

WATER-SOLUBLE, SURFACE-FUNCTIONALIZED NANOPARTICLE FOR
BIOCONJUGATION VIA UNIVERSAL SILANE COUPLING

FIELD OF THE INVENTION

This invention relates to a process for the preparation of a
5 surface-functionalized nanoparticle, to the surface-
functionalized nanoparticle prepared therefrom, and to a
bioconjugate comprising the surface-functionalized
nanoparticle.

BACKGROUND OF THE INVENTION

10 Nanoparticles of 1-100 nm have broad applications in
biology, such as in labelling, imaging, drug delivery,
separation and optical sensing. The size of nanoparticles
plays an important role in determining their properties as
well as their effectiveness in bioapplications. The
15 synthesis of monodisperse nanoparticles is critical to the
tailoring and optimization of size-dependent
characteristics.

Nanoparticles also require suitable surface functional
groups in order to conjugate with biomolecules. Most
20 biomolecules have carboxylic acid, primary amine, alcohol,
phosphate, or thiol groups, and nanoparticles that are
functionalized with primary amines, carboxylic acids and
thiol surface groups can be covalently conjugated with
biomolecules via amide, disulfide and ester bonds.

25 Many methods are currently available for nanoparticle
synthesis. Most synthetic approaches are based on organic
solvent routes where particles are coated with
hydrophobic/lipophilic organic stabilizer molecules. The
nanoparticles as synthesized cannot be used directly for
30 biofunctionalization/bioapplication because they are

insoluble in water, they do not have required functional groups for bioconjugation, and/or they are unstable toward various processing steps for bioapplications.

Currently, several strategies are available to solve these problems, for example by exchanging the original stabilizer with surfactant/ligand molecules or polymers, silanization, and dendron bridging. The key issue is to deal with the sensitive surface chemistry of the nanoparticles, and the colloidal stability of the nanoparticles in aqueous phase. Ligand-exchanged nanoparticles are less stable and often lead to irreversible aggregates as the nanoparticles lose their organic shells. To solve this problem, ligand-exchange followed by cross-coupling is used to provide covalent bridging surrounding the particles. Silanization and dendron bridging are unique alternative schemes to generate covalent bridging of stabilizer shell surrounding the nanoparticle. Using this approach, stable quantum dot and metallic nanoparticle dispersions can be prepared. However, these approaches often produce water-insoluble nanoparticles, because of interparticle bridging.

BRIEF SUMMARY OF THE INVENTION

In one aspect, the present invention provides a process for the preparation of a surface-functionalized nanoparticle comprising:

- (a) reacting a nanoparticle with a functionalized silane and a base in a substantially non-aqueous solvent to obtain a partially conjugated silanated nanoparticle, wherein the functionalized silane and the base are present in relative amounts such that said functionalized silane undergoes substantially only a single hydrolysis reaction;

(b) reacting the partially conjugated silanated nanoparticle formed in step (a) with a base in a solvent in which the partially conjugated silanated nanoparticle is substantially insoluble and in which the base is
5 substantially soluble.

In further aspects, the present invention provides a surface-functionalized nanoparticle prepared by the process described herein, and a bioconjugate comprising said surface-functionalized nanoparticle.

10 BRIEF DESCRIPTION OF THE DRAWINGS

Embodiments of the invention will be discussed with reference to the following Figures:

Figure 1 is a flow chart illustrating a two-step silane conjugation on the surface of a nanoparticle.

15 Figure 2 is a flow chart illustrating a process for biotinylating amine- and phosphonate-functionalized nanoparticle surfaces.

Figure 3 shows chemical structures of various silanes that can be utilized to prepare a surface-functionalized
20 nanoparticle.

Figure 4 shows a reaction scheme of silane conjugation on a hydroxide-terminated oxide nanoparticle surface.

Figure 5 shows a reaction scheme of silane conjugation chemistry on a metal nanoparticle surface via a linker
25 mercaptosilane.

Figure 6 shows a transmission electron microscopy (TEM) of iron oxide nanoparticles of two different sizes before and after silane coating.

Figure 7 shows an excitation and emission spectra (a) and a TEM micrograph (b) of biotinylated ZnO nanoparticles.

Figure 8 shows a UV-visible absorption spectra and TEM of silanized gold and silver nanoparticles in aqueous buffer.

- 5 Figure 9 shows optically clear aqueous solutions of various silanized nanoparticles under (a) visible and (b) ultraviolet light.

DETAILED DESCRIPTION OF THE INVENTION

This invention discloses a two-step silanization method to
10 derive soluble nanoparticles with different types of surface functionality.

In previous attempts to silanize nanoparticle surfaces, results were difficult to reproduce and nanoparticle aggregates were often obtained. In practice, most covalent
15 bridging schemes for nanoparticle surfaces have this aggregation problem due to competitive interparticle bridging processes. This detrimental process was further complicated with silanization reactions, as silane groups are sensitive to reaction conditions such as moisture,
20 solvents and residual silanol, which induce particle-particle aggregation. This problem is solved herein by performing the silanization reaction in non-aqueous media via a two-step process. In some embodiments, the silanized nanoparticles obtained are completely soluble in aqueous
25 solvents, and they remain stable for long periods of time. Bioconjugates, such as biotinylated nanoparticles, can be prepared using these surface-functionalized nanoparticles, and these bioconjugates are also stable and soluble. The method described herein is general, efficient and highly
30 reproducible when compared to previous methods.

In order to avoid the interparticle reactions that lead to aggregation of nanoparticles, the process according to the present invention proceeds in a two-step manner, which provides some control on the silazination reactions.

5 In a first step, a nanoparticle is reacted with a functionalized silane in the presence of a controlled amount of base such that the functionalized silane undergoes substantially only a single hydrolysis reaction. As such, the functionalized silane forms a single bond with the
10 nanoparticle to obtain a partially conjugated silanated nanoparticle. The degree and rate of silane conjugation can be controlled by varying the temperature and the amount of base in the reaction system. In some embodiments where a hydroxide base is used, the ratio of functionalized silane
15 to base is about 1:1. In other embodiments where a non-hydroxide base is used, the ratio of functionalized silane to base can be less than 1.

In some embodiments, the base used in the first step is soluble in organic solvents. Examples of suitable bases
20 include hydroxide bases, such as tetra-methyl ammonium hydroxide, tetra-butyl ammonium hydroxide, or sodium hydroxide, and non-hydroxide bases such as an alkyl amine. Examples of suitable alkyl amines include C₁-C₁₆ alkyl amines and C₁-C₆ alkyl amines. For certain alkyl amines, the
25 silanization reaction can be fairly slow, although the reaction rate can be increased by heating. Water can also help silanization, as it can provide hydroxide groups to the system. The presence of small amounts of water can perform silanization without any other bases. In some embodiments,
30 small amounts of water can come from organic solvents.

The silane conjugation in the first step is preferably performed in an organic solvent and substantially in the

absence of water. Examples of suitable solvents include organic-like alcohols, hydrocarbons, and benzene derivatives. Specific examples of suitable solvents include toluene, cyclohexane, methanol, ethanol, mixtures of ethanol and toluene, DMSO, DMF, and liquid ammonia. Toluene is a preferred solvent for this step, as partially conjugated silanated nanoparticle precipitate once formed so that unbound silane molecules can be removed. Preferably, the partially conjugated nanoparticle intermediate is somewhat insoluble in the solvent used, which aids in the separation of the intermediate. The removal of unbound silanes is preferred at this point, as free silanes give rise to thick silica shells and/or interparticle silanization in the second step of the reaction. Removal of unbound silanes can be assisted by the addition of solvents that induce the precipitation of silane bound particles.

In the second step of the reaction, the intermediate isolated from the first step is placed in a solvent in which it is substantially insoluble, where it is then reacted with an excess of a base that is soluble in the solvent, to complete the intraparticle silanization of the functionalized silane moieties. Examples of suitable bases for the second reaction step include hydroxide bases, such as tetra-methyl ammonium hydroxide and tetra-butyl ammonium hydroxide, and non-hydroxide bases such as an alkyl amine. Again, Examples of suitable alkyl amines include C₁-C₁₆ alkyl amines and C₁-C₆ alkyl amines.

Examples of suitable solvents for the second step include organic-like alcohols, hydrocarbons, and benzene derivatives. Specific examples of suitable solvents include toluene, cyclohexane, methanol, ethanol, mixtures of ethanol and toluene, DMSO and DMF. As the partially conjugated silanated nanoparticle is substantially insoluble in the

solvent used in the second step, there is minimal contact between each nanoparticle, greatly diminishing the amount of interparticle reactions and substantially avoiding the productions of nanoparticle aggregates.

5 Control experiments were carried out to determine the effectiveness of the process described herein. In some of these control experiments, where the second step was omitted, it was found that the nanoparticles were highly soluble, but they would slowly precipitate with time and
10 that complete precipitation would occur after several hours or days.

In another embodiment, the residual silanol/alkoxysilane in the partially conjugated silanated nanoparticles formed in step (a) are rendered unreactive, for example by conversion
15 to alkylsilane groups. This can be achieved, for example, by reacting the partially conjugated silanated nanoparticles with a chlorosilane, examples of which include chlorotrialkyl silanes such as chlorotrimethyl silane. As the silanol/alkoxysilane are rendered unreactive, inter
20 particle coupling via these groups can be blocked.

Silanes

The process of the present invention can be carried out with various types of silanes. In one embodiment, the silanes comprise a silyl group substituted by 1, 2 or 3 C₁-C₆ alkoxy
25 groups or silanol groups. Examples of suitable silanes are shown in Figure 3. In Figure 3, the silanes have trimethoxy silyl, methoxy silyl, or silanol groups at one end, which groups can hydrolyze in a basic medium to form a silica shell around a nanoparticle. The silanes also have
30 organic functional groups, examples of which include phosphonate groups, amine groups, thiol groups, C₁-C₂₀ alkyl,

C₁-C₂₀ alkene, C₁-C₂₀ alkyne, azido groups, or epoxy groups. These groups can be found on the functionalized silanes prior to the first step of the reaction, or they can be added to the silane groups after they have been conjugated with the nanoparticle. Silanes with phosphonate functional groups can render the nanoparticles water-soluble. Silanes with amine functional groups are useful for bioconjugation via amide bond formation, and they render nanoparticles water-soluble, but only at acidic pH. Silanes with thiol groups can be used for bioconjugation via disulfide bond formation. Mercaptosilanes can be used as linker molecules for preparing silane/silica coatings on metallic nanoparticles, and such mercaptosilane bearing nanoparticles show increased solubility in alcohols. Silanes terminated with long hydrophobic chains can be used to render nanoparticles more hydrophobic, and therefore more soluble in organic solvents such as toluene. When preparing functionalized nanoparticles, a single type of silane or a mixture of silanes can be employed. As such, nanoparticles that are water-soluble and stable over a wide range of pH, as well as reactive to biological molecules, can be obtained, for example, by using a mixture of phosphonate silanes and aminosilanes.

Nanoparticles

The nanoparticles used in the present invention are preferably metal oxides, semiconductors or metallic nanoparticles. In some embodiments, the nanoparticles used to prepare the surface-functionalized nanoparticle are near-monodisperse, and they can comprise, for example, iron oxide, zinc oxide, manganese oxide, nickel oxide or chromium oxide, cadmium-selenium (CdSe), silver or gold. In some embodiments, the nanoparticles have a diameter or from about 2 to about 50 nm, while in other embodiments, their diameter

is from about 2 to about 20 nm. In further embodiments, the nanoparticles can have diameters of from about 2 to about 3 nanometers.

While silanization of compounds such as iron oxide and zinc oxide can be carried out without using any linker silane, silanization of gold, silver and CdSe nanoparticles can involve a linker silane, such as mercaptosilane. When metal oxides are present in the nanoparticle, the presence of a base, for example tetra-methyl ammonium hydroxide, forms hydroxide-terminated nanoparticles which can react with functionalized silanes. When a non-oxide nanoparticle is used, an additional reaction step may be carried out where a linker silane is attached to the nanoparticle to provide a reactive group on the surface of the nanoparticle. An example of a suitable linker silane is mercaptosilane, which chemisorbs on the surface of metallic nanoparticles. The reaction of mercaptosilane with the nanoparticles can be carried out prior to the first step of the reaction. Alternatively, this reaction can be carried out at the same time as the first step.

In most nanoparticle synthesis, excess surfactants and ligands are used. In such cases, the nanoparticles are separated from the free ligands to insure that the silanization reaction in the first step leads to products that are partially or completely insoluble. This is particularly important when no linker silanes are used, for example in the cases of the functionalization of nanoparticles comprising iron oxide or zinc oxide. Free ligands can be removed, for example, via multiple precipitations in methanol.

Bioconjugation

Once water-soluble functionalized nanoparticles are prepared, they can be conjugated with biological molecules. Examples of suitable biological molecules include, for
5 example, biotin, low molecular weight sugars such as Dextran, short peptides, single strand DNA, or Concanavalin A. The surface-functionalized nanoparticle and the biological molecules can be conjugated, for example, through an amide, a disulfide or an ester bond.

10 An example of such bioconjugation is the biotinylation of nanoparticles, which process can be carried out in a simple one-step mechanism. Biotinylation of nanoparticles is important for selective targeting of biologically active substance via biotin-avidin interaction. Earlier results in
15 the art show that biotinylated particles often aggregate due to excessive biotin loading per particle. With the functionalized nanoparticles of the present invention, the biotinylated particles can remain stable and soluble as the presence of phosphonate groups provides increased water-
20 solubility. Figure 9b shows the fluorescence property of a biotinylated zinc oxide nanoparticle solution.

The process of the present invention also provides some control on the number of biological molecules attached to each nanoparticle, by controlling the amount of
25 functionalized silane groups that bear the required reactive functionality to bind with the biomolecules (such as amine or thio groups). In some embodiments, the combination of amine and phosphonate groups has proven to be useful for bioconjugation, as the bioconjugated particles show good
30 water-solubility characteristics and the number of biomolecules of the particle surface can be controlled.

EXAMPLES

The following examples are provided to illustrate the invention. It will be understood, however, that the specific details given in each example have been selected for purpose of illustration and are not to be construed as limiting the scope of the invention. Generally, the experiments were conducted under similar conditions unless noted.

General Procedures for Nanoparticle Preparation

- 10 Near-monodisperse gold and silver nanoparticles in the size range of 2-20 nm were prepared in toluene according to standard procedure (Jana et al., J. Am. Chem. Soc. (2003) 125, 14280). Metal salts were dissolved in toluene in the presence of long-chain tetra-alkyl ammonium salts, amines or
15 acids, and then reduced using a mixture of weak and strong reducing agents. Strong reducing agents induce particle nucleation and weak reducing agents promote particle growth. By varying the ratio of the two reducing agents, particle size can be controlled.
- 20 Near-monodisperse iron oxide nanoparticles were prepared according to a known procedures (Jana et al., Chem. Mater(2004) 16, 3931-3935). Long-chain carboxylic acid salts of iron(II) were heated in the presence of the respective carboxylic acid in octadecene at 300°C. Particle
25 size was controlled between 8 nm and 40 nm by varying the amount of free carboxylic acid. Particles of 2-5 nm were prepared under similar conditions with octadecylamine as the additional capping agent.

Zinc oxide nanoparticles were prepared using a modified
30 literature approach (Meulenkamp et al., J. Phys. Chem. B (1998) 102, 5566; Abdullah et al., Adv. Func. Mater. (2003)

13, 800). To a boiling ethanol solution of zinc acetate and long-chain carboxylic acids, a boiling ethanol solution of tetra-methyl ammonium hydroxide was injected. The zinc oxide nanoparticles started to grow within minutes and the
5 reaction was stopped by rapidly quenching the reaction temperature. Following this approach, monodisperse particles of 5-10 nm were prepared, depending on growth duration.

General Procedures for Silane Conjugation

- 10 All silane solutions were prepared in toluene (100 mM) except for phosphonate silane, which was prepared in methanol. Fresh stock solutions were prepared daily, as silanes are reactive to moisture. Tetra-methyl ammonium hydroxide solution (100 mM) was prepared in methanol.
- 15 Optically clear nanoparticle solutions were prepared in toluene for all silane conjugation experiments. Iron oxide and zinc oxide nanoparticles were purified from free ligands, using standard precipitation-redispersion protocol, prior to preparing their toluene solution.
- 20 In the silanization experiments, either individual silane or mixtures of silanes were used. In most of the mixed silane experiments, phosphonate silane and aminosilane were used and their molar ratio was varied from about 1:100 to about 100:1. In the silanization of metallic nanoparticles,
- 25 mercaptosilane (linker silane) and other silane mixtures were used. In the first step of silane conjugation, 4-5 mL of nanoparticle solution was mixed with a silane solution, and in specific cases, mixed with an equivalent amount (with respect to total silanes) of tetra-methyl ammonium hydroxide
- 30 solution. The mixture was heated to 60°C for 15-30 minutes for iron oxide and zinc oxide. For gold and silver, the mixture was either stirred for a long time (4-5 hr) or

heated to 60°C. Precipitation was observed in most cases, with a clear supernatant. When there was partial precipitation or no precipitation, minimum methanol was added for complete precipitation. The precipitate was
5 separated from the supernatant, and washed with methanol/toluene.

In the second step of conjugation, the solid precipitate was mixed with toluene and tetra-methyl ammonium hydroxide solution, and then heated (for oxides) or stirred for 30-60
10 minutes. Intraparticle silane conjugation took place in this step.

The solid precipitate was finally washed repeatedly in toluene and/or methanol to remove free silanes and bases. Particles can be prepared from milligram to gram scale with
15 this approach. Finally, particles were dispersed in water, aqueous buffer or organic solvent. All the solutions were optically clear, and the particle concentration could be varied from $\mu\text{g/mL}$ to mg/mL .

General Procedures for Bioconjugation

20 In order to show that the surface-functionalized nanoparticles of the invention are useful for bioconjugation, biotinylated particles were prepared using commercially available activated biotin. The carboxylic acid group in biotin was activated with n-hydroxy
25 succinamide (NHS) to react with primary amines. Amine-phosphonate bifunctionalized nanoparticles were used for bioconjugation. In a typical experiment, the dimethylformamide solution of activated biotin was mixed with the particle solution in phosphate buffer and stirred
30 for 12 hr. After biotinylation, free biotin was separated using a Sephadex column.

Example 1 - Preparation of amine-functionalized 5-nm Fe₃O₄ nanoparticles using aminosilane

Iron oxide nanoparticles of 5 nm were prepared in octadecene using a mixture of octadecylamine and iron(II) stearate.

- 5 After preparation, the nanoparticles were purified via methanol precipitation and toluene redispersion. The toluene dispersion was then used in two-step silanization with aminopropyl trimethoxysilane at 60°C. Final solid nanoparticles were dispersed in water using dilute
- 10 formic/acetic acid. The nanoparticles were partially soluble in water without acid.

- TEM images of 5-nm oxide nanoparticles, before and after silanization, are shown in Figure 6. From the TEM images, it can be seen that particle size does not change
- 15 substantially during silanization, that the silane coating is too thin to be seen, and that the particles are isolated after being coated.

Example 2 - Preparation of amine- and phosphonate-bifunctionalized 15-nm Fe₃O₄ nanoparticles using mixed

20 silanes

- Iron oxide nanoparticles of 15 nm were prepared in octadecene using a mixture of iron(II) oleate and oleic acid. After preparation, the nanoparticles were purified via methanol precipitation and toluene redispersion. The toluene
- 25 dispersion was then used in two-step silanization with a mixture of phosphonate silane and bis(aminoethyl) aminopropyl trimethoxysilane at 60°C. Final solid nanoparticles were dispersed in water using dilute formic/acetic acid.

- 30 TEM images of 15-nm oxide nanoparticles, before and after silanization, are shown in Figure 6. Again, the TEM images

support the earlier observations that particle size does not change substantially during silanization, that the silane coating is too thin to be seen, and that the particles are isolated after being coated.

5 Example 3 - Preparation of hydrophobic 5-nm Fe₃O₄ nanoparticles using octadecylsilane

Iron oxide nanoparticles of 5 nm were prepared as described in Example 1. The purified nanoparticles were dispersed in toluene and used in two-step silanization with hexadecyl
10 trimethoxysilane at 60°C. The silanized nanoparticles were dispersed in toluene/hexane.

Example 4 - Preparation of amine-phosphonate-bifunctionalized 8-nm ZnO nanoparticles using mixed silanes

Zinc oxide nanoparticles of 8 nm were prepared in ethanol
15 using zinc(II) acetate as precursor and oleic acid as particle stabilizer. They were purified from free ligands via centrifuging, and were redispersed in toluene. The optically clear toluene dispersion was used for two-step silanization with a mixture of bis(aminoethyl) aminopropyl
20 trimethoxysilane and phosphonate silane at 60°C. The silanized nanoparticles were dispersed in an aqueous buffer.

Example 5 - Preparation of amine-functionalized 2-nm Ag nanoparticles using aminosilane

Silver nanoparticles of 2 nm were prepared in toluene using
25 silver(I) acetate as precursor, decanoic acid-dodecylamine as ligand, and tetrabutyl ammonium borohydride as reducing agent. The as-prepared nanoparticle solutions were used directly for silanization. The optically clear toluene dispersion was treated with a base and a linker silane,
30 bis(aminoethyl) aminopropyl trimethoxysilane and stirred for

4-5 hr without heating so as to prevent particle growth. Particles were centrifuged and separated from free ligands and treated with excess base for 4-5 hr. The silanized nanoparticles were dispersed in an aqueous buffer using a
5 small amount of organic acid.

Example 6 - Preparation of amine-phosphonate-bifunctionalized 10-nm Ag nanoparticles using mixed silanes

Silver nanoparticles of 10 nm were prepared in toluene using silver(I) acetate as precursor, decanoic acid-dodecylamine
10 as ligand, and a mixture of tetrabutyl ammonium borohydride and hydrazine as reducing agents. The as-prepared nanoparticle solution was used directly for silanization. The optically clear toluene dispersion was mixed with a linker silane, methoxydimethylaminopropyl silane,
15 phosphonate silane and a base, and stirred for 4-5 hr without heating. The nanoparticles were centrifuged and separated from free ligands, and treated with excess base for 4-5 hr. The silanized nanoparticles were isolated and dispersed in an aqueous buffer.

20 Example 7 - Preparation of amine-functionalized 8-nm Au nanoparticles using mixed silanes

Gold nanoparticles of 8 nm were prepared in toluene using gold (III) chloride-didodecyldimethylammonium bromide as precursor, decanoic acid as ligand, and tetrabutyl ammonium
25 borohydride as reducing agent. As prepared nanoparticle solution was used directly for silanization. The optically clear toluene dispersion was mixed with linker silane and bis(aminoethyl) aminopropyl trimethoxysilane, and heated to 60°C for 30 min. Particles were centrifuged and separated
30 from free ligands, and again heated with excess base for

another 30 min. Solid nanoparticles were isolated and dispersed in water using a small amount of organic acid.

An UV-visible absorption spectra and a TEM micrograph of silanized gold and silver nanoparticles in aqueous buffer are shown in Figure 7. Again, this figure shows that the silane coating is too thin to be seen, and that the particles are isolated after silane coating.

Example 8 - Biotinylation of amine-phosphonate-bifunctionalized 8-nm ZnO nanoparticles

10 ZnO nanoparticles modified with phosphonate silane and aminosilane were prepared in a phosphate buffer. Dimethylformamide solution of activated biotin (NHS-biotin) was mixed and stirred for 12 hr and then passed through a Sephadex G-25 column three times for separation of free
15 biotin. The optically clear biotinylated nanoparticles were preserved at 4°C.

An excitation and emission spectra and a TEM micrograph of biotinylated ZnO nanoparticles are shown in Figure 8. It is seen from this figure that the biotinylated particles are
20 isolated and fluorescent.

All publications, patents and patent applications cited in this specification are herein incorporated by reference as if each individual publication, patent or patent application were specifically and individually indicated to be
25 incorporated by reference. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

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Although the foregoing invention has been described in some

detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made
5 thereto without departing from the spirit or scope of the appended claims.

It must be noted that as used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates
10 otherwise. Unless defined otherwise all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs.

CLAIMS:

1. A process for the preparation of a surface-functionalized nanoparticle comprising:

5 (a) reacting a nanoparticle with a functionalized silane and a base in a substantially non-aqueous solvent to obtain a partially conjugated silanated nanoparticle, wherein the functionalized silane and the base are present in relative amounts such that said functionalized silane
10 undergoes substantially only a single hydrolysis reaction;

(b) reacting the partially conjugated silanated nanoparticle formed in step (a) with a base in a solvent in which the partially conjugated silanated nanoparticle is substantially insoluble and in which the base is
15 substantially soluble.

2. A process according to claim 1, wherein the nanoparticle comprises a metal oxide.

3. A process according to claim 1, wherein the nanoparticle comprises zinc oxide, iron oxide, manganese
20 oxide, nickel oxide or chromium oxide.

4. A process according to claim 1, wherein prior to, or during, step (a) the nanoparticle is reacted with a mercaptosilane to obtain a mercaptosilane-functionalized nanoparticle.

25 5. A process according to claim 4, wherein the nanoparticle is metallic.

6. A process according to claim 4, wherein the nanoparticle comprises gold, silver or CdSe.

7. A process according to any one of claims 1 to 6, wherein the nanoparticle has a diameter of from about 2 to about 50 nm

8. A process according to any one of claims 1 to 7,
5 wherein the functionalized silane comprises a silyl group substituted by 1, 2 or 3 C₁-C₆ alkoxy groups or silanol groups

9. A process according to any one of claims 1 to 7,
10 wherein the functionalized silane comprises a trimethoxy silyl group, a methoxy silyl group, an ethoxy silyl group or a silanol group.

10. A process according to any one of claims 1 to 9,
wherein the functionalized silane comprises a phosphonate group, an amine group, a thiol group, a C₁-C₂₀ alkyl, a C₁-C₂₀
15 alkene, a C₁-C₂₀ alkyne, an azido group, or an epoxy group.

11. A process according to any one of claims 1 to 10, wherein the base in step (a) is a hydroxide base.

12. A process according to any one of claims 1 to 10, wherein the base in step (a) is a non-hydroxide base.

20 13. A process according to any one of claims 1 to 10, wherein the base in step (a) is tetra-methyl ammonium hydroxide, tetra-butyl ammonium hydroxide, sodium hydroxide or an alkyl amine.

14. A process according to any one of claims 1 to 10,
25 wherein the base is a hydroxide base, and the functionalized silane and the hydroxide base are present in a molar ratio of about 1:1.

15. A process according to any one of claims 1 to 10, wherein the base is a non-hydroxide base, and the

functionalized silane and the non-hydroxide base are present in a molar ratio less than 1.

16. A process according to any one of claims 1 to 15, wherein the non-aqueous solvent is selected from the group consisting of organic-like alcohols, hydrocarbons, and benzene derivatives.

17. A process according to any one of claims 1 to 15, wherein the non-aqueous solvent is selected from the group consisting of toluene, cyclohexane, methanol, ethanol, mixtures of ethanol and toluene, DMSO, DMF, and liquid ammonia.

18. A process according to any one of claims 1 to 15, wherein the non-aqueous solvent is toluene.

19. A process according to any one of claims 1 to 18, wherein the base in step (b) is a hydroxide base.

20. A process according to any one of claims 1 to 18, wherein the base in step (b) is a non-hydroxide base.

21. A process according to any one of claims 1 to 18, wherein the base in step (b) is tetra-methyl ammonium hydroxide, tetra-butyl ammonium hydroxide or an alkyl amine.

22. A process according to any one of claims 1 to 21, wherein the solvent in step (b) is selected from the group consisting of organic-like alcohols, hydrocarbons, and benzene derivatives.

23. A process according to any one of claims 1 to 21, wherein the solvent in step (b) is selected from the group consisting of toluene, cyclohexane, methanol, ethanol, mixtures of ethanol and toluene, DMSO, and DMF.

24. A process according to any one of claims 1 to 21, wherein the solvent in step (b) is toluene.

25. A surface-functionalized nanoparticle obtained by or obtainable by a process according to any one of claims 1
5 to 24.

26. A surface-functionalized nanoparticle according to claim 25, wherein the surface-functionalized nanoparticle comprises one or more functional groups selected from the group consisting of phosphonate groups, amine groups, thiol
10 groups, C₁-C₂₀ alkyl, C₁-C₂₀ alkene, C₁-C₂₀ alkyne, azido groups, and epoxy groups.

27. A surface-functionalized nanoparticle comprising a silver nanoparticle to which is attached functionalized silanes, the functionalized silanes comprising one or more
15 functional groups selected from the group consisting of phosphonate groups, amine groups, thiol groups, C₁-C₂₀ alkyl, C₂-C₂₀ alkene, C₂-C₂₀ alkyne, azido groups, and epoxy groups.

28. A surface-functionalized nanoparticle comprising an iron nanoparticle having a diameter of from about 2 to
20 about 3 nm, to which nanoparticle is attached functionalized silanes, the functionalized silanes comprising one or more functional groups selected from the group consisting of phosphonate groups, amine groups, thiol groups, C₁-C₂₀ alkyl, C₂-C₂₀ alkene, C₂-C₂₀ alkyne, azido groups, and epoxy groups.

25 29. A surface-functionalized nanoparticle comprising an iron nanoparticle to which is attached functionalized silanes, the functionalized silanes comprising two or more functional groups selected from the group consisting of phosphonate groups, amine groups, thiol groups, C₁-C₂₀ alkyl,
30 C₂-C₂₀ alkene, C₂-C₂₀ alkyne, azido groups, and epoxy groups.

30. A surface-functionalized nanoparticle comprising a zinc oxide nanoparticle to which is attached a functionalized silane, the functionalized silane comprising one or more functional groups selected from the group
5 consisting of phosphonate groups, amine groups, thiol groups, C₁-C₂₀ alkyl, C₂-C₂₀ alkene, C₂-C₂₀ alkyne, azido groups, and epoxy groups.

31. A bioconjugate comprising a surface-functionalized nanoparticle according to any one of claim 25 to 30 that is
10 conjugated to a biological molecule.

32. A bioconjugate according to claim 31, wherein the biological molecule is biotin, a low molecular weight sugar, a short peptide, a single strand DNA, or Concanvalin A.

33. A bioconjugate according to claim 32, wherein the
15 low molecular weight sugar is Dextran.

34. A bioconjugate according to any one of claims 31 to 33, wherein the surface-functionalized nanoparticle and the biological molecules are conjugated through an amide, a disulfide or an ester bond.

20 35. A process for the preparation of a surface-functionalized nanoparticle comprising:

(a) reacting a nanoparticle with a functionalized silane and a base in a substantially non-aqueous solvent to obtain a partially conjugated silanated nanoparticle,
25 wherein the functionalized silane and the base are present in relative amounts such that said functionalized silane undergoes substantially only a single hydrolysis reaction;

(b) reacting the partially conjugated silanated nanoparticle formed in step (a) with a chlorosilane.