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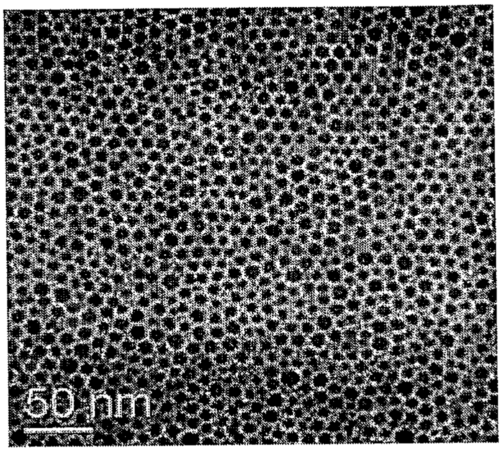
(54) Title: METHOD OF PREPARING NANO-STRUCTURED MATERIAL(S) AND USES THEREOF



(57) Abstract: The present invention provides a method of preparing at least one nano- structured material of formula $M_1M_2X_n$ comprising the step of treating: at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_1$; and at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$; wherein each X is the same or different and is selected from the group consisting of : halogens, O, S, Se, Te, N, P and As ; each n is the same or different and is $0 \leq n \leq 10$; each m is the same or different and is $0 \leq m \leq 10$; each p is the same or different and is $1 \leq p \leq 5$; each M_1 is the same or different and is selected from the group consisting of: Li, Na, K, Rb, Cs, Fr, Be, Mg, Ca, Sr, Ba, Ra and NH_4 ; each M_2 is the same or different and is a metal ion. The present invention also provides uses of the nano-structured material prepared according to the method of the present invention.

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Method of preparing nano-structured material(s) and uses thereof

Field of the invention

The present invention relates to nano-structured materials and a method of preparing the nano-structured materials. The present invention also provides uses of the prepared nano-structured materials.

Background of the invention

The search for ultra sensitive fluorescent bio-probes for analytical and biophysical applications is very active (Bruchez M. et al, 1998; van de Rijke et al, 2001). To date, the commonly used fluorescent bio-probes are organic dyes, such as rhodamine, fluorescein isothiocyanates (FITC), and cyanine dyes (Cy3, Cy5 and Cy7). More recently, semiconductor nanoparticles (quantum dots, QDs) have been employed as bio-probes (Gerion D et al, 2001; Dubertret B et al, 2002). These down-conversion fluorescent bio-probes emit one lower energy fluorescent photon after absorbing another higher energy UV or visible photon. The main problem of these probes in bio-applications is the autofluorescence (noise) from the analytes under UV and visible light. It decreases the signal-to-noise ratio, limiting the sensitivity.

The use of infrared-to-visible up-conversion phosphors as bio-probe, which is able to absorb and combine two or more near-infrared (NIR) photons with lower energy to produce a higher energy photon in the visible spectrum, is a promising approach to solve the problem of autofluorescence. This concept was first disclosed in US Patent 5,674,698. Compared with the current bio-probes including organic dyes, fluorescent proteins and quantum dots, the advantages include: improved signal-to-noise ratio due to absence of autofluorescence and reduction of light scattering, the non-invasive 980 nm NIR excitation falls within the "water window" (a gap in the absorption spectrum of tissue between chromophores (<800 nm) and water (> 1200 nm)). In vivo imaging can be easily achieved as a function of the strong tissue penetration ability of the NIR. Photo-bleaching can be greatly reduced because of the resistance to photo-bleaching of these inorganic nanoparticles. Multiple labelling can also be achieved by the fluorescent nanoparticles at various optical wavelengths under the same 980 nm NIR excitation.

Currently, the fabrication of up-conversion fluorescent inorganic nanoparticles suitable as bio-probe remains as the key technological issue. As bio-probes, the targeted molecules (such as proteins, oligonucleotides and other biomolecules in cells or tissues) are in the range from several nanometers to tens of nanometers. An optimal universal bio-probe therefore should be small in size with narrow size distribution. It should yield high fluorescent efficiency and must be water re-dispersible (Dubertret B et al, 2002).

The most efficient infrared-to-visible up-conversion phosphors are Yb-Er or Yb-Tm co-doped fluorides such as $NaYF_4$, $BaYF_5$, $NaLaF_4$, $NaGdF_4$, YF_3, LaF_3, GdF_3 and oxysulphides like Y_2O_2S (Basse G and Grabmaier BC, 1994), where fluorides and oxysulphide are the hosts, ytterbium (Yb) acts as the sensitizer and erbium (Er) or thulium (Tm) acts as the fluorescent centre. Under the 980 nm NIR excitation, they give off different colours of visible up-conversion fluorescence, depending on the different doping ions. Among them, rare earth doped hexagonal phase $NaYF_4$ is one of the most efficient material for green and blue up-conversion. However, all these commercially available phosphors are in bulk form usually prepared by high-temperature solid-state reactions. Making these bulk phosphors into nanoparticles, which simultaneously satisfy the bio-probe criteria mentioned above, remains a big challenge. Several research groups have sought alternative approaches and synthesized the up-conversion fluorescent nanoparticles for bio-probes. 400 nm Yb-Er and Yb-Tm co-doped Y_2O_2S up-conversion fluorescent particles have been adopted for detection of nucleic acid (van de Rijke, F et al, 2001). The particles were prepared using the method disclosed in US patent 6,039,894. Their synthesized particles were, however, too large for application as bio-probes; and the efficiency of Y_2O_2S was less than that of the hexagonal phase $NaYF_4$ phosphors. Fabrication of smaller particles is being researched on (Corstjens P et al. 2005). Several other research groups focused on the synthesis of doped $NaYF_4$ nanoparticles.

In WO 03/087259 the synthesis of 37 nm $NaYF_4$:Yb,Er up-conversion nanoparticles, by the room-temperature reaction of rare earth-EDTA complex with sodium fluoride in aqueous solution has been disclosed (Yi GS et al, 2004). The synthesis and multicolour up-conversion emission of Yb-Er and Yb-Tm co-doped $NaYF_4$ nanoparticles has been reported (Heer S, et al, 2004). The 15 nm nanoparticles were acquired by the reaction of rare earth *N*-(2-hydroxyethyl)ethylenediamine salt, sodium alkoxide of *N*-(2-hydroxyethyl) ethylenediamine and *N*-(2-hydroxyethyl)ethylenediamine fluoride at 200°C under dry N_2 atmosphere for 2 h. Recently, Wang et al, 2005, reported a liquid-solid-solution (LSS) method for the synthesis of $NaYF_4$:Yb,Er up-conversion nanoparticles. However, all of the above efforts produced cubic-phase nanoparticles, with efficiency of at least one order of magnitude less than the desirable hexagonal phase. Although Zeng JH et al, 2005, reported the synthesis of hexagonal phase NaYF4:Yb,Er(Tm) nanoparticles, the bigger size of approximately 50 nm was not sufficiently small for them to be used as bio-probes for smaller molecules.

Yi GS and Chow GM, 2005, described the synthesis of LaF_3:Yb,Er, LaF_3:Yb,Ho and LaF_3:Yb,Tm nanoparticles of size 5.4 nm having potential applications as bio-probes, by reacting $LaCl_3$, $YbCl_3$, $ErCl_3$/$HoCl_3$/$TmCl_3$ and NaF at a temperature of 72°C. The particles could be dispersed in organic solutions and formed a transparent colloid. With the 980 nm NIR excitation, the nanoparticles yielded different fluorescent emissions in the visible range. However, their up-conversion fluorescent was not efficient.

Accordingly, there is a need in this field of technique of improved fluorescent nano-structured materials.

Summary of the invention

The present invention addresses the problems above, and in particular provides a method for the preparation of nano-structured material(s). The nano-structured material prepared from the method of the present invention may be used for applications such as in bio-imaging and bio-detection of biomolecules.

According to a first aspect, the present invention provides a method of preparing at least one nano-structured material of formula $M_1M_2X_n$ comprising the step of treating:

- at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_1$; and

- at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$;

wherein

each X is the same or different and is selected from the group consisting of: halogens, O, S, Se, Te, N, P and As;

each n is the same or different and is $0 \leq n \leq 10$;

each m is the same or different and is $0 \leq m \leq 10$;

each p is the same or different and is $1 \leq p \leq 5$;

each M_1 is the same or different and is selected from the group consisting of: Li, Na, K, Rb, Cs, Fr, Be, Mg, Ca, Sr, Ba, Ra and NH_4;

each M_2 is the same or different and is a metal ion.

According to a particular aspect, the present invention provides a method for preparing at least one nano-structured material of formula $M_1M_2X_n{:}M_q$ comprising the step of treating:

- at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_1$;
- at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$; and
- at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_q$,

wherein

each M_q is the same or different and is selected from the group consisting of Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu;

n, m, p, X, M_1 and M_2 are as defined above; and

each q is the same or different and is $0 \leq q \leq 10$.

According to a further aspect, the treating in the method may be carried out in the presence of at least one source of energy. Any suitable source of energy may be used. For example, the source of energy may be, but not limited to, any one of the following: light source, electric source, thermal source, magnetic source, or a combination thereof. In particular, the source of energy is a thermal source. The treating may be carried out at a temperature of up to about 1000°C. For example, the treating may be carried out at a temperature of 200°C - 400°C. In particular, the treating is carried out at a temperature of 300°C - 350°C. Even more in particular, the treating is carried out at a temperature of 330°C.

According to another aspect, the present invention provides a method of preparing at least one nano-structured material of formula M_2X_n comprising the step of treating at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$, in the presence of at least one source of energy, wherein

each X is the same or different and is selected from the group consisting of: halogens, O, S, Se, Te, N, P and As;

each n is the same or different and is $0 \leq n \leq 10$;

each m is the same or different and is $0 \leq m \leq 10$;

each p is the same or different and is $1 \leq p \leq 5$; and

each M_2 is the same or different and is a metal ion.

According to another particular aspect, the present invention provides a method for preparing at least one nano-structured material of formula M_2X_n:M_q comprising the step of treating:

- at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$; and
- at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_q$;

in the presence of at least one source of energy, wherein

each M_q is the same or different and is selected from the group consisting of Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu;

n, m, p, X and M_2 are as defined above; and

each q is the same or different and is $0 \leq q \leq 10$.

Any suitable source of energy may be used. For example, the source of energy may be, but not limited to, any one of the following: light source, electric source, thermal source, magnetic source or a combination thereof. In particular, the source of energy is a thermal source. The treating may be carried out at a temperature of up to about 1000°C. For example, the treating may be carried out at a temperature of 200°C - 400°C. In particular, the treating is carried out at a temperature of 300°C - 350°C. Even more in particular, the treating is carried out at a temperature of 330°C.

M_2, according to any aspect of the present invention, may be the same or different and may be selected from the group consisting of: transition metal ions, inner transition metal ions, and Group I to Group VI metal ions. In particular, each M_2 may be the same or different and may be Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb or Lu.

The nano-structured material according to any aspect of the present invention may be selected from the group consisting of: NaM_2F_4, LiM_2F_4, KM_2F_4, RbM_2F_4, CsM_2F_4, BeM_2F_5, $Be(M_2)_2F8$, MgM_2F_5, $Mg(M_2)_2F_8$, CaM_2F_5, $Ca(M_2)_2F_8$, SrM_2F_5, $Sr(M_2)_2F_8$, $BaLnF_5$, $Ba(M_2)_2F_8M_2F_3$, M_2F_3, M_2Cl_3, M_2Br_3, M_2I_3, M_2FClBr, M_2OF, M_2OCl, M_2OBr, M_2OS, $(M_2)_2S_3$, wherein each M_2 is as defined above. In particular, the nano-structured material is $NaYF_4$, $LiYF_4$, $BaYF_5$, $NaLaF_4$, LaF_3, YF_3, BaY_2F_8, $NaGdF_4$ or GdF_3. Even more in particular, the nano-structured material is $NaYF_4$.

The at least one compound of formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_1$ may be selected from the group consisting of: CF_3COONa and CF_3COOLi. The at least one compound of formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$ may be selected from the group consisting of: $(CF_3COO)_3Y$ and $(CF_3COO)_3La$. For example, the at least one compound of formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_q$ may be selected from the group consisting of: $(CF_3COO)_3Yb$, $(CF_3COO)_3Er$, $(CF_3COO)_3Tm$ and $(CF_3COO)_3Ho$.

The at least one nano-structured material prepared according to any method of the present invention may be selected from the group consisting of: $NaM_2F_4:M_q$, $LiM_2F_4:M_q$, $KM_2F_4:M_q$, $RbM_2F_4:M_q$, $CsM_2F_4:M_q$, $BeM_2F_5:M_q$, $Be(M_2)_2F_8:M_q$, $MgM_2F_5:M_q$, $Mg(M_2)_2F_8:M_q$, $CaM_2F_5:M_q$, $Ca(M_2)_2F_8:M_q$, $SrM_2F_5:M_q$, $Sr(M_2)_2F_8:M_q$, $BaM_2F_5:M_q$, $Ba(M_2)_2F_8:M_q$, $M_2F_3:M_q$, $M_2Cl_3:M_q$, $M_2Br_3:M_q$, $M_2I_3:M_q$,

$M_2FClBr:M_q$, $M_2OF:M_q$, $M_2OCl:M_q$, $M_2OBr:M_q$, $M_2OS:M_q$, $(M_2)_2S_3:M_q$, wherein each M_2 and M_q are as defined above. In particular, each M_q is the same or different and is selected from the group consisting of: Yb, Er, Tm and Ho. In particular, the at least one nano-structured material of formula $M_1M_2X_n:M_q$ is $NaYF_4$:Yb,Er; $NaYF_4$:Yb,Tm; $NaYF_4$:Yb,Ho; LiYF4:Yb,Er; $BaYF_5$:Yb,Er; $NaLaF_4$:Yb,Er; or YOF:Yb,Er. Even more in particular, the nano-structured material is $NaYF_4$:Yb,Er, $NaYF_4$:Yb,Tm or $NaYF_4$:Yb,Ho.

According to a further aspect, the mixing or treating in the method according to any aspect of the present invention may be carried out in the presence of at least one polar or non-polar solvent, or a mixture thereof. Any suitable polar or non-polar solvent may be used. For example, the polar solvent may be water, methanol, ethanol, propyl alcohol, butanol, pentanol, hexanol, ketone, ethylene glycol, glycerol, propylene glycol, polyethylene glycol, ethyl acetate and a combination thereof. The non-polar solvent may be oleylamine, octadecene, oleic acid, alkyl amine, dialkyl amine, trialkyl amine, alkenyl amine, dialkenyl amine, trialkenyl amine, alkyl acid, alkenyl acid, trialkyl phosphine, trialkyl phosphine oxide, trialkylphosphate, alkane, alkene, alkyl ether, alkenyl ether or a combination thereof.

The at least one nano-structured material prepared by the method according to any aspect of the present invention may have a structure selected from one of the following: hexagonal, cubic, tetragonal, rhombohedral, orthorhombic, monoclinic, triclinic and a combination thereof. For example, the nano-structured material prepared from the method according to any aspect of the present invention may have a lattice structure selected from the group consisting of: hexagonal, cubic, tetragonal, rhombohedral, orthorhombic, monoclinic, triclinic and a combination thereof. In particular, the nano-structured material has a hexagonal lattice structure. Even more in particular, the nano-structured material is hexagonal phase $NaYF_4$, hexagonal phase $NaYF_4$:Yb,Er, hexagonal phase $NaYF_4$:Yb,Tm or hexagonal phase $NaYF_4$:Yb,Ho.

The nano-structured material prepared by the method according to any aspect of the present invention may comprise at least one dimension having size $\leq$1000 nm. For example, $\leq$100 nm, in particular, less than 50 nm. In particular, the nano-structured material comprises at least one dimension of size $\leq$25 nm. Even more in particular, the at least one dimension is of size $\leq$10 nm.

The nano-structured material prepared by the method according to any aspect of the present invention may be in the form of: nanoparticle(s), nanofilm or monolith. In particular, the at least one nano-structured material may be at least one nanoparticle and the average diameter of the nanoparticle is $\leq$1000 nm. For example, $\leq$100 nm, in particular, less than 50 nm. Even more in particular, the average diameter of the nanoparticle is $\leq$10 nm. The nanoparticle(s) may comprise a core nanoparticle(s) or a core-shell nanoparticle(s).

According to a further aspect, the present invention provides a method of preparing a nano-structured material as described above, wherein the nanoparticle is in the form of a core nanoparticle, and the method further comprises applying at least one organic and/or inorganic material (shell) on the core, to obtain a core-shell nanoparticle(s). According to another further aspect, the present invention provides a method of preparing a nano-structured material as described above, wherein the nanoparticle is in the form of a core nanoparticle, and the nanoparticle further comprises at least one organic and/or inorganic material (shell) applied on the core, to obtain a core-shell nanoparticle(s). The shell may be applied continuously or discontinuously on the core.

The shell of the core-shell nanoparticle may comprise an organic shell material or an inorganic shell material. The shell may comprise a material of the formula $M_1M_2X_n$ or $M_1M_2X_n$:M_q, wherein each M_1, M_2, X, n and M_q are as defined above. For example, the organic shell material may comprise at least one polymer, a surfactant or a lipid, or a combination thereof. Any suitable polymer, surfactant or lipid may be used for the purposes of the present invention.

The inorganic shell material may comprise NaM_2F_4, LiM_2F_4, KM_2F_4, RbM_2F_4, CsM_2F_4, BeM_2F_5, $Be(M_2)_2F8$, MgM_2F_5, $Mg(M_2)_2F_8$, CaM_2F_5, $Ca(M_2)_2F_8$, SrM_2F_5, $Sr(M_2)_2F_8$, $BaLnF_5$, $Ba(M_2)_2F_8M_2F_3$, M_2F_3, M_2Cl_3, M_2Br_3, M_2I_3, M_2FClBr, M_2OF, M_2OCl, M_2OBr, M_2OS, $(M_2)_2S_3$, wherein each M_2 is the same or different and is selected from the group consisting of: Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu; SiO_2; TiO_2; ZnS; or a combination thereof.

According to a further aspect, the surface of the nano-structured material may be modified by adding at least one surfactant, lipid, polymer, inorganic material, or a mixture thereof. Any suitable surfactant, lipid, polymer, inorganic material may be used. For example, the surfactant may have Formula (I):

$$R^5-\underset{R^2}{\overset{R^1}{|}}(C_JH_K)_s-\underset{R^4}{\overset{R^3}{|}}(C_ZH_W)_y-R^6 \qquad \text{(Formula I)}$$

wherein

each J is the same or different, and $1 \leq J \leq 9$;

each K is the same or different, and $0 \leq K \leq 9$;

each s is the same or different, and $0 \leq s \leq 9$;

each Z is the same or different, and $1 \leq Z \leq 9$;

each W is the same or different, and $0 \leq W \leq 9$;

each y is the same or different, and $0 \leq y \leq 9$;

each R^1, R^2, R^3, R^4 and R^5 is the same or different, and is independently selected from the group consisting of: H, substituted or unsubstituted C_1-C_6 alkyl, substituted or unsubstituted C_1-C_6 aryl, HS, COOH, NH_2 and OH;

each R_6 is the same or different, and is selected from the group consisting of: COOH, NH_2, OH, P=0 and P;

with the proviso that $s + y < 10$.

The surfactant may be hydrophilic, hydrophobic and/or amphiphilic.

According to a further aspect, the present invention provides a method of preparing a nano-structured material as described above, wherein a biomolecule is attached to the nano-structured material. The biomolecule may be any suitable biomolecule. For example, the biomolecule is selected from the group consisting of: protein, nucleic acid, nucleosides, nucleotides, DNA, hormone, amino acid, peptide, peptidomimetic, RNA, lipid, albumin, antibody, phospholipids, glycolipid, sterol, vitamins, neurotransmitter, carbohydrate, sugar, disaccharide, monosaccharide, oligopeptide, polypeptide, oligosaccharide, polysaccharide and a mixture thereof.

The present invention also provides a nano-structured material obtainable by the method according to any aspect described above.

According to another aspect, the present invention provides a nano-structured material of formula $M_1M_2X_n$:M_q, wherein M_1, M_2, X, n and M_q are as defined above. The nano-structured material may have a hexagonal lattice structure and may comprise at least one dimension of < 50 nm. In particular, the at least one dimension is $\leq$ 10 nm.

According to a particular aspect, the present invention provides a nano-structured material of formula $M_1M_2X_n$:M_q, wherein M_1, M_2, X, n and M_q are as defined above, and wherein the nano-structured material has a hexagonal lattice structure, comprising at least one dimension of < 50 nm, with the proviso that the nano-structured material is not LaF_3:Yb,Er, LaF_3:Yb,Ho or LaF_3:Yb,Tm. In particular, the at least one dimension is ≤ 10 nm or ≤ 5 nm. Even more in particular, the nano-structured material is hexagonal phase $NaYF_4:M_q$. For example, $NaYF_4:M_q$ is $NaYF_4$:Yb,Er, $NaYF_4$:Yb,Ho or $NaYF_4$:Yb,Tm.

The present invention also provides an article of manufacture comprising any nano-structured material described above. The article of manufacture may be at least one of the following: a display device, a solar cell, an optical data storage, a bio-probe, a carrier for drug delivery, a lamp, a LED, a LCD, a wear resistance, a laser, optical amplifier, and/or a device for bio-imaging.

Another aspect of the present invention is a kit comprising at least one nano-structured material described above and at least one biomolecule. The biomolecule may be any suitable biomolecule. For example, the biomolecule may be selected from the group consisting of: protein, nucleic acid, nucleosides, nucleotides, DNA, hormone, amino acid, peptide, peptidomimetic, RNA, lipid, albumin, antibody, phospholipids, glycolipid, sterol, vitamins, neurotransmitter, carbohydrate, sugar, disaccharide, monosaccharide, oligopeptide, polypeptide, oligosaccharide, polysaccharide and a mixture thereof.

The present invention also provides a bio-imaging and/or bio-detection system comprising:

- at least one nano-structured material prepared from the method according to any aspect of the invention;
- at least one biomolecule;
- at least one source of excitation; and
- at least one means for delivery of the source of excitation to the system.

The biomolecule may be any suitable biomolecule. For example, the biomolecule may be selected from the group consisting of: protein, nucleic acid, nucleosides, nucleotides, DNA, hormone, amino acid, peptide, peptidomimetic, RNA, lipid, albumin, antibody, phospholipids, glycolipid, sterol, vitamins, neurotransmitter, carbohydrate, sugar, disaccharide, monosaccharide, oligopeptide, polypeptide, oligosaccharide, polysaccharide and a mixture thereof.

The source of excitation may be any suitable source. For example, the source of excitation is NIR. The NIR may be at 980 nm. The means for delivery of the source of excitation to the system may be any suitable means for delivery. For example, the means for delivery may be optical fibres, endoscopes, external light and/or external laser.

Another aspect of the present invention is a method of modifying at least one hydrophobic structure to make it hydrophilic, comprising applying at least one modifier on the structure surface to make a structure-modifier complex, and wherein the modifier is a surfactant, lipid, polymer and/or inorganic material. The structure may be the nano-structured material as described above. Any suitable surfactant, lipid, polymer and/or inorganic material may be used. The method may comprise applying at least one first modifier on the structure surface, and further a second hydrophilic modifier to make a structure-first modifier-second modifier complex, and wherein the first and/or second modifier is a surfactant, lipid, polymer and/or inorganic material. The method may also comprise applying at least one hydrophilic surfactant on the structure to make a structure-surfactant complex.

Brief description of the figures

Figure 1 shows an illustration of (a) core nanoparticles and (b) core/shell nanoparticles and films.

Figure 2 shows an illustration of (a) the core and (b) core/shell structured $NaYF_4$:Yb,Er(Tm)/$NaYF_4$ nanoparticles.

Figure 3 shows an illustration of (a) poly acrylic acid (PAA) caped $NaYF_4$:Yb,Er(Tm)/$NaYF_4$ nanoparticles and (b) PEG-phospholipids caped $NaYF_4$:Yb,Er(Tm)/$NaYF_4$ nanoparticles.

Figure 4 shows the structure of three examples of lipids: (a) 18:0 mPEG2000PE, (b) DSPE-PEG(2000) carboxylic acid and (c) DSPE-PEG(2000) Biotin.

Figure 5 shows the TEM pictures of the hexagonal phase $NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm nanoparticles at a magnification of (a) 50K (b) 150K and, (c) XRD pattern and (d) SAED pattern of the hexagonal phase $NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm nanoparticles.

Figure 6 shows (a) the fluorescence spectra and (b) fluorescence picture of the hexagonal phase (i) $NaYF_4$:Yb,Er and (ii) $NaYF_4$:Yb,Tm nanoparticles. The excitation is 980 nm NIR laser.

Figure 7 shows (a) the TEM picture and (b) the XRD pattern of the cubic phase $NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm nanoparticles.

Figure 8 shows (a) the TEM photographs of $NaYF_4$:Yb,Er core, (b) $NaYF_4$:Yb,Er(Tm)/$NaYF_4$ core/shell, and (c) XRD pattern of the core, core/shell nanoparticles.

Figure 9 shows (a) fluorescence spectra of core, core/shell and PAA coated core/shell $NaYF_4$:Yb,Er and (b) $NaYF_4$:Yb,Tm nanoparticles. The excitation is 980 nm NIR laser.

Figure 10 shows (a) the fluorescence pictures of core, core/shell and PAA coated core/shell $NaYF_4$:Yb,Er and (b) $NaYF_4$:Yb,Tm nanoparticles. The excitation is 980 nm NIR laser.

Figure 11 shows (a) the TEM picture and (b) XRD pattern of $LiYF_4$ nanoparticles.

Figure 12 shows (a) the TEM picture and (c) SAED pattern of $BaYF_5$ nanoparticles. Figure 12 (b) shows the TEM picture of (a) at a greater magnification.

Figure 13 shows (a) the TEM picture and (c) SAED pattern of $NaLaF_4$ nanoparticles. Figure 13 (b) shows the TEM picture of (a) at a greater magnification.

Figure 14 shows the XRD patterns of $LiYF_4$, $BaYF_5$ and $NaLaF_4$ nanoparticles.

Figure 15 shows the fluorescence spectra of Yb-Er co-doped $LiYF_4$, $BaYF_5$ and $NaLaF_4$ nanoparticles.

Figure 16 shows the TEM picture of LaF_3 nanoparticles.

Figure 17 shows the TEM picture of YF_3 nanoparticles.

Figure 18 shows (a) the TEM picture of the $NaYF_4$:20%Yb,2%Er nanoparticles, and (b) up-conversion fluorescence of the nanoparticles in hexane. The excitation is a 980 nm NIR laser with power density of 1W.

Figure 19 shows the TEM picture of the $NaYF_4$:Yb,Er nanoparticles obtained in 1-octadecene with oleylamine as surfactant, at temperature of 300°C for 1h.

Figure 20 shows the TEM picture of the $NaYF_4$:Yb,Er nanoparticles prepared in tetracosane with octadecaneamine as surfactant, at temperature of 340°C for 30min.

Figure 21 shows the $NaYF_4$:Yb,Er thin film on glass substrates. Under 980 nm NIR excitation, green emission (as shown by arrow) was observed.

Figure 22 shows YOF:Yb,Er thin film on glass substrates. Under 980 nm NIR excitation, red emission (as shown by arrow) was observed.

Figure 23 shows the size distribution of the PAA polymer coated $NaYF_4$:Yb,Er/$NaYF_4$ nanoparticles, obtained from the dynamic light scattering technique.

Figure 24 shows XRD patterns of the $NaYF_4$:20%Yb,2%Er nanoparticles synthesized at 300°C, 320°C and 330°C, respectively, in oleylamine for 1h. The particles in cubic phase are peaks marked with asterisk. The line pattern in the lower part is the calculated hexagonal phase of $NaYF_4$.

Figure 25 shows (a) TEM image of $NaYF_4$:Yb,Er nanoparticles prepared in 2ml oleic acid and 8 ml oleylamine solution at 330°C for 1h, and (b) shows the XRD pattern of dominant cubic phase (marked with asterisk) and hexagonal phase. The line pattern in (b) is the calculated hexagonal phase of $NaYF_4$.

Detailed description of the invention

Bibliographic references mentioned in the present specification are for convenience listed in the form of a list of references and added at the end of the examples. The whole content of such bibliographic references is herein incorporated by reference.

The present invention provides a method for preparing nano-structured material of suitable size for use in applications such as bio-imaging and bio-detection. In order to obtain suitable sized nano-structured material, it would be useful to control the crystal structure of the nano-structured material prepared and the size distribution of the nano-structured material. A narrow size distribution would be preferred. For example, the nano-structured material may be used in the bio-imaging and bio-detection of biomolecules.

According to a first aspect, the present invention provides a method of preparing at least one nano-structured material of formula $M_1M_2X_n$ comprising the step of treating (mixing):

- at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_1$; and
- at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$;

wherein

each X is the same or different and is selected from the group consisting of: halogens, O, S, Se, Te, N, P and As;

each n is the same or different and is $0 \leq n \leq 10$;

each m is the same or different and is $0 \leq m \leq 10$;

each p is the same or different and is $1 \leq p \leq 5$;

each M_1 is the same or different and is selected from the group consisting of: Li, Na, K, Rb, Cs, Fr, Be, Mg, Ca, Sr, Ba, Ra and NH_4;

each M_2 is the same or different and is a metal ion.

In particular, the step of treating comprises reacting at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_1$ and at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$.

According to a further aspect, the present invention provides a method for preparing at least one nano-structured material of formula $M_1M_2X_n{:}M_q$ comprising the step of treating (mixing):

- at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_1$;
- at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$; and
- at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_q$,

wherein

each M_q is the same or different and is selected from the group consisting of Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu;

n, m, p, X, M_1 and M_2 are as defined above; and

each q is the same or different and is $0 \leq q \leq 10$.

In particular, the step of treating comprises reacting at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_1$; at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$ and at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_q$.

The treating in the method according to any aspect of the present invention may be carried out in the presence of at least one source of energy. Accordingly, the method may also be referred to as a method according to any aspect of the invention wherein the method comprises treating the compounds (or the mixing) in the presence of at least one source of energy. For example, the source of energy may be light source, electric source, thermal source, magnetic source, heat source, or a combination thereof. The at least one source of energy may also comprise microwave assisted heating, NIR assisted heating, IR assisted heating, laser heating, X-ray heating. According to a particular aspect, the at least one source of energy is thermal source. The treating may be carried out at a temperature up to 1000ºC. In particular, the treating is carried out at a temperature 200°C - 400°C, even more in particular, at a temperature 300°C - 350°C. For example, the treating is carried out at a temperature of 330°C.

According to another aspect, the present invention provides a method of preparing at least one nano-structured material of formula M_2X_n comprising the step of treating (mixing) at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$, optionally with at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_q$, in the presence of at least one source of energy,

wherein

each X is the same or different and is selected from the group consisting of: halogens, O, S, Se, Te, N, P and As;

each n is the same or different and is $0 \leq n \leq 10$;

each m is the same or different and is $0 \leq m \leq 10$;

each p is the same or different and is $1 \leq p \leq 5$;

each M_2 is the same or different and is a metal ion; and

each M_q is as defined as below.

According to another further aspect, the present invention provides a method for preparing at least one nano-structured material of formula M_2X_n:M_q comprising the step of treating (mixing):

- at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$; and
- at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_q$,

in the presence of at least one source of energy, wherein

each M_q is the same or different and is selected from the group consisting of Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu;

n, m, p, X, M_1 and M_2 are as defined above; and

each q is the same or different and is $0 \leq q \leq 10$.

In particular, the step of treating comprises reacting at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$ and at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_q$.

Each M_2, according to any aspect of the present invention, may be the same or different, and may be any suitable metal ion. For example, each M_2 may be the same or different and may be a transition metal ion, inner transition metal ion, or any one of Group I to Group VI metal ion. In particular, each M_2 may be selected from the group consisting of: Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu.

According to another aspect, the present invention provides a method which comprises the steps as shown:

$$\begin{array}{l}[CX_3(CX_2)_{n1}(CH_2)_{m1}COO]_pM_1 + \\ [CX_3(CX_2)_{n2}(CH_2)_{m2}COO]_pM_2 \\ + \ldots\ldots + [CX_3(CX_2)_{nq}(CH_2)_{mq}COO]_pM_q \\ + \text{Surfactant (optionally)}\end{array} \xrightarrow[\text{Solvent}]{\text{Energy source}} \begin{array}{l}M_1M_2\ldots M_{m-1}MX_n{:}M_q \\ \text{nano-structured} \\ \text{material}\end{array}$$

wherein each X may be the same or different and refers to halogen, such as F, Cl, Br and I; each M_1 may be the same or different and refers to ions which may be selected from the group consisting of: NH_4, Group I and Group II metal ions such as Li, Na, K, Rb, Cs, Be, Mg, Ba, Ca, Sr; M_2 to M_q each may be the same or different metal ion and may be selected from the group consisting of rare earth metal ions such as Y, La, Ce Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu.

Further, $0 \leq n \leq 10$, $0 \leq m \leq 10$, $1 \leq p \leq 5$, $0 \leq q \leq 10$. The surfactant is optional in the method described. Any suitable surfactant, energy source and solvent may be used for the present invention, as will be described below.

According to another aspect, the present invention also provides a method comprising the following reaction:

$$CF_3COOM_1 + CF_3COOM_2 + CF_3COOM_q + \text{Surfactant} \xrightarrow[\text{High-boiling organic solvent}]{\text{Heating in}} M_1M_2F_n{:}M_q \text{ nano-structured material}$$

M_1, M_2, n and M_q may be as described above. For example, each M_1 may be the same or different and is selected from the group consisting of: Na, K, Ba, Li and NH_4. each M_2 may be the same or different and is selected from the group consisting of: Y, La, Gd, Lu. Each M_q may be the same or different and is selected from the group consisting of: Yb, Er, Ho, Tm and a combination thereof, such as Yb-Er, Yb-Ho and Yb-Tm.

M_q according to any aspect of the present invention may act as the dopant. A dopant may be an impurity which is added to a compound in low concentrations to alter some properties of the compound. For example, a dopant may be added in a concentration ranging from one part in a thousand to one part in ten million. It would be understood that a dopant does not alter the crystal structure of the compound it is added to. For example, a dopant may be added to a nano-structured material prepared according to the method of any aspect of the present invention so that the nano-structured material can have additional or enhanced properties. The properties include, but are not limited to, optical properties, magnetic properties, electrical properties and fluorescence.

According to a particular aspect, the nano-structured material prepared according to the method of any aspect of the invention comprising M_q may have fluorescence properties. Fluorescence refers to the emission of light in any wavelength excited with energy source. The energy source may be a light source, electric source, thermal source, magnetic source or a combination thereof. The light source may be at least one of UHV, UV, NIR, visible or X-ray. The light can be of any wavelength. The wavelength of the source may be shorter than the emission. For example, UV excitation with emission in the visible range. The wavelength may be longer than the emission, e.g. NIR excitation with visible emission. The energy source may also be referred to as the excitation source. In particular, the nano-structured materials can be excited with NIR. The NIR may be emitted at visible wavelength. The NIR may be emitted at 980 nm. The excitation source may be a laser source e.g. 980 nm NIR laser.

The compounds used for the method according to any aspect of the present invention may be solid or liquid. The at least one compound of formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_1$ may be selected from the group consisting of: CF_3COONa and CF_3COOLi. The at least one compound of formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$ may be selected from the group consisting of: $(CF_3COO)_3Y$ and $(CF_3COO)_3La$. The at least one compound of formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_q$ may be selected from the group consisting of: $(CF_3COO)_3Yb$, $(CF_3COO)_3Er$, $(CF_3COO)_3Tm$ and $(CF_3COO)_3Ho$.

In order to achieve suitable nano-structured material prepared according to the method of any aspect of the invention, it may be useful to control the parameters involved in the preparation of the nano-structured material. For example, the condition(s) at which the treating step of the method is carried out may affect the nano-structured material prepared by the method. The conditions may include the source of energy and/or any solvent used in the method according to any aspect of the present invention.

For the purposes of the present invention, a nano-structured material is defined as being one comprising constituents which has at least one dimension in the nanoscale.

The nano-structured material prepared from the method according to any aspect of the present invention may comprise at least one dimension having size ≤ 1000 nm. For example, ≤ 100nm, in particular, ≤ 50nm and even more in particular, less than 50 nm. More in particular, the nano-structured material may comprise at least one dimension of size ≤ 25 nm, and even more in particular the nano-structured material may comprise at least one dimension of size ≤ 10 nm or ≤ 5 nm. According to a particular aspect, the nano-structured material prepared according to any method of the invention, may comprise one, two, three, four, five, six or even more dimension(s), each dimension of size ≤ 1000 nm, ≤100 nm, ≤ 50 nm, less than 50 nm, ≤ 25 nm, ≤ 10 nm or ≤ 5 nm. According to a more particular aspect, the nano-structured material prepared according to method of the invention, may comprise one, two, three, four, five, six or even more dimension(s), each dimension of size less than 50 nm, ≤ 25 nm, ≤10 nm or ≤ 5 nm. The dimension may refer to the average diameter of the nano-structured material.

The treating in the method according to any aspect of the present invention may be carried out in the presence of at least one source of energy. Accordingly, the method may also be referred to as a method according to any aspect of the invention wherein the method comprises treating the compounds in the presence of at least one source of energy. For example, the source of energy may be light source, electric source, thermal source, magnetic source, heat source, or a combination thereof. The at least one source of energy may also comprise microwave assisted heating, NIR assisted heating, IR assisted heating, laser heating, X-ray heating. According to a particular aspect, the at least one source of energy is thermal source. The treating may be carried out at a temperature up to 1000°C. In particular, the treating is carried out at a temperature 200°C - 400°C, even more in particular, at a temperature 300°C - 350°C. For example, the treating is carried out at a temperature of 330°C.

The treating in the method according to any aspect of the present invention may be carried out in the presence of at least one solvent. Solvent may be defined as being a fluid phase (such as liquid, gas or plasma) that dissolves a solid, liquid or gaseous compound, resulting in a solution. The at least one solvent may be a polar solvent, a non-polar solvent, or a mixture thereof. Any suitable polar solvent and non-polar solvent may be used for the present invention.

For example, the polar solvent may be selected from the group consisting of: water, methanol, ethanol, propyl alcohol, butanol, pentanol, hexanol, ketone, ethylene glycol, glycerol, propylene glycol, polyethylene glycol, ethyl acetate, esters and a combination thereof.

The non-polar solvent may be selected from the group consisting of: oleylamine, octadecene, oleic acid, alkyl amine, dialkyl amine, trialkyl amine, alkenyl amine, dialkenyl amine, trialkenyl amine, alkyl acid, alkenyl acid, trialkyl phosphine, trialkyl phosphine oxide, trialkylphosphate, alkane, alkene, alkyl ether, alkenyl ether and a combination thereof.

The at least one solvent may be an organic solvent, inorganic solvent or a mixture thereof. Examples of organic solvent include solvents which contain carbon atom(s), such as acetone, alcohol, benzene, benzol, carbon disulphide, carbon tetrachloride, chloroform, ether, ethyl acetate, furfural, gasoline, toluene, turpentine, xylene, xylol, octadecane, tetracosane, oleylamine or oleic acid. Inorganic solvents are solvents which do not contain carbon atom(s), such as water.

The nano-structured material prepared from the method according to any aspect of the present invention may be selected from the group consisting of: NaM_2F_4, LiM_2F_4, KM_2F_4, RbM_2F_4, CsM_2F_4, BeM_2F_5, $Be(M_2)_2F8$, MgM_2F_5,

$Mg(M_2)_2F_8$, CaM_2F_5, $Ca(M_2)_2F_8$, SrM_2F_5, $Sr(M_2)_2F_8$, $BaLnF_5$, $Ba(M_2)_2F_8M_2F_3$, M_2F_3, M_2Cl_3, M_2Br_3, M_2I_3, M_2FClBr, M_2OF, M_2OCl, M_2OBr, M_2OS and $(M_2)_2S_3$. M_2 may be as defined above. In particular, each M_2 is the same or different and is selected from the group consisting of: Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu. In particular, the nano-structured material is $NaYF_4$, $LiYF_4$, $BaYF_4$, $NaLaF_4$, LaF_3, YF_3, CeF_3, GdF_3 or YOF. Even more in particular, the nano-structured material is $NaYF_4$.

According to a particular aspect, the nano-structured material prepared from the method according to any aspect of the present invention may be selected from the group consisting of: $NaM_2F_4{:}M_q$, $LiM_2F_4{:}M_q$, $KM_2F_4{:}M_q$, $RbM_2F_4{:}M_q$, $CsM_2F_4{:}M_q$, $BeM_2F_5{:}M_q$, $Be(M_2)_2F_8{:}M_q$, $MgM_2F_5{:}M_q$, $Mg(M_2)_2F_8{:}M_q$, $CaM_2F_5{:}M_q$, $Ca(M_2)_2F_8{:}M_q$, $SrM_2F_5{:}M_q$, $Sr(M_2)_2F_8{:}M_q$, $BaM_2F_5{:}M_q$, $Ba(M_2)_2F_8{:}M_q$, $M_2F_3{:}M_q$, $M_2Cl_3{:}M_q$, $M_2Br_3{:}M_q$, $M_2I_3{:}M_q$, $M_2FClBr{:}M_q$, $M_2OF{:}M_q$, $M_2OCl{:}M_q$, $M_2OBr{:}M_q$, $M_2OS{:}M_q$, $(M_2)_2S_3{:}M_q$, wherein each M_2 and each M_q are as defined above. In particular, each M_2 is the same or different and is selected from the group consisting of: Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu, and each M_q is the same or different and is selected from the group consisting of: Yb, Er, Tm and Ho. In particular, the nano-structured material is $NaYF_4$:Yb,Er, $NaYF_4$:Yb,Tm, $NaYF_4$:Yb,Ho, $LiYF_4$:Yb,Er, $BaYF_5$:Yb,Er, $NaLaF_4$:Yb,Er, LaF_3:Yb,Er, CeF_3:Yb,Er, GdF_3:Yb,Er, YF_3:Yb,Er, YOF:Yb,Er, LaF_3:Yb,Tm, CeF_3:Yb,Tm, GdF_3:Yb,Tm, YF_3:Yb,Tm or YOF:Yb,Tm. Even more in particular, the nano-structured material is $NaYF_4$:Yb,Er, $NaYF_4$:Yb,Tm or $NaYF_4$:Yb,Ho.

The nano-structured material prepared from the method according to any aspect of the present invention may have a structure selected from the group consisting of: hexagonal, cubic, tetragonal, rhombohedral, orthorhombic, monoclinic, triclinic and a combination thereof. In particular, the nano-structured material prepared from the method according to any aspect of the present invention may have a lattice structure selected from the group consisting of:

hexagonal, cubic, tetragonal, rhombohedral, orthorhombic, monoclinic, triclinic and a combination thereof. It will be understood that for the purposes of the present invention, the lattice structure of nano-structured material describes the grouping of the material according to the axial system. Each lattice structure consists of a set of three axes in a particular geometrical arrangement. The nano-structured material's lattice structure may play a role in determining some of its properties, such as its electric properties and optical properties.

In particular, the nano-structured material has a hexagonal lattice structure. For example, the nano-structured material may be hexagonal phase $NaYF_4$. Even more in particular, the nano-structured material is hexagonal phase $NaYF_4$:Yb,Er, hexagonal phase $NaYF_4$:Yb,Tm or hexagonal phase $NaYF_4$:Yb,Ho.

The nano-structured material prepared from the method according to any aspect of the present invention may be in the form of: nanoparticle(s), nanofilm, or monolith. For example, the nano-structured material may be at least one nanoparticle and the average diameter of the nanoparticle(s) is $\leq$ 1000 nm, $\leq$100 nm, $\leq$ 50 nm, < 50 nm, $\leq$ 25 nm, $\leq$ 10 nm or $\leq$5 nm. In particular, the average diameter of the nanoparticle(s) is < 50 nm, $\leq$ 25 nm, $\leq$ 10 nm or $\leq$ 5 nm. More in particular, the average diameter of the nanoparticle(s) is $\leq$ 10 nm.

The nano-structured material may be at least one nanofilm. The nanofilm may have a thickness between 0.1 nm to 1 mm. In particular, the nanofilm thickness may be the same or less than 500 nm, 400 nm, 300 nm, 200 nm, 100 nm, 50 nm, 25 nm, 20 nm, 15 nm, 10 nm or 5 nm. The nanofilm may be a single layer or multiple layers, and wherein each layer of the nanofilm is the same or different from the other layer. The nanofilms may be prepared by depositing particles using methods such as dip coating or spin coating.

The nanoparticle(s) may comprise core nanoparticle(s) and/or core-shell nanoparticle(s). The shell may be the same or different material as the core. An illustration of the core nanoparticle and core-shell nanoparticle is shown in Figures 1(a) and 1(b) respectively Figure 1(a) shows a core nanoparticle with at least one kind of surfactant on its surface. Figure 1(b) shows a core-shell nanoparticle with at least one kind of surfactant on the shell. Figure 2 shows a nano-structured material where the core and the shell are of the same material, $NaYF_4$. For example, the nanoparticle may be a core nanoparticle and the nanoparticle further comprises at least one organic and/or inorganic material (shell) applied on the core, to obtain a core-shell nanoparticle(s). Accordingly, the method according to any aspect of the present invention may comprise a further step of applying at least one organic and/or inorganic material (shell) on the core to obtain a core-shell nanoparticle(s).

As mentioned above, the nanoparticle may comprise an organic and/or inorganic material (shell). The organic and/or inorganic material (shell) may be applied continuously or discontinuously on the core. According to a particular aspect, the shell material has the formula $M_1M_2X_n$ or $M_1M_2X_n$:M_q, wherein M_1, M_2, X, n and M_q are as defined above. For example, the shell material may comprise a material selected from the group consisting of: NaM_2F_4, LiM_2F_4, KM_2F_4, RbM_2F_4, CsM_2F_4, BeM_2F_5, $Be(M_2)_2F8$, MgM_2F_5, $Mg(M_2)_2F_8$, CaM_2F_5, $Ca(M_2)_2F_8$, SrM_2F_5, $Sr(M_2)_2F_8$, $BaLnF_5$, $Ba(M_2)_2F_8M_2F_3$, M_2F_3, M_2Cl_3, M_2Br_3, M_2I_3, M_2FClBr, M_2OF, M_2OCl, M_2OBr, M_2OS, $(M_2)_2S_3$, wherein M_2 is as defined above. In particular, each M_2 is the same or different and is selected from the group consisting of: Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu.

According to another particular aspect, the organic shell material may comprise at least one polymer, a surfactant, a lipid, or a combination thereof. For example, the polymer may be selected from the group consisting of: polystyrene (PS), polyethylene (PE), polymethyl methacrylate (PMMA), polylactic acid (PLA) and a combination thereof. For the purposes of the present invention, a surfactant will be understood to be on which is a surface active agent that lowers the surface tension. The surfactant may contain both hydrophilic and hydrophobic components and may be semi-soluble in both organic and aqueous solvents. For example, surfactants tend to clump up when in solution, forming a surface between fluid and air with hydrophobic tails in the air and the hydrophilic heads in the fluid.

The inorganic shell material may comprise any one of the following: NaM_2F_4, LiM_2F_4, KM_2F_4, RbM_2F_4, CsM_2F_4, BeM_2F_5, Be(M_2)2F8, MgM_2F_5, $Mg(M_2)_2F_8$, CaM_2F_5, $Ca(M_2)_2F_8$, SrM_2F_5, $Sr(M_2)_2F_8$, $BaLnF_5$, $Ba(M_2)_2F_8M_2F_3$, M_2F_3, M_2Cl_3, M_2Br_3, M_2I_3, M_2FClBr, M_2OF, M_2OCl, M_2OBr, M_2OS, $(M_2)_2S_3$, wherein each M_2 is the same or different and is selected from the group consisting of: Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu; SiO_2; TiO_2; ZnS; or a combination thereof.

The shell material may confer certain properties onto the nano-structured material. For example, the shell may make the nano-structured material more hydrophilic, hydrophilic or amphiphilic. In particular, the nano-structured material may be made hydrophilic in order to better attach the nano-structured materials to biomolecules such as proteins and DNA.

According to a further aspect, the nano-structured material prepared from the method according to any aspect of the present invention may have its surface modified. The surface of the nano-structured material may be modified by adding at least one surfactant, lipid, polymer, inorganic material, or a mixture thereof. The surface of the nano-structured material may be modified to confer certain properties onto the nano-structured material. For example, the surface of the nano-structured material may be modified to make the nano-structured material more hydrophilic, hydrophilic or amphiphilic. In particular, the nano-structured material may be made hydrophilic in order to better attach the nano-structured materials to biomolecules such as proteins and DNA. The nano-structured material may be made more hydrophilic by surfactant(s) and/or lipid(s).

According to a particular aspect, the nano-structured material may be surface modified by any one of the following ways:

(a) Surfactant/lipids modification:

Nano-structured material + surfactant(s) ⟶ nano-structured material-Surfactants complex

(b) Surfactant(s)/lipid(s) replacement:

Nano-structured material-Surfactant(1) + Surfactant (2) ⟶ Nano-structured material-surfactant(2)

(c) Surfactant attached on the surfactant on the nano-structure material surface

Nanoparticles-Surfactant(1) + Surfactant (2) ⟶ Nano-structured material-Surfactant(1)-Surfactant(2)

It will be understood that in the above, surfactant and lipid may be used interchangeably. An illustration of surface modified nano-structured material is shown in Figure 3.

The surface of the nano-structured material may be modified by at least one lipid. The lipid may be any suitable lipid. For example, the lipid may be phospholipid, long-chain aliphatic hydrocarbon, lipid multichain, comb-shaped lipid-polymer steroid, fullerene, polyaminoacid, native or denatured protein, aromatic hydrocarbon, or partially or completely fluorinated lipid. In particular, the lipid may have the structure as shown in Figure 4 (a), (b) and (c).

In particular, the surface is modified by at least one surfactant. The at least one surfactant may be adsorbed onto the surface of the nano-structured material.

The surfactant according to any aspect of the present invention may be hydrophilic, hydrophobic and/or amphiphilic. The surfactant may have the following formula:

$$\begin{array}{ccccc} & R^1 & & R^3 & \\ & | & & | & \\ R^5 - (& C_JH_K &)_s\text{-}(& C_ZH_W &)_y - R^6 \\ & | & & | & \\ & R^2 & & R^4 & \end{array} \qquad \text{(Formula I)}$$

wherein

each J is the same or different, and $1 \leq J \leq 9$;

each K is the same or different, and $0 \leq K \leq 9$;

each s is the same or different, and $0 \leq s \leq 9$;

each Z is the same or different, and $1 \leq Z \leq 9$;

each W is the same or different, and $0 \leq W \leq 9$;

each y is the same or different, and $0 \leq y \leq 9$;

each R^1, R^2, R^3, R^4 and R^5 is the same or different, and is independently selected from the group consisting of: H, substituted or unsubstituted C_1-C_6 alkyl, substituted or unsubstituted C_1-C_6 aryl, HS, COOH, NH_2 and OH;

each R_6 is the same or different, and is selected from the group consisting of: COOH, NH_2, OH, P=0 and P;

with the proviso that $s + y < 10$.

As used herein, the term “alkyl” refers to a straight or branched, monovalent, saturated aliphatic chain of preferably 1 to 6 carbon atoms, including normal, iso, neo and tertiary. “Alkyl” includes, but is not limited to, methyl, ethyl, propyl, isopropyl, butyl, iso-butyl, sec butyl, tert butyl, amyl, isoamyl, neoamyl, hexyl, isohexyl, neohexyl, and the like; cycloalkyl group such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and the like, the cycloalkyl group may be substituted. The alkyl may be optionally substituted with substituents selected from the group consisting of lower alkyl, lower alkoxy, lower alkylsulfanyl, lower alkylsulfenyl, lower alkylsulfonyl, oxo, hydroxy, mercapto, amino optionally substituted by alkyl, carboxy, carbamoyl optionally substituted by alkyl, aminosulfonyl optionally substituted by alkyl, silyloxy optionally substituted by alkoxy, alkyl, or aryl, silyl optionally substituted by alkoxy, alkyl, or aryl, nitro, cyano, halogen, or lower perfluoroalkyl, multiple degrees of substitution being allowed. Such an "alkyl" group may contain one or more O, S, S(O), or $S(O)_2$, P, P(O), $P(O)_2$ atoms.

The term "aryl" refers to a benzene ring or to an optionally substituted benzene ring system fused to one or more optionally substituted benzene rings, optionally substituted with substituents selected from the group consisting of lower alkyl, lower alkoxy, lower alkylsulfanyl, lower alkylsulfenyl, lower alkylsulfonyl, oxo, hydroxy, mercapto, amino optionally substituted by alkyl, carboxy, tetrazolyl, carbamoyl optionally substituted by alkyl, aminosulfonyl optionally substituted by alkyl, acyl, aroyl, heteroaroyl, acyloxy, aroyloxy, heteroaroyloxy, alkoxycarbonyl, silyloxy optionally substituted by alkoxy, alkyl, or aryl, silyl optionally substituted by alkoxy, alkyl, or aryl, nitro, cyano, halogen, or lower perfluoroalkyl, multiple degrees of substitution being allowed. Examples of aryl include, but are not limited to, phenyl, biphenyl, naphthyl, furanyl, pyrrolyl, thiophenyl, pyridinyl, indolyl, benzofuranyl, benzothiophenyl, quinolinyl, isoquinolinyl, imidazolyl, thiazolyl, pyrazinyl, pyrimidinyl, purinyl and pteridinyl and the like.

The term "lower" refers to a group having between one to six carbon atoms.

Any suitable surfactant as described in the Sigma Aldrich catalogue, 2004-2005 may be used for the present invention. In particular, the surfactant used may be at least one or a mixture of the following:

(i) a surfactant, comprising thiol and carboxylic acid functional groups, selected from mercaptosuccinic acid, mercaptobenzoic acid, penicillamine, mercaptopropioinyl glycine, thioldiacetic acid, thiodipropionic acid, and cysteine hydrochloride;

(ii) a surfactant, comprising thiol and amine functional groups, selected from cysteine, mercaptoethylamine, thioguanine, and thioacetamide;

(iii) a surfactant, comprising thiol and hydroxyl groups, selected from mercaptoethanol, thiodiethanol, thioglucose, thioglycerol and cysteine-OH;

(iv) cysteine; and/or

(v) a peptide comprising cysteine.

For example, in the surfactant(s) of Formula (I), s + y has the following ranges: 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, or 1. In particular, s + y is 1-4, preferably 1 or 2, and each of R^1, R^2, R^3, and R^4, independently, is not present or is H. More in particular, surfactant(s) according to any aspect of the present invention may be $HSCH_2COOH$ and/or $HS(CH_2)_2COOH$. The cysteine-containing peptide of (v) may be a peptide of the following sequence: CDPGYIGSR (SEQ ID NO:1). SEQ ID NO:1 refers to the 925-933 laminin fragment. In particular, the surfactant is polyacrylic acid, polyethylene glycol 600 (HOOC-PEG-COOH), 11-aminoundecanoic acid (AUA) or a mixture thereof.

According to a further aspect, a biomolecule may be attached to the nano-structured material prepared from the method according to any aspect of the present invention. Therefore, the method according to any aspect of the present invention may comprise a further step of attaching a biomolecule to the nano-structured material. The biomolecule may be attached to the nano-structured material by chemical or physical conjugation. Any suitable biomolecule may be attached to the nano-structured material. For example, the biomolecule is selected from the group consisting of: protein, nucleic acid, nucleosides, nucleotides, DNA, hormone, amino acid, peptide, peptidomimetic, RNA, lipid, albumin, antibody, phospholipids, glycolipid, sterol, vitamins, neurotransmitter, carbohydrate, sugar, disaccharide, monosaccharide, oligopeptide, polypeptide, oligosaccharide, polysaccharide and a mixture thereof. In particular, the biomolecule is streptavidin, an antibody, DNA or a combination thereof. Other biomolecules with free amine, hydroxyl or carboxyl groups which could be attached to surfactants as described above include anti-cancer drugs such as carboplatin, nedaplatin, JM216, methotrexate and doxorubicin, as well as proteins and glycoproteins such as herceptin.

According to another aspect, the present invention provides a nano-structured material obtainable by any method described above. The nano-structured material may further comprise at least one surfactant, lipid and/or polymer. A suitable biomolecule, as described above, may be attached to the nano-structured material.

The nano-structured material according to any aspect of the present invention may provide a fluorescent dye that can be embedded or mixed or coated with another semiconductor matrix for the generation of electrical current. For example, the absorption of NIR, visible or UV light to generate electrical current in a dye-sensitized solar cell.

According to yet another aspect, the present invention provides a nano-structured material of formula $M_1M_2X_n$:M_q, wherein each of M_1, M_2, M_q, X and n are as defined above, and wherein the nano-structured material has a hexagonal (lattice) structure and comprises at least one dimension of size ≤1000 nm, ≤100 nm, ≤ 50 nm, < 50 nm, ≤25 nm, ≤10 nm or ≤ 5 nm. In particular, the nano-structured material comprises at least one dimension of ≤ 10 nm.

In particular, the present invention provides a nano-structured material of formula $M_1M_2X_n$:M_q, wherein M_1, M_2, X, n and M_q are as defined above, and wherein the nano-structured material has a hexagonal lattice structure, comprising at least one dimension of < 50 nm, with the proviso that the nano-structured material is not LaF_3:Yb,Er, LaF_3:Yb,Ho or LaF_3:Yb,Tm. Even more in particular, the nano-structured material is hexagonal phase $NaYF_4$:M_q. For example, $NaYF_4$:M_q is $NaYF_4$:Yb,Er, $NaYF_4$:Yb,Ho or $NaYF_4$:Yb,Tm. The nano-structured material may have a hexagonal (lattice) structure and comprise at least one dimension of size ≤1000 nm, ≤100 nm, ≤ 50 nm, < 50 nm, ≤25 nm, ≤10 nm or ≤5 nm. In particular, the nano-structured material comprises at least one dimension of ≤ 10 nm.

The present invention also provides an article of manufacture comprising the any nano-structured material as described above. For example, the article of manufacture may be at least one of the following: a display device, a solar cell, an optical data storage, a bio-probe, a carrier for drug delivery, a lamp, a LED, a LCD, a wear resistance, a laser, optical amplifier, low density IR imaging, mercury-free fluorescent lamps, plasma display panel (PDP) and/or a device for bio-imaging. For example, nano-structured material such as $BaYF_5$, BaY_2F_8, $LiYF_4$, NaM_2F_4 and M_2F_3, wherein each M_2 is the same or different and is as described above, are efficient hosts for near-infrared emission ions such as Nd^{3+}, Er^{3+}, Ho^{3+} and Pr^{3+}. Because the emissions fall in the telecommunication windows, the nano-structured materials can be used as optical amplifiers in telecommunication.

$BaYF_5$, BaY_2F_8, $LiYF_4$, NaM_2F_4 and M_2F_3, wherein each M_2 is the same or different and is as defined above, are also the efficient hosts for quantum cutting phosphors, which can absorb a high energy VUV photon, giving off two or more visible photons. The total quantum efficiency is therefore more than 100%. Such nano-structured materials have applications which include mercury-free fluorescent lamp and plasma display panel (PDP).

Oil dispersible $BaYF_5$, BaY_2F_8, $LiYF_4$, NaM_2F_4 and M_2F_3, wherein each M_2 is the same or different and is as defined above, are nano-structured materials which are anti-wear and may be used as extreme pressure additives due to their tribological and lubricating behaviour.

According to another aspect, the present invention provides a kit comprising at least one nano-structured material as described above and at least one biomolecule. The biomolecule may be any suitable biomolecule. For example, the biomolecule is selected from the group consisting of: protein, nucleic acid, nucleosides, nucleotides, DNA, hormone, amino acid, peptide, peptidomimetic, RNA, lipid, albumin, antibody, phospholipids, glycolipid, sterol, vitamins, neurotransmitter, carbohydrate, sugar, disaccharide, monosaccharide, oligopeptide, polypeptide, oligosaccharide, polysaccharide, and a mixture thereof. In particular, the biomolecule is streptavidin, an antibody, DNA or a combination thereof. The kit may further comprise at least one surfactant as described above. The kit may also comprise a suitable excitation source and/or means for delivery of the excitation source as described herein. In particular, the excitation source is NIR. More in particular, the excitation source is NIR laser at 980 nm. The means for delivery of the excitation source may be optical fibres or endoscopes. The kit may be used in bio-imaging and/or bio-detection. For example, the kit may be used for labelling a tissue sample. When the tissue is labelled with the nano-structured material of the present invention, and a source of excitation is provided, the tissue which is affected by cancer, for example, will emit fluorescence, indicating the presence of cancer.

Another aspect of the present invention is a bio-imaging and/or bio-detection system comprising:

- at least one nano-structured material prepared from the method described above;
- at least one biomolecule;
- at least one source of excitation; and
- at least one means for delivery of the source of excitation to the system.

For example, the system may be used for detecting cancer. In particular, a tissue sample is labelled with the nano-structured material of the present invention. When a source of excitation is provided, the tissue which is affected by cancer, for example, will emit fluorescence, indicating the presence of cancer. The system may also be used in the detection of specific viruses or biomolecules in blood, for example, by detecting the fluorescence of the nano-structured material when a source of excitation is provided.

The biomolecule may be any suitable biomolecule. For example, the biomolecule is selected from the group consisting of: protein, nucleic acid, nucleosides, nucleotides, DNA, hormone, amino acid, peptide, peptidomimetic, RNA, lipid, albumin, antibody, phospholipids, glycolipid, sterol, vitamins, neurotransmitter, carbohydrate, sugar, disaccharide, monosaccharide, oligopeptide, polypeptide, oligosaccharide, polysaccharide and a mixture thereof. In particular, the biomolecule is streptavidin, an antibody, DNA or a combination thereof.

The source of excitation may be any suitable source. For example, the source of excitation may be a microwave source, NIR source, IR source, laser source, X-ray source or a combination thereof. In particular, the source may be NIR source. In particular, the NIR source is at 980 nm.

Any suitable means for delivery of the source of excitation to the system may be used. For example, the means for delivery may be selected from the group consisting of: optical fibres, endoscopes, external light, external laser and a combination thereof. The optical fibres may be inserted in a needle. The source of excitation may also be delivered through the skin surface.

The present invention also provides a nano-structured material prepared according to a method as described above for use as fluorescent sensors for bio-imaging and/or detection of biomolecule(s). For example, the nano-structured material prepared may be used for labelling cells or tissues and to observe the image via a fluorescent microscopy. The nano-structured material may also be used for labelling biomolecules such as proteins and detecting the fluorescent signal.

The present invention also provides a method of modifying the surface of a nano-structured material to alter, change or modify its properties. For example, the surface modification may be carried out by adding at least one surfactant, lipid, polymer, inorganic material, or a mixture thereof. The nano-structured material may be the nano-structured material as described above or any other nano-structured material. This surface modification may also be applied to any compound, molecule, structure or material. The surface modification may be as described above. For example, the surface modification may be used for making a hydrophobic material hydrophilic. The present invention also provides a method of modifying at least one hydrophobic structure to make it hydrophilic, comprising applying at least one modifier on the structure surface to make a structure-modifier complex, and wherein the modifier is a surfactant, lipid, polymer and/or inorganic material. The structure may be the nano-structured material as described above. For example, the method may comprise applying at least one first modifier on the structure surface, and further a second hydrophilic modifier to make a structure-first modifier-second modifier complex, wherein the first and/or second modifier is a surfactant, lipid, polymer and/or inorganic material. Any suitable surfactant, lipid, polymer and/or inorganic material may be used. For example, the surfactants, lipids, polymers and/or inorganic material described above may be used as the first and/or second modifier. According to a particular aspect, the method may comprise applying at least one hydrophilic surfactant on the structure to make a structure-surfactant complex.

The present invention also provides a method of varying the length of the at least one surfactant(s) on the nano-structured material so as to modulate the efficiency of loading of the biomolecule(s) on the surfactant(s), as well as to modulate the NIR optical properties of the NIR-sensitive nano-structured material. In particular, the modulation is obtained by varying the chain length of the at least one surfactant and determining the improved efficiency of loading biomolecule(s) on the surface modified nano-structured material, and/or improved efficiency of releasing biomolecule(s) from the surface modified nano-structured material following light irradiation.

The surface modification of the nano-structured material with surfactants facilitates the binding of functional molecules, such as biomolecules. Suitable surfactants have functional groups reactive to both the nano-structured material and desired biomolecules. The inorganic-organic surface interactions between the surfactants and the nano-structured material may be used to modulate (manipulate) the optical properties of the biomolecule delivery system in which the nano-structured material is used. For the same number of functional groups on each surfactant, the reactivity and number of binding sites may be modified by altering the surfactant intermolecular and surfactant-particle interactions through differences in the surfactant chain length. The alteration of surfactant interactions to control the binding intensity would be applicable to systems surface functionalised with biomolecules.

Accordingly, the present invention provides a method of modulating the biomolecule loading efficiency on the nano-structured material which has been surface modified by at least one surfactant according to any embodiment of the invention, comprising varying the chain length of the at least one surfactant adsorbed on the nano-structured material.

In particular, the surfactant is at least one surfactant or a mixture thereof of the surfactant of Formula (I).

In particular, the method is a method for increasing the biomolecule loading efficiency comprising varying the chain length of the at least one surfactant as described above, so that the biomolecule loading efficiency is higher (increased). Even more in particular, the surfactant is at least one surfactant or a mixture thereof of the surfactant of Formula (I).

Further, the present invention provides a method of modulating binding affinity and/or binding recognition of the surface modified nano-structured material ligand(s) (sensors) with the respective receptor(s). The nano-structured material may be surface modified having at least one surfactant adsorbed onto the surface of the nano-structured material. The modulation of the binding affinity and/or binding recognition of particular kind of biomolecule(s), that is, ligand(s) (sensors) loaded on the surface modified nano-structured material according to any embodiment of the invention can be carried out, for example, by altering the surfactant interaction.

The nano-structured material as described above may be used as bio-probes. Accordingly, the expensive Ti-Sa laser system may be replaced with a cheaper 980 nm semiconductor laser system instead. The price of a multi-photon microscope may be comparable or even lower than that of the traditional confocal microscope.

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention.

EXAMPLES

Characterisation Techniques

TEM images of the nano-structured materials were collected on a JEOL JEM 3010 transmission electron microscope. Powder X-ray diffraction spectra were acquired with a D8 advance x-ray diffractometer, with Cu Ka radiation at 1.5406 Å. Size distribution and zeta-potentials of the nano-structured materials were determined using a dynamic light scattering instrument (Nano-ZS, Malvern Instruments). Up-conversion fluorescence spectra were obtained on a LS-55 luminescence spectrometer (Perkin-Elmer) with an external 980 nm laser diode (1W, continuous wave with 1 m fibre, Beijing Viasho Technology Co.) as the excitation source in place of the xenon lamp in the spectrometer. The spectrometer operated at the bioluminescence mode, with gate time 1 ms, delay time 1 ms, cycle 20 ms and flash count 1. For comparison, colloidal solutions of core, core-shell in chloroform, and core-shell-polymer in water with the same concentration were used.

Example 1: Synthesis of nanoparticles (core or core/shell structure)

Example 1.1: Synthesis of hexagonal phase $NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm nanoparticles

For the synthesis of hexagonal $NaYF_4$:20%Yb,2%Er and $NaYF_4$:20%Yb,2%Tm nanoparticles, a mixture of 2 mmol CF_3COONa, 0.78 mmol of $(CF_3COO)_3Y$, 0.2 mmol of $(CF_3COO)_3Yb$ and 0.02 mmol of $(CF_3COO)_3Er/(CF_3COO)_3Tm$ was dissolved in 10 ml of oleylamine, and then passed through a 0.22μm filter (Millipore) to remove any residue. Under vigorous stirring in a 50 ml flask, the mixture was heated to 330°C in the presence of argon for protection from oxidation. After 1 h, heating was stopped. The transparent yellowish reaction mixture obtained was allowed to cool to 80°C before 20 ml of ethanol was added to the mixture. The nanoparticles were isolated by centrifuging. They were washed 3 times with hexane and 3 times with de-ionized water to remove any NaF residue. The scheme of the reaction was as follows:

$$CF_3COONa \longrightarrow NaF$$

$$+$$

$$(CF_3COO)_3Y + (CF_3COO)_3Yb + (CF_3COO)_3Er/Tm \longrightarrow YF_3 + YbF_3 + ErF_3/TmF_3$$

$$\downarrow$$

$$NaYF_4\text{:Yb,Er(Tm)}$$

Figures 5a and 5b show TEM images of the $NaYF_4$:20%Yb,2%Er nanoparticles. The well-dispersed particles suggest that the long chain oleylamine ligand on the crystal surface prevented aggregation. The average diameter of the nanoparticles, obtained from measuring 200 particles randomly from 5 TEM micrographs, was 10.5 nm, with a standard derivation of ±0.7 nm. Figure 5c shows that these nanoparticles were in hexagonal phase. The peak positions and intensities of these nanoparticles agreed well with calculated hexagonal $NaYF_4$:Yb,Er nanoparticles (line pattern in the lower part of Figure 5c). There were two unknown small peaks at $2\theta \sim 64°$ and 73°. There was a residual amount of cubic phase as indicated by the dashed arrow in Figure 5c.

Combined with the TEM results, the particles were confirmed to be single crystals. Similar TEM results were also observed for $NaYF_4$:20%Yb,2%Tm nanoparticles (results not shown).

The selected area electron diffraction (SAED) pattern (Figure 5d) shows the polycrystalline diffraction rings corresponding to the (100), (110), (111), (201), (311) and (321) of the hexagonal $NaYF_4$ lattice. The SAED pattern as shown in Figure 5d showed six of the diffraction rings corresponding to the hexagonal phase $NaYF_4$ nanoparticles.

Figure 6a shows the room-temperature up-conversion fluorescence spectra of the $NaYF_4$:20%Yb,2%Er and $NaYF_4$:20%Yb,2%Tm nanoparticles. For $NaYF_4$:20%Yb,2%Er nanoparticles, under 980 nm NIR excitation (continuous wave, CW), there were three emission peaks at 522.5, 541.5 and 655.5 nm, which were assigned to $^4H_{11/2}$ to $^4I_{15/2}$, $^4S_{3/2}$ to $^4I_{15/2}$ and $^4F_{9/2}$ to $^4I_{15/2}$ transitions of erbium, respectively (GS Yi et al, 2004). For $NaYF_4$:20%Yb,2%Tm nanoparticles, the blue emission band at 450.5 and 475 nm corresponded to the transitions from $^1G_4$ to $^3H_6$ and $^1G_4$ to $^3H_6$ of thulium, respectively (GS Yi and GM Chow, 2005). A strong near infrared emission at 801.5 nm was attributed to the transition from $^1G_4$ to $^3H_5$ (S Heer et al, 2003). As-synthesized $NaYF_4$:20%Yb,2%Er and $NaYF_4$:20%Yb,2%Tm nanoparticles were easily dispersed in organic solvents like hexane and formed a transparent colloidal solution. Figure 6b (i) and (ii) show the $NaYF_4$:20%Yb,2%Er and $NaYF_4$:20%Yb,2%Tm nanoparticles colloidal solution (0.1 wt.-%) respectively under 980 nm NIR excitation (CW). The laser power for excitation was 1W with a power density < 0.1 W/mm^2. The bands of light in Figure 6b(i) refer to the 522.5 nm and 541.5 nm emission of the $NaYF_4$:20%Yb,2%Er nanoparticles, while the bands of light in Figure 6b(ii) corresponds to the 450.5 nm and 475 nm emission of the $NaYF_4$:20%Yb,2%Tm nanoparticles, respectively.

Example 1.2: Synthesis of cubic phase $NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm nanoparticles

For the synthesis of cubic $NaYF_4$:20%Yb,2%Er and $NaYF_4$:20%Yb,2%Tm nanoparticles, a mixture of 2 mmol CF_3COONa, 0.78 mmol of $(CF_3COO)_3Y$, 0.2 mmol of $(CF_3COO)_3Yb$ and 0.02 mmol of $(CF_3COO)_3Er/(CF_3COO)_3Tm$ was dissolved in 10 ml of 1-octadecene, with 2 ml of oleic acid. The solution was passed through a 0.22 µm filter (Millipore) to remove any residue. Under vigorous stirring in a 50 ml flask, the mixture was heated to 300°C in the presence of argon for protection from oxidation. After 1 h, heating was stopped. The transparent reaction mixture was allowed to cool to 80°C before 20 ml of ethanol was added. The nanoparticles were isolated by centrifuging. They were washed 3 times with hexane and 3 times with de-ionized water to remove any NaF residue.

The average diameter of the nanoparticles obtained was 22 nm, with narrow size distribution, as shown in Figure 7a. X-ray diffraction (XRD) results showed that these nanoparticles were in the cubic phase (see Figure 7b). The up-conversion fluorescence of the nanoparticles obtained was 7.5 times lower than that of the hexagonal phase nanoparticles described in Example 1.1 above.

Example 1.3: Synthesis of core and core/shell $NaYF_4$:Yb,Er/$NaYF_4$ and $NaYF_4$:Yb,Tm/$NaYF_4$ nanoparticles

For the synthesis of hexagonal $NaYF_4$:20%Yb,2%Er and $NaYF_4$:20%Yb,2%Tm core nanoparticles, a mixture of 0.5 mmol CF_3COONa, 0.195 mmol of $(CF_3COO)_3Y$, 0.05 mmol of $(CF_3COO)_3Yb$ and 0.005 mmol of $(CF_3COO)_3Er/(CF_3COO)_3Tm$ was dissolved in 5 ml oleylamine, and passed through a 0.22 µm filter (Millipore) to remove any residue. Under vigorous stirring in a 25 ml flask, the mixture was heated to 340°C in the presence of argon protection to produce core nanoparticles. After 30 min, 0.5 ml of the core nanoparticle product was taken out for reference. 1 ml shell precursor solution containing 0.5 mmol of CF_3COONa and 0.25 mmol of $(CF_3COO)_3Y$ in oleylamine was slowly added into the reaction. The reaction was allowed to continue for another 30 min. The reaction mixture was allowed to cool down to room temperature and kept as stock solution.

The average diameter of the core nanoparticles obtained was 8.5 nm with a standard derivation of ±0.8 nm (Figure 8a). The diameter of the core-shell (CS) nanoparticles was 11.1±1.5nm as shown in Figure 8b. X-ray diffraction (XRD) results showed that these nanoparticles were in hexagonal phase (see Figure 8c). The fluorescent intensity of the core-shell nanoparticles was much stronger than the core nanoparticles, as shown in Figures 9(a), 9(b), 10(a) and 10(b). In particular, the fluorescent intensity was 7.4 times stronger for $NaYF_4$:Yb,Er and 29.6 times for $NaYF_4$:Yb,Tm.

Example 1.4: Synthesis of $LiYF_4$ nanoparticles

0.5 mmol of CF_3COOLi and 0.5 mmol of $(CF_3COO)_3Y$ were dissolved in 10 ml of oleylamine, passed through a 0.22 µm filter (Millipore) to remove any residue. Under vigorous stirring in a 50 ml flask, 0.5 ml of CF_3COOH was added. The solution was heated to reflux (~343°C) under the protection of dry argon gas. After 1 h, heating was stopped. The reaction mixture was allowed to cool down to 80°C before 20 ml of ethanol was added. The nanoparticles were isolated by centrifuge, rinsed with ethanol, and then stored.

Most of the $LiYF_4$ particles took on rhombus shape, with equal side edge length of 49.3±4.5 nm, long axial length of 90.9±6.1 nm and short axial length of 51.4±3.3 nm, respectively (obtained from measuring 50 particles from 5 TEM micrographs). The obtuse angle between the two side edges was 130°. The nanoparticles were confirmed to be cuboid shape. The $LiYF_4$ nanoparticles were in tetragonal phase as shown in Figure 11(a) with each particle as a single crystal (Figure 11(b)).

Example 1.5: Synthesis of $BaYF_5$ nanoparticles

Precursor of $Ba(acac)_2$ and $(CF_3COO)_3Y$ were first dissolved in oleic acid in a separate vial (0.25 mmol/ml), and passed through a 0.22 µm filter (Millipore) to remove any of the residues. For the synthesis of $BaYF_5$ nanoparticles, 2 ml of $Ba(acac)_2$ and 2 ml of $(CF_3COO)_3Y$ in oleic solution were introduced into 6 ml of octadecene. Under vigorous stirring in a 50 ml flask, the mixture was heated to 300°C under the protection of dry argon gas. After 1 h, heating was stopped. The reaction mixture was allowed to cool down to 80°C before 20 ml of ethanol was added. The nanoparticles were isolated by centrifuge, washing three times with ethanol, and stored.

The average diameter of the $BaYF_5$ nanoparticles was 6.7±0.5 nm. XRD results confirmed that the $BaYF_5$ nanoparticles were real tetragonal phase crystals (Figure 12 and Figure 14).

Example 1.6: Synthesis of $NaLaF_4$ nanoparticles

1 mmol of CF_3COONa and 0.5 mmol of $(CF_3COO)_3La$ were dissolved in 10 ml of oleylamine, passed through a 0.22µm filter (Millipore) to remove any residue. Under vigorous stirring in a 50 ml flask, 0.5 ml of CF_3COOH was added. The solution was heated to reflux (~ 345°C) under the protection of dry argon gas. After 1 h, heating was stopped. The reaction mixture was allowed to cool down to 80°C before 20 ml of ethanol was added. The nanoparticles were isolated by centrifuge, washed three times with hexane, and another 3 times with de-ionized water to remove any NaF residue.

The average diameter of the $NaLaF_4$ nanoparticles was 8.8±1.2 nm. XRD results confirmed that the $NaLaF_4$ nanoparticles were real hexagonal phase crystals (Figure 13 and Figure 14).

Example 1.7: Synthesis of 20%Yb, 3%Er co-doped $LiYF_4$, $BaYF_5$ and $NaLaF_4$ nanoparticles

The synthesis procedure was the same as that used to prepare undoped $LiYF_4$, $BaYF_5$ and $NaLaF_4$ nanoparticles, except that 78% $(CF_3COO)_3Y$, 20%(CF3COO)$_3$Yb and 2% $(CF_3COO)_3Er$ mixture was used as the rare earth source, instead of using a single source of $(CF_3COO)_3Y$ for the synthesis of $LiYF_4$, $BaYF_5$ in examples 1.4 and 1.5 respectively. For the synthesis of doped $NaLaF_4$, 78% $(CF_3COO)_3La$, 20%$(CF_3COO)_3Yb$ and 2% $(CF_3COO)_3Er$ mixture were used instead of using single source of $(CF_3COO)_3La$ as in example 1.6. The fluorescence spectra of the doped nanoparticles prepared is shown in Figure 15.

Example 1.8: Synthesis of LaF_3 nanoparticles

1 mmol of (CF_3COO) $_3La$ was dissolved in 10 ml of oleylamine, and passed through a 0.22 μm filter (Millipore) to remove any residue. Under vigorous stirring in a 50 ml flask, the solution was heated to reflux (~ 345°C) under the protection of dry argon gas. After 1 h, heating was stopped. The reaction mixture was allowed to cool down to 80°C before 20 ml of ethanol was added. The nanoparticles were isolated by centrifuge, and washed three times with hexane.

The average diameter of the LaF_3 nanoparticles obtained was 4.3 nm. XRD results confirmed that the LaF_3 nanoparticles were in the hexagonal phase (Figure 16).

Example 1.9: Synthesis of YF_3 nanoparticles

1 mmol of (CF_3COO) $_3Y$ was dissolved in 10 ml of oleylamine, and passed through a 0.22 μm filter (Millipore) to remove any residue. Under vigorous stirring in a 50 ml flask, 0.5 ml of CF_3COOH was added. The solution was heated to reflux (~ 345°C) under the protection of dry argon gas. After 1 h, heating was stopped. The reaction mixture was allowed to cool down to 80°C

before 20 ml of ethanol was added. The nanoparticles were isolated by centrifuge, and washed three times with hexane.

The average diameter of the YF_3 nanoparticles obtained was 8.7 nm. XRD results confirmed that the YF_3 nanoparticles were in the tetragonal phase (Figure 17).

Example 1.10: Synthesis of $NaYF_4$:20%Yb,2%Er nanoparticles in tetracosane with trioctylphosphine (TOP) as surfactant

Synthesis of $NaYF_4$:20%Yb,2%Er nanoparticles was performed according to the following steps:

A) Preparation of $(CF_3COO)_3Y$, $(CF_3COO)_3Yb$, $(CF_3COO)_3Er$ and CF_3COONa

A rare earth chloride solution containing 0.78 mmol of YCl_3, 0.2 mmol of $YbCl_3$ and 0.02 mmol of $ErCl_3$ was mixed with 2 ml 25% ammonium hydroxide solution. White precipitates of rare earth hydroxide were then formed. They were separated by centrifugation, washed several times with de-ionized water. The precipitates were then mixed with 1 mmol (0.106 g) sodium carbonate (Na_2CO_3) and dissolved completely in 2 ml trifluoroacetic acid (CF_3COOH). After drying at 80°C for 24 h, a mixture of $(CF_3COO)_3Y$, $(CF_3COO)_3Yb$, $(CF_3COO)_3Er$ and CF_3COONa was obtained.

B) The mixture of $(CF_3COO)_3Y$, $(CF_3COO)_3Yb$, $(CF_3COO)_3Er$ and CF_3COONa was dissolved in 4 ml of trioctylphosphine (TOP).

C) In a typical procedure for the synthesis of $NaYF_4$:20%Yb,2%Er nanoparticles, 6.4 g tetracosane was heated to 385°C under argon protection. 2 ml (0.5 mmol) of the solution obtained in step B was quickly injected into it through a 5 ml syringe. A sudden decrease of temperature to 340°C occurred. Heating was restored to 360°C and maintained for 30 min. After the reaction, the mixture was allowed to cool down to around 70°C before adding 20 ml of hexane to dissolve tetracosane. $NaYF_4$:20%Yb,2%Er nanoparticles were collected by centrifugation. The product was washed several times with hexane and a white powder obtained was left to dry in open air at room temperature overnight.

The reaction can be illustrated as:

$$\begin{array}{l} CF_3COONa + (CF_3COO)_3Y \\ + (CF_3COO)_3Yb + (CF_3COO)_3Er \\ + \text{Trioctylphosphine} \end{array} \xrightarrow[\text{In Tetracosane}]{360^{\circ}C \text{ for 30min}} \begin{array}{l} NaYF_4{:}Yb,Er \\ \text{nanoparticles} \end{array}$$

Figure 18(a) shows the TEM picture of the $NaYF_4$:Yb,Er nanoparticles obtained and Figure 18(b) shows the up-conversion fluorescence of the nanoparticles in hexane. The excitation was with NIR laser source at 980 nm with a power density of 1W. Average size of the nanoparticles was 11 nm with narrow size distribution. Most of the nanoparticles were in the hexagonal phase.

Example 1.11: Synthesis of $NaYF_4$:20%Yb,2%Er nanoparticles in tetracosane with oleylamine as surfactant

6.4 g tetracosane was heated to 300°C under argon protection. 0.5 mmol of $(CF_3COO)_3Y$, $(CF_3COO)_3Yb$, $(CF_3COO)_3Er$ and CF_3COONa in 2 ml of oleylamine was quickly injected into it through a 5 ml syringe. After 1 h of reaction, the mixture was allowed to cool down to around 70°C before adding 20 ml of hexane to dissolve tetracosane. $NaYF_4$:20%Yb,2%Er nanoparticles were collected by centrifugation. The product was washed several times with hexane and the white powder obtained was left to dry in open air at room temperature overnight. Figure 19 shows the TEM picture of the $NaYF_4$:Yb,Er nanoparticles obtained. The average particle size was 9 nm. Most of the nanoparticles obtained were in the cubic phase.

Example 1.12: Synthesis of $NaYF_4$:20%Yb,2%Er nanoparticles in tetracosane with octadecaneamine as surfactant

6.4 g tetracosane was heated to 340°C under argon protection. 0.5 mmol of $(CF_3COO)_3Y$, $(CF_3COO)_3Yb$, $(CF_3COO)_3Er$ and CF_3COONa in 2 ml of octadecaneamine was added in. After 1 h reaction, the mixture was allowed to cool down to around 70°C before adding 20 ml of hexane to dissolve tetracosane. $NaYF_4$:20%Yb,2%Er nanoparticles were collected by centrifugation. The product was washed several times with hexane and the white powder obtained was left to dry in open air at room temperature overnight. Figure 20 is the TEM picture of the $NaYF_4$:Yb,Er nanoparticles obtained. Most of the nanoparticles were agglomerated and were in the hexagonal phase.

Example 2: Synthesis of thin films

Example 2.1: Synthesis of $NaYF_4$:Yb,Er thin film with yellow fluorescent emission

For the synthesis of $NaYF_4$:20%Yb,2%Er thin film, a mixture of 1 mmol CF_3COONa, 0.78 mmol of $(CF_3COO)_3Y$, 0.2 mmol of $(CF_3COO)_3Yb$ and 0.02 mmol of $(CF_3COO)_3Er$ was dissolved in butanol and water, and then passed through a 0.22 μm filter (Millipore) to remove any residue. The precursor was dipped onto a glass substrate. A spin-coater was used to spread the precursors on the glass substrate. After drying, it was sintered in an oven at 500°C for 10 min to obtain the $NaYF_4$:20%Yb,2%Er thin film. Yellow emission was observed when excited with 980 nm NIR laser as shown by the arrow in Figure 21.

Example 2.2: Synthesis of $NaYF_4$:Yb,Tm thin film with blue fluorescent emission

For the synthesis of $NaYF_4$:20%Yb,2%Tm thin film, a mixture of 1 mmol CF_3COONa, 0.78 mmol of $(CF_3COO)_3Y$, 0.2 mmol of $(CF_3COO)_3Yb$ and 0.02 mmol of $(CF_3COO)_3Tm$ was dissolved in butanol and water, and then passed through a 0.22μm filter (Millipore) to remove any residue. The precursor was dipped onto a glass substrate. A spin-coater was used to spread the precursors on the glass substrate. After drying, it was sintered in an oven at 500°C under argon protection for 10min to get the $NaYF_4$:20%Yb,2%Tm thin film. Blue emission was observed when excited with 980 nm NIR laser (result not shown).

Example 2.3: Synthesis of YOF:Yb,Er thin film with red fluorescent emission

For the synthesis of YOF:20%Yb,2%Er thin film, a mixture of 0.78 mmol of $(CF_3COO)_3Y$, 0.2 mmol of $(CF_3COO)_3Yb$ and 0.02 mmol of $(CF_3COO)_3Er$ was dissolved in butanol and water, and then passed through a 0.22μm filter (Millipore) to remove any residue. The precursor was dipped onto a glass substrate. A spin-coater was used to spread the precursors on the glass substrate. After drying, it was sintered in an oven at 500°C under argon protection for 10min to get the YOF:20%Yb,2%Er thin film. Red emission was observed when excited with 980 nm NIR laser, as shown by the arrow in Figure 22.

Example 2.4: Synthesis of $NaYF_4$:Yb,Er thin film without using solvent

For the synthesis of $NaYF_4$:20%Yb,2%Er thin film, a powder mixture of 1 mmol CF_3COONa, 0.78 mmol of $(CF_3COO)_3Y$, 0.2 mmol of $(CF_3COO)_3Yb$ and 0.02 mmol of $(CF_3COO)_3Er$ was pressed onto a glass substrate. It was sintered in an oven at 500°C for 10min to get the $NaYF_4$:20%Yb,2%Er film. Bright green fluorescence was observed when excited with a 980 nm NIR laser (result not shown).

Example 3: Synthesis of water-soluble nanoparticles

The nanoparticles obtained by the methods of Example 1 may be made water-soluble through surface modification of the nanoparticles by surface exchange of, replacement of or attachment by a water soluble molecule. Two methods are described in the examples below.

Example 3.1: Synthesis of water soluble $NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm nanoparticles by surface exchange

The As-synthesized hydrophobic $NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm nanoparticles with oleylamine surfactant or shell on their surface as described in Example 1.3, were modified to have a hydrophilic surface by replacing oleyamine on the particles' surface with a bipolar regent, polyethylene glycol 600 (HOOC-PEG-COOH). 10 mg of the prepared nanoparticles in 1 ml hexane and 10 mg of the ligand to be exchanged in 1 ml of ethanol were mixed and vortexed for 48 h. After surface exchange, the nanoparticles remained as a clear colloid in deionized water, without any noticeable settling or precipitation after two weeks.

Example 3.2: Synthesis water soluble $NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm nanoparticles by surface exchange

The As-prepared hydrophobic $NaYF_4$:Yb,Er(Tm) nanoparticles were transformed into hydrophilic nanoparticles by replacing the oleylamine on the nanoparticles' surface with a bipolar surfactant, 11-aminoundecanoic acid (AUA). The modification of the nanoparticles was achieved by dispersing them with AUA in boiling 0.1 mol/L NaOH. The product, after surfactant exchange, was clear and stable colloidal in deionized water or Tris buffer.

Example 3.3: Synthesis water soluble PAA (poly acrylic acid) coated $NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm nanoparticles

100 μl each of the core and core-shell (CS) nanoparticles stock solutions as described in Example 1.3 were taken into two centrifuge tubes. The core and CS nanoparticles were precipitated by centrifugation. The supernatant was discarded, and the nanoparticles were rinsed twice with ethanol, dispersed in 4 ml of chloroformed. A second solution of 50mg PAA (poly acrylic acid)_polymer in 2ml chloroform was then added. The chloroform was slowly evaporated via vacuum, and the residue was dissolved in 5ml of ethanol or water, resulting in

an optically clear solution. The obtained nanoparticles had the structure as shown in Figure 3(a).

The diameter of the PAA coated nanoparticles was obtained via a dynamics light scattering technique using a dynamic light scattering instrument (Nano-ZS from Malvern Instruments). The diameter of the obtained nanoparticles were 30.2±5.24 nm (Figure 23).

Example 3.4: Synthesis water soluble PEG-phospholipid coated $NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm nanoparticles

1,2-Distearoyl-*sn*-Glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000] (Ammonium Salt), also called 18:0 PEG2 PE, may be used as a surface coating to make the nanoparticles of the present invention water soluble. Its structure is as seen in Figure 4a.

100 µL of $NaYF_4$:Yb,Er and NaYF4:Yb,Tm core-shell nanoparticles as described in Example 1.3, were precipitated with ethanol and dried under vacuum. The nanoparticles were then suspended in 2 mL chloroform with 20 mg of phospholipids. After complete evaporation of the chloroform, the residue was heated at 80°C and 1 mL of water was added to obtain an optically clear suspension containing PEG-PE micelles.

Since this suspension contained both empty micelles and those containing nanoparticles, the empty micelles were removed with centrifugation. The micelles containing nanoparticles formed a pellet while the empty micelles stayed suspended. The supernatant was discarded and the nanoparticles-micelles were re-suspended in water. This can be illustrated as seen in Figure 3.

Example 3.5: Synthesis water soluble PEG-phospholipid coated $NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm nanoparticles with functional groups such as –COOH and -Biotin

The procedure is similar to that described in Example 3.4, except that a mixture of 18:0 PEG2 PE and PEG2PE carboxylic acid (or biotin, or amino) was used, instead of single 18:0 PEG2 PE. Their structures are as shown in Figures 4a and 4b respectively.

Example 4: Conjugation of biomolecules with fluorescent nanoparticles

For the methods under Example 4, the core-shell (CS) nanoparticles obtained by the method in Example 1.3 were used.

Example 4.1: Conjugation of $NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm nanoparticles with streptavidin

Conjugation of PAA coated nanoparticles (with carboxylic acid group) with streptavidin was obtained by EDC (1-ethyl-3-(3-dimethylamino propyl)carbodiimide)-mediated coupling. The CS nanoparticles were dissolved in 10 mM sodium phosphate, pH 7.4. 10 mg streptavidin was added to the nanoparticles suspension followed by 10 mg EDC per milliliter of the nanoparticle/streptavidin mixture. The carbodiimide was made soluble using a vortex mixer and allowed to react for 2 h at room temperature. The conjugate was then purified by gel filtration using a column of Sephadex G-75.

Example 4.2: Conjugation of $NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm nanoparticles with antibody

Conjugation of nanoparticle-micelles (with carboxylic groups on them) with antibody was performed using a method similar to that for obtaining PAA-coated nanoparticles. A carboxyl-PEG-DSPE was incorporated in the CS nanoparticles during preparation. It was then activated with water-soluble carbodiimide (EDC) in the presence of N-hydroxysulfosuccinimide in mildly acidic pH, and the desired antibody added in mildly alkaline HEPES buffer.

Example 4.3: Conjugation of $NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm nanoparticles with DNA

Conjugation of CS nanoparticle-micelles with DNA was obtained by replacing 20% of the mPEG-2000 PE with an amino PEG-PE. DNA contains a disulfide group at the 5' end. This disulfide bonds were cleaved with dithiothreitol (DTT) and the oligonucleotide was purified of excess DTT. The DNA was then coupled to the QD-micelle using Sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate.

Example 5: Effect of temperature

The reaction temperature plays an important role in the synthesis of hexagonal phase $NaYF_4$ nanoparticles. Figure 24 shows the XRD patterns of the $NaYF_4$:20%Yb,2%Er nanoparticles synthesized in a 1 h reaction at 300°C, 320°C and 330°C, respectively. At 300°C, 80% of the nanoparticles were in the cubic phase (peaks marked with asterisk) with the remaining in hexagonal phase. At 320°C, the intensity of hexagonal phase significantly increased. At 330°C, nearly all the nanoparticles were in the hexagonal phase. Corresponding sharp up-conversion fluorescence enhancement was observed with increasing temperature in synthesis of nanoparticles. The line pattern in the lower part of Figure 24 shows the calculated hexagonal phase of $NaYF_4$.

Example 6: Effect of solvent

Oleylamine, simultaneously as ligand and solvent, was critical in controlling the formation and size of hexagonal phase $NaYF_4$ nanoparticles. Hexagonal $NaYF_4$ nanoparticles, as the major phase, were synthesized with 10 ml of oleylamine. However, when a mixture of 8 ml oleylamine and 2 ml oleic acid was used as ligand/solvent at 330°C for 1 h, mono-dispersed $NaYF_4$:20%Yb,2%Er nanoparticles with a smaller size of 8 nm were obtained. However, most of particles were in the cubic phase, as shown in Figure 25 (a) and (b). In the absence of oleylamine, when 2 ml of oleic acid was used as ligand and 8 ml non-chelating 1-octadecene as solvent, only cubic $NaYF_4$:Yb,Er nanoparticles with much larger size (~ 28 nm) were synthesized (Figure 7 (a) and (b)). The fluorescence efficiency of these larger cubic $NaYF_4$:Yb,Er nanoparticles, synthesized ≥ 300 °C was still 7.5 times smaller than that of hexagonal nanoparticles. Differential thermal analysis of the precursors in oleylamine confirmed the onset of a phase transition at 310°C, consistent with the XRD results of formation of hexagonal phase. No such phase transition was observed for the precursors in oleic acid.

Discussion

In summary, infrared-to-visible up-conversion fluorescent nanoparticles, in particular, hexagonal phase $NaYF_4$:20%Yb,2%Er and $NaYF_4$:20%Yb,2%Tm were synthesized by decomposition of multi-precursors of CF_3COONa, $(CF_3COO)_3Y$, $(CF_3COO)_3Yb$ and $(CF_3COO)_3Er$ / $(CF_3COO)_3Tm$ in oleylamine at 330°C. The average particle size was 10.5 nm with a narrow size distribution of ±0.7 nm. Green or blue emission was observed by doping with Er or Tm, respectively, even with only a 5 mW, 980 nm laser pointer. These small nanoparticles were easily dispersed in organic solvents producing a transparent colloidal solution. The up-conversion fluorescence intensity of the hexagonal nanoparticles was high compared to other cubic nanoparticles. In particular, the fluorescence intensity far exceeded that of As-synthesized cubic $NaYF_4$:Yb,Er (37 nm) nanoparticles that required subsequent high temperature annealing to achieve the hexagonal phase transformation (GS Yi et al, 2004). These features of the nanoparticles may be exploited for application as bio-probes.

Compared to corresponding bulk phosphors (prepared using hydrothermal method), the up-conversion fluorescence output as determined by the integrated intensity of the emission peak of the nanoparticles was smaller by one order of magnitude. The decrease of up-conversion fluorescence have been reported and studied previously for Y_2O_2S:Yb,Er up-conversion phosphors (XY Chen et al, 2003). When the size of the Y_2O_2S:Yb,Er phosphors decreased to 30 nm, the up-conversion efficiency was reduced to be about 22% of their bulk counterparts. When the size decreased from 30 nm to 2 nm, the efficiency rapidly reduced. The decrease in fluorescence efficiency was attributed to the lack of low frequency phonon modes and restricted excitation migration in nano-phosphors. The reduction of up-conversion fluorescence was also attributed to the organic ligand on the surface that quenched the ions for fluorescence (S Heer et al, 2004; S Heer et al, 2003).

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Claims

1. A method of preparing at least one nano-structured material of formula $M_1M_2X_n$ comprising the step of treating:

   - at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_1$; and

   - at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$;

   wherein

   each X is the same or different and is selected from the group consisting of: halogens, O, S, Se, Te, N, P and As;

   each n is the same or different and is $0 \leq n \leq 10$;

   each m is the same or different and is $0 \leq m \leq 10$;

   each p is the same or different and is $1 \leq p \leq 5$;

   each M_1 is the same or different and is selected from the group consisting of: Li, Na, K, Rb, Cs, Fr, Be, Mg, Ca, Sr, Ba, Ra and NH_4;

   each M_2 is the same or different and is a metal ion.

2. The method according to claim 1, wherein M_2 is selected from the group consisting of: Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu.

3. The method according to claim 1 or claim 2, wherein the method is for preparing at least one nano-structured material of formula $M_1M_2X_n$:M_q comprising the step of treating:

   - at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_1$;

- at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$; and

- at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_q$, wherein

each M_q is the same or different and is selected from the group consisting of Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu;

n, m, p, X, M_1 and M_2 are as defined in claim 1 or claim 2; and

each q is the same or different and is $0 \leq q \leq 10$.

4. The method according to any one of the preceding claims, wherein M_2 is selected from the group consisting of: transition metal ions, inner transition metal ions, and Group I to Group VI metal ions.

5. The method according to any one of the preceding claims, wherein the treating is carried out in the presence of at least one source of energy.

6. The method according to claim 5, wherein the source of energy is selected from at least one of the following: light source, electric source, thermal source and magnetic source.

7. The method according to at least one of the preceding claims, wherein the treating is carried out at a temperature up to 1000°C.

8. The method according to any one of the preceding claims, wherein the treating is carried out at a temperature of 200°C - 400°C.

9. The method according to claim 8, wherein the treating is carried out at a temperature of 300°C - 350°C.

10. The method according to claim 8 or claim 9, wherein the treating is carried out at a temperature of 330°C.

11. The method according to claim any one of the preceding claims, wherein the treating is carried out in the presence of at least one polar or non-polar solvent, or a mixture thereof.

12. The method according to claim 11, wherein the polar solvent is selected from the group consisting of: water, methanol, ethanol, propyl alcohol, butanol, pentanol, hexanol, ketone, ethylene glycol, glycerol, propylene glycol; polyethylene glycol, ethyl acetate and a combination thereof.

13. The method according to claim 11, wherein the non-polar solvent is selected from the group consisting of: oleylamine, octadecene, oleic acid, alkyl amine, dialkyl amine, trialkyl amine, alkenyl amine, dialkenyl amine, trialkenyl amine, alkyl acid, alkenyl acid, trialkyl phosphine, trialkyl phosphine oxide, trialkylphosphate, alkane, alkene, alkyl ether, alkenyl ether.

14. The method according to any one of the preceding claims, wherein the nano-structured material is selected from the group consisting of: NaM_2F_4, LiM_2F_4, KM_2F_4, RbM_2F_4, CsM_2F_4, BeM_2F_5, $Be(M_2)_2F8$, MgM_2F_5, $Mg(M_2)_2F_8$, CaM_2F_5, $Ca(M_2)_2F_8$, SrM_2F_5, $Sr(M_2)_2F_8$, $BaLnF_5$, $Ba(M_2)_2F_8M_2F_3$, wherein each M_2 is the same or different and is selected from the group consisting of: Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu.

15. The method according to claim 14, wherein the nano-structured material is $NaYF_4$.

16. The method according to any one of claims 3 to 15, wherein the nano-structured material is selected from the group consisting of: $NaM_2F_4{:}M_q$, $LiM_2F_4{:}M_q$, $KM_2F_4{:}M_q$, $RbM_2F_4{:}M_q$, $CsM_2F_4{:}M_q$, $BeM_2F_5{:}M_q$, $Be(M_2)_2F_8{:}M_q$, $MgM_2F_5{:}M_q$, $Mg(M_2)_2F_8{:}M_q$, $CaM_2F_5{:}M_q$, $Ca(M_2)_2F_8{:}M_q$, $SrM_2F_5{:}M_q$, $Sr(M_2)_2F_8{:}M_q$, $BaM_2F_5{:}M_q$, $Ba(M_2)_2F_8{:}M_q$, wherein each M_2 is the same or different and is selected from the group consisting of: Sc, Y, La, Ce, Pr, Nd,

Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu, and each M_q is the same or different and is selected from the group consisting of: Yb, Er, Tm and Ho.

17. The method according to claim 16, wherein the nano-structured material is $NaYF_4$:Yb,Er, $NaYF_4$:Yb,Tm or $NaYF_4$:Yb,Ho.

18. The method according to any one of the preceding claims, wherein the at least one compound of formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_1$ is selected from the group consisting of: CF_3COONa and CF_3COOLi.

19. The method according to any one of the preceding claims, wherein the at least one compound of formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$ is selected from the group consisting of: $(CF_3COO)_3Y$ and $(CF_3COO)_3La$.

20. The method according to any one of claims 3 to 19, wherein the at least one compound of formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_q$ is selected from the group consisting of: $(CF_3COO)_3Yb$, $(CF_3COO)_3Er$, $(CF_3COO)_3Tm$ and $(CF_3COO)_3Ho$.

21. The method according to any one of the preceding claims, wherein the nano-structured material has a structure selected from the group consisting of: hexagonal, cubic, tetragonal, rhombohedral, orthorhombic, monoclinic, triclinic and a combination thereof.

22. The method according to claim 21, wherein the nano-structured material has a hexagonal structure.

23. The method according to any one of the preceding claims, wherein the nano-structured material comprises at least one dimension of size < 50 nm.

24. The method according to any one of the preceding claims, wherein the nano-structured material comprises at least one dimension of size ≤ 25 nm.

25. The method according to any one of the preceding claims, wherein the nano-structured material comprises at least one dimension of size ≤ 10 nm.

26. The method according to any one of the preceding claims, wherein the nano-structured material comprises at least one dimension of size ≤ 5 nm.

27. The method according to any one of the preceding claims, wherein the nano-structured material is in the form of: nanoparticle(s), nanofilm or monolith.

28. The method according to claim 27, wherein the nanoparticle(s) comprise core nanoparticle(s) and/or core-shell nanoparticle(s).

29. The method according to claim 27 or claim 28, wherein the nanoparticle is in the form of a core nanoparticle, and the method further comprises applying at least one organic and/or inorganic material (shell) on the core, to obtain a core-shell nanoparticle(s).

30. The method according to claim 27 or claim 28, wherein the nanoparticle is in the form of a core nanoparticle, and the nanoparticle further comprises at least one organic and/or inorganic material (shell) applied on the core, to obtain a core-shell nanoparticle(s).

31. The method according to claim 29 or claim 30, wherein the shell material has the formula $M_1M_2X_n$ or $M_1M_2X_n$:M_q, wherein M_1, M_2, X, n and M_q are as defined in any one of claims 1 to 3.

32. The method according to claim 29 or claim 30, wherein the organic shell material comprises at least one polymer, a surfactant or a lipid, or a combination thereof.

33. The method according to claim 32, wherein the at least one polymer is selected from the group consisting of: polystyrene (PS), polyethylene (PE), polymethyl methacrylate (PMMA) and polylactic acid (PLA).

34. The method according to any one of claims 29 to 31, wherein the inorganic shell material comprises: NaM_2F_4, LiM_2F_4, KM_2F_4, RbM_2F_4, CsM_2F_4, BeM_2F_5, $Be(M_2)_2F8$, MgM_2F_5, $Mg(M_2)_2F_8$, CaM_2F_5, $Ca(M_2)_2F_8$, SrM_2F_5, $Sr(M_2)_2F_8$, $BaLnF_5$, $Ba(M_2)_2F_8M_2F_3$, M_2F_3, M_2Cl_3, M_2Br_3, M_2I_3, M_2FClBr, M_2OF, M_2OCl, M_2OBr, M_2OS, $(M_2)_2S_3$, wherein each M_2 is the same or different and is selected from the group consisting of: Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu; SiO_2; TiO_2; ZnS; or a combination thereof.

35. The method according to any one of claims 28 to 34, wherein the shell is applied continuously or discontinuously on the core.

36. The method according to any one of the preceding claims, wherein the at least one nano-structured material is at least one nanoparticle and the average diameter of the nanoparticle(s) is < 50 nm.

37. The method according to any one of the preceding claims, wherein the at least one nano-structured material is at least one nanoparticle and the average diameter of the nanoparticle(s) is ≤ 10 nm.

38. The method according to any one of the preceding claims, wherein the nano-structured material is hexagonal phase $NaYF_4$.

39. The method according to any one of the preceding claims, wherein the nano-structured material is hexagonal phase $NaYF_4$:Yb,Er, hexagonal phase $NaYF_4$:Yb,Tm or hexagonal phase $NaYF_4$:Yb,Ho.

40. The method according to any one of the preceding claims, wherein the surface of the nano-structured material is modified by adding at least one surfactant, lipid, polymer, inorganic material, or a mixture thereof.

41. The method according to any one of the preceding claims, wherein the surface of the nano-structured material is modified by adding at least one surfactant.

42. The method according to claim 41, wherein the at least one surfactant has Formula (I):

$$\begin{array}{ccccc} & R^1 & & R^3 & \\ & | & & | & \\ R^5 - & (C_JH_K)_s & - & (C_ZH_W)_y & - R^6 \\ & | & & | & \\ & R^2 & & R^4 & \end{array} \qquad \text{(Formula I)}$$

wherein

each J is the same or different, and $1 \leq J \leq 9$;

each K is the same or different, and $0 \leq K \leq 9$;

each s is the same or different, and $0 \leq s \leq 9$;

each Z is the same or different, and $1 \leq Z \leq 9$;

each W is the same or different, and $0 \leq W \leq 9$;

each y is the same or different, and $0 \leq y \leq 9$;

each R^1, R^2, R^3, R^4 and R^5 is the same or different, and is independently selected from the group consisting of: H, substituted or unsubstituted C_1-C_6 alkyl, substituted or unsubstituted C_1-C_6 aryl, HS, COOH, NH_2 and OH;

each R_6 is the same or different, and is selected from the group consisting of: COOH, NH_2, OH, P=0 and P;

with the proviso that s + y < 10.

43. The method according to claim 41 or claim 42, wherein the surfactant is hydrophilic, hydrophobic and/or amphiphilic.

44. The method according to any one of the preceding claims, wherein a biomolecule is attached to the nano-structured material.

45. The method according to claim 44, wherein the biomolecule is selected from the group consisting of: protein, nucleic acid, nucleosides, nucleotides, DNA, hormone, amino acid, peptide, peptidomimetic, RNA, lipid, albumin, antibody, phospholipids, glycolipid, sterol, vitamins, neurotransmitter, carbohydrate, sugar, disaccharide, monosaccharide, oligopeptide, polypeptide, oligosaccharide, polysaccharide and a mixture thereof.

46. A nano-structured material obtainable by the method according to any one of claims 1 to 45.

47. A nano-structured material of formula $M_1M_2X_n$:M_q as defined in any one of claims 1 to 3, wherein the nano-structured material has a hexagonal lattice structure and comprises at least one dimension of size < 50 nm, with the proviso that the nano-structured material is not LaF_3:Yb,Er, LaF_3:Yb,Ho or LaF_3:Yb,Tm.

48. The nano-structured material according to claim 47, wherein the at least one dimension is of size $\leq$ 10 nm.

49. The nano-structured material according to claim 48, wherein the at least one dimension is of size $\leq$ 5 nm.

50. The nano-structured material according to any one of claims 47 to 49, wherein the nano-structured material is hexagonal phase $NaYF_4:M_q$.

51. The nano-structured material according to claim 50, wherein $NaYF_4:M_q$ is $NaYF_4$:Yb,Er, $NaYF_4$:Yb,Ho or $NaYF_4$:Yb,Tm.

52. An article of manufacture comprising the nano-structured material according to any one of claims 46 to 51.

53. The article of manufacture according to claim 52, wherein the article of manufacture is at least one of the following: a display device, a solar cell, an optical data storage, a bio-probe, a carrier for drug delivery, a lamp, a LED, a LCD, a wear resistance, a laser, optical amplifier, and/or a device for bio-imaging.

54. A kit comprising at least one nano-structured material according to any one of claims 46 to 51 and at least one biomolecule.

55. The kit according to claim 54, wherein the biomolecule is selected from the group consisting of: protein, nucleic acid, nucleosides, nucleotides, DNA, hormone, amino acid, peptide, peptidomimetic, RNA, lipid, albumin, antibody, phospholipids, glycolipid, sterol, vitamins, neurotransmitter, carbohydrate, sugar, disaccharide, monosaccharide, oligopeptide, polypeptide, oligosaccharide, polysaccharide and a mixture thereof.

56. A bio-imaging and/or bio-detection system comprising:

- at least one nano-structured material prepared from the method according to any one of claims 1 to 45;

- at least one biomolecule;

- at least one source of excitation; and

- at least one means for delivery of the source of excitation to the system.

57. The system according to claim 56, wherein the biomolecule is selected from the group consisting of: protein, nucleic acid, nucleosides, nucleotides, DNA, hormone, amino acid, peptide, peptidomimetic, RNA, lipid, albumin, antibody, phospholipids, glycolipid, sterol, vitamins, neurotransmitter, carbohydrate, sugar, disaccharide, monosaccharide, oligopeptide, polypeptide, oligosaccharide, polysaccharide and a mixture thereof.

58. The system according to claim 56 or claim 57, wherein the source of excitation is NIR.

59. The system according to claim 58, wherein the NIR is at 980 nm.

60. The system according to any one of claims 56 to 59, wherein the means for delivery of the source of excitation to the system is selected from the group consisting of: optical fibres, endoscopes, external light and external laser.

61. A method of modifying at least one hydrophobic structure to make it hydrophilic, comprising applying at least one modifier on the structure surface to make a structure-modifier complex, and wherein the modifier is a surfactant, lipid, polymer and/or inorganic material.

62. The method according to claim 61, comprising applying at least one first modifier on the structure surface, and further a second hydrophilic modifier to make a structure-first modifier-second modifier complex, and wherein the first and/or second modifier is a surfactant, lipid, polymer and/or inorganic material.

63. The method according to claim 61 or claim 62, comprising applying at least one hydrophilic surfactant on the structure to make a structure-surfactant complex.

64. The method according to any one of claims 61 to 63, wherein the structure is the nano-structured material according to any one of claims 46 to 51.

65. A method of preparing at least one nano-structured material of formula M_2X_n comprising the step of treating at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$ in the presence of at least one source of energy,

wherein

each X is the same or different and is selected from the group consisting of: halogens, O, S, Se, Te, N, P and As;

each n is the same or different and is $0 \leq n \leq 10$;

each m is the same or different and is $0 \leq m \leq 10$;

each p is the same or different and is $1 \leq p \leq 5$; and

each M_2 is the same or different and is a metal ion.

66. The method according to claim 65, wherein each M_2 is the same or different and is selected from the group consisting of: Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu.

67. The method according to claim 65 or claim 66, wherein the method is for preparing at least one nano-structured material of formula $M_2X_n{:}M_q$ comprising the step of treating:

- at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$; and
- at least one compound having the formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_q$;

in the presence of at least one source of energy and wherein each M_q is the same or different and is selected from the group consisting of Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu;

n, m, p, X and M_2 are as defined in claim 65 or claim 66;

each q is the same or different and is $0 \leq q \leq 10$.

68. The method according to any one of claims 65 to 67, wherein each M_2 is the same or different and is selected from the group consisting of: transition metal ions, inner transition metal ions, and Group I to Group VI metal ions.

69. The method according to any one of claims 65 to 68, wherein the at least one source of energy is selected from at least one of the following: light source, electric source, thermal source and magnetic source.

70. The method according to any one of claims 65 to 69, wherein the treating is carried out at a temperature up to 1000°C.

71. The method according to claim 70, wherein the treating is carried out at a temperature of 200°C - 400°C.

72. The method according to claim 71, wherein the treating is carried out at a temperature of 300°C - 350°C.

73. The method according to claim 72, wherein the treating is carried out at a temperature of 330°C.

74. The method according to claim any one of claims 65 to 73, wherein the treating is carried out in the presence of at least one polar or non-polar solvent, or a mixture thereof.

75. The method according to claim 74, wherein the polar solvent is selected from the group consisting of: water, methanol, ethanol, propyl alcohol,

butanol, pentanol, hexanol, ketone, ethylene glycol, glycerol, propylene glycol, polyethylene glycol, ethyl acetate and a combination thereof.

76. The method according to claim 74, wherein the non-polar solvent is selected from the group consisting of: oleylamine, octadecene, oleic acid, alkyl amine, dialkyl amine, trialkyl amine, alkenyl amine, dialkenyl amine, trialkenyl amine, alkyl acid, alkenyl acid, trialkyl phosphine, trialkyl phosphine oxide, trialkylphosphate, alkane, alkene, alkyl ether, alkenyl ether and a combination thereof.

77. The method according to any one of claims 65 to 76, wherein the nano-structured material is selected from the group consisting of: M_2F_3, M_2Cl_3, M_2Br_3, M_2I_3, M_2FClBr, M_2OF, M_2OCl, M_2OBr, M_2OS, $(M_2)_2S_3$, wherein each M_2 is the same or different and is selected from the group consisting of: Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu.

78. The method according to claim 77, wherein the nano-structured material is LaF_3, CeF_3, GdF_3, YF_3 or YOF.

79. The method according to any one of claims 67 to 78, wherein the nano-structured material is selected from the group consisting of: $M_2F_3:M_q$, $M_2Cl_3:M_q$, $M_2Br_3:M_q$, $M_2I_3:M_q$, $M_2FClBr:M_q$, $M_2OF:M_q$, $M_2OCl:M_q$, $M_2OBr:M_q$, $M_2OS:M_q$, $(M_2)_2S_3:M_q$, wherein each M_2 is the same or different and is selected from the group consisting of: Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu, and M_q is selected from the group consisting of: Yb, Er, Tm and Ho.

80. The method according to claim 79, wherein the nano-structured material is YOF:Yb,Er, LaF_3:Yb,Er, CeF_3:Yb,Er, GdF_3:Yb,Er, YF_3:Yb,Er, YOF:Yb,Tm, LaF_3:Yb,Tm, CeF_3:Yb,Tm, GdF_3:Yb,Tm or YF_3:Yb,Tm.

81. The method according to any one of claims 65 to 80, wherein the at least one compound of formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_2$ is selected from the group consisting of: $(CF_3COO)_3Y$ and $(CF_3COO)_3La$.

82. The method according to any one of claims 67 to 81, wherein the at least one compound of formula $[CX_3(CX_2)_n(CH_2)_mCOO]_pM_q$ is selected from the group consisting of: $(CF_3COO)_3Yb$ and $(CF_3COO)_3Er$.

83. The method according to any one of claims 65 to 82, wherein the nano-structured material has a structure selected from the group consisting of: hexagonal, cubic, tetragonal, rhombohedral, orthorhombic, monoclinic, triclinic and a combination thereof.

84. The method according to any one of claims 65 to 83, wherein the nano-structured material comprises at least one dimension of size < 50 nm.

85. The method according to any one of claims 65 to 84, wherein the nano-structured material comprises at least one dimension of size ≤ 25 nm.

86. The method according to any one of claims 65 to 85, wherein the nano-structured material comprises at least one dimension of size ≤ 10 nm.

87. The method according to any one of claims 65 to 86, wherein the nano-structured material is in the form of: nanoparticle(s), nanofilm or monolith.

88. The method according to claim 87, wherein the nanoparticle(s) comprise core nanoparticle(s) and/or core-shell nanoparticle(s).

89. The method according to claim 87 or claim 88, wherein the nanoparticle is in the form of a core nanoparticle, and the method further comprises applying at least one organic and/or inorganic material (shell) on the core, to obtain a core-shell nanoparticle(s).

90. The method according to claim 87 or claim 88, wherein the nanoparticle is in the form of a core nanoparticle, and the nanoparticle further comprises at least one organic and/or inorganic material (shell) applied on the core, to obtain a core-shell nanoparticle(s).

91. The method according to any one of claims 88 to 90, wherein the shell material has the formula $M_1M_2X_n$ or $M_1M_2X_n$:M_q, wherein M_1, M_2, X, n and M_q are as defined in any one of claims 1 to 3 or claims 65 to 67.

92. The method according to claim 89 or claim 90, wherein the organic shell material comprises at least one polymer, a surfactant or a lipid, or a combination thereof.

93. The method according to claim 92, wherein the at least one polymer is selected from the group consisting of: polystyrene (PS), polyethylene (PE), polymethyl methacrylate (PMMA) and polylactic acid (PLA).

94. The method according to any one of claims 89 to 91, wherein the inorganic shell material comprises: NaM_2F_4, LiM_2F_4, KM_2F_4, RbM_2F_4, CsM_2F_4, BeM_2F_5, $Be(M_2)_2F8$, MgM_2F_5, $Mg(M_2)_2F_8$, CaM_2F_5, $Ca(M_2)_2F_8$, SrM_2F_5, $Sr(M_2)_2F_8$, $BaLnF_5$, $Ba(M_2)_2F_8M_2F_3$, M_2F_3, M_2Cl_3, M_2Br_3, M_2I_3, M_2FClBr, M_2OF, M_2OCl, M_2OBr, M_2OS, $(M_2)_2S_3$, wherein each M_2 is the same or different and is selected from the group consisting of: Sc, Y, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu; SiO_2; TiO_2; ZnS; or a combination thereof.

95. The method according to any one of claims 88 to 94, wherein the shell is applied continuously or discontinuously on the core.

96. The method according to any one of claims 65 to 95, wherein the at least one nano-structured material is at least one nanoparticle and the average diameter of the nanoparticle(s) is < 50 nm.

97. The method according to any one of claims 65 to 96, wherein the at least one nano-structured material is at least one nanoparticle and the average diameter of the nanoparticle(s) is $\leq$ 10 nm.

98. The method according to any one of claims 65 to 97, wherein the surface of the nano-structured material is modified by adding at least one surfactant, lipid, polymer, inorganic material, or a mixture thereof.

99. The method according to any one of claims 65 to 98, wherein the surface of the nano-structured material is modified by adding at least one surfactant.

100. The method according to claim 99, wherein the at least one surfactant has Formula (I):

$$R^5 - (\overset{\displaystyle R^1 \atop |}{\underset{\displaystyle | \atop R^2}{C_JH_K}})_s - (\overset{\displaystyle R^3 \atop |}{\underset{\displaystyle | \atop R^4}{C_ZH_W}})_y - R^6 \qquad \text{(Formula I)}$$

wherein

each J is the same or different, and $1 \leq J \leq 9$;

each K is the same or different, and $0 \leq K \leq 9$;

each s is the same or different, and $0 \leq s \leq 9$;

each Z is the same or different, and $1 \leq Z \leq 9$;

each W is the same or different, and $0 \leq W \leq 9$;

each y is the same or different, and $0 \leq y \leq 9$;

each R^1, R^2, R^3, R^4 and R^5 is the same or different, and is independently selected from the group consisting of: H, substituted or unsubstituted C_1-C_6 alkyl, substituted or unsubstituted C_1-C_6 aryl, HS, COOH, NH_2 and OH;

each R_6 is the same or different, and is selected from the group consisting of: COOH, NH_2, OH, P=0 and P;

with the proviso that $s + y < 10$.

101. The method according to claim 99 or claim 100, wherein the surfactant is hydrophilic, hydrophobic and/or amphiphilic.

102. The method according to any one of claims 65 to 101, wherein a biomolecule is attached to the nano-structured material.

103. The method according to claim 102, wherein the biomolecule is selected from the group consisting of: protein, nucleic acid, nucleosides, nucleotides, DNA, hormone, amino acid, peptide, peptidomimetic, RNA, lipid, albumin, antibody, phospholipids, glycolipid, sterol, vitamins, neurotransmitter, carbohydrate, sugar, disaccharide, monosaccharide, oligopeptide, polypeptide, oligosaccharide, polysaccharide and a mixture thereof.

104. A nano-structured material obtainable by the method according to any one of claims 65 to 103.

105. An article of manufacture comprising the nano-structured material according to claim 104.

106. The article of manufacture according to claim 105, wherein the article of manufacture is at least one of the following: a display device, a solar cell, an optical data storage, a bio-probe, a carrier for drug delivery, a lamp, a LED, a LCD, a wear resistance, a laser, optical amplifier, and/or a device for bio-imaging.

107. A kit comprising at least one nano-structured material according to claim 104 and at least one biomolecule.

108. The kit according to claim 107, wherein the biomolecule is selected from the group consisting of: protein, nucleic acid, nucleosides, nucleotides, DNA, hormone, amino acid, peptide, peptidomimetic, RNA, lipid, albumin, antibody, phospholipids, glycolipid, sterol, vitamins, neurotransmitter, carbohydrate, sugar, disaccharide, monosaccharide, oligopeptide, polypeptide, oligosaccharide, polysaccharide and a mixture thereof.

109. A bio-imaging and/or bio-detection system comprising:

- at least one nano-structured material prepared from the method according to any one of claims 65 to 103;

- at least one biomolecule;

- at least one source of excitation; and

- at least one means for delivery of the source of excitation to the system.

110. The system according to claim 109, wherein the biomolecule is selected from the group consisting of: protein, nucleic acid, nucleosides, nucleotides, DNA, hormone, amino acid, peptide, peptidomimetic, RNA, lipid, albumin, antibody, phospholipids, glycolipid, sterol, vitamins, neurotransmitter, carbohydrate, sugar, disaccharide, monosaccharide, oligopeptide, polypeptide, oligosaccharide, polysaccharide and a mixture thereof.

111. The system according to claim 109 or claim 110, wherein the source of excitation is NIR.

112. The system according to claim 111, wherein the NIR is at 980 nm.

113. The system according to any one of claims 109 to 112, wherein the means for delivery of the source of excitation to the system is selected from the group consisting of: optical fibres, endoscopes, external light and external laser.

FIGURE 1

(a)

(b)

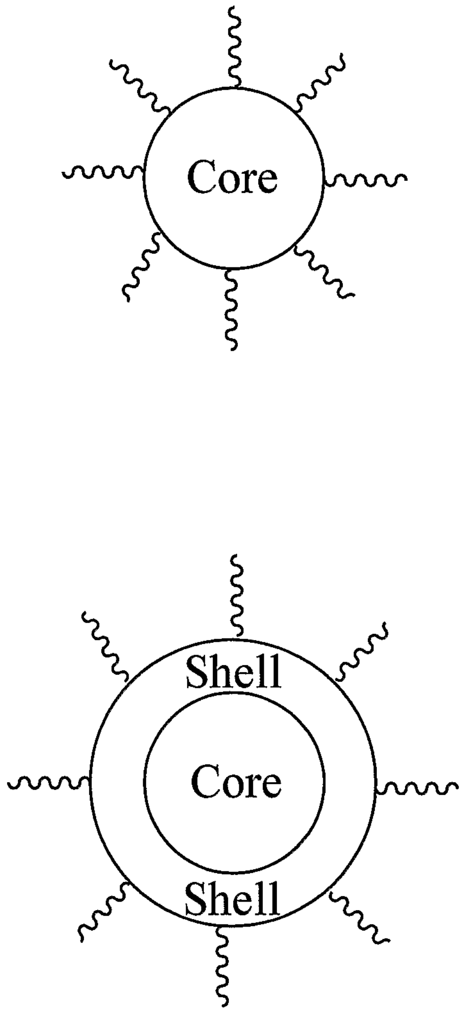
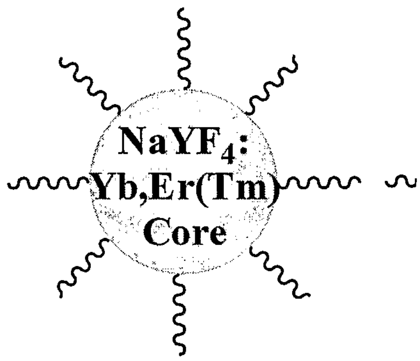


FIGURE 2

(a)



(b)

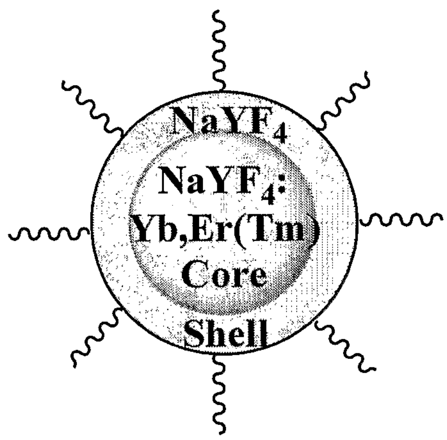
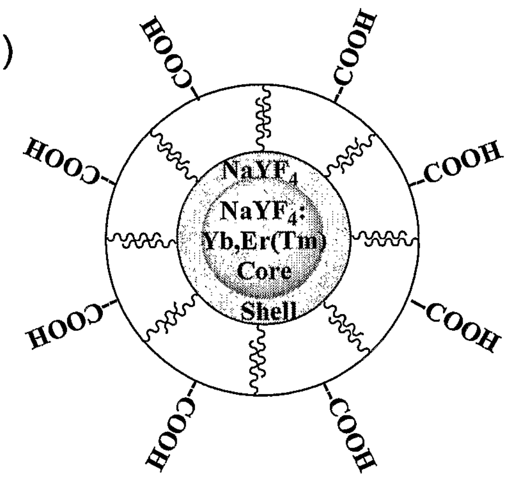


FIGURE 3

(a)


COOH
COOH
COOH
COOH
COOH
COOH
COOH
COOH
NaYF4
NaYF4:
Yb,Er(Tm)
Core
Shell

(b)

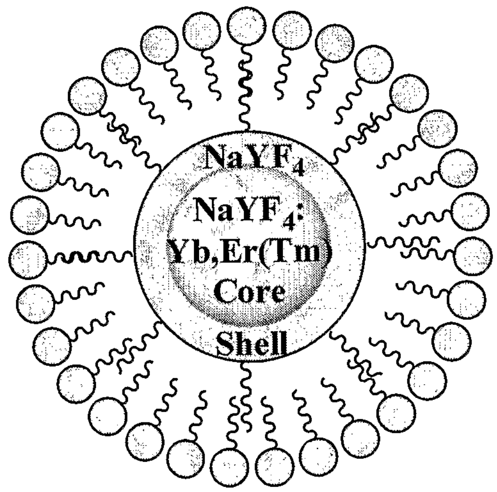
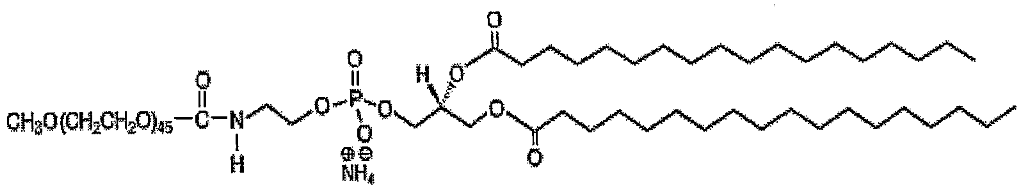
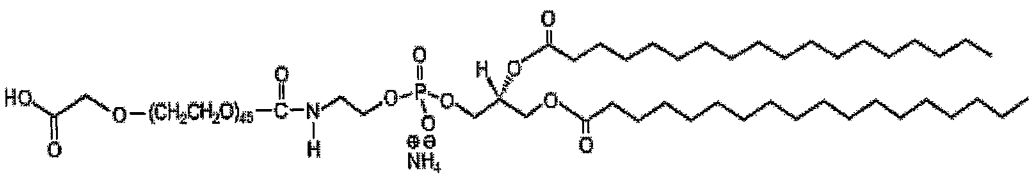

NaYF4
NaYF4:
Yb,Er(Tm)
Core
Shell

FIGURE 4

(a)



(b)



(c)

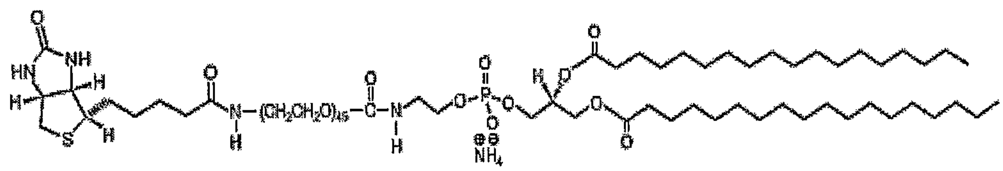
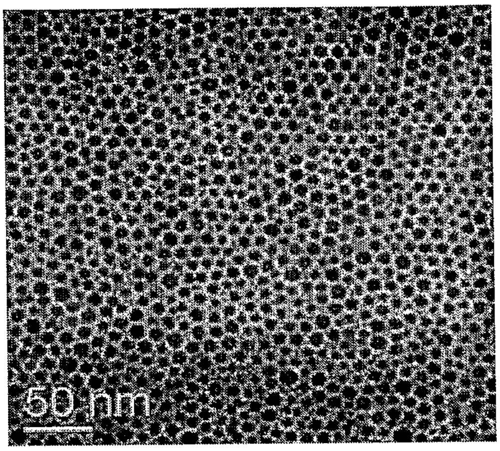


FIGURE 5

(a)



(b)

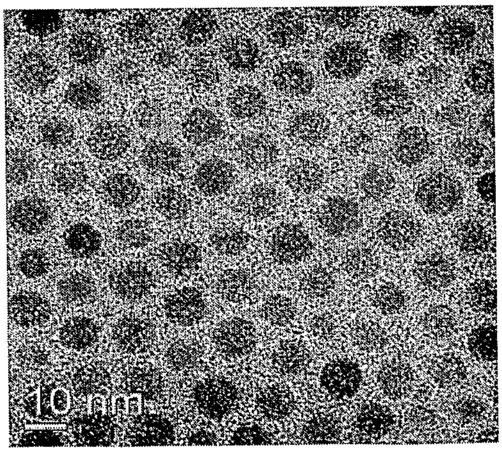
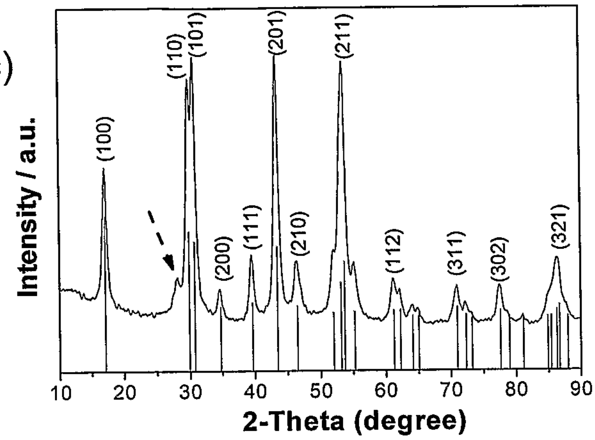


FIGURE 5 (cont.)

(c)



(d)

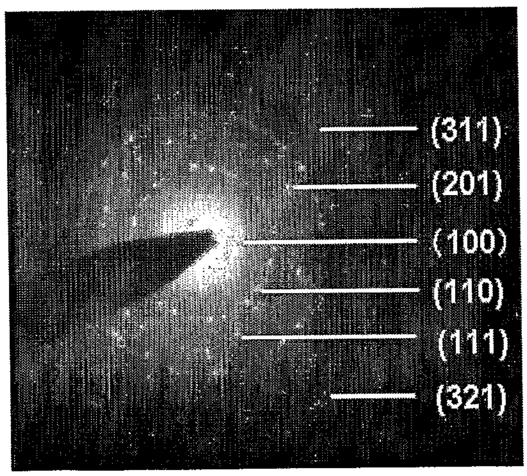
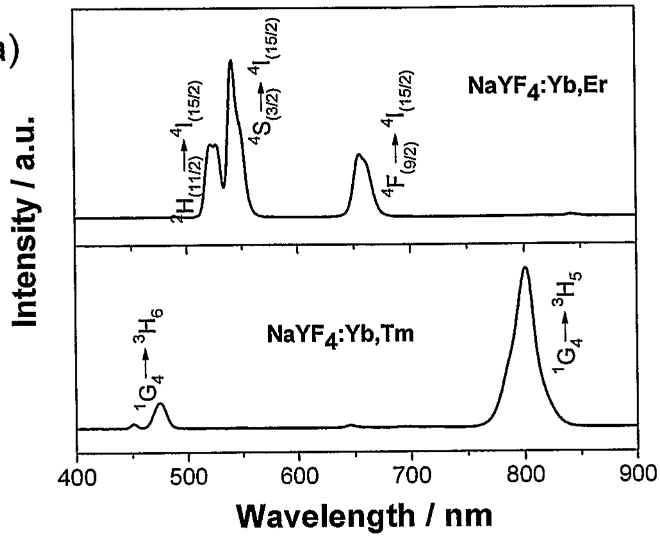
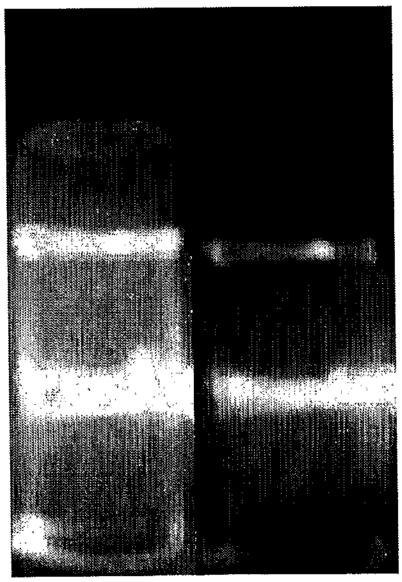


FIGURE 6

(a)



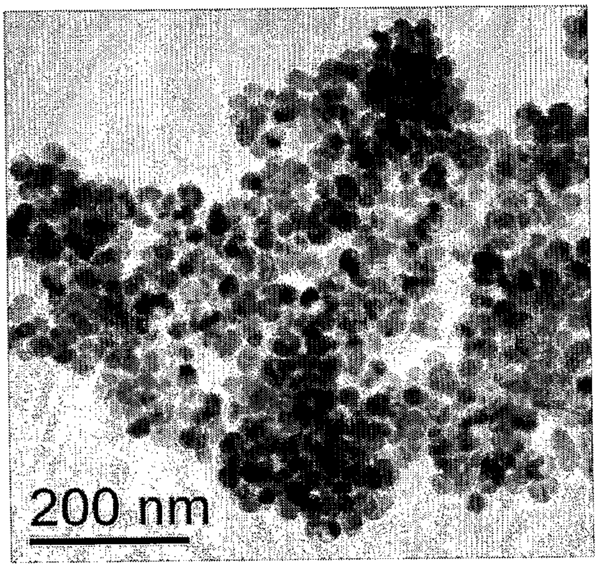
(b)



(i) (ii)

FIGURE 7

(a)


200 nm

(b)

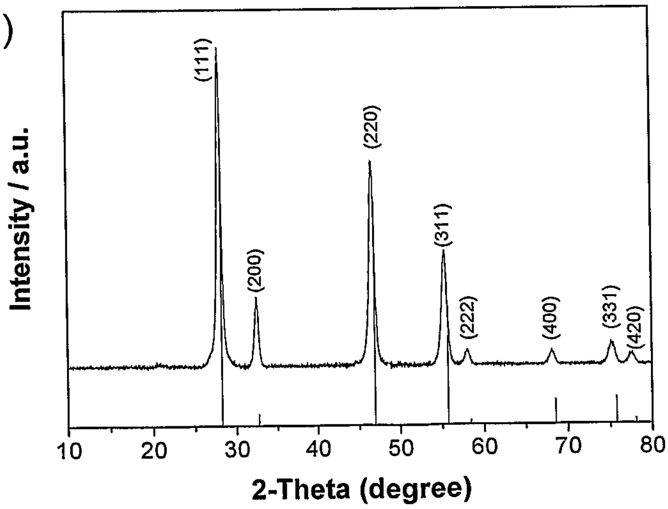

(111)
(200)
(220)
(311)
(222)
(400)
(331)
(420)
Intensity / a.u.
10
20
30
40
50
60
70
80
2-Theta (degree)

FIGURE 8

(a)

(b)

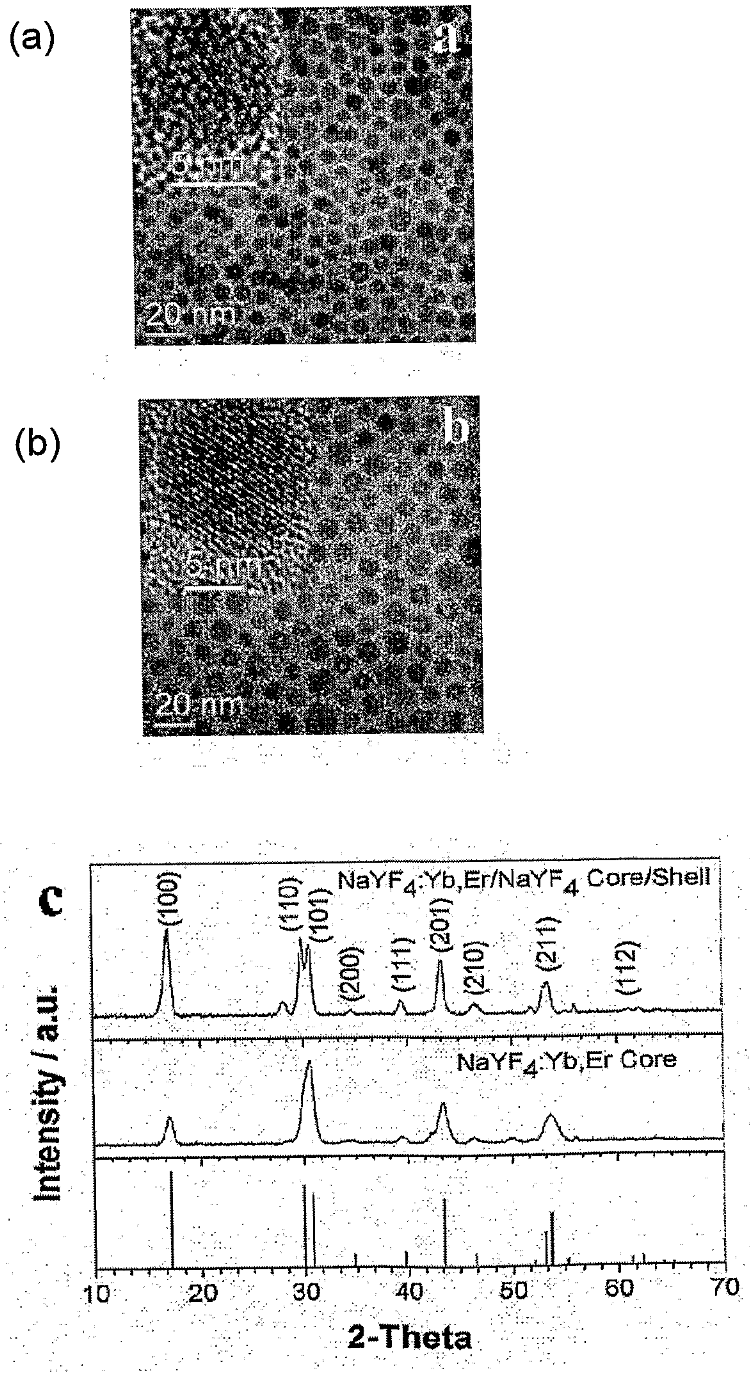
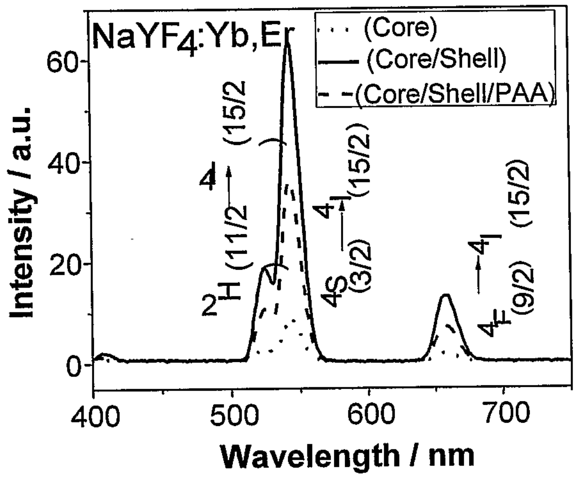

a
5 nm
20 nm
b
5 nm
20 nm
c
Intensity / a.u.
$NaYF_4$:Yb,Er/$NaYF_4$ Core/Shell
(100)
(110)
(101)
(200)
(111)
(201)
(210)
(211)
(112)
$NaYF_4$:Yb,Er Core
10
20
30
40
50
60
70
2-Theta

FIGURE 9

(a)


NaYF4:Yb,Er
(Core)
(Core/Shell)
(Core/Shell/PAA)
Intensity / a.u.
Wavelength / nm

(b)

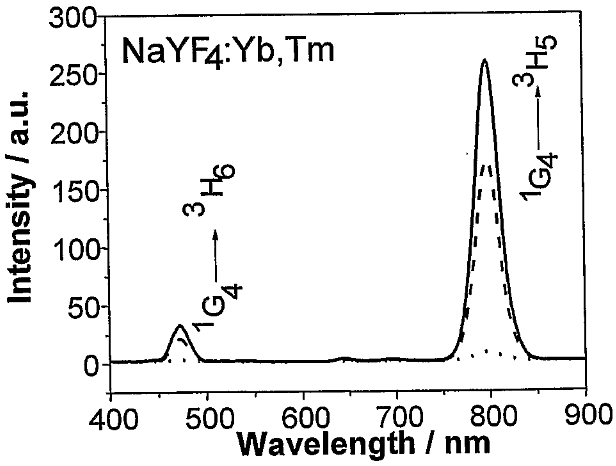
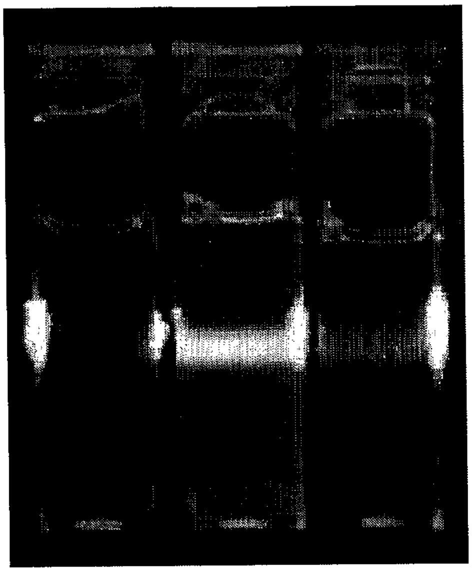

NaYF4:Yb,Tm
Intensity / a.u.
Wavelength / nm

FIGURE 10

(a)



(b)

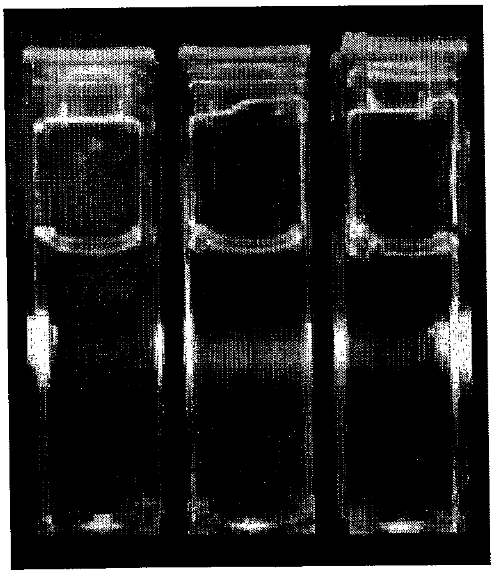
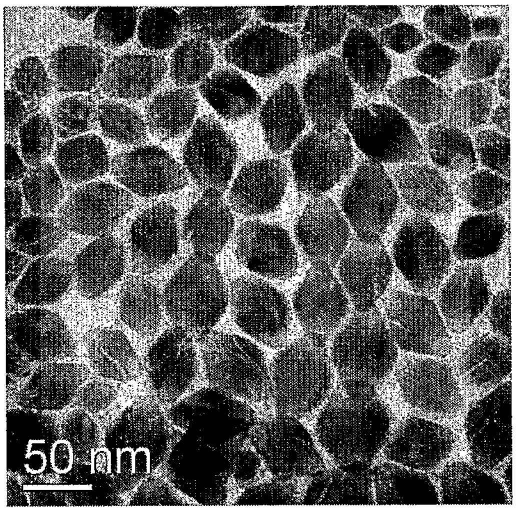


FIGURE 11

(a)


50 nm

(b)

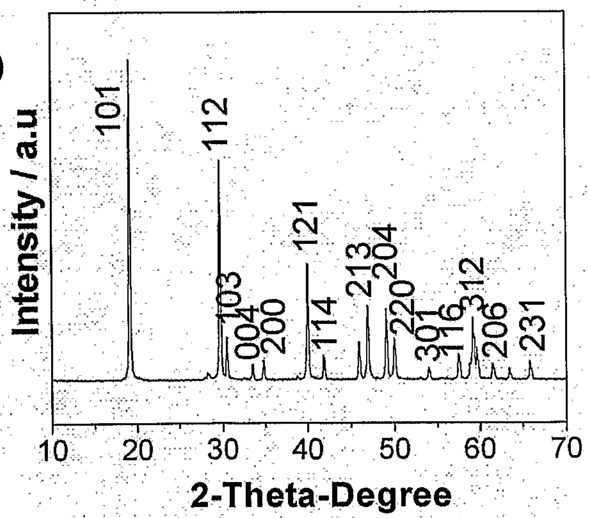

101
112
103
004
200
121
114
213
204
220
301
116
312
206
231
Intensity / a.u
10
20
30
40
50
60
70
2-Theta-Degree

FIGURE 12

(a)

(b)

(c)

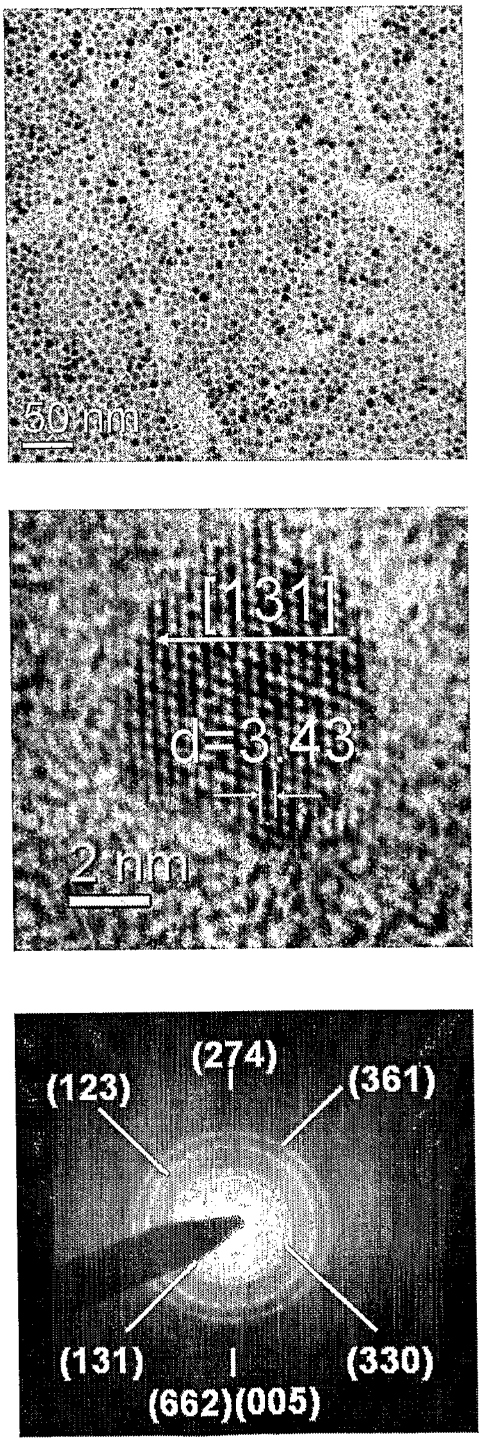


FIGURE 13

(a)

(b)

(c)

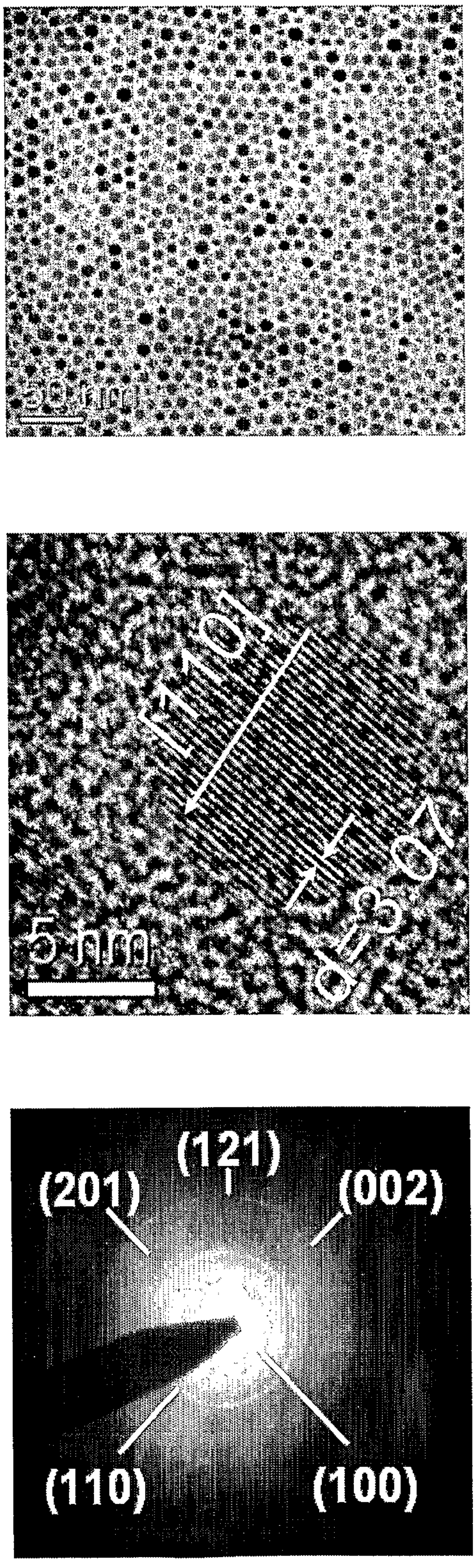


FIGURE 14

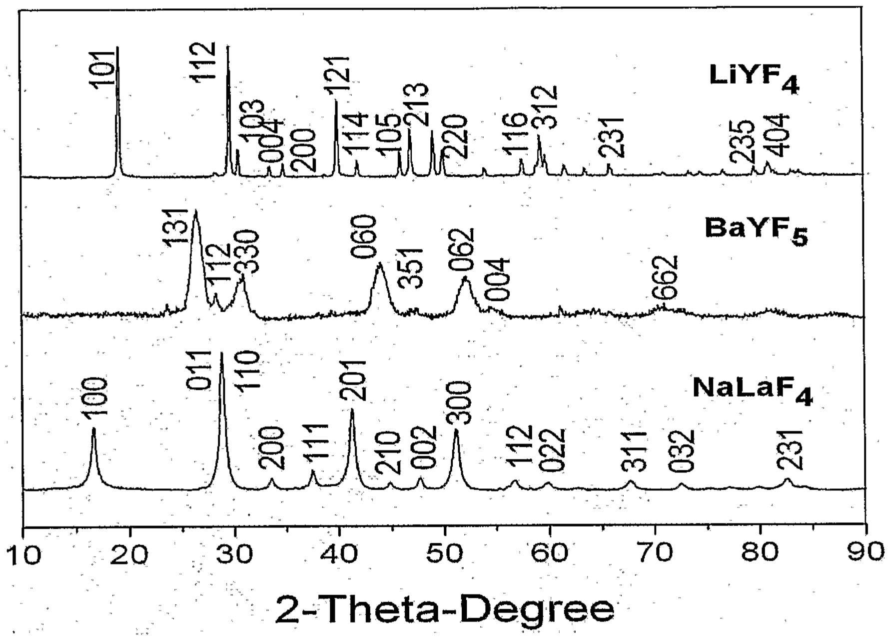


FIGURE 15

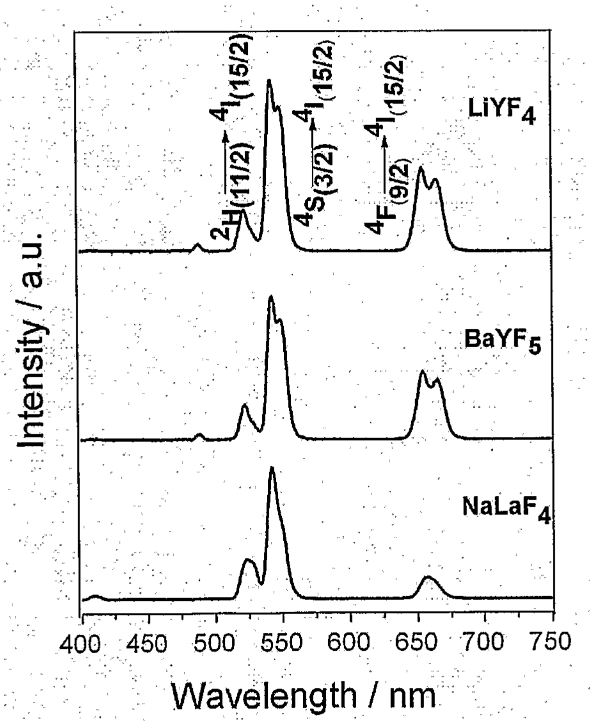


FIGURE 16

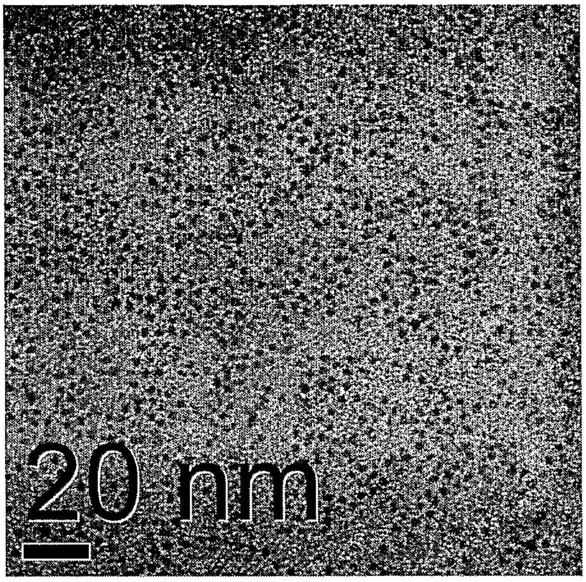


FIGURE 17

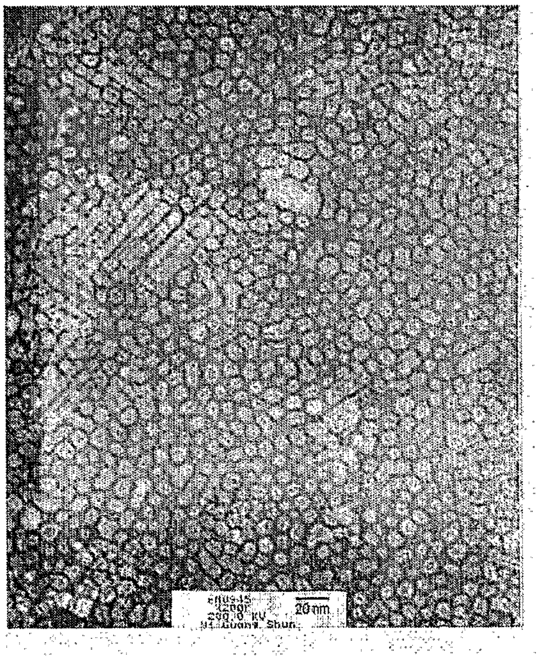
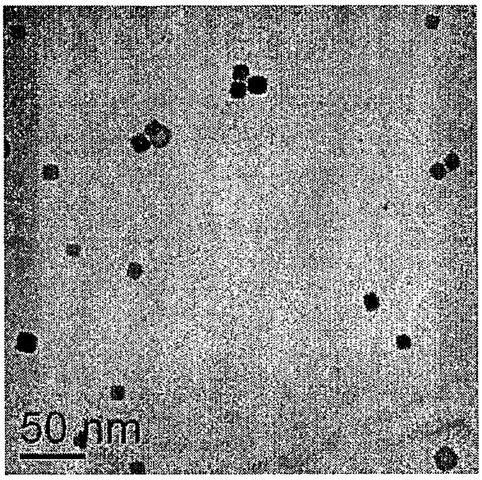


FIGURE 18

(a)



(b)

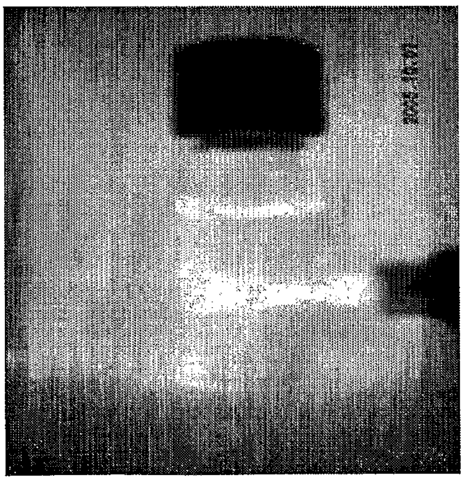


FIGURE 19

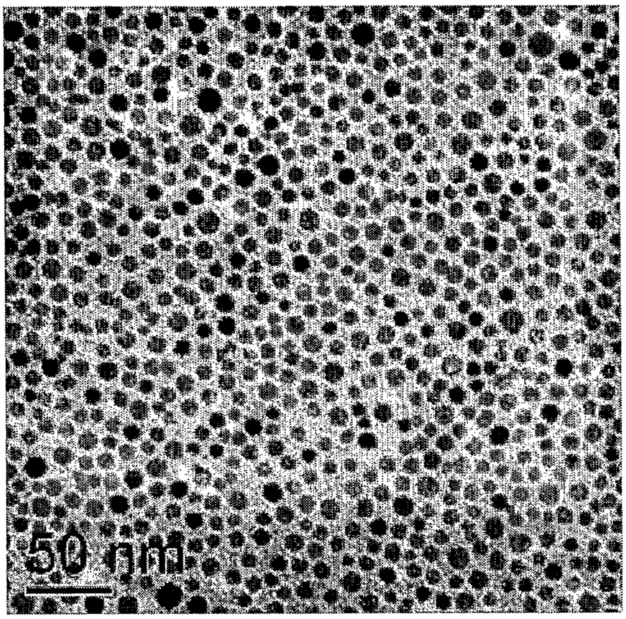


FIGURE 20

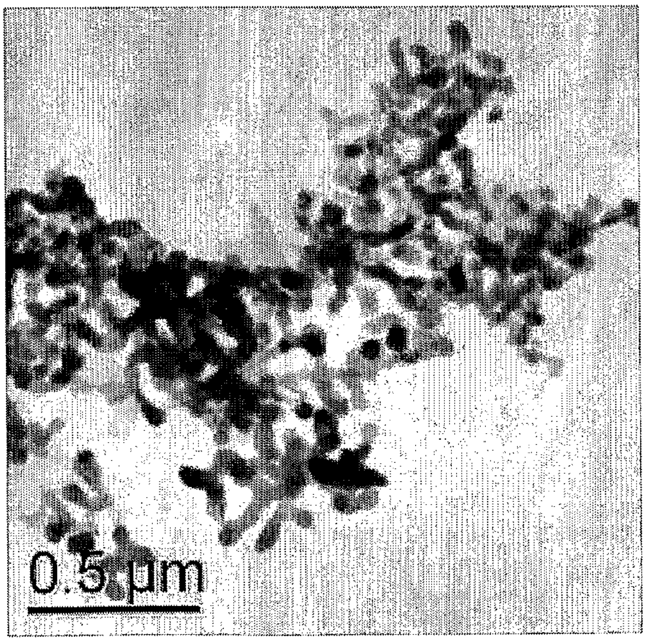


FIGURE 21

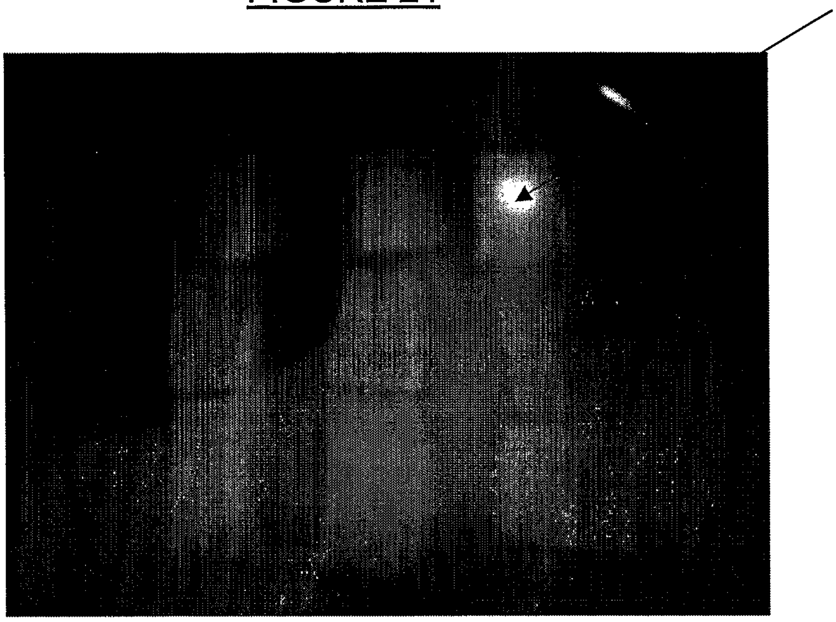
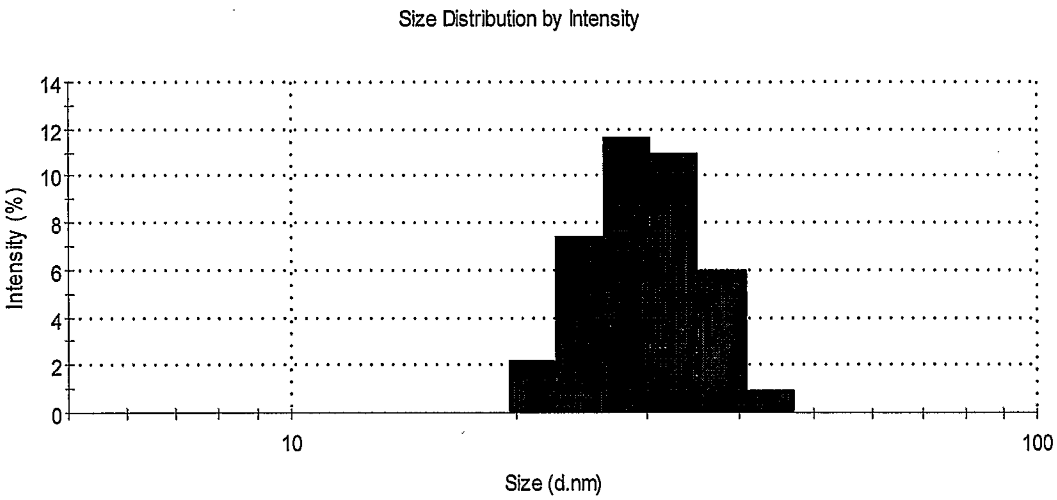


FIGURE 22

FIGURE 23



Size Distribution by Intensity
Intensity (%)
14
12
10
8
6
4
2
0
10
100
Size (d.nm)

FIGURE 24

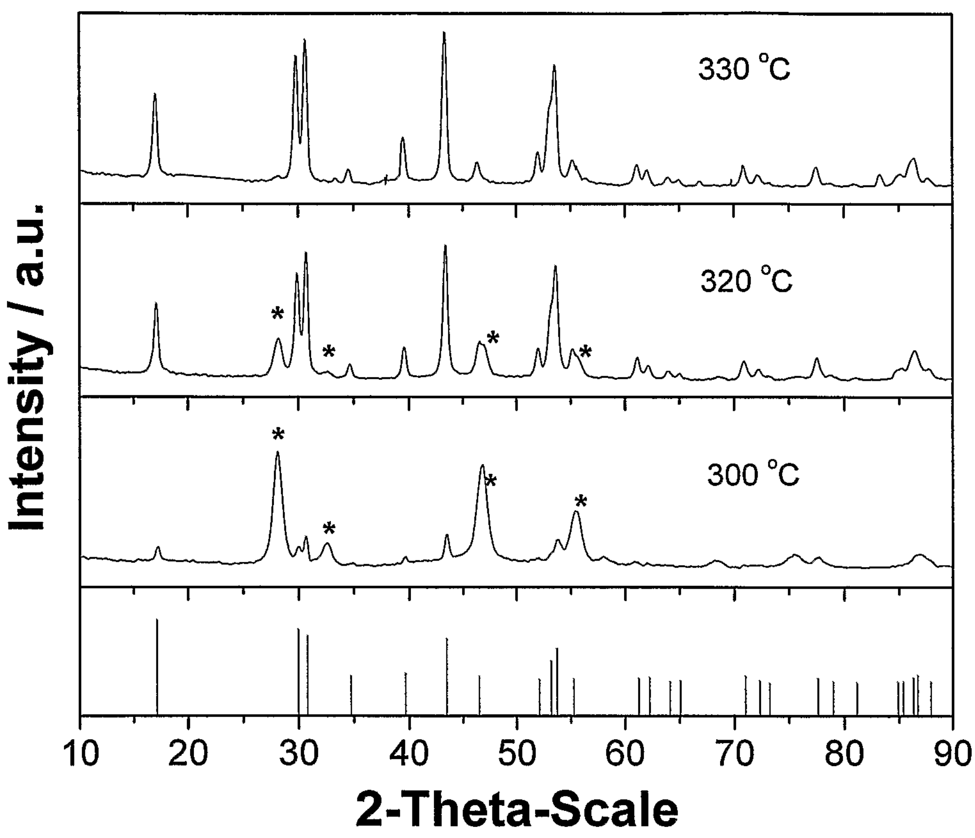
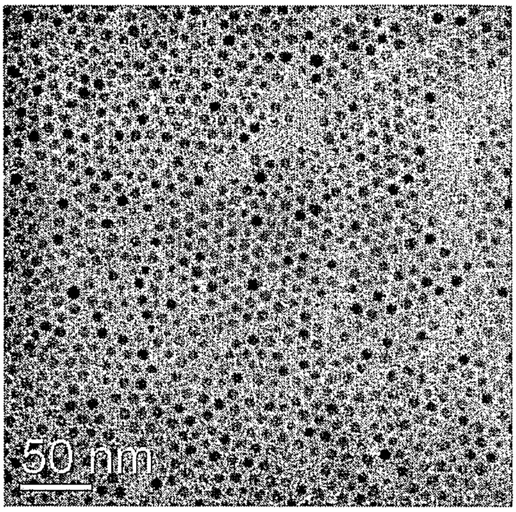
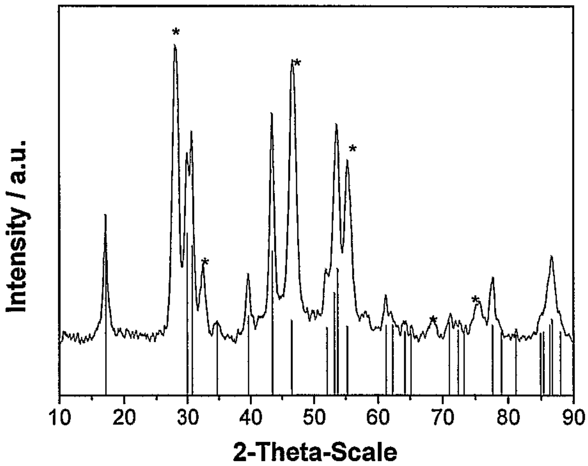

330 °C
320 °C
300 °C
*
Intensity / a.u.
10
20
30
40
50
60
70
80
90
2-Theta-Scale

FIGURE 25

(a)



(b)



SEQUENCE LISTING

<110> National University of Singapore

<120> Method of preparing nano-structured material(s) and uses thereof

<130> FP3417

<150> US 60/756,557
<151> 2006-01-06

<160> 1

<170> PatentIn version 3.3

<210> 1
<211> 9
<212> PRT
<213> Artificial

<220>
<223> 925 to 933 laminin fragment

<400> 1

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Cys Asp Pro Gly Tyr Ile Gly Ser Arg
1               5
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International application No.
PCT/SG2007/000003

PCT/SG2007/000003

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

C09K 11/85 (2006.01)	***C01D 17/00*** (2006.01)	***C01F 11/22*** (2006.01)
C01D 3/02 (2006.01)	***C01F 1/00*** (2006.01)	***C01F 15/00*** (2006.01)
C01D 3/22 (2006.01)	***C01F 5/28*** (2006.01)	***C01F 17/00*** (2006.01)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPIDS, JAPIO, CAPLUS: IPC C09K, C01F, C01D, & keywords based on nano, fluorine, dope, temperature and the exemplified compounds.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO2005/015213 A1 (TSINGHUA UNIVERSITY *et al*) 17 February 2005 See examples 1-7 starting on page 13	46, 54-55, 104, 107-108
X	Tanabe, S. *et al.* (2002) *Optical Mater.* **19**:343-349 See the abstract on page 343	104
X	Yi, G. *et al.* (2004) *Nano. Lett.* **4(11)**:2191-2196 Whole document	46-47, 54-55
X	Yan, R. and Li, Y. (2005) *Adv. Funct. Mater.* **15(5)**:763-770 See results sections 2.2.1 to 2.2.3 starting on page 765	104-108

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search 06 February 2007	Date of mailing of the international search report 13 MAR 2007
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized officer **GEORGE HEARDER** Telephone No : (02) 6283 2553

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. Claims 1-60 and 64-113 define methods for the preparation of nano-structured material, the products of said methods, articles of manufacture comprising said products, kits comprising said products and imaging systems comprising said products.
2. Claims 61-63 define methods of modifying a hydrophobic structure to make it hydrophilic

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
   1-60 and 64-113

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	2005015213	AU	2003257368	CN	1580775	EP	1651957

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX