez et al, Science, Vol. 281, pages 2013–2015, 2001; Chan & Nie, Science, Vol. 281, pages 2016–2018, 2001; US Patent Number 6,207,392, summarized in Klarreich, Nature, Vol. 43, pages 450-452, 2001; see also Mitchell, Nature Biotechnology, pages 1013–1017, 2001, and US Patent Numbers 6,423,551, 6,306,610, and 6,326,144.

[0003] The remarkable luminescent properties of quantum dots arise from quantum size confinement, which occurs when metal and semiconductor core particles are smaller than their excitation Bohr radii, about 1 to 5 nm. (Alivisatos, Science, 271, 933-37, 1996; Alivisatos, J. Phys. Chem. 100, 13226-39, 1996; Brus, Appl Phys., A53, 465-74, 1991; Wilson et al., Science, 262, 1242-46, 1993.)

[0004] Among the most studied semiconductor nanocrystal materials have been the chalcogenide II-VI materials and III-V materials. The primary reason for the interest in these semiconductor nanocrystal materials is their size-tunable photoluminescence emission spanning the whole visible spectrum.

[0005] Considering the aforementioned, it is desired to find further applications for nanocrystals.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

5 [0007] **Fig. 1** shows an orchid, namely *Dendrobium* White Fairy which was dip coated in a solution comprising CdZnSe nanocrystals. The picture shows the orchid under a UV lamp with an excitation at 302/345 nm. The flower of the orchid appears in orange after dipping in the solution compared to its natural white/purple colour shown in smaller picture at the upper left of Figure 1.

10 [0008] **Fig. 2** shows in the upper left picture a vial with a solution comprising CdZnSe nanocrystals dissolved in octadecene. The upper right picture shows the orchid *Dendrobium* White Fairy in its white colour before uptake of the CdZnSe nanocrystals via the plant stem. The lower picture shows the orchid after uptake of CdZnSe nanocrystals and illumination with a UV lamp with an excitation at 302/345 nm. The coloured orchid has the same colour
15 as the orchid shown in Figure 1, namely orange.

[0009] **Fig. 3** shows in the upper left picture a vial with an aqueous solution comprising fluorescein. The upper right picture shows the orchid *Dendrobium* White Fairy in its white colour before uptake of fluorescein via the roots of the plant. The lower picture shows the orchid after uptake of fluorescein and illumination with a UV lamp with an excitation at
20 302/345 nm. The coloured orchid appears in a green colour wherein the colour concentrates in the vascular strands, also called leave vein or leave vein network.

[0010] **Fig. 4a** shows a multicoloured *Dendrobium* White Fairy after uptake of fluorescein and CdZnSe nanocrystals. The orange colour of the nanocrystals is visible in Figure 4a in the ovary region and slightly in the base of petals and sepals while the leave veins appear green
25 in the fluorescence light of fluorescein. **Fig. 4b** shows a transverse cross section of stem. **Fig. 4b** shows a real colour image which was based on 32 channels / 32 color range. The 6 gray dots (better seen in Figure 4c) surrounding the centre of the cut stem appear green in the fluorescence light of fluorescein while the surrounding part appears orange due to the light emission from the CdZnSe nanocrystals. **Fig. 4c** shows the same section of the stem but
30 using a filter only showing the light emitted by fluorescein (c) while **Fig. 4D** shows only the light emitted by CdZnSe nanocrystals.

DETAILED DESCRIPTION

[0011] In a first aspect, the present invention refers to a plant comprising or incorporating at least one type of nanocrystal (quantum dot). Plants comprising or incorporating semiconductor nanocrystal materials emit light of a specific wavelength depending on the nanocrystals (quantum dots) used for colouring the plants.

[0012] The term “plant” includes those organism whose cells comprise in addition to a cell nucleus (with double nucleus membrane and several chromosomes) also plastids. Plastides exist either in the form of chloroplasts or can develop to them under suitable conditions. Green plants are phototrophic in contrast to animals and all other heterotrophic organisms. The term plant as used in the present invention is not supposed to include single cell organisms, such as cyanobacteria, which are sometimes also added to the plants, or single cells. The present invention of plants also excludes moss, lichen and fungi, the latter one are also sometimes allocated to plants even though fungi do not have plastides.

[0013] In another aspect of the present invention the nanocrystals can also be comprised in or on dried plant, or artificial plants. That means that the term plant as used herein does not refer only to a living plant but can also refer to non-living or a dead plant. Those plants comprise a dried plant as well as an artificial plant. The dried plants can be chosen from the plants referred to further below or parts of them as also specified further below. Artificial plants are generally used for decorative purposes and can be made of a polymer or mixture of polymers, paper or board (laminated or unlaminated), a metal foil or mixtures of the aforementioned materials. The artificial plants can be a replica of one of the below listed plants or a new creation of a new form of plant which does not exist in that form in nature.

[0014] Artificial plants can be made of silk; artificial silk, made for example of rayon, mercerized cotton, polyester or blends of these materials or blend of rayon and silk; polymer modified silk, such as collagen modified silk; polymer modified cotton, such as mercerized cotton; or a plain polymer, such as nylon, a polyamide or a polyester.

[0015] A plant comprises several organs which can develop in great number outwardly. Such organs include roots, leaves and flowers. In one aspect of the present invention, the nanocrystals are comprised or incorporated in an aerial part of the plant, meaning a part of the plant lying above the earth in which the plant is growing, such as stem, branches, flower and leave. In one example this aerial part refers to the flower(s) or the leave(s) of the plant.

[0016] In one example, the plant of the present invention is a flowering plant, i.e. a plant which develops at least at a certain stage of its life a flower or florescence. The phrase

“flowering plants” refers in general to any of the more than 250,000 species of angiosperms (division Magnoliophyta) having roots, stems, leaves, and well-developed conductive tissues (xylem and phloem). They are often differentiated from gymnosperms by their production of seeds within a closed chamber (the ovary) within the flower, but this distinction is not always clear-cut. The division of angiosperms is composed of two classes: monocots and dicots. Monocots have flower parts in threes, scattered conducting strands in the stem, and usually prominent parallel veins in the leaves, and they lack a cambium. Dicots have flower parts in fours or fives, conducting strands arranged in a cylinder, a net-veined pattern in the leaves, and a cambium. Flowering plants reflect an immense diversity in habit, size, and form; they account for more than 300 families growing on every continent, including Antarctica. Flowering plants have adapted to almost every habitat. Most reproduce sexually by seeds via the specialized reproductive organs that are present in all flowers.

[0017] In one aspect of the present invention, the flowering plants include, but are not limited to plants from the family of rosaceae, hydrangeaceae, orchidaceae, hydrangeaceae, hyacinthaceae, begoniaceae, lamiaceae, including, e.g., the genus *lavandula*; asteraceae, including, e.g., the genus *dahlia* and *gerbera*; geraniaceae, including, e.g., the genus *geranium*; solanaceae, including, e.g., the genus *petunia*; liliaceae, including, e.g., the genus *tulipa*; primulaceae, including, e.g., the genus *primula* and *cyclamen*; onagraceae, violaceae, gesneriaceae, malvaceae, including, e.g., the genus *hibiscus*; bromeliaceae, araceae, including, e.g., the genus *anthurium*; ericaceae, including, e.g., the genus *rhododendron* (including *azaleas*); theaceae, including, e.g., the genus *camellia* or *nyctaginaceae*, including, e.g., the genus *bougainvillea*.

[0018] In one example, the plant includes, but is not limited to roses, orchids, hydrangea, hyacinth, begonia, lavender, dahlia, geranium, petunia, tulip, lily, primrose, viola, gerbera, gloxinia, hibiscus, bromeliad, cyclamen, anthurie, azalea, bougainvillea or camellia.

[0019] The family of orchidaceae includes the subfamily of Orchidoideae, Epidendroideae, Vanilloideae, Cypridioideae, and Apostasioideae. The subfamily of Cypridioideae includes, for example, the tribes Cypridieae, Mexipodieae, Phragmipodieae, and Selenipodieae. The subfamily of Epidendroideae includes, for example, the tribes Arethuseae, Calypsoeae, Cryptarrheneae, Coelogyneae, Epidendreae, Epipogieae, Gastrodieae, Malaxideae, Neottieae, Podochilaceae, Tropidieae and Xerorchideae. The subfamily of Vanilloideae includes, for example, the subfamily of Pogoniinae and Vanilleae.

The subfamily of Orchidoideae includes, for example, the tribes Diceratosteleae, Codonorchideae, Cranichideae, Diseae, Diurideae and Orchideae.

[0020] The tribe Podichileae of the subfamily Epidendroideae includes, for example, the subtribe Dendrobiinae which includes for example the genera *Cadetia*, *Dendrobium*,
 5 *Diplocaulobium*, *Epigeneium*, *Flickingeria* and *Pseuderia*. For example, one of the orchid plants “*Dendrobium* White Fairy” used in an example of the present invention belongs to the genus *Dendrobium*.

[0021] The types of nanocrystals used in the present invention can include or consist of semiconducting matter or a magnetic oxide, including in ternary or higher systems, e.g. a
 10 metal ferrite. The emission of these nanocrystals (quantum dots) can be tunable by composition and/or size.

[0022] The at least one type of nanocrystal used for colouring of the plant can include, but are not limited to

- a rare earth metal doped metal oxide of the chemical formula $\text{MeAl}_2\text{O}_4\text{:Re}$ or
 15 $\text{Me}_4\text{Al}_{14}\text{O}_{25}\text{:R}$; or
- a binary nanocrystal of the chemical formula M1A or M1O ; or
- a ternary nanocrystal of the chemical formula M1M2A or M1AB or M1M2O ; or
- a quaternary nanocrystal of the chemical formula M1M2AB ;

wherein M1 and M2 of the binary, ternary and quaternary nanocrystals are independently a
 20 metal, such as one from Group II, Group III, Group IV or Group VIII of the PSE; and, if present, A and B are independent from each other an element, such as from Group VI or Group V of the PSE; and

wherein Me of the rare earth metal doped metal oxide can include, for example Ca, Sr or Ba; and

25 Re of the rare earth metal doped metal oxide is at least one element that can include, Tb, Dy, Nd, Eu or Tm.

[0023] Rare earth metal doped metal oxides are known in the art and are described in detail for example in US 6,264,855, JP H9-143,463, JP 2543852, JP 2697733, JP 2697688, JP H10-273654, JP 2929162. In brief, rare earth metal doped metal oxides as the one
 30 described above can be water resistant and can be prepared, for example, by an acid treatment of a luminous pigment, as a starting material, with at least one acidic compound which can be an acid or acid generating compound under the condition of pH of not more than 3, and

subsequently by an alkaline treatment with at least one alkaline compound which can be an alkalis or alkali generating compound under the condition of pH 4-9.

[0024] A luminous pigment as an object of an acid treatment can be of any type if it is a luminous pigment in which an oxide matrix comprising at least one element, such as Mg, Ca, Sr, Ba and Zn, and at least one element, such as B, Al, P or Ga is doped with at least one rare-earth metal element. An amount of the rare-earth element to be doped is not particularly restricted, but it is enough in 0.0001 to 30% by weight, or in one example 0.1 to 10% by weight relative to the total amount of the luminous pigment.

Examples for luminous pigments include compounds of the general formula $\text{MeAl}_2\text{O}_4\cdot\text{Re}$ or $\text{Me}_4\text{Al}_{14}\text{O}_{25}\cdot\text{R}$. In this formula, Me can be Ca, Sr and Ba; and Re of the rare earth metal doped metal oxide is at least one element such as Tb, Dy, Nd, Eu and Tm. The manufacture of such rare earth metal doped metal oxides is described in detail, e.g., US 6,264,855.

[0025] Examples for binary nanocrystal of the chemical formula M1A or M1O are known in the art. M1 in this formula can be a metal of Group II, Group III, Group IV, Group VII or Group VIII of the Periodic System of Elements (PSE) according to the traditional IUPAC system. According to the new IUPAC system the corresponding groups (traditional system in brackets) are group 2/ group 12 (group II), group 13 (group III), group 14 (group IV), group 7 (group VII). Suitable examples of group II are (group 12 of the new IUPAC system) Cd, Zn, as well as (group 2 of the new IUPAC system) Mg, Sr, Ca, and Ba. Suitable examples of group III are (group 13 of the new IUPAC system) Al, Ga, and In. Two illustrative examples of a suitable element of group IV are (group 14 of the new IUPAC system) Pb and Sn. An illustrative example of a suitable element of group VII is (group 7 of the new IUPAC system) Mn. Suitable examples of elements of group VIII are (group 8 of the new IUPAC system) Fe, Co, Ni, and Ir. In one example of the present invention Pb and Cd are excluded from being used for the manufacture of a binary nanocrystal.

[0026] Generally, M1, or where applicable each of M1 and M2, may for instance be of the Group II, such as Cd, Zn, Mg, Sr, Ca, or Ba, of the Group III, such as Al, Ga, and In, of the Group IV, such as Pb, Sn, of the Group VII, such as Mn or of the Group VIII, such as Fe, Co, Ni or Ir.

[0027] A in the above formula can be a chalcogen or a pnictogen, i.e. an element of Group VI or Group V of the PSE according to the historic IUPAC nomenclature or of group 16 or group 15 according to the new IUPAC nomenclature. A reaction mixture for the manufacture of such a binary nanocrystal can include in this case a metal precursor containing the metal

M1, as well as the element A. A metal precursor can include a salt, that provides the corresponding metal in the formation of a nanocrystal. It may for instance be an inorganic (e.g. a carbonate) or an organic (e.g. an acetate, a stearate or an oleate) salt of the corresponding metal. Where two metals or metal precursors are used for the manufacture of such a binary nanocrystal system, e.g. cadmium and zinc or oxides thereof, the two metals/precursors are present in any desired ratio.

[0028] Element A and, where applicable, element B can be independently selected of Group VI of the PSE, such as of S, Se, Te, O, or Group V of the PSE, such as P, Bi or As.

[0029] Metal precursors used for the manufacture of such binary nanocrystals can be a metal oleate. Such a metal oleate can include, but is not limited to cadmium oleate, cadmium zinc oleate, lead oleate, manganese oleate, magnesium oleate, cadmium lead oleate, magnesium ferrous oleate, manganese ferrous oleate, calcium ferrous oleate, zinc ferrous oleate, strontium ferrous oleate, cobalt ferrous oleate, or nickel ferrous oleate.

[0030] Examples for binary nanocrystals include, but are not limited to CdSe, CdTe, CdS, PbSe, PbTe, PbS, SnSe, ZnS, ZnSe, or ZnTe. A binary nanocrystal of general formula M1O can for example be of the formulas CdO, PbO, MnO, CoO, ZnO, or FeO. In this context it is noted that the representation M1A and M1O should only illustrate that this nanocrystals are binary, meaning that they comprise two elements. The representation M1A and M1A does not necessarily represent the stoichiometry of the nanocrystals (even though it can in the case of CdSe or CdO, to recite only two examples) but can also include nanocrystals of the stoichiometry MO₂ (for example MnO₂), M₂O₃ (for example, Al₂O₃), or M₃O₄ (for example Fe₃O₄).

[0031] Some studies have shown that improved luminescence of nanocrystals can be achieved by capping a size-tunable lower bandgap core particle with a higher band gap inorganic materials shell. For example, CdSe quantum dots passivated with a ZnS layer are strongly luminescence at room temperature, and their emission wavelength can be tuned from blue to red by changing the particle size. Moreover, the ZnS capping layer passivates surface nonradiative recombination sites and leads to greater stability of the nanocrystal (quantum dot). (Dabbousi et al., J. Phys. Chem. B101, 9463-75, 1997. Kortan, et al., J. Am. Chem. Soc. 112, 1327-1332, 1990.)

[0032] A ternary nanocrystal can be of the general formula M1M2A. M1 and M2 in this formula can independently be a metal of Group II, Group III, Group IV, Group VII or Group VIII of the PSE. A can be an element from Group VI or V of PSE. In other examples, a

ternary nanocrystal can have the general formula M_1AB . M_1 can in this formula be a metal of Group II, Group III, Group IV Group VII or Group VIII of the PSE. A and B can independently be an element of Group V or Group VI of the PSE. In yet another example, a ternary nanocrystal formed according to a method of the present invention may be of the general formula M_1M_2O . M_1 and M_2 can be independently a metal of Group II, Group III, Group IV, Group VII or Group VIII of the PSE. O is oxygen. A ternary nanocrystal may be of any structure. It may for instance be of layered structure, such as a core/shell structure, or it may be homogenous, e.g. of uniform composition or of gradually or stepless varying composition.

[0033] A quaternary nanocrystal formed can be of the general formula M_1M_2AB . As defined above, M_1 and M_2 can be a metal of Group II, Group III, Group IV, Group VII or Group VIII of the PSE. A and B can independently be an element of Group V or Group VI of the PSE.

[0034] Illustrative examples of a ternary nanocrystal of the general formula M_1M_2A include, but are not limited to, a ternary nanocrystal of formulas $ZnCdSe$, $CdZnS$, $CdZnSe$, $CdZnTe$, $SnPbS$, $SnPbSe$, and $SnPbTe$. Illustrative examples of a ternary nanocrystal of general formula M_1AB can include a nanocrystal of formulas $CdSeS$, $CdSeTe$, $CdSTe$, $ZnSeS$, $ZnSeTe$, $ZnSTe$, $SnSeS$, $SnSeTe$, $SnSTe$, $PbSeS$, $PbSeTe$, and $PbSTe$. Illustrative examples of a ternary nanocrystal of formula M_1M_2O can include a nanocrystal of the general formula $M_1M_2O_4$ such as the ferrites $MgFe_2O_4$, $CaFe_2O_4$, $SrFe_2O_4$, $ZnFe_2O_4$, $NiFe_2O_4$, $CoFe_2O_4$, and $MnFe_2O_4$. In this context it is noted that also the representations M_1M_2A , M_1AB , and M_1M_2O merely serve in illustrating the fact that these nanocrystals are ternary, meaning that they include three different elements. Accordingly, from the representation M_1M_2A , M_1AB and M_1M_2O no conclusion in terms of the stoichiometry of the nanocrystal can be drawn, even though in some examples by coincidence, for example in embodiments of $CdZnSe$, $CdSeTe$ or $MgFe_2O_4$, the stoichiometry of the nanocrystal could be rather accurately read into the general formula. Hence, the above general formulas M_1M_2A , M_1AB , and M_1M_2O also include for instance nanocrystals of the stoichiometry $M_{1-x}M_2_xA$ (for example $Cd_{1-x}Zn_xSe$), $M_{1-x}M_2_{1-x}A$ (for example, $Zn_xCd_{1-x}Se$), or $M_{1-x}A_yB_{1-y}$ (for example $CdSe_yS_{1-y}$), $M_{1-x}A_{1-y}B_y$ (for example $CdSe_{1-y}S_y$) or $M_{1-x}M_2_xO$ (for example, or $Mg_{1-x}Fe_xO$) or $M_{1-x}M_2_{1-x}O$ (for example, $Fe_xMn_{1-x}O$).

[0035] Illustrative examples of a quaternary nanocrystal of general formula M1M2AB can include a nanocrystal of CdZnSeS, CdZnSeTe, CdZnSTe, CdHgSeS, CdHgSeTe, CdHgSTe, HgZnSeS, HgZnSeTe and HgZnSTe. In this context it is noted that also the representation M1M2AB, should only illustrate that this nanocrystals are quaternary, meaning that they
 5 comprise four elements. The representation M1M2AB does not necessarily represent the stoichiometry of the nanocrystals (even though it can in the case of CdZnSeS, to recite only one example) but also includes nanocrystals of the stoichiometry $M1_{1-x}M2_xA_yB_{1-y}$ (for example $Cd_{1-x}Zn_xSe_yS_{1-y}$), or $M1_{1-x}M2_xA_{1-y}B_y$ (for example $Cd_{1-x}Zn_xSe_{1-y}S_y$).

[0036] Examples of homogenous ternary ($M1_{1-x}M2_xA$) or quaternary ($M1_{1-x}M2_xA_yB_{1-y}$)
 10 nanocrystals have been described, for instance, in Zhong et al, J. Am. Chem. Soc, 2003 125, 8598-8594, Zhong et al, J. Am. Chem. Soc, 2003 125, 13559-13553, or the International patent application WO 2004/054923.

[0037] It is further noted that independent of whether a binary, a ternary or a quaternary nanocrystal is used those nanocrystals can be of any desired structure. A nanocrystal can for
 15 instance be of a layered structure, e.g. a core/shell structure or a core-mantle-shell structure, (Hines, M.A., & Guyot-Sionnest, P., *J. Phys. Chem.* (1996) 100, 468; Dabbousi, B.O., et al., *J. Phys. Chem. B* (1997) 101, 9463; Peng, X., et al., *J. Am. Chem. Soc.* (1997) 119, 7019 16-18) or of any alloy structure (see for example US patent 7,056,471), including alloy -gradient structure (Foley, J., et al., *Materials Science Forum*, vols. 225-227 (1996) pp. 323-328) or a
 20 "mantel structure" having a core surrounded by a relatively thin alloyed layer and a shell as described in co-pending PCT application PCT/SG2008/000290 "Process Of Forming A Cadmium And Selenium Containing Nanocrystalline Composite And Nanocrystalline Composite Obtained Therefrom".

[0038] Those nanocrystals can be manufactured by using a method comprising:

- 25 (i) forming a homogenous reaction mixture comprising a metal precursor containing the metal M1, the element A, and a non-polar solvent of low boiling point, and
 (ii) bringing the homogenous reaction mixture under elevated pressure to an elevated temperature suitable for forming a nanocrystal.

[0039] In this method the non-polar solvent of low boiling point has a boiling point of less
 30 than about 100 °C or 80 °C at atmospheric pressure (1013 mbar). Examples of such solvents include, but are not limited to hexane, chloroform, toluene, benzene, heptane, cyclohexane, dichloromethane, pyridine, carbon tetrachloride, carbon disulfide, dioxane, diethyl ether, diisopropylether, and tetrahydrofuran and mixtures thereof.

[0040] In this method it is also possible to add a surfactant, such as an organic carboxylic acid, an organic phosphate, an organic phosphonic acid, an organic phosphine oxide, an organic amine or mixtures thereof. Examples for the manufacture of such nanocrystals are given in Example 2.

5 [0041] **Water soluble nanocrystals**

[0042] In one example of the present invention, the nanocrystals used are water soluble. Water soluble nanocrystals are known in the art and are described, for example in WO 2006/118543, WO2006/118542 and WO 2006/075974.

[0043] Also, the PCT publication WO 00/17656 discloses core-shell nanocrystals which
10 are capped with a carboxyl acid or sulfonic acid compound of the formula $\text{SH}(\text{CH}_2)_n\text{-COOH}$ and $\text{SH}(\text{CH}_2)_n\text{-SO}_3\text{H}$, respectively in order to render the nanocrystals water soluble. Similarly, the PCT application WO 00/29617 and British patent application GB 2342651 describe that organic acids such as mercaptoacetic acid or mercapto-undecanoic acid are attached to the surface of nanocrystals to render them water soluble and suitable for
15 conjugation of biomolecules such as proteins or nucleic acids. The British patent application 2342651 also describes the use of trioctylphosphine as capping material that is to be supposed to confer water solubility of the nanocrystals.

[0044] In another document, PCT publication WO 00/27365 reports the use of
20 diaminocarboxylic acids or amino acids as water-solubilising agents for nanocrystals. PCT publication WO 00/17655 discloses nanocrystals that are rendered water-soluble by the use of a solubilising agent that has a hydrophilic moiety and a hydrophobic moiety. The solubilising agent attaches to the nanocrystal via the hydrophobic group, whereas the hydrophilic group, such as a carboxylic acid or methacrylic acid, provides for water solubility. In a further PCT application (WO 02/073155) water soluble semiconductor nanocrystals are described in
25 which various molecules such as trioctylphosphine oxide hydroxamates, derivatives of hydroxamic acid or multidentate complexing agents such as ethylenediamine are directly attached to the surface of a nanocrystal to render them water-soluble. These nanocrystals can then be linked to a protein via EDC. In another approach, the PCT application WO 00/58731 discloses nanocrystals which are used for the analysis of blood cell populations and in which
30 amino-derived polysaccharides having a molecular weight from about 3,000 to about 3,000,000 are linked to the nanocrystals.

[0045] US Patent 6,699,723 discloses the use of silane-based compounds as linking agent to facilitate the attachment of biomolecules such as biotin and streptavidin to luminescent

nanocrystal probes. US Patent Application No. 2004/0072373 A1 describes a method of biochemical labeling using silane-based compounds. Silane-linked nanoparticles are bonded to template molecules by molecular imprinting, and then polymerized to form a matrix. Thereafter, the template molecules are removed from the matrix. The cavity produced in the matrix due to the removal of the template molecule has properties that can be used for labeling.

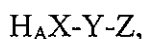
[0046] Recently, the use of synthetic polymers to stabilize water soluble nanocrystals has been reported. US Patent Application No. 2004/0115817 A1 describes that of amphiphilic, diblock polymers can be attached non covalently via hydrophobic interactions to a nanocrystal, the surface of which is coated with agents such as trioctylphosphine or trioctylphosphine oxide. Similarly, Gao et al. (Nature Biotechnology, Vol. 22, 969-976, August 2004) disclose water soluble semiconductor nanocrystals that are encapsulated with amphiphilic, tri-block copolymers via non covalent hydrophobic interactions.

[0047] 1. In one example, in order to obtain water soluble nanocrystals, a capping reagent is attached to the surface of the nanocrystals. The capping reagent forms a host guest complex with a water soluble molecule.

[0048] Every nanocrystal whose surface can be reacted with a capping reagent which has a (terminal) group that has affinity for (the surface of) the core nanocrystal can be used herein to obtain a water soluble nanocrystal. Accordingly, the capping reagent typically forms a covalent bond with the surface of the nanocrystal. In case of a core-shell nanocrystal, the covalent bond is usually formed between the capping reagent and the shell of the nanocrystal. In case a homogenous ternary or quaternary nanocrystal as described in WO 2004/054923 is used, the covalent bond is formed between the surface of the homogenous core and the capping reagent. The capping reagent can be either of substantially hydrophilic or substantially hydrophobic nature, depending, for example, on the hydrophobicity (or hydrophily) of the inner cavity of the host molecule. In this respect it is noted that within the meaning of term "(substantially) hydrophobic molecule" is also a molecule that in addition to hydrophobic parts can also comprise hydrophilic parts as long as these hydrophilic parts do not interfere with the formation of the host guest complex by the hydrophobic parts of the molecule (i.e. capping reagent) with a host molecule having a hydrophobic internal cavity. Likewise, the term "(substantially) hydrophilic molecule" include a molecule that in addition to hydrophilic parts can comprise hydrophobic parts as long as these hydrophobic parts do

not interfere with the formation of the host guest complex by the hydrophilic parts of the molecule (i.e. capping reagent) with a host molecule having a hydrophilic internal cavity.

[0049] In one example the capping reagent that is used for the "surface capping" has the formula



wherein X is a terminal group selected from S, N, P, or O=P, A is an integer from 0 to 3, Y is a moiety having at least three main chain atoms, and Z is a hydrophobic ending group that can form a host-guest inclusion complex with a suitable host molecule.

[0050] Typically, the moiety Y of the capping reagent comprises 3 to 50 main chain atoms. The moiety Y can principally comprise any suitable moieties that confer a predominantly hydrophobic character to this reagent. Examples of suitable moieties which can be used in Y comprise alkyl moieties such as CH₂- groups, cycloalkyl moieties such as cyclohexyl groups, ether moieties such as -OCH₂CH₂-groups, or aromatic moieties such as a benzene ring or a naphthalene ring, to name a few of them. The moiety Y can be straight chained, branched and can also have substitutions to the main chain atoms. Z may be a -CH₃ group, a phenyl group (-C₆H₅), a -SH group, a hydroxyl group (OH), an acid group (for example, -SO₃H, PO₃H or a -COOH), a basic group (for example, NH₂ or NHR₁ with R=CH₃ or -CH₂-CH₃), a halogen (-Cl, -Br, -I, -F) -OH, -C≡CH, -CH=CH₂, a trimethylsilyl group (-Si(Me)₃), a ferrocene group, or an adamantane group to name a few examples.

[0051] In some examples, compounds such as CH₃(CH₂)_nCH₂SH, CH₃O(CH₂CH₂O)_nCH₂SH, HSCH₂CH₂CH₂(SH)(CH₂)_nCH₃, CH₃(CH₂)_nCH₂NH₂, CH₃O(CH₂CH₂O)_nCH₂NH₂; P((CH₂)_nCH₃)₃, O=P((CH₂)_nCH₃)₃, wherein n is an integer 30 $\geq$ n $\geq$ 8 are used as capping agent. In other embodiments n is an integer 30 $\geq$ n $\geq$ 8.

[0052] In this regard it is noted that examples of capping reagents that provide more hydrophobic or substantially hydrophobic properties include, but are not limited to, 1-mercapto-6-phenyl hexane acid (HS-(CH₂)₆-Ph), 1,16-dimercapto-hexadecane (HS-(CH₂)₁₆-SH), 18-mercapto-octadecylamine (HS-(CH₂)₁₈-NH₂), trioctylphosphine, or 6-mercapto-hexane (HS-(CH₂)₅-CH₃).

[0053] Exemplary capping reagents that provide more hydrophobic or substantially hydrophilic properties include, but are not limited to, 6-mercapto-hexanoic acid (HS-(CH₂)₆-COOH), 16-mercapto-hexadeconic acid (HS-(CH₂)₁₆-COOH), 18-mercapto-octadecylamine

(HS-(CH₂)₁₈-NH₂), 6-mercapto-hexylamine (HS-(CH₂)₆-NH₂), or 8-hydroxy-octylthiol HO-(CH₂)₈-SH.

[0054] Any host molecule can be used, as long it is able to react with the capping agent and confers water solubility to the complex formed between the capped nanocrystal and the host molecule. Typically, the host molecule is a water soluble compound that contains solvent exposed polar groups such as hydroxyl groups, carboxylate groups, sulfonate groups, phosphate groups, amine groups, carboxamide groups or the like.

[0055] Examples of suitable host molecules include, but are not limited to carbohydrates, cyclic polyamines, cyclic peptides, crown ethers, dendrimers and the like.

[0056] Examples of cyclic polyamines that can be used as host molecules include tetraaza macrocyclic molecules such as 1,4,8,11-tetraazacyclotetradecane (also known as cyclam) and derivatives thereof such as 1,4,7,11-tetraazacyclotetradecane (isocyclam), 1-(2-aminomethyl)-1,4,8,11-tetraazacyclotetradecane (scorpiand), 1,4,8,11-tetraazacyclotetradecane-6,13-dicarboxylate which are described in Sroczynski and Grzejda, J. Incl. Phenom. Macrocyclic Chem. 35, 251-260, 1999, or Bernhardt et al., J. Aus. Chem, 56, 679-684, 2003, hexaza macrocyclic complexes, (Hausmann, J. et al., Chemistry, A European Journal, 2004, 10, 1716; Piotrowski, T. et al., Electroanalysis, 2000, 12, 1397), or octaza macrocyclic compounds (Kobayashi, K. et al., J. Am. Chem. Soc. 1992, 114, 1105), for example. The octaza macrocyclic compounds described by Kobayashi, K. et al., *supra* are also one example of compounds that are suitable for accommodation of polar guest molecules (for example, hydrophilic capping agents). It is also possible to employ a cyclic polyamine which may only be water soluble to a limited extent, for example, 5,5,7,12,14,14-hexamethyl-1,4,8,11-tetraazacyclotetradecane(Me₆cyclam) and to modify it with substituents that provide polar groups such as carboxylate or sulfonate groups. Other examples of macrocyclic amines that can be used as host molecule are the compounds described in Odashima, K., Journal of Inclusion phenomena and molecular recognition in chemistry, 1998, 32, 165.

[0057] Examples of suitable calixarenes include 4-*tert*-Butylcalix[4]arene tetraacetic acid tetraethyl ester, tetragalactosylcalixarene as described in Dondoni et al, Chem. Eur., J. 3, 1774, 1997, tetragalactosylcalixarene (Davis, AP. et al., Angew. Chem. Int. Edit., 1999, 38, 2979.) octaaminoamide resorcin[4]-arenes (Kazakov, E.K. et al., Eur. J. Org. Chem., 2004, 3323.), 4-sulphonic calyx[n]-arenes (Yang, W.Z., J. Pharm. Pharmacology, 2004, 56, 703.), sulfonated thiacalix[4 or 6]-arene (Kunsasgi-Mate S., Tetrahedron Letters, 2004, 45, 1387),

the calixarenes described in Kobayashi et al., J. Am. Chem. Soc. 116, 6081, 1994 and Yanagihara et al., J. Am. Chem. Soc. 114, 10307, 1992.

[0058] Examples of cyclic peptides that can be used as host molecule in the present invention include, but are not limited to, the dicyclodipeptide bearing calixarenes that are described in Guo, W et al., Tetrahedron Letters, 2002, 43, 5665; or Peng Li et al., Current Organic Chemistry, 2002, 6.

[0059] Crown ether that can employed as host molecule can have any ring size, for example, have a ring system comprising 8, 9, 10, 12, 14, 15, 16, 18 or 20 atoms of which some are typically heteroatoms such as O or S. Typical crown ethers used here include, but are not limited to, water soluble 8-Crown-4 compounds (wherein 4 indicates the number of heteroatoms), 9-Crown-3 compounds, 12-Crown-4 compounds, 15-Crown-5 compounds, 18-Crown-6 compounds, and 20-Crown-8 compounds. Examples of such suitable crown ethers include (18-Crown-6)-2,3,11,12 tetracarboxylic acid or 1,4,7,10-tetrazaacylcododecane-1,4,7,10 tetracarboxylic acid) to name only a few.

[0060] In principal, every water soluble dendrimer that provides a hydrophilic or hydrophobic cavity (depending on whether a hydrophobic or hydrophilic capping reagent is used) that is able to at least partially accommodate the capping reagent used herein. Suitable classes of dendrimers include, but are not limited to, polypropylene imine dendrimers, polyamido amine dendrimers, poly aryl ether dendrimers, polylysine dendrimers, carbohydrate dendrimers and silicon dendrimers (reviewed in Boas and Heegard, Chem. Soc. Rev. 33, 43-63, 2004, for example).

[0061] In one example, the nanocrystal comprises a carbohydrate as host molecule. This carbohydrate host molecule may be, but is not limited to, an oligosaccharide, starch or a cyclodextrin molecule (cf. Davis and Wareham, Angew. Chem. Int. Edit. 38, 2979-2996, 1999).

[0062] In some examples, in which the host molecule is an oligosaccharide, this oligosaccharide may comprise between 2, for example 6, and 20 monomer units in the main chain. These oligomers may be straight or branched chained. Examples of suitable oligosaccharides include, are not limited to 1,3-(dimethylene)benzenediyl-6,6'-O-(2,2'-oxydiethyl)-bis-(2,3,4-tri-O-acetyl- β -D-galactopyranoside), 1,3-(dimethylene)benzenediyl-6,6'-O-(2,2'-oxydiethyl)-bis-(2,3,4-tri-O-methyl- β -D-galactopyranoside) Shizuma *et al.*, J. Org. Chem. 2002, 67, 4795), cyclotriakis-(1,2,3,4,5,6)-[α -D-glucopyranosyl]-(1,2,3,4)- α -D-glucopyranosyl], (Cescutti et al., Carbohydrate Research, 2000, 329, 647),

acetylenosaccharides (Burli *et al.*, Angew. Chem. Int. Edit. 1997, 36, 1852), or cyclic fructo-oligosaccharides (Takai *et al.*, J. Chem. Soc. Chem. Commun., 1993, 53.).

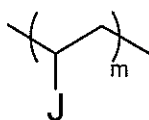
[0063] If starch is used as host molecule the starch may have a molecular weight Mw of about 1,000 to about 6,000 Da. In some examples, the starch has a molecular weight Mw of about 4,000 Da $\geq$ Mw $\geq$ about 2,000 Da. Starches that can be used also include amylose, for example α -amylose or β -amylose.

[0064] Examples of cyclodextrins that are suitable as host molecule include α -cyclodextrin, β -cyclodextrin, γ -cyclodextrin, Dimethyl- α -cyclodextrin, Trimethyl- α -cyclodextrin, Dimethyl- β -cyclodextrin, Trimethyl- β -cyclodextrin, Dimethyl- γ -cyclodextrin, and Trimethyl- γ -cyclodextrin.

[0065] The process for manufacturing such water soluble nanocrystals is known in the art and is described for example in WO 2006/075974. In brief, such a method comprises reacting a nanocrystal having a nanocrystal core with a capping reagent, thereby attaching the capping agent to the surface of the core of the nanocrystal, and then contacting the so obtained nanocrystal with a host molecule to form a host guest complex between the reagent and the water soluble host molecule. The (capping) reagent can either be of hydrophilic or hydrophobic nature. In case a pure metal nanocrystal or a homogenous ternary or quaternary nanocrystal is used, the same reaction can be carried out to prepare such a nanocrystal. Examples for the preparation of such water soluble nanocrystals are provided in Example 3.

[0066] 2. In another example, the at least one nanocrystal comprises a capping reagent which is attached to the surface of the nanocrystals forming a first layer, the capping reagent having at least one coupling group. In addition, a polymer forming a second layer and having at least one coupling moiety is covalently coupled to the at least one coupling group of the capping reagent.

[0067] In one example, the polymer comprising at least one coupling moiety that is reactive towards the coupling group of the capping reagent has the formula:



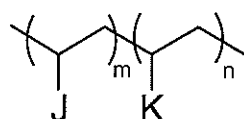
where J is a coupling moiety that is reactive towards the at least one coupling group of the capping reagent, and m is an integer of at least 1.

[0068] To illustrate this example, if for instance the first layer has amino-terminated groups, the polymer forming the second layer can have carboxyl groups for covalently

coupling with the amino groups of the first layer. In practical, it is possible that not all coupling moieties and coupling groups present are involved in covalent coupling. For example, 50% carboxyl groups may be polymerized with amino groups in the first layer.

[0069] In another example, if the first layer have carboxyl-terminated surface, the second layer polymer can have amino groups which covalently couple with the carboxyl groups of the first layer. It is also possible that not all coupling moieties and coupling groups present are involved in covalent coupling. For instance, 50% amino groups may be polymerized with amino groups in the first layer.

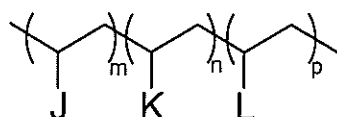
[0070] In another example, the polymer comprises at least two coupling moieties that are reactive towards the at least one coupling group of the capping reagent. In this case, the polymer may have the formula:



where J and K are coupling moieties, said J and K are the same or different, and each of m and n is an integer of at least 1.

[0071] In general, if the capping reagent also has both J and K terminating groups, the polymer can have one or both K and J groups for covalent coupling with the capping reagent. For example, if the first layer has both carboxyl- and amino-terminated surface, the second layer polymer may have only one of or both amino- and carboxyl- groups, respectively, for covalent coupling with the carboxyl groups and amino groups of the first layer. It is sufficient that some of the coupling moieties are covalently coupled to the coupling groups, and it is not necessary for the coupling moieties to be present in exact stoichiometric ratio as the coupling groups.

[0072] In yet another example, the polymer comprises at least three coupling moieties that are reactive towards the at least one coupling group of the capping reagent. In this example, the polymer may have the formula:



wherein J, K and L are coupling moieties, said J, K and L are the same or different, and each of m, n and p is an integer of at least 1. In a further embodiment, the polymer can have 3 or

more different functional groups (NH_2 , COOH , NCO , CHO , etc) for providing water-solubility as well as surface coupling with the first layer.

[0073] The polymer forming the second layer would come into contact with the solvent into which the nanocrystal is placed. Therefore, in order for the nanocrystal to be soluble in the solvent, which may comprise water, for example, at least one of said coupling moieties J, K or L preferably comprises a hydrophilic group which confers water solubility to the water-soluble shell. For this purpose, the polymer may also comprise at least one moiety having a hydrophilic group that confers water solubility to water-soluble shell. The moiety may be present either separately from the coupling moiety or on the coupling moiety itself.

[0074] In one example, the coupling moieties J, K and L each comprises a functional group selected from amino, hydroxyl, carbonyl, carboxyl, nitrile, isocyanate and halide groups. If it is desired to have a homofunctional polymer, the coupling moieties of the polymer may be made up solely of, for instance, hydroxyl groups, or carboxyl groups, or amino groups. In such a case, the polymer is, respectively, a polyvinyl alcohol, a polycarboxylic acid, and a polyamine.

[0075] In order to obtain nanocrystals with differing properties (e.g. solubility in water), other types of polymers having more than one type of monomer may be used. For example, it is possible to use a diblock copolymer, tri-block copolymer or a mixed random polymer as the polymer for forming the second layer. Specific examples include poly(acrylic acid-b-methyl methacrylate), poly(methyl methacrylate-b-sodium acrylate), poly(t-butyl methacrylate-b-ethylene oxide), poly(methyl methacrylate-b-sodium methacrylate), and poly(methyl methacrylate-b-N,N-dimethyl acrylamide).

[0076] The coupling moiety J in the polymer of formula (III) can comprise any suitable functional group that is reactive towards the coupling group present in the capping reagent.

The hydrophilic moiety K can comprise any functional group that accords a predominantly hydrophilic character to the polymer, thereby enabling the polymer to be water soluble. Examples of functional groups which are suitable include carboxyl, amino, hydroxyl, amide, ester, anhydride and aldehyde moieties, for example.

[0077] In one example, the polymer is selected from the group consisting of a polyamine, a polyacetyl acid, or a polyol. The molecular weight of the polymer may range from less than about 500 (about 400) to more than about 1,000,000. In one of these embodiments, the molecular weight range may be between about 600 to about 1,400,000, and more preferably

between about 2000 to about 750,000. For *in vivo* applications, the lower limit being of about 2000 may be chosen to minimize the potential toxicity to the human body.

[0078] If the capping reagent present comprises polymerizable unsaturated groups as coupling groups, unsaturated polymers can be used for forming the second layer of the water souble shell, including polyacetylene, polyacrylic acid, polyethylenimine.

[0079] In still another example, in order to obtain water soluble nanocrystals, the at least one nanocrystal comprises:

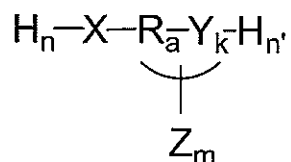
a capping reagent which is attached to the surface of the nanocrystals forming a first layer, the capping reagent having at least two coupling group;

a low molecular weight coating reagent forming a second layer and having at least two coupling moieties covalently coupled with the coating reagent; and

at least one water soluble group for conferring water solubility to the second layer.

[0080] The capping reagent can comprise a terminal group having affinity for the surface of the core of the nanocrystal. The terminal group can include, but is not limited to, sulfhydryl, amino, amine-oxide and phosphino groups. The at least two coupling groups of the capping reagent can be spaced apart from the terminal group by a hydrophobic region. Each of the at least two coupling groups can comprise a functional group independently selected from amino, hydroxyl, carbonyl, carboxyl, nitrile, nitro, isocyanate, epoxide, anhydride and halide groups.

[0081] The capping reagent can comprise two coupling groups spaced apart from a terminal group by a hydrophobic region, as illustrated in the following general formula:



[0082] In this formula, X represents a terminal group that has affinity for the surface of the nanocrystal, or nanocrystal core as it is sometimes called. X may be selected from S, N, P, or O=P. Specific examples of the moiety Hn-X- may include any one of the following: H-S-, O=P-, and H2N-, for example. Ra is a moiety comprising at least 2 main chain carbon atoms, and thus possesses hydrophobic character. If Ra is predominantly hydrophobic in character, e.g. a hydrocarbon, it then provides a hydrophobic region separating moiety Z from the

nanocrystal core. The moiety Y is selected from N, C, -COO-, or -CH₂O-. Z is a moiety that comprises at least one coupling moiety for subsequent polymerization, and which thus confers a predominantly hydrophilic character to a portion of the hydrophilic capping reagent. Exemplary polar functional groups include, but are not limited to -OH, -COOH, -NH₂, -CHO, -CONHR, -CN, -NCO, -COR and halides. The numerals in the formula are represented by the symbols k, n, n' and m. k is 0 or 1. The numeral n is an integer from 0 to 3 and n' is an integer from 0 to 2; both are selected in order to satisfy the valence requirement of X and Y respectively. The numeral m is an integer from 1 to 3. The numeral k is 0 or 1. The condition applies that if k is 0, Z will be bonded to Ra. The value of k=0 caters to the case where the coupling moiety Z is directly bonded to Ra where, for example, Ra is a cyclic moiety, e.g. aliphatic cycloalkanes, aromatic hydrocarbons or heterocycles. However, it is possible that Ra is a cyclic moiety when k=1, e.g. a tertiary amino group bonded to a benzene ring, or a cyclic hydrocarbon. In the present formula, Z is a functional group selected from the group consisting of amino, hydroxyl, carbonyl, carboxyl, nitrile, nitro, isocyanate, epoxide, anhydride and halide groups. Either Y or Z can function as a coupling group. If Z is present as a coupling group, then Y may function as a structural component for attaching coupling group Z. If Z is absent, Y may then form part of the coupling group.

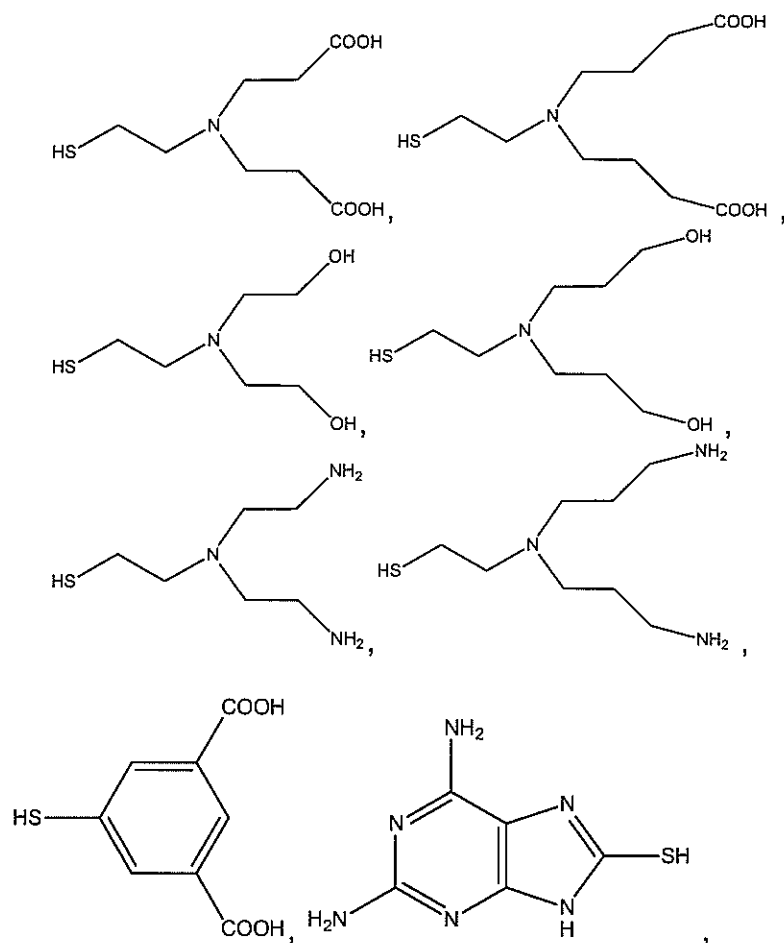
[0083] The moiety Ra in the above formula may comprise between several tens to several hundred main chain atoms. In one particular embodiment, each of Ra and Z independently comprises 2 to 50 main chain atoms. Z may comprise one or more amide or ester linkages. Examples of suitable moieties which can be used for Ra include alkyl, alkenyl, alkoxy and aryl moieties.

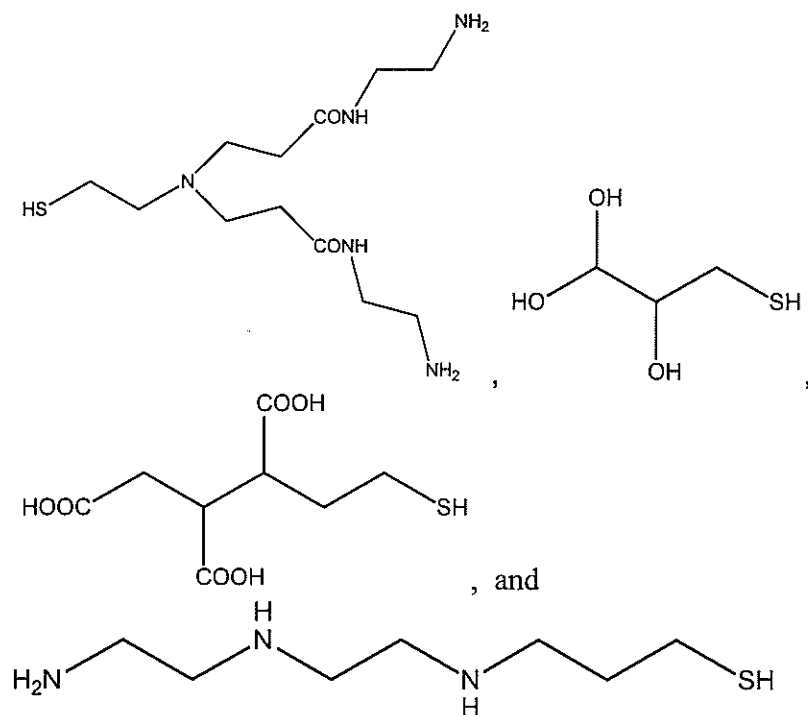
[0084] The term "alkyl" as used herein refers to a branched or unbranched, straight-chained or cyclic saturated hydrocarbon group, generally comprising 2 to 50 carbon atoms, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, t-butyl, octyl, decyl, tetradecyl, hexadecyl, eicosyl, tetracosyl, as well as cycloalkyl groups such as cyclopentyl, cyclohexyl, for instance. The term "alkenyl" as used herein refers to a branched or unbranched hydrocarbon group generally comprising 2 to 50 carbon atoms and containing at least one double bond, typically containing one to six double bonds, more typically one or two double bonds, e.g. ethenyl, n-propenyl, n-butenyl, octenyl, decenyl, as well as cycloalkenyl groups, such as cyclopentenyl, cyclohexenyl, for instance. The term "alkoxy" as used herein refers to a substituent -O-R wherein R is alkyl as defined above. The term "aryl" as used herein, and unless otherwise specified, refers to an aromatic moiety containing one or more aromatic

rings. Aryl groups are optionally substituted with one or more inert, non-hydrogen substituents on the aromatic ring, and suitable substituents include, for example, halo, haloalkyl (preferably halo-substituted lower alkyl), alkyl (preferably lower alkyl), alkenyl (preferably lower alkenyl), alkynyl (preferably lower alkynyl), alkoxy (preferably lower alkoxy), alkoxycarbonyl (preferably lower alkoxycarbonyl), carboxy, nitro, cyano and sulfonyl. In all embodiments, R_a may include heteroaromatic moieties which generally comprise heteroatoms such as nitrogen, oxygen or sulfur.

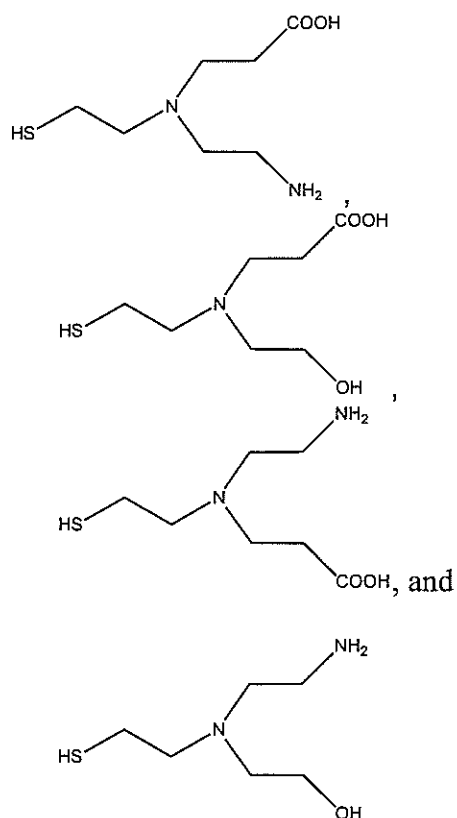
[0085] In some examples, R_a can include, but is not limited to ethyl, propyl, butyl and pentyl, cyclopentyl, cyclohexyl, cyclo-octyl, ethoxy, propoxy, butoxy, benzyl, purine, pyridine, imidazole, moieties.

[0086] In another example, the at least two coupling groups of the capping reagent may be homo-bifunctional or hetero-bifunctional, meaning that they may comprise at least two identical coupling groups or two different coupling groups, respectively. Illustrative examples of some suitable capping reagents with two or three coupling groups have respective structures as follows:





- 5 [0087] Exemplary capping reagents in which the coating reagent is hetero-bifunctional, i.e. 2 different coupling groups are present, include, but is not limited to



[0088] In another example, the capping reagent couples with the coating reagent via polymerizable unsaturated groups, such as C=C double bonds, via any free radical

polymerization mechanism. Specific examples of such capping reagents include, but are not limited to ω -thiol terminated methyl methacrylate, 2-butenethiol, (E)-2-Butene-1-thiol, S-(E)-2-butenyl thioacetate, S-3-methylbutenyl thioacetate, 2-quinolinemethanethiol, and S-2-quinolinemethyl thioacetate.

[0089] The second component of the water-soluble shell surrounding the nanocrystal core is formed by coupling of a low molecular weight coating reagent bearing water-soluble groups to the capping reagent. A coupling agent may be optionally used to activate the coupling groups present in the capping reagent. The coupling agent and the coating reagent bearing the coupling moieties may be added sequentially, i.e. the coating reagent is added after the activation has been carried out; alternatively, the coating reagent may be added simultaneously along with the coupling agent.

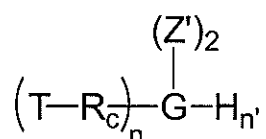
[0090] The determination of an appropriate coupling agent is within the knowledge of the person of average skill in the art. One example of a suitable coupling reagent is 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide (EDC) used in combination with sulfo-N-hydroxysuccinimide (NHS). Other types of coupling reagents may be used, including, but not limited to, imides and azoles. Some examples of imides which can be used are carbodiimides, succinimides and phthalimides. Some explicit examples of imides include 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide (EDC), sulfo-N-hydroxysuccinimide, N,N'-Dicyclohexylcarbodiimide (DCC), N,N'-dicyclohexyl carbodiimide, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide, used in connection with N-hydroxysuccinimide or any other activation molecule.

[0091] The coating reagent which is used for forming the second layer of the water-soluble shell may comprise one or more suitable coupling moieties that has coupling moieties which will react with activated coupling groups on the capping reagent. Typically, suitable coating reagents have at least 2 coupling moieties, i.e. in some examples, there are 2, 3 or 4 functional groups, for example, that are reactive towards the activated coupling groups of the capping reagent. When at least two coupling moieties of the coating reagent are reacted with the capping reagent, the coating reagent becomes covalently coupled ("cross-linked") to the capping reagent via the formation of, for example, ester or amide linkages, thereby forming a water soluble shell that surrounds the nanocrystal core.

[0092] The term 'low molecular weight coating reagent' as used to form the second layer of the water-soluble shell includes non-polymeric ('small') molecules. The molecular weight of the coating reagent can be low or high, depending on the type of groups present in the

coating reagent molecule. If the coating reagent has, for example, small side chains, then the molecular weight of the coating reagent will be low. In the case of a coating reagent in which bulky side chains are present, then molecular weight of such a coating reagent molecule will be higher. Accordingly, in some embodiments, the upper limit of molecular weight of the coating reagent may be about 200, about 400, about 600 Daltons or about 1000. In other embodiments in which a high molecular weight or large spatial volume capping reagent is used, the upper limit may be higher, for example, about 1200, or about 1500 or about 2000 Daltons. In accordance with this definition, the term 'low molecular weight coating reagent' also includes oligomeric compounds which have a molecular weight of up to about 2000 Daltons, for example. The terms "coupling" and "covalent coupling" refer generally to any type of reaction which joins two molecules together to form one single, bigger entity, such as the coupling of an acid and an alcohol to form an ester, or the coupling of an acid and an amine to form an amide. Accordingly, any reaction that can couple the coupling groups and the coupling moieties present in the capping reagent and the coating reagent are within the meaning of the term. 'Coupling' also includes reacting one or more unsaturated groups (e.g. –C=C– double bonds) present as the coupling group in the capping reagent with a corresponding coupling moiety in the coating reagent via free radical coupling to covalently bond the coating reagent to the capping reagent layer.

[0093] The capping reagent and the coating reagent may each possess functional groups that are mutually reactive in order for polymerization to be carried out. In one example, the coating reagent is a water soluble molecule comprising at least 2 coupling moieties, each having at least one co-polymerizable functional group that can react with the coupling group on the capping reagent. In one example, the coating reagent may be a water soluble molecule having the formula:



wherein

T is a moiety for adjusting solubility,

R_c is a moiety comprising at least 3 main chain carbon atoms,

G is selected from N, or C,

Z' is a copolymerisable moiety,

n is an integer of 1 or 2, and

n' is 0 or 1, wherein n' is selected to satisfy the valence requirement of G.

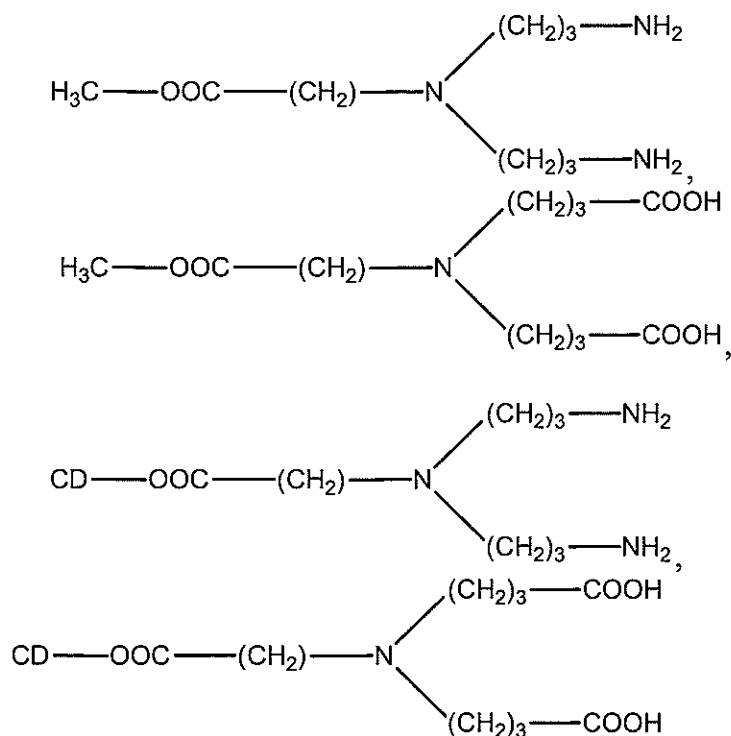
[0094] Water-soluble shells with desirable properties can be obtained with capping reagents in which the moiety Rc has less than 30, preferably less than 20, or more preferably less than 12 main chain carbon atoms. In a preferred embodiment, Rc comprises between 3 to 12 main chain carbon atoms. Under specific experimental conditions, this range provided high coupling efficiency during the synthesis of the nanocrystal. The moiety T can be a polar/hydrophilic functional group for adjusting the solubility of the nanocrystal in the environment in which it is placed. Accordingly, it may impart hydrophilic, or hydrophobic characteristics to the shell, thus allowing the nanocrystal to be soluble in an aqueous environment as well as a non-aqueous environment. T may selected from polar groups such as hydroxyl groups, carboxyl groups, carbonyl groups, sulfonate groups, phosphate groups, amino groups, carboxamide groups, for example. The moiety T may also be hydrophobic, such as any aliphatic or aromatic hydrocarbon (e.g. fatty acid or benzene derivative), or any other organic moiety that is insoluble in water, in order to obtain a nanocrystal that is insoluble in an aqueous environment. Where T is hydrophobic, it can also be modified through the incorporation of hydrophilic moieties after the coating reagent has been copolymerised with the capping reagent. The moiety Z' is a copolymerisable moiety that has functional groups that can copolymerise with the coupling moiety on the capping reagent. Suitable functional groups include, but are not limited to, -NH₂, -COOH or -OH, -Br, -C=C-, for example. Z' may additionally comprise an aliphatic or cyclic carbon chain, preferably with at least 2 main chain carbon atoms.

[0095] In one example, T may be derived from a cyclodextrin molecule. Cyclodextrin molecules have a large number of hydroxyl groups which improves water solubility of the resulting copolymer, and can also conjugate easily to biomolecules for biological labeling purposes. Examples of suitable cyclodextrins that are suitable include α -cyclodextrin, β -cyclodextrin, γ -cyclodextrin, Dimethyl- α -cyclodextrin, Trimethyl- α -cyclodextrin, Dimethyl- β -cyclodextrin, Trimethyl- β -cyclodextrin, Dimethyl- γ -cyclodextrin, and Trimethyl- γ -cyclodextrin.

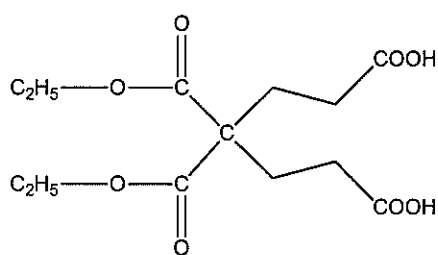
[0096] In yet another example, the coating reagent is a water soluble molecule selected from amino acids, preferably diamino acids or dicarboxylic amino acids. Specific examples of diamino-acids that are presently contemplated include 2,4-diaminobutyric acid,

2,3-diaminopropionic acid or 2,5-diaminopentanoic acid, to name only a few. Dicarboxylic acids that are contemplated in the present invention include but are not limited to aspartic acid and glutamic acid.

[0097] In other examples, the coating reagent is a water soluble molecule selected from the group consisting of:



wherein CD is cyclodextrin, and



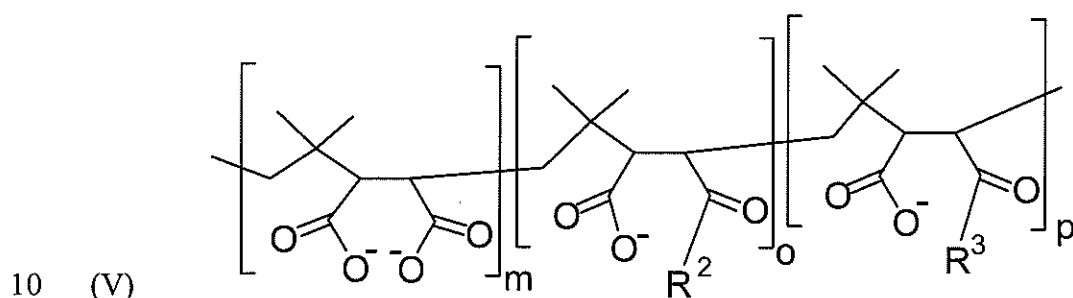
[0098] In another example in which the capping reagent comprises unsaturated group (e.g. C=C double bonds), suitable coating reagents that may be used for coupling include dienes and tri-enes such as 1,4-butadiene, 1,5-pentadiene, and 1,6-hexadiene.

[0099] A method for the manufacture of one of the above mentioned water soluble nanocrystals (item 2) is described for example in WO 2006/118543 and WO 2006/118542.

Some examples for the manufacture of such water soluble nanoparticles are described in Example 4 and 5.

[00100] Another option to obtain water soluble nanocrystals is described in PCT/SG2008/000356. The nanocrystals described in this application include on their surface
5 via non-covalent or covalent interaction an amphiphilic polymer.

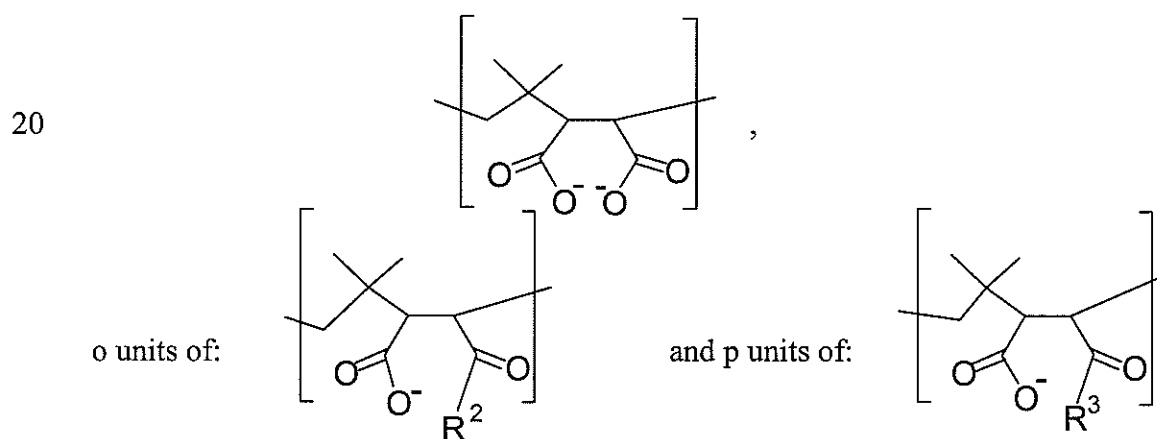
[00101] The amphiphilic polymer can be of the following general formula (V):



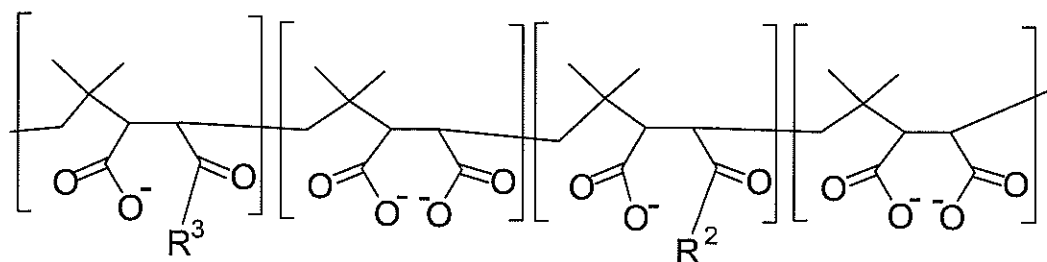
[00102] In this formula each of m, o and p is an independently selected integer from 0 to about 400, including from 1 to about 400 or about 2 to about 400, such as about 0 to about 400, about 0 to about 350, about 0 to about 300, about 3 to about 300, about 0 to about 250,
15 about 0 to about 200, about 2 to about 200, about 0 to about 150, about 2 to about 150, about 0 to about 200, about 1 to about 200, about 3 to about 100, about 2 to about 100, about 0 to about 100, about 3 to about 50, about 2 to about 50, about 1 to about 50 or about 0 to about 50. As further illustrations, m may in some examples be selected in the range from about 5 to about 50, such as about 10 to about 45 including about 10 to about 43, whereas p may for
20 instance be selected in the range from about 3 to about 40, such as about 3 to about 35 or about 4 to about 30, and p may for example be selected in the range from 0 to about 30, such as from 0 to about 25 or from 0 to about 20. The sum of m + o + p is selected in the range from about 10 to about 10000, including about 10 to about 8000, about 10 to about 6000, about 10 to about 5000, about 10 to about 4000, about 10 to about 2000, about 10 to about
25 1000, about 10 to about 750, about 10 to about 600, about 10 to about 400, about 10 to about 250, about 10 to about 150, about 10 to about 100, about 15 to about 150, about 20 to about 150, about 15 to about 100, or about 20 to about 100. In some examples each of m, o and p is an independently selected integer from about 2 to about 300, including from about 3 to about 300, about 3 to about 250, about 3 to about 200, about 3 to about 150 or about 2 to about 200,

about 3 to about 100, about 2 to about 100, about 3 to about 80, about 2 to about 80, about 3 to about 40 or about 2 to about 40 and the sum of $(m + o + p)$ is selected in the range from about 6 to about 400, including from about 10 to about 400, from about 10 to about 350, from about 10 to about 300, from about 10 to about 250, from about 10 to about 200, from about 6 to about 200, from about 10 to about 150, from about 6 to about 150, from about 10 to about 100, from about 6 to about 100, from about 10 to about 50 or from about 6 to about 50. In one embodiment the sum of $(m + o + p)$ is 32. In another example the sum of $(m + o + p)$ is 48. The ratio of $p / (m + o)$ may be selected in the range from 0 to about 25, such as from 0 to about 20, from 0 to about 15, from 0 to about 12, from 0 to about 10, from 0 to about 8, from about 0 to about 6, to about 4, to about 3 or to about 2. In one example the ratio of $p / (m + o)$ is about 1.

[00103] R^2 is the first aliphatic moiety described above, with a main chain of about 3 to about 20 carbon atoms and 0 to about 3 heteroatoms. The heteroatoms are selected from the group N, O, S, Se and Si. R^3 is the second aliphatic moiety described above, with a main chain of about 3 to about 80 carbon atoms and 0 to about 40 heteroatoms. The heteroatoms are selected from N and O. The second aliphatic moiety R^3 has a copolymerisable group. It is understood that the individual units indicated in the above formula may be arranged in any, including random, order – rather than in the form of blocks. Thus, above general formula merely defines that m units of:



are present in the polymer. Such a polymer may therefore encompass any sequence of these units. As an illustrative example a respective sequence may include the following arrangement of units:



5

[00104] An amphiphilic polymer may be prepared by the process described in PCT/SG2008/000356. The amphiphilic polymer is typically at least essentially free of cross-links. Accordingly, in the amphiphilic polymer the copolymerisable group (*supra*) of the second aliphatic moiety R³ is available for any crosslinking or copolymerization reaction.

10 [00105] For example, in the process of manufacturing such nanocrystals coated with amphiphilic polymers a maleic anhydride polymer is used as a reactant, which forms the hydrophilic backbone on the amphiphilic polymer. The maleic anhydride polymer may be the commercially available poly(isobutylene-alt-maleic anhydride) of Chemical Abstracts No. 26426-80-2, also termed isobutylene-maleic acid anhydride copolymer. It is inter alia

15 available under the names BM 30AE20, FibersorbTM SA 7200H, IB 6, KI Gel and Isobam[®]. It is also available from e.g. Sigma-Aldrich (St. Louis, MO, USA) or SinoChemexper Company (Shanghai, PRC). The maleic anhydride polymer may also be poly(ethylene-alt-maleic anhydride) of Chemical Abstracts No. 106973-21-1, also termed ethylene-maleic anhydride alternating copolymer. It is for example available from Rutherford Chemicals (Bayonne, NJ)

20 under product code 27109P, as well as under the names ZeMac[®] E 400 or ZeMac[®] E 60. In the above described formula (V) n may be any integer from about 10 to about 10000, such as about 10 to about 5000, about 10 to about 2000, about 10 to about 1000, about 20 to about 1000, about 10 to about 800, about 20 to about 800, such as about 10 to about 400. In one example n is 32. The above examples of maleic anhydride polymers are not to be considered as being

25 limiting but every available maleic anhydride polymer (and also those yet to be synthesized), in particular a maleic anhydride polymer which may be prepared according to standard procedures as known in the art are suitable to be used. Respective maleic anhydride polymers may for instance be formed following the procedures described in US patents 3846383 and 6316554. A general standard procedure used in the art is also summarized in the

30 abstract of Frank, H.P., *Makromolekulare Chemie* (1968) 114, 113-121 and involves the free-

radical copolymerisation of maleic anhydride with an olefin in the presence of e.g. a peroxide.

[00106] As further reactants a monofunctional compound and an at least bifunctional compound are used. In the course of the process the monofunctional compound is converted to the first aliphatic moiety R^2 described above. The functional group of the monofunctional compound is capable of forming a linkage with an anhydride. The at least bifunctional compound is converted into the second aliphatic moiety R^3 (supra). The at least bifunctional compound may have two, three, four or more functional groups. One of the functional groups is capable of forming a linkage with an anhydride. Another of the functional groups of the at least bifunctional compound is copolymerisable. The reaction of a fraction of the anhydride rings with the monofunctional compounds leads to the formation of the hydrophobic side chains that can interact with the hydrophobic surface of nanoparticles as described herein. Another fraction of the anhydride rings is used to link the at least bifunctional compounds to the backbone. Control over the number of hydrophobic units with respect to the number of carboxyl groups in the backbone of polymers may be achieved by changing the amount of nucleophilic reactants used for the reaction with the polyanhydride chain. Thus, the monofunctional compound of the invention may react with at least about 25% of the available anhydride rings, such as at least about 35%, at least about 50%, at least about 70% or at least about 75%. The at least bifunctional compound of the invention may react with at least about 5% of the available anhydride rings, such as at least about 8%, at least about 10%, at least about 15%, at least about 20% or at least about 25%.

[00107] The functional group of the monofunctional compound may be selected from, but is not limited to, an amino group, a hydroxyl group, a thiol group, a selenol group, a halogen group, an ether group, a thioether group or the like. The monofunctional compound has an alkyl chain of about 2 to about 20 carbon atoms, such as about 3 to about 20 carbon atoms, including about 5 to about 20 carbon atoms, about 5 to about 15 carbon atoms, about 7 to about 20 carbon atoms, such as 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 carbon atoms. Further, the monofunctional compound has 0 to about 2 heteroatoms, such as one heteroatom. The heteroatoms may for instance be N, O, S, Se or Si. Examples of monofunctional compounds may be, but are not limited to, alkylamines, wherein the alkyl group is as defined above. In one embodiment the alkylamin may be n-propylamine, n-butylamine, n-pentylamine, n-hexylamine, n-octylamine or n-dodecylamine.

[00108] Generally, the at least bifunctional group has two or more different functional groups. Any functional group may be selected for each of the at least two functionalities as long as one, typically merely one, of them is capable of forming a linkage with an anhydride. One group, which reacts with the maleic anhydride moiety may be selected from, but is not limited to, an amino group, a hydroxyl group, a thiol group, a selenol group, a halogen group, an ether group, a thioether group or the like. The second functional group, which does not react with the maleic anhydride moiety, may be a copolymerisable group. Copolymerizable means that this group may be polymerized with another functional group. Examples of a suitable copolymerisable group include, but are not limited to, an amino group, a hydroxyl group, an allyl glycidyl ether group, an epoxide group, an oxetane group, and a C=C bond such as an allyl group.

[00109] The at least bifunctional compound has an alkyl chain of about 3 to about 80 carbon atoms, including of about 3 to about 70 carbon atoms, about 3 to about 60 carbon atoms, of about 3 to about 40 carbon atoms, of about 10 to about 80 carbon atoms, of about 10 to about 60 carbon atoms, of about 25 to about 60 carbon atoms, of about 10 to about 40 carbon atoms, of about 3 to about 20 carbon atoms or about 3 to about 10 carbon atoms, such as 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43 or 44 carbon atoms. Further, the at least bifunctional compound has 0 to about 45 heteroatoms, including 0 to about 40 heteroatoms, 1 to about 40 heteroatoms, about 2 to about 40 heteroatoms, about 2 to about 30 heteroatoms or about 0 to about 3 heteroatoms such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42 or 43 heteroatoms, such as N or O.

[00110] In the process of forming an amphiphilic polymer as described above the reaction between the maleic anhydride polymers of formula (V) (*supra*), the monofunctional compound and the at least bifunctional compound may be carried out in the presence of a base. Generally, any base suitable for the intended purpose may be used. In one embodiment the base is a nucleophilic base. A nucleophilic base is a base having basic properties as well as nucleophilic properties. Illustrative examples include, but are not limited to, lithium diisopropylamide, lithium tetramethylpiperidide, diisopropylethyl amine (Hünig's base), 1,5-diazabicyclo[4.3.0]-non-5-ene, 1,8-diazabicyclo[5.4.0]undec-7-ene, a bis(trimethylsilyl)amide, a hexamethyldisilazane or bismesitylmagnesium.

[00111] During the process of forming an amphiphilic polymer, typically a reaction of anhydride groups of the maleic anhydride polymer results in the formation of an amide or an ester group thereby providing a non-polar side chain. This side chain includes the moiety formed from the monofunctional compound, e.g. an alkyl chain, as well as at least one
5 heteroatom, which is the respective atom of the ester or amido group to which the moiety formed from the monofunctional compound is bonded. The first aliphatic moiety thus includes the moiety formed from the monofunctional compound as well as the group COO-, CO-NH- or CO-N-. In embodiments where the group is CO-N- a secondary amide is formed and the first aliphatic moiety accordingly includes 2 moieties formed from the
10 monofunctional compound.

[00112] It is noted in this regard that in examples where a secondary amide is formed the first aliphatic moiety may also include one moiety formed from the monofunctional compound and one moiety formed from the at least bifunctional compound. For the sake of clarity any aliphatic moiety that includes a moiety formed from the at least bifunctional
15 compound shall be considered a second aliphatic moiety. Accordingly an aliphatic moiety that includes both a moiety formed from the monofunctional compound and a moiety formed from the at least bifunctional compound shall be considered a branched second aliphatic moiety.

[00113] Further, each anhydride group of the maleic anhydride polymer may react with one
20 or two monofunctional compounds or with one or two at least bifunctional compounds (see also below). Typically an anhydride group undergoes a reaction with maximally one reactant, whether mono- or at least bifunctional compound. As a result a carboxylic acid group is formed, which typically provides a negative charge in solution. Additionally, a number of anhydride groups do not undergo a reaction. These anhydride groups are generally being
25 hydrolysed in the course of the process of the invention, thereby providing further carboxylic acid groups. Those skilled in the art will appreciate that the process of the invention of forming an amphiphilic polymer does not require the use of any coupling agents such as cross-linkers.

[00114] While it has been previously shown that poly(isobutylene-alt-maleic anhydride) is
30 capable of undergoing a reaction with an aliphatic amine (abstract of Japanese patent application JP 57016004, Fernandez-Arguelles, M.T., et al., *Nano Letters* (2008) 7, 9, 2613-2617), it has been found that furthermore bi-, tri- and higher functionalized compounds can be used as additional reactants in a one-pot synthesis. It is generally possible to control the

reaction conditions in such a manner that the bi- or higher functionalized compound is allowed to undergo only one reaction, thereby forming only one link to the hydrocarbon backbone of the polymer. Remaining functional groups of the bi- or higher functionalized compound are subsequently available for coupling and cross-linking reactions. In this respect it should be noted that the hydrocarbon backbone of the amphiphilic polymer may carry polar side groups and side chains having an alkyl chain of about 3 to about 80 carbon atoms and 0 to about 40 heteroatoms selected from N and O, the side chains having a copolymerisable group. As stated above, the copolymerisable group may be an amino group, a hydroxyl group or a group containing a terminal or internal C=C or C≡C bond, such as a terminal group -CH=CH₂, a terminal group -C≡CH, an internal -CH=CH- or an internal group -C≡C-. As indicated above, the copolymerisable group may also include a terminal or internal C=C or C≡C bond as well as an additional functional group or a vicinal or geminal heteroatom, for instance of the general structure -G-CH=CH- or G-C≡C- with G being N, O, or a group -CH=CH- or -C≡C-, for example.

[00115] The copolymerisable group may be the same or different from remaining groups of the at least bifunctional compound. In examples where the functional groups are the same, those functional groups that are not allowed to react with the maleic acid anhydride polymer may be shielded from participating in a polymerisation process. A large number of protecting groups, which are well known to those skilled in the art, is available for various functional groups. As an illustrative example, hydroxyl groups may be protected by an isopropylidene group. Such a protecting group may be removed after polymerisation and thus the functional group(s) that is/are no longer shielded are available for a coupling-, crosslinking or copolymerisation reaction. For example, the isopropylidene protecting group shielding a hydroxyl group may be removed by acid treatment. Those skilled in the art will furthermore be aware that such protecting groups may have to be introduced well in advance during the synthesis of the respective at least bifunctional compound.

[00116] Examples for the manufacture of such amphiphilic polymers and nanocrystals coated with them are described in PCT/SG2008/000356. Some examples are also listed in Example 6. Other amphiphilic nanocrystals which can be used in the present invention are described for example in the article of Fernandez-Argüelles, M.T., Yakovlev, A., et al. (2007, Nano Lett., vol.7, no.9, pp.2613) and Cheng-An, J.L., Ralph, A., et al. (2008, small, vol.4, no.3, pp.334). The amphiphilic nanocrystals described by Cheng-An, J.L., Ralph, A., et al.

are based on a poly(maleic anhydride) backbone. Reaction of a fraction of the anhydride rings with alkylamines leads to the formation of the hydrophobic side chains that are needed for intercalation with the hydrophobic surfactant layer on the nanoparticle surface. Another fraction of the anhydride rings is used to link function organic molecules to the backbone.

5 The amphiphilic nanocrystals described by Fernandez-Argüelles, M.T., Yakovlev, A., et al. comprise an amphiphilic polymer which is synthesized by linking amino-functionalized hydrophobic hydrocarbone chains (dodecylamine) to a polar backbone (poly(isobutylene-*alt*-maleic anhydride) under reaction of the anhydride rings of the polymer backbone.

[00117] Further water soluble nanocrystals are described in Dubertret et al. (*Science* (2002) 10 298, 1759). Dubertret and his colleagues have encapsulated the nanocrystals in the hydrophobic core of a micelle composed of a mixture of 40% 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] and 60% of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine. The micelles provided a hydrophobic interface for the nanoparticles and maintain high colloidal stability. The presence of PEG layer was a prerequisite 15 to solubilize the nanocrystal.

[00118] Other authors reported about further possibilities which do not rely on a polymer micelle formation as in the method of Dubertret and his colleagues but rather on the direct coating of the nanocrystals with amphiphilic polymers. Amphiphilic, alkyl modified (octylamine or isopropylamine) low molecular weight polyacrylic acids were successfully 20 shown to coat TOPO-protected nanocrystals and solubilize the nanocrystals in water (Wu, X., et al., *Nat. Biotechnol.* (2003) 21, 41; Mattheakis, L.C., et al., *Anal. Biochem.* (2004) 327, 200; Luccardini C., et al., *Langmuir* (2006) 22, 2304). In one study Luccardini et al. (ibid.) have reported on the pH dependent interactions of the coated nanocrystals with lipid membranes. They found that in biological buffers the interaction between the polymer coated 25 nanocrystal and the membrane can be controlled by the pH of the buffer. In the work of Gao et al. high molecular mass amphiphilic ABC triblock copolymers consisting of a polybutylacrylate part (hydrophobic), polyethylacrylate part (hydrophobic), and polymethacrylic acid part (hydrophilic), were used to directly encapsulate QDs (Gao X., et al., *Nat. Biotechnol.* (2004) 22, 969).

30 [00119] A different class of polymers used to transfer hydrophobic QDs directly into water is poly(maleic anhydride *alt*-1-tetradecene) (Pellegrino, T., et al., *Nano Lett.* (2004) 4, 703; Yu, W.W., et al., *J. Am. Chem. Soc.* (2007) 129, 2871). The stability of the polymer shell was increased by addition of the bis(6-aminohexyl)amine crosslinker.

[00120] In addition to the nanocrystal the plant of the present invention can also comprise an organic dye, such as a fluorophore. Examples for a fluorophore include, but are not limited to Hydroxycoumarin (blue color), such as 7-Hydroxycoumarin-3-carboxylic acid; methoxycoumarin (blue color), such as 7-Methoxycoumarin-4-acetic acid; cyanine dyes, such as Cyanine (Cy2, green dark), Indocarbocyanine (Cy3), Indodicarbocyanine (Cy5); aminocoumarin (blue color), such as 7-amino coumarin; FAM (green dark color), such as 5-Carboxyfluorescein (5-FAM); Alexa fluor, such as Alexa Fluor 350, 405, 430, 488, 500, 514, 532, 546, 555, 568, 594, 610, 633, 647, 660, 680, 700 and 750; Fluorescein (FITC; CAS number: 2321-07-5) (light green color); HEX fluorophore (light green color), such as 6-carboxy-2',4,4',5',7,7'-hexachlorofluorescein; TRITC (yellow color), such as Tetramethylrhodamine 6-isothiocyanate chloride (6-TRITC); R-phycoerythrin (PE) (yellow color); Texas Red ($C_{31}H_{29}S_2N_2O_6Cl_1$; CAS No.: 82354-19-6) (orange color); carboxytetrametyl Rhodamine (TAMARA) (red color); 6-Carboxy-X-rhodamine (6-Rox) (red color); or Allophycocyanin (A-PC) (red color) to name only a few. In one example, fluorescein has been used as fluorophore which can be mixed with a nanocrystal (see Figure 4).

[00121] An organic dye can also include, but is not limited to a chromophore, such as azo compounds, lycopene, β -carotene, anthocyanin, fluorescein and luciferin.

[00122] Such mixtures of nanocrystal and organic dye can result in different colors of the plant comprising such a mixture of at least one type of nanocrystal and at least one type of organic dye.

[00123] In another aspect, the present invention refers to a method of coloring a plant with at least one type of a nanocrystal, comprising:

- providing a solution comprising at least one type of nanocrystal dissolved therein;
- contacting the plant with said solution.

[00124] In the above method of the present invention first the nanocrystal is provided in a suitable solvent or mixtures of such solvents, i.e. the solution is a mixture of a solvent and at least one type of nanocrystal, wherein the nanocrystal is dissolved in the solvent.

[00125] Suitable in this respect means that the nanocrystal should be soluble in the respective solvent. Examples of such solvents are, but not limited to, aprotic solvents and/or non-polar solvents, such as an aprotic non-polar solvent. The latter may include, but is not limited to a mineral oil, hexane, heptane, cyclohexane, benzene, toluene, pyridine, dichloromethane, chloroform, carbon tetrachloride, carbon disulfide, dioxane, diethyl ether,

1-octadecene, 9-octadecene, diisopropylether, ethylene glycol monobutyl ether, tetrahydrofuran or mixtures thereof. In one example, 1-octadecene has been used to dissolve the nanocrystal.

[00126] In another example, a protic solvent is used. A protic solvent is a solvent that has, for example, a hydrogen atom bound to an oxygen as in a hydroxyl group or a nitrogen as in an amino group. More generally, any molecular solvent which contains dissociable H^+ , such as hydrogen fluoride, is called a protic solvent. The molecules of such solvents can donate an H^+ (proton). Examples for polar protic solvents include, but are not limited to, water, methanol, ethanol, butyl alcohol, tert.-butyl alcohol, phenol, cyclohexanol, formic acid, acetic acid, dimethylarsinic acid $[(CH_3)_2AsO(OH)]$, aniline, N,N-dimethyl-formamide, N,N-diisopropylethylamine, or chlorophenol. In particular when water-soluble nanocrystals are used, protic solvents, such as water, can be used.

[00127] A further example for a suitable non-polar solvent is a non-polar ionic liquid. Examples of a non-polar ionic liquid include, but are not limited to, 1-ethyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]amide bis(triflyl)amide, 1-ethyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]amide trifluoroacetate, 1-butyl-3-methylimidazolium hexafluorophosphate, 1-hexyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, trihexyl(tetradecyl)phosphonium bis[oxalato(2-)]borate, 1-hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate, 1-butyl-3-methylimidazolium hexafluorophosphate, tris(pentafluoroethyl)trifluorophosphate, trihexyl(tetradecyl)phosphonium, N"-ethyl-N,N,N',N'-tetramethylguanidinium, 1-butyl-1-methylpyrrolidinium tris(pentafluoroethyl)trifluorophosphate, 1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide, 1-butyl-3-methylimidazolium hexafluorophosphate, 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide and 1-n-butyl-3-methylimidazolium. Examples of polar aprotic solvent include, but are not limited to, methyl ethyl ketone, methyl isobutyl ketone, acetone, cyclohexanone, ethyl acetate, isobutyl isobutyrate, ethylene glycol diacetate, dimethylformamide, acetonitrile, N,N-dimethylacetamide, nitromethane, acetonitrile, N-methylpyrrolidone, and dimethylsulfoxide.

[00128] In one example, the solvent is a solvent with a high boiling point. Examples of such solvents include 1-octadecene or 9-octadecene. Their boiling point is above 250°C. Other examples include, but are not limited to Dowtherm A which is a eutectic mixture of two very stable compounds, biphenyl ($C_{12}H_{10}$) and diphenyl oxide ($C_{12}H_{10}O$) with a boiling point of 275.1°C, or polychlorinated biphenyls of the general formula $C_{12}H_{10-x}Cl_x$, such as

Therminol 66 with a boiling point of 359°C. In another embodiment, a low boiling point solvent such as hexane, cyclohexane, chloroform or the like can be used.

[00129] Water soluble nanocrystals can be easily mixed or dissolved in an aqueous solution. Such an aqueous solution is suitable to be taken up by a plant via the roots or the cut stem. In case non-water soluble, i.e. hydrophobic, nanocrystals are used, they have to be dissolved in a suitable solvent, such as a hydrophobic or amphiphilic solvent, which can be taken up by the plant via the roots or a cut stem. Besides octadecene which was used in one of the Examples described herein (see Example 1), other hydrophobic solvents can be used to. Examples include plant oils, such as soy oil, rape oil, sun flower oil, olive oil, to name only a few. The hydrophobic nanocrystals can be dissolved in such oils first before being taken up by the plant via the roots or a cut stem.

[00130] It is also possible to disperse hydrophobic nanoparticle in non-hydrophobic solvents. For example, it is possible to produce a water-oil emulsion by dispersing hydrophobic nanocrystals in a polar or aqueous solvent as described above.

[00131] In another aspect of the present invention the roots of the plant are immersed in the solution. In another aspect, the stem of a plant is cut and the plant with the cut stem is placed in the solution. In such cases, since plant cell walls and epidermis materials are largely made up of long chain fatty acids, emissive nanocrystal are dispersed in a long chain hydrocarbon, such as 1-octadecene or 9-octadecene, to name only two examples.

[00132] In one example, an orchid, namely *Dendrobium* White Fairy was subjected to the uptake of nanocrystals which were dissolved in octadecene. In the absence of specialized translocation channels for organic media in a plant, diffusion of nanocrystals into the flower takes place at a much slower rate. An example of a plant coloured by immersing the cut stem of the plant in a solution of an organic solvent with a nanocrystal is shown in Figure 2. In another example, a mixture of a nanocrystal together with a fluorophore has been dissolved in an organic dye. Afterwards, the cut stem of the plant has been immersed in this solution and after uptake into the plant, the plant showed was multicoloured (see, e.g. Figure 4a).

[00133] In another aspect of the present invention an aerial part or the whole plant has been contacted with the solution comprising at least one type of nanocrystal or a mixture of a nanocrystal with an organic dye which have been dissolved in a suitable solvent.

[00134] Contacting of the plant with this solution can take place by dipping or spraying the solution over an aerial part of the plant or the whole plant. Figure 1 shows an example of an orchid, namely *Dendrobium* White Fairy, which has been dip coated with the solution. The

nanocrystals or mixture of nanocrystals and organic dye are adsorbed at the surface of the plant and are also partly taken up by the plant cells of the epidermis/cuticular.

[00135] The concentration of the at least one type of nanocrystal is between about 0.001 to 1 mmol/l or between about 0.01 to 1 mmol/l or between about 0.1 to 0.5 mmol/l. The concentration of the organic dye that can be used is in the range of 0.001-1.0 mol/L or between about 0.01 to 1 mmol/l or between about 0.1 to 0.5 mmol/l.

[00136] The plant of the present invention can appear in the colour conferred by the uptake or contacting with the at least one type of nanocrystal when being exposed to light of a certain wavelength. This can be in the visible spectrum as well as under UV light. Most nanocrystals emit a specific colour when exposed to UV light.

[00137] The inventions illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[00138] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[00139] Other embodiments are within the following claims and non-limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

EXAMPLES

[00140] 1.1 Surface adsorption/uptake of emissive nanocrystals

There are various methods in achieving flora and foliage color modifications. Physical methods are attractive as compared to genetic modifications due to the facile techniques.

- 5 Surface adsorption/uptake of emissive nanocrystals by dip coating an orchid, such as *Dendrobium* White Fairy in a solution of CdZnSe nanocrystals demonstrated highly emissive flower under UV excitation 302/345 nm excitation (Figure 1).

[00141] Root uptake of emissive nanocrystals in octadecene

- [00142] As plant cell walls and epidermis materials are largely made up of long chain fatty acids, we designed emissive nanocrystal dispersed in a long chain hydrocarbon, such as octadecene. The orchid *Dendrobium* White Fairy was then subjected to the same uptake of CdZnSe nanocrystals (quantum dots) dissolved in octadecene. In the absence of specialized translocation channels for organic media, diffusion of quantum dots into the flower took place at a much slower rate. A minimum 1 hour of uptake was required to have observable glow under UV excitation and 3 hours for a substantial uptake. In contrast to the vein-like fluorescein diffusion, nanocrystal uptake was observed to take place at a higher rate along the periphery of the sepals. Subsequently, the diffusion from the center took place, in a less directional manner, towards edge of the petals and sepals. This can be interpreted by the highly compatible octadecene with the plant cuticular and cell wall chemistry. 15 hours of uptake resulted in full colour emission of the flower (Figure 2, lower picture).
- 10
15
20

- [00143] In general, it can also be said that colouring of a plant can be controlled by varying the uptake time for a colouring agent, such as different types of nanocrystals or another organic dye or mixtures of both. In one example, a mixture of CdZnSe nanocrystals and fluorescein has been used. A short uptake time of the solution in which both colouring agents are comprised will result in fluorescein colouring the veins while the CdZnSe nanocrystals colour the ovary region, base and periphery of the petals and sepals.
- 25

[00144] Root uptake of emissive quantum dots and dyes sequentially

- [00145] Taking a step further, the sequential uptake utilizing CdZnSe nanocrystals dissolved in octadecene and an aqueous fluorescein dye is demonstrated. The sequential uptake of the nanocrystals and the organic dye can result in multicolor modification. 4 hours uptake of nanocrystals via the cut stem was followed by 15 minutes uptake of fluorescein into the flower (also via the cut stem). The result was a combined fluorescein-nanocrystal
- 30

emission in the ovary region that branched out into distinct emission contrast in the petals and sepals, (Figure 4a) despite the minimal change in brightfield appearance of the flower.

[00146] Transverse cross-sections of the stem corresponding to the uptake described above were observed under confocal microscope (Figure 4b). In addition to standard confocal
5 imaging, spectral confocal images were utilized to confirm the presence of CdZnSe nanocrystals (quantum dots) and fluorescein. Ensuring no crosstalk information by selecting appropriate wavelength sampling increment, and minimizing autofluorescence, the confocal image was then spectrally unmixed.

[00147] The specific adsorption of the dye and the nanocrystals on the cell walls were
10 further studied on the longitudinal cross-sections of the stem. Consistently, distinct water-based regions were observed along the vascular bundles and hydrophobic regions surrounding these bundles. Spectroscopic analyses of different regions of the cross section compared the data of fluorescein and nanocrystal emission. Based on the emission behaviour of the dye and nanocrystals, the expanded segment was digitally reconstructed using the
15 confocal software into corresponding hydrophobic and hydrophilic cells indications.

[00148] Uptake of the dye and nanocrystals were carried out using commercially obtained white *Dendrobium*. The stem was cut and left dipped in either 0.001-1.0 mmol/L or as in this specific example 0.03 mol/l CdZnSe nanocrystals in octadec-1-ene or 0.001-1.0 mol/L or as in this specific example 0.03 mol/l fluorescein sodium (Fluka 46960) in water. In case a
20 visible colouring of the whole plant is desired, the stem was dipped in the solution comprising CdZnSe nanocrystals for about 4 h, followed by 15 minutes of dipping in a solution comprising fluorescein. Next, the stem was washed with respective octadecene or water before wiped dry with KimWipes cleansing tissue. Images under UV then captured using digital camera Sony Cybershot DSC-T1.

[00149] For confocal imaging, sections of the stems were carefully sliced using surgical blades. Individual sections of the sliced stem were then carefully transferred onto a microscope glass coverslip. Imaging was carried out with the sample placed facing the objective using Nikon C1si Laser Scanning Confocal Microscope equipped with spectral imaging and fluorescent probe un-mixing.

[00150] Manufacture of CdZnSe nanocrystals was carried out as follows

[00151] Nanocrystals were manufacture using liquid oleic acid and liquid octadecene for preparing luminescent or magnetic nanocrystals. The procedure for the preparation of luminescent nanocrystals as an example is given below: 1.0-mmol cadmium oxide, 0.5-mmol

zinc oxide, 3.1-mmol selenium were loaded into a flask containing 3.1-mmol oleic acid and 10-mL octadecene followed by degassing and heating to 300 °C with stirring, until a clear solution formed. The reaction was remained for 30 min, the reactant is cooled down naturally to room temperature. The obtained nanocrystals can be used directly for uptaking into flowers.

[00152] Instead of oleic acid, soya oil can also be used. Nanocrystals can be formed in these medium as described in the previous paragraph or in Example 1 above. It is also possible to disperse ready made nanocrystals in the solution after purification of nanocrystals produced by other methods.

[00153] 2. Synthesis of a binary metal oxide in a homogenous mixture using a non-polar solvent of low boiling point

[00154] 2.1 Synthesis of Binary metal oxide ZnO

[00155] 3.0 mmol ZnO was dissolved in 7.5 mmol oleic acid at 260 °C to form a clear solution. After it was cooled down to room temperature, 18 ml hexane and 6 mmol oleylamine were added, then it was transferred to 100 mL Parr reactor 4950 and purged with N₂ gas. The mixture was quickly heated to 320 °C under stirring and maintained at the same temperature for 30 minutes. The reaction was then stopped by simply removing the heat and cooling down. The final product was purified by a simple centrifugation dispersion process, i.e. the precipitated nanocrystals can be collected and dried or be re-dissolved in an organic solvent such as hexane for storage.

[00156] 2.2 Synthesis of Ternary quantum dots ZnCdSe

[00157] 0.3 mmol ZnO and 0.3 mmol CdO were dissolved in 2.4 mmol oleic acid at 320 °C to form a clear homogeneous solution. After it was cooled down to 60 °C, 2.4 mL Se solution (1 M TOP-Se solution) and 5 g hexadecylamine (HDA) together with 2 g trioctylphosphineoxide (TOPO) and 20 mL hexane were added; then it was transferred to the 100mL Parr reactor 4950 and purged with N₂ gas. The mixture was quickly heated to 320 °C under stirring and maintained at the same temperature for 30 mins to 3 h. The reaction was stopped by removing the heat and cooling down. The obtained nanocrystals were purified by a simple centrifugation/dispersion process, i.e. the precipitated nanocrystals can be collected and dried or the precipitated products can be re-dissolved in an organic solvent such as hexane for storage.

[00158] 2.3 Synthesis of ternary quantum dots MgFe₂O₄

[00159] 0.4 mmol magnesium carbonate and 0.4 mmol ferrous acetate were dissolved in 3.0mmol oleic acid at 320 °C to form a clear homogeneous solution. After it was cooled down to 60 °C, 5 g oleylamine and 20mL hexane were added; then it was transferred to the 100mL Parr reactor 4950 and purged with N₂ gas. The mixture was quickly heated to 320 °C under stirring and maintained at the same temperature for 30 minutes to 3 h. The reaction was stopped by removing the heat and cooling down. After centrifugation, the precipitated products were either collected and dried or re-dissolved in an organic solvent such as hexane for storage.

[00160] **2.4 Synthesis of NiFe₂O₄ nanocrystals**

[00161] 1.0 mmol nickel acetate and 2.0 mmol iron (III) acetylacetonate were dissolved in 9.0 mmol oleic acid at 150 °C to form a homogeneous solution. After it was cooled down to 60 °C, 5 mL trioctylamine and 20 mL hexane were added; then it was transferred to the 100 mL Parr reactor 4950 and purged with N₂ gas. The mixture was quickly heated to 320 °C under stirring and maintained at the same temperature for 30 min to 1 h. The reaction was stopped by removing the heat and cooling down. In which, 1.0 mmol nickel acetate and 2.0 mmol iron (III) acetylacetonate were dissolved in 9.0mmol oleic acid at 150 °C together with 5 mL trioctylamine and 5 mL ODE to form a homogeneous solution. Then it was quickly heated to 320 °C under 1 atm with stirring and maintained at the same temperature for 30 min to 1 h. The reaction was stopped by removing the heat and cooling down. The obtained mixture was exposed to centrifugation and the obtained nanocrystals collected and dried or re-dissolved in an organic solvent such as hexane for storage purpose.

[00162] **3.1 Preparation of TOPO Capped (CdSe)-ZnS nanocrystals (Quantum Dots, QD)**

[00163] Trioctylphosphine (TOP)/Trioctylphosphine oxide (TOPO) capped CdSe nanocrystals were prepared as follows. TOPO (30 g) was placed in a flask and dried under vacuum (~ 1 Torr) at 180°C for 1 hour. The flask was then filled with nitrogen and heated to 350°C. In an inert atmosphere drybox the following injection solution was prepared: CdMe₂ (200 ml), 1 M TOPSe solution (4.0 ml), and TOP (16 ml). The injection solution was thoroughly mixed, loaded into a syringe, and removed from the drybox.

[00164] The heat was removed from the reaction and the reaction mixture was transferred into vigorously stirring TOPO with a single continuous injection. Heating was resorted to the reaction flask and the temperature was gradually raised to 260-280°C. After the reaction, the reaction flask was allowed to cool to ~ 60°C, and 20 ml of butanol were added to prevent solidification of the TOPO. Addition of large excess of methanol causes the particles to

flocculate. The flocculate was separated from the supernatant liquid by centrifugation; the resulting powder can be dispersed in a variety of organic solvents to produce an optically clear solution.

[00165] A flask containing 5 g of TOPO was heated to 190°C under vacuum for several hours then cooled to 60°C after which 0.5 ml trioctylphosphine (TOP) was added. Roughly 0.1-0.4 μ mol of CdSe dots dispersed in hexane were transferred into the reaction vessel via syringe and the solvent was pumped off. Diethyl zinc (ZnEt_2) and hexamethyldisilathiane ($(\text{TMS})_2\text{S}$) were used as the Zn and S precursors, respectively. Equimolar amount of the precursors were dissolved in 2-4 ml TOP inside an inert atmosphere glove box. The precursor solution was loaded into a syringe and transferred to an additional funnel attached to the reaction flask. After the addition was completed, the mixture was cooled to 90°C and left stirring for several hours. Butanol was added to the mixture to prevent the TOPO from solidifying upon cooling to room temperature.

[00166] **3.2 Preparation of Water-Soluble Nanocrystals by Formation of Host-Guest Complex with γ -Cyclodextrin**

[00167] The nanocrystals obtained in 3.1 having a hydrophobic capping with TOP/TOPO were dissolved into 200 μ l of a mixture of chloroform/hexane (1:1). About 0.5 g of γ -cyclodextrin and the nanocrystal solution were added to a solution of 20 ml deionized water. The mixture was refluxed for about 8 hour until a cloudy solution formed. A rotary evaporator was used to remove most of the water, and then the formed host-guest inclusion complex was isolated by centrifugation. The collected solid was further washed with water to remove the free cyclodextrins molecules. The so obtained nanocrystals that had formed a host guest complex with cyclodextrins via TOP/TOPO were stored in solid state. They can easily be transferred into water by dissolving them in water by means of ultrasonic treatment. The nanocrystals which are protected by the host/guest complex were found to be stable in the solid state for a relatively long time.

[00168] The formation of the water soluble γ -CD modified quantum dots by formation of a host guest complex could be followed optically. When adding γ -cyclodextrin to a chloroform solution containing TOP/TOPO-capped CdSe/ZnS core shell nanocrystals, the formed nanocrystals migrated from the organic chloroform phase to the aqueous solution. The formation of γ -CD modified quantum dots was also confirmed by ^1H -NMR, FT-IR spectroscopy and XRD measurement (data not shown). Transmission electron microscopy (TEM) and fluorescence images show that the quantum dots having formed host guest

complexes with γ -cyclodextrin form high mono-dispersed particles. It has been shown that the CdSe/ZnS core shell nanocrystals possess a higher fluorescence intensity after formation of the host complex (measured in water) than the unmodified TOP/TOPO-capped core shell nanocrystals (measured in CHCl_3), whereas the wavelengths of the emission maximum remained unchanged. The photoluminescence measurements showed that the CdSe/ZnS core shell nanocrystals that have formed a host guest complex with γ -cyclodextrin are very stable in PBS puffer of pH 7.4 (i.e. under physiological conditions), and even show a satisfying stability in aqueous solution of pH 5.0 (upwards pointing triangles) and pH 3.0, respectively (downwards pointing triangles). Finally, the CdSe/ZnS core shell nanocrystals after having formed a host guest complex with γ -cyclodextrin show a good thermal stability in aqueous solution when heated to 50°C.

[00169] **4.1 Preparation of Water-Soluble Nanocrystals with Coupled Polymers in aqueous solution**

[00170] TOPO coated quantum dots are manufactured as described under 3.1. Afterwards, TOPO coated quantum dots were dissolved in chloroform, along with a large amount of aminoethylthiol. The mixture was ultrasonicated for 2 hours and then left at room temperature until the formation of precipitate was completed. The obtained solid was washed with chloroform several times and collected by centrifugation. Then the amino capped quantum dots were dissolved into a buffer solution with pH value of 8 and then added dropwise into a solution of the poly(acrylic acid) polymer (Average Molecular Weight: 2,000 based on GPC), with EDC and sulfo-NHS present as coupling agents to activate the coupling groups on the capping reagent, and stirred at room temperature for 30 mins.

[00171] The reaction mixture was first stirred at 0 °C for 4 hours and then left to react at room temperature overnight. The obtained solution was dialyzed overnight and stored after degassing with nitrogen. Further purification was carried out by first washing the reaction solution with ether twice and centrifugation of the acidic (pH adjusted to about 4-5) polymer coated nanocrystal solution. The collected nanocrystals were then re-dissolved into water by adjustment of the pH value (to 7-8).

[00172] The physical-chemical properties of the polymer shell nanocrystals of the invention were compared to those of (CdSe)-ZnS core shell nanocrystals capped with only mercaptopropionic acid (MCA) or aminoethanethol (AET) as follows: To an aqueous solution of the nanocrystals, H_2O_2 was added in a final concentration of 0.15 mol/l and the chemical behaviour followed photospectroscopically. For the nanocrystals that were coated

only with MCA or AET oxidation of the nanocrystals was immediately detected and the nanocrystals precipitated within 30 minutes. In contrast, the shelled nanocrystals were significantly more stable against chemical oxidation which occurred only slowly.

[00173] **4.2** Preparation of Water-Soluble Nanocrystals with Coupled Polymers in organic solution

[00174] TOPO capped nanocrystals were prepared in accordance with Example 4.1 and dissolved in chloroform, along with excess of 3-mercaptopropionic acid. The mixture was first sonicated for about 1 hour and then left at room temperature overnight until a large amount of precipitate was formed in the solution. The precipitate was collected by centrifugation and free 3-mercaptopropionic acid was removed by washing with acetone for several times. The obtained 3-mercaptopropionic acid capped quantum dots were dried briefly with argon gas and then dissolved into anhydrous DMF. To this solution, excess of EDC and NHS was added and then stirred at room temperature for about 30 minutes for activation and formation of the cross-linking interface. From an additional funnel, polyethylenimine (Sigma-Aldrich Pte Ltd) with a molecular weight of 1200 (a MW of 400 to 60,000 is generally suitable), dissolved in anhydrous DMF was added dropwise with strong stirring. After the entire polyethylenimine solution was added, the reaction was continued at room temperature overnight for coupling of the polymer second layer to the capping reagent. Then, the DMF solvent was removed by rotary evaporation under reduced pressure and then dissolved into water. Further purification of the polymer coated quantum dots was carried out by washing with ether twice.

[00175] **5.1** Preparation of Water-Soluble Nanocrystals with Cross-Linked Shell in aqueous solution

[00176] TOPO coated quantum dots are manufactured as described under 2.1. The (CdSe)-ZnS core shell nanocrystals thus formed were dissolved in chloroform with large excess of the 3-mercaptopropionic acid with a few drops of pyridine. The mixtures were subjected to ultrasonication for about 2 hours and were kept stirred at room temperature overnight. The formed precipitate was collected by centrifugation and washed with acetone to remove the excess of the acid. The residue was briefly dried with a stream of argon. The resultant nanocrystals, coated with molecules of carboxylic acid forming the first layer covering/surrounding the nanocrystal core, were then dissolved in water or buffer solution. The nanocrystals in aqueous solution were centrifuged once more, filtered through a 0.2 μm filter, degassed with argon and stored at 25°C before use.

[00177] For the formation of the cross-linking interface and subsequently the polymerization with the coating reagent layer comprised in the second layer, the carboxylic acid-capped nano crystals were dissolved in an aqueous buffer system. EDC (1-ethyl-3-[3-dimethylaminopropyl] carbodiimide) and sulfoNHS (sulfo-N-hydroxysuccinimide) were added as cross-linking agents to the nano-crystals solution in 500-1000 time excess. The resulting solution was stirred at room temperature for 30 minutes for activation of the functional groups involved in the formation of the cross-linking interface. The mixture containing the carboxylic acid-capped nanocrystals, EDC and sulfoNHS was added was added drop-wise with stirring to a solution of diamino-carboxyl methyl ester in the same buffer. The mixture was stirred for 2 hours at room temperature and then left at 4°C overnight for formation of the cross-linking interface and covalently coupling the coating reagent that is comprised in the second layer to the first layer. For releasing the water soluble carboxyl groups of the diamino-carboxyl ester (i.e. hydrolysis of methyl ester bond) and thus forming the second water-soluble layer, then 0.1 N NaOH and ethanol was added and the solution was kept stirred at room temperature for another 6 hours. The solution was centrifuged to remove any solids and stored in aqueous solution as stock solution at 4 °C.

[00178] The obtained quantum dots can also be purified by organic solvent extraction. After the reaction (formation of the cross-linking interface and covalently coupling the coating reagent that is comprised in the second layer to the first layer) was completed, the solution was extracted with ethyl acetate to extract the polymer-shelled quantum dots with ester surface from the organic solvent. The organic solvents thus obtained were combined and dried, and then removed by rotary evaporator and dissolved in ethanol and 0.1 N NaOH for hydrolysis of the ester bond and formation of the water soluble nanocrystals. The solution was kept constantly stirred at room temperature for 4 hours, and then neutralized. The obtained clear solution was centrifuged to remove any trace amounts of solid and stored in aqueous solution at room temperature after degassing.

[00179] The physical-chemical properties of the obtained cross-linked water soluble shelled nanocrystals of the invention were compared to those of (CdSe)-ZnS core shell nanocrystals with were capped only with mercaptopropionic acid (MCA) or aminoethanol (AET) as follows: To an aqueous solution of the nanocrystals, H₂O₂ was added in a final concentration of 0.15 mol/l and the chemical behaviour followed photospectroscopically. For the nanocrystals that were coated only with MCA or AET oxidation of the nanocrystals was immediately detected and the nanocrystals precipitated within 30 minutes. In contrast, the

shelled nanocrystals of the invention were significantly more stable against chemical oxidation which occurred only slowly.

[00180] In a further experiment (data not shown), when 0.1 M CdSO₄ solution was added to either (CdSe)-ZnS core shell nanocrystals capped with MCA only or to shelled nanocrystals of the invention, the MCA capped nanocrystals precipitated quickly from the solution. Contrasting, the nanocrystals of the invention maintained stable in the solution meaning the addition of cadmium ions has no significant effect on their stability.

[00181] Similarly, the photochemical stability of the shelled nanocrystals was also significantly improved in comparison to the MCA capped nanocrystals (data not shown).

When exposed to UV light with a wavelength of 254 nm, the MCA capped nanocrystals were found to precipitate from the solution in 48 hours whereas the shelled nanocrystals of the invention were stable for 4 days. The fluorescence intensity was also found to be stable for a long time.

[00182] **5.2 Preparation of Water-Soluble Nanocrystals with Cross-Linked Shell in organic solution**

[00183] TOPO capped nanocrystals were prepared in accordance with Example 4.1 and dissolved in chloroform, along with excess amount of pentane-(3-N-ethylthiol)-1,5-diamine for formation of the first layer. The mixture was left at room temperature overnight. Formed precipitates were collected by centrifugation and then washed with methanol, dried briefly with argon gas. The obtained nanocrystals were dissolved in anhydrous DMF (50 ml).

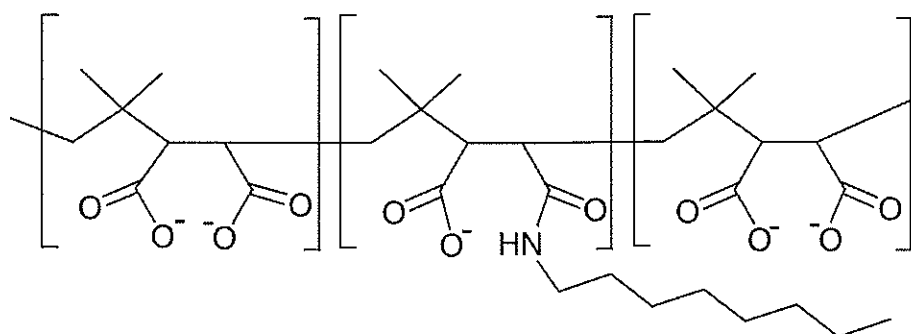
[00184] In another flask, pentane-3,3-diethyl-carboxylic ester-1,5-dicarboxylic acid (as coating agent comprised in the second layer) was dissolved in DMF with 5 equiv. of EDC and NHS, and stirred at room temperature for 20 minutes under nitrogen protection. The solution was added slowly to the nanocrystal solution for covalent coupling with the coating agent. After stirring the resulting solution for 2 hours at room temperature, the DMF solvent was evaporated under reduced pressure by using a rotavapor system. The obtained slurry was dissolved in 5 ml of water and then 5 ml of 1 M EtONa/EtOH solution was added and stirred at room temperature for another 2 hours to form the solvent-exposed water soluble bonds in the second layer. The resulting solution was washed twice (5 ml × 2) with ether to remove any trace of the additives or un-reacted starting materials. It was then neutralized with 0.1 N HCl aqueous solution for storage. Further purification was carried out by centrifugation of the polymer-coated nanocrystals in acidic solution and re-dissolving the nanocrystals in water by adjustment of the pH value of the solution.

[00185] **6.1** Manufacture of amphiphilic polymers

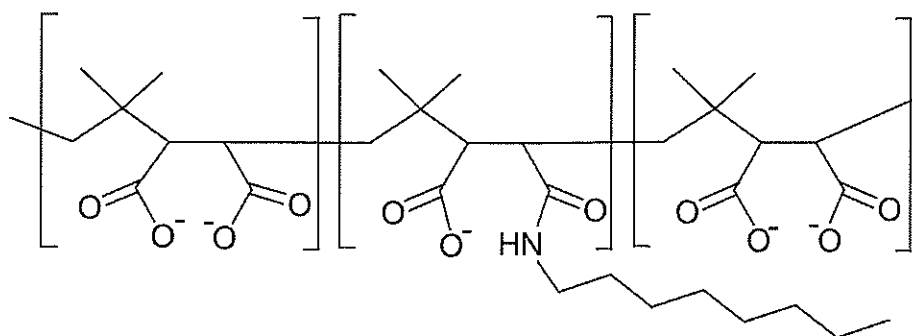
[00186] To the solution of 1g poly(isobutylene-alt-maleic anhydride) in 300 mL of dry CHCl_3 the respective amine followed by DIPEA (1 mL) were added and the mixture was stirred for 16 h in 50 °C. After evaporation of CHCl_3 the material was suspended in water with small excess of NaOH toward the carboxylic groups in the polymer backbone. After evaporation of water and DIPEA, remain was dissolved in water and dialyzed against water for a few days. The solution after alkalization to pH 11 with 1 M NaOH and subsequent evaporation resulted with products. The poly(isobutylene-alt-maleic anhydride) reacts spontaneously with high yield with both the monofunctional compound and the at least bifunctional compound (here the second component).

[00187] In the following exemplary data on the formation of a series of amphiphilic polymers of interest 1-5 are provided. Following the foregoing protocol these amphiphilic polymers were synthesised by grafting *n*-octylamine to poly(isobutylene-alt-maleic anhydride) at different molar ratios.

Polymer 1

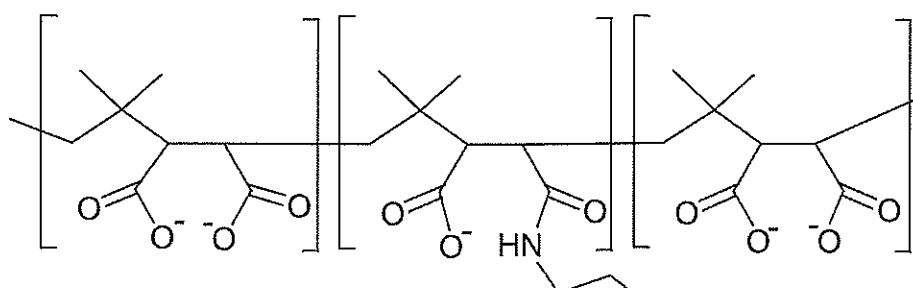


[00188] Reaction of poly(isobutylene-alt-maleic anhydride 6000 with octylamine (0.05g, 0.4 mmol) resulted with crystalline product 1 (0.86 g, 59 %). $^1\text{H-NMR}$ (400MHz, D_2O) δ : 3.25 - 2.90 (m, 9 H), 2.59 (bs, 39 H), 2.08 (bs 39 H), 1.95 - 1.35 (m, 81 H), 1.34 (bs, 62 H), 1.13 - 0.65 (m, 238 H). Target composition: carboxylic 97%, octyl amide 3%; observed: carboxylic 96 %, octyl amide 4%.

Polymer 2

5

[00189] Reaction of poly(isobutylene-alt-maleic anhydride 6000 with octylamine (0.15 g, 1.2 mmol) resulted with crystalline product 2 (0.97 g, 64 %). $^1\text{H-NMR}$ (400MHz, D_2O) δ : 3.30 - 2.90 (m, 23 H), 2.59 (bs, 38 H), 2.42 - 1.40 (m, 152 H), 1.23 (bs, 116 H), 1.15 - 0.70 (m, 238 H). Target composition: carboxylic 91%, octyl amide 9%; observed: carboxylic 89 %
10 %, octyl amide 11 %. Polymer 3

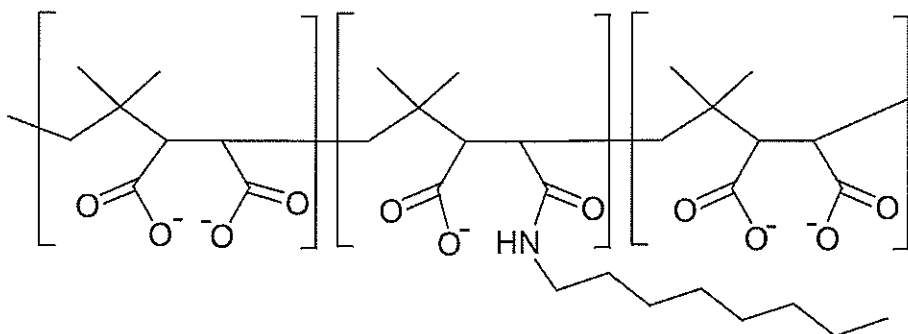


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[00190] Reaction of poly(isobutylene-alt-maleic anhydride 6000 with octylamine (0.42 g, 3.2 mmol) resulted with crystalline product 3 (1.06g, 63 %). $^1\text{H-NMR}$ (400 MHz, D_2O) δ : 3.28 - 2.85 (m, 47 H), 2.60 (bs, 37 H), 2.41 - 1.60 (m, 81 H), 1.47 (bs, 68 H), 1.24 (bs, 253 H), 1.12 - 0.75 (m, 286 H). Target composition: carboxylic 75%, octyl amide 25%; observed: carboxylic 75%, octyl amide 25%.

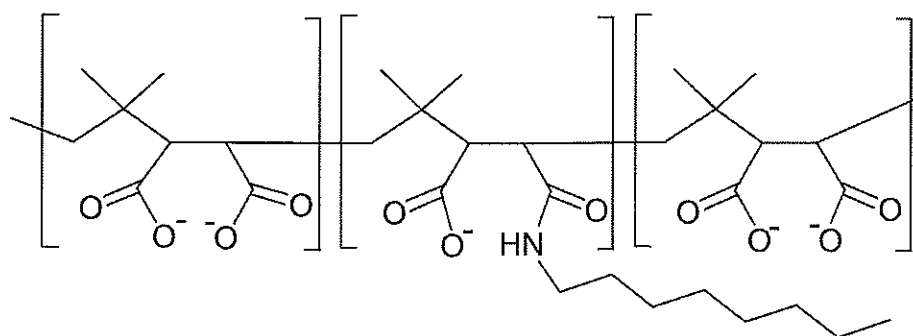
Polymer 4

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[00191] Reaction of poly(isobutylene-alt-maleic anhydride 6000 with octylamine (0.84 g, 6.5 mmol) resulted with crystalline product 4 (1.63g, 88%). $^1\text{H-NMR}$ (400 MHz, D_2O) δ : 3.25 - 2.85 (m, 72 H), 2.60 (bs, 34 H), 2.40 - 1.65 (m, 73 H), 1.48 (bs, 87 H), 1.24 (bs, 379 H), 1.12 - 0.65 (m, 317 H). Target composition: carboxylic 50 %, octyl amide 50 %;
 5 observed: carboxylic 61 %, octyl amide 39 %.

Polymer 5



[00192] **6.2** Synthesis of nanocrystal (quantum dots (QD) / polymer assemblies

[00193] 2 mg of purified QDs were dissolved in THF (2 mL) and 0.5 mL of 1.4 mM polymer solution was added followed by 5 mL of water. The mixture was concentrated with a
 15 rotary evaporator to 1 mL. Turbid water suspension was filtered through a 0.8 μm filter and the filter was washed with 5 mL of pure water. The supernatant was filtered again according to the same procedure through a hydrophilic filter (0.2 μm) resulting in a clear QDs water solution. Excess of water can be removed with a rotary evaporator to reach desirable concentration.

[00194] The above described polymers **1-5** were used to transfer nanocrystals into water. Based on the absorption of QD/polymer assemblies it is estimated that from 10 to 80% of dots is passing the suspension procedure. This yield is however strongly dependent on polymer used and even more dependent on the purity of the QDs (excess of hydrophobic ligands lead to formation of aggregates). The suspending efficiency of polymers **1-5** was
 25 subsequently tested. Starting with the same initial amount of QDs in THF, the same concentration of polymers **1-5** in water has been applied, but different concentrations of QDs in water (calculated from optical absorption) were obtained. The final concentration of QDs in water depends on the ability of a given polymer to suspend QDs in water. It is found that polymer with higher number of n-octyl groups attached to the polymer backbone is more

favourable, and this follows a general observation that a higher number of anchoring points (in this case hydrophobic n-octyl chains) gives higher stability to an assembly based on hydrophobic-hydrophobic interactions. For the polymer **5**, with a 10 times higher molar mass value than for **1-4**, low concentration of QDs in the final aqueous solution can be attributed to the formation of aggregates (few dots attracted to the same polymer chain), and to problems related to the filtration step. Careful purification to remove TOPO, TOP and hexadecylamine in the QDs solution is critical for the effective formation of stable QD/polymer assemblies. Probably the remaining alkyl ligands prevent the formation of monodispersed micelles, as well as drive flocculation. Coating the QDs with polymers and transferring them into water did not show any significant effects on their basic luminescence properties. Although the absorption spectrum shows minor changes, the wavelength at the maximum of the emission and the widths of the emission spectra remain essentially unchanged.

[00195] **6.2.1 Nanocrystal coating**

[00196] Covering hydrophobic nanocrystal (quantum dots (QD)) (for example TOPO coated CdSe/ZnS Quantum Dots) is carried out by suspending them in THF and subsequent addition of aqueous polymer solution. Removing of THF by evaporation results with stable colloidal solutions of polymer coated Quantum Dots. The resulting solutions are clear, transparent, and display long-term optical and colloidal stability. It is noted that the coating process can generally be carried out on any nanoparticulate matter, regardless of the surface thereof.

[00197] **6.3 Example 4: NIPAM quantum dots copolymerization**

[00198] Polymerization is carried by direct mixing of acrylic monomer and QDs coated with polymer in water or by dosing one of the ingredient to the solution of another in presence of appropriate initiator. Depending on the polymer used and reaction conditions (for example temperature, initiator, concentration of ingredients) different size of microspheres with different number of embedded QDs covalently attached to the polymer chains are possible to obtain.

[00199] Representative polymerization procedure:

[00200] In the 50 mL three neck flask NIPAM (0.0503 g), solution of QDs (8 mg) coated with acrylic ester polymer - ammonium salt form and 20 mL of water was deoxidized by 15 times vacuum / argon evacuation. Temperature was increased to 70 °C and initiator $K_2S_2O_8$

(0.0319 g in 1 mL of H₂O) was added. Subsequently solution of crosslinker MBAAM (0.0285 g) and NIPAM (0.2526 g) in 4 mL of H₂O was dosed within 75 min. After dosing mixture was kept in 71°C for additional 4h and cooled to r.t. Purification was carried out by dialysis (50 kD membrane) and centrifuged to remove bigger particles. As a result, water solution of submicroparticles of PNIPAM bearing QDs were obtained.

[00201] Direct cross-polymerization of quantum dots

[00202] Polymerization is carried by addition of initiator (like K₂S₂O₈) to water solution of dots coated with polymerizable amphiphilic polymer. Dialysis of the crude reaction mixture results in water-processable and suspendable solid polymeric film carrying high content of QDs.

[00203] Representative polymerization procedure:

[00204] In 50mL flask solution of QDs (8 mg) coated with acrylic amide polymer – sodium salt form and 20 mL of water was deoxidized by 15 times vacuum / argon evacuation. Temperature was increased to 70 °C and initiator K₂S₂O₈ (0.0319 g in 1 mL of H₂O) was added. Temperature of the reaction was maintained for 6 h. Purification was carried by dialysis (50 kD membrane). Purification resulted with water soluble polymeric network bearing high concentration of QDs.

[00205] **6.4** Micro-emulsion polymerization and copolymerization of QDs

[00206] Polymeric material bearing coated QDs (such as described in Examples 5.1 to 5.2) with polymerizable functions (such as described in Example 5.3) is used for self polymerization and copolymerization with other water soluble monomers in reversed microemulsion process. Microsphere with controllable diameters in the range between 400 nm and 10 μm are obtained. The microspheres are luminescent over an extended period of time.

[00207] Representative polymerization procedure of microemulsion polymerization:

[00208] Argon was passed through solution of sorbitan monooleate (Span 80, 0.23 g) in 10 mL of parafin oil for 1 h. Water solution 1 mL in volume, containing NIPAM (0.15 g, 1.32 mmol), N,N' methylenebisacrylamide (0.013 g, 0.06 mmol), 8 mg of QDs and 10 mg of polymers (liken in 1.3.2) were added to oil phase and argon bubbling was continued for next 1h. Emulsion was sonicated for 1 min in 40 °C and then was shaken for 1h in r.t. with laboratory vortex shaker. Stable homogeneous emulsion was casted onto a Petri dish and irradiated with UV lamp under nitrogen-ventilated crosslinker device for 4 h at a temperature

between 5 and 15°C and subsequently left without irradiation for 16 h in the inert atmosphere. Purification of PNIPAM latex was carried out by several washing steps and centrifugation with hexane, and subsequently several washing steps and centrifugation with water.

What is claimed is

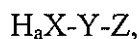
1. A plant comprising at least one type of nanocrystal.
- 5 2. The plant according to claim 1, wherein the at least one type of nanocrystal is selected from the group of types of nanocrystals consisting of
 - a rare earth metal doped metal oxide of the chemical formula $\text{MeAl}_2\text{O}_4\cdot\text{Re}$ or $\text{Me}_4\text{Al}_{14}\text{O}_{25}\cdot\text{R}$;
 - 10 ▪ a binary nanocrystal of the chemical formula M1A or M1O ;
 - a ternary nanocrystal of the chemical formula M1M2A or M1AB or M1M2O ; and
 - a quaternary nanocrystal of the chemical formula M1M2AB ;

wherein M1 and M2 of the binary, ternary and quaternary nanocrystals are independently a metal selected from one of Group II, Group III, Group IV and Group VIII of the PSE; and, if present, A and B are independent from each other an element selected from Group VI or Group V of the PSE; and

wherein Me of the rare earth metal doped metal oxide is selected from the group consisting of Ca, Sr and Ba; and

20 Re of the rare earth metal doped metal oxide is at least one element selected from the group consisting of Tb, Dy, Nd, Eu and Tm.
3. The plant according to claim 1 or 2, wherein the at least one type of nanocrystal does not comprise cadmium (Cd).
- 25 4. The plant according to any of claims 1 to 3, wherein the at least one type of nanocrystals is water soluble.
5. The plant according to claim 4, wherein a capping reagent is attached to the surface of the nanocrystals, and wherein the capping reagent forms a host guest complex with a water soluble molecule.
- 30

6. The plant according to claim 5, wherein the capping reagent is molecule having the formula:



wherein

- 5 X is a terminal group selected from S, N, P, or O=P,
a is an integer from 0 to 3,
Y is a moiety having at least three main chain atoms, and
Z is a hydrophobic ending group.

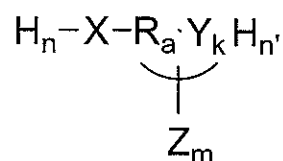
- 10 7. The plant according to claim 5, wherein the water soluble molecule is a compound containing solvent exposed polar groups.

8. The plant according to claim 7, wherein the water soluble molecule is selected from the group consisting of carbohydrates, cyclic polyamines, cyclic peptides, calixarenes,
15 crown ethers and dendrimers.

9. The plant according to claim 4, wherein the at least one nanocrystal comprises:
a capping reagent which is attached to the surface of the nanocrystals forming a first layer, the capping reagent having at least two coupling group;
20 a low molecular weight coating reagent forming a second layer and having at least two coupling moieties covalently coupled with the coating reagent; and
at least one water soluble group for conferring water solubility to the second layer.

10. The plant according to claim 4, wherein the at least one nanocrystal comprises:
25 a capping reagent which is attached to the surface of the nanocrystals forming a first layer, the capping reagent having at least one coupling group;
a polymer forming a second layer and having at least one coupling moiety covalently coupled to the at least one coupling group of the capping reagent.

- 30 11. The plant according to claim 9 or 10, wherein the capping reagent is molecule having the formula:



wherein

X is a terminal group selected from S, N, P, or O=P,

R_a is a moiety comprising at least 2 main chain carbon atoms,

5 Y is selected from N, C, -COO-, or -CH₂O-,

Z is a moiety comprising a polar functional group,

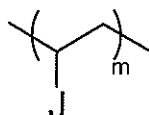
k is 0 or 1,

m is an integer from 1 to 3,

n is an integer from 0 to 3, and

10 n' is an integer from 0 to 2, wherein n' is selected to satisfy the valence requirement of Y.

12. The plant according to claim 10, wherein the polymer has the formula:

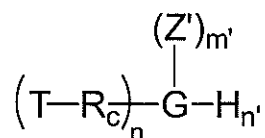


15 wherein

J is a coupling moiety that is reactive towards the at least one coupling group of the capping reagent, and

m is an integer of at least 1.

20 13. The plant according to claim 9, wherein the low molecular weight coating reagent comprised in the second layer comprises a water soluble molecule having the general formula:



wherein

25 T is a hydrophilic moiety,

R_c is a moiety comprising at least 2 main chain carbon atoms,

G is selected from N, P or C, or Si

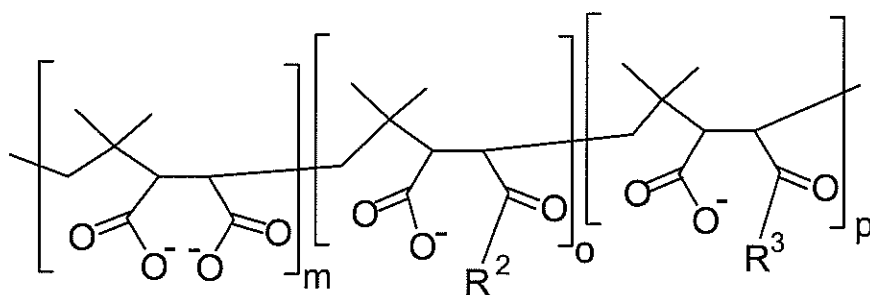
Z' is a coupling moiety,

m' is 2 or 3,

n is 1 or 2, and

n' is 0 or 1, wherein n' is selected to satisfy the valence requirement of G.

14. The plant according to claim 4, wherein at least one of the at least one type of nanocrystal comprises on its surface via non-covalent or covalent interaction an amphiphilic polymer of the general formula:



wherein each of m, o and p is an independently selected integer from about 3 to about 400 and wherein the sum of m + o + p is selected in the range from about 10 to about 10000,

R² is a first aliphatic moiety with a main chain of about 3 to about 20 carbon atoms and 0 to about 3 heteroatoms selected from the group N, O, S, Se and Si, and

R³ is a second aliphatic moiety with a main chain of about 3 to about 80 carbon atoms and 0 to about 40 heteroatoms selected from N and O,

wherein R³ has a copolymerisable group.

15. The plant according to any of claims 1 to 14, wherein the nanocrystal is comprised on an aerial part or all of the outer surface of the plant.
16. The plant according to claim 15, wherein the nanocrystals are comprised in an aerial part of the plant.
17. The plant according to claim 15 or 16, wherein the aerial part of the plant is a flower and/or a leave of the plant.

18. The plant according to any of claims 1 to 15 or 17, wherein the plant is an artificial plant, which is made of at least one polymer or paper or a metal foil or a mixture of the aforementioned materials, or a dried plant.
- 5 19. The plant according to any of claims 1 to 18, wherein the plant is a flowering plant.
20. The plant according to claim 19, wherein the plant is selected from the group consisting of plants from the family rosaceae, hydrangeaceae, orchidaceae, hydrangeaceae, hyacinthaceae, begoniaceae, lamiaceae; asteraceae; geraniaceae, 10 solanaceae, liliaceae, primulaceae, onagraceae, violaceae, gesneriaceae, malvaceae, bromeliaceae, araceae, ericaceae, theaceae, and nyctaginaceae.
21. The plant according to claim 19, wherein the plant is selected from the group of plants consisting of roses, orchids, hydrangea, hyacinth, begonia, lavender, dahlia, geranium, 15 petunia, tulip, lily, primrose, viola, gerbera, gloxinia, hibiscus, bromeliad, cyclamen, anthurie, azalea, bougainvillea, and camellia.
22. The plant according to claim 20, wherein an orchid is selected from the subfamily of the family of orchidaceae selected from the group consisting of Orchidoideae, Epidendroideae, Vanilloideae, Cypripedioideae, and Apostasioideae.
- 20 23. The plant according to any of claims 1 to 22, further comprising an organic dye.
- 25 24. The plant according to claim 23, wherein the organic dye is a fluorophore.
25. The plant according to claim 23, wherein the organic dye is selected from the group consisting of ink, a chromophore, such as azo compounds, lycopene, β -carotene, and anthocyanins; fluorescein and luciferin.
- 30 26. A method of coloring a plant with at least one type of a nanocrystal, comprising:
 - providing a solution comprising at least one type of nanocrystal dissolved therein;

- contacting the plant with said solution.

27. The method according to claim 26, comprising:

- immersing the roots of the plant in the solution or cutting a stem of the plant and immersing the end of the cut stem in the solution.

28. The method according to claim 26, comprising:

- contacting an aerial part or the whole plant with the solution.

29. The method according to claim 28, wherein the contacting comprises dipping or spraying the solution over an aerial part of the plant or the whole plant.

30. The method according to claims 26 or 27, wherein the at least one type of nanocrystal is a nanocrystal as defined in any of claims 1 to 14 or a mixture of a nanocrystal and an organic dye as defined in any of claims 23 to 25.

31. The method according to any of claims 26 to 30, wherein the solution comprises a suitable solvent or mixture of solvents in which the at least one type of nanocrystal is dissolved.

32. The method according to claim 31, wherein the solvent is an aprotic solvent and/or a non-polar solvent or a protic solvent.

33. The method according to claim 32, wherein the non-polar solvent is selected from the group consisting of mineral oil, hexane, heptane, cyclohexane, benzene, toluene, pyridine, dichloromethane, chloroform, carbon tetrachloride, carbon disulfide, dioxane, diethyl ether, 1-octadecene, 9-octadecene, diisopropylether, ethylene glycol monobutyl ether, tetrahydrofuran and mixtures of the aforementioned solvents.

34. The method according to any of claims 26 to 33, wherein the concentration of the at least one type of nanocrystal is between about 0.001 to 1 mol/l.

35. The method according to any of claims 26 to 34, wherein the solution further comprises an organic dye.
36. The method according to claim 35, wherein the organic dye is a fluorophore.
- 5 37. The method according to claim 35, wherein the organic dye is selected from the group consisting of ink, a chromophore, such as azo compounds, lycopene, β -carotene, and anthocyanins; fluorescein and luciferin.
- 10 38. The method according to claim 31, wherein the solvent is a solvent having a high boiling point.
39. The method according to claim 38, wherein the solvent is selected from the group consisting of 1-octadecene and 9-octadecene.
- 15 40. Use of a nanocrystal as defined in any of claims 1 to 14 or a mixture of a nanocrystal and an organic dye as defined in any of claims 23 to 25 for colouring a plant.
41. The use according to claim 40, wherein an aerial part of the plant is coloured.
- 20 42. The use according to claim 41, wherein the aerial part is coloured by contacting a plant with a solution comprising the nanocrystal dissolved in a suitable solvent.
43. The use according to claim 42, wherein the contacting includes immersing the roots of the plant in the solution or cutting a stem of the plant and immersing the end of the cut stem in the solution.
- 25 44. The use according to claim 43, wherein the contacting comprises dipping or spraying the solution over an aerial part of the plant or the whole plant.
- 30