

# A generalized approach for the surface engineering of nanoparticles in suspension for highly efficient hybrid capture of bio-molecules

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## ABSTRACT

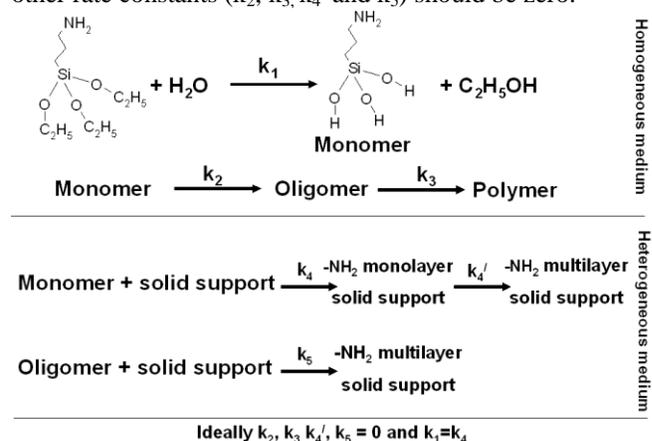
Surface engineering of nanoparticles with hydrophilic surfaces has been carried out in suspension by a strict control of the surface ad-layer of water surrounding the nanoparticles using a generalized strategy called tri-phasic reverse emulsion (TPRE). This approach produces an optimal density of surface amine groups with monolayer patterns on the nanoparticles surfaces. Surface functionalized nanoparticles when chemically conjugated to oligonucleotide (5'NH<sub>2</sub>-dC<sub>6</sub>-dT<sub>25</sub>), the oligonucleotide conjugated bio-nanoparticles exhibited exceptional hybridization efficiency to fluorescence labeled complementary oligonucleotide (dA<sub>25</sub>). This strategy has overcome the problem associated with the hydrolysis and condensation of aminosilane molecules during the surface functionalization of nanoparticles in suspension and can have improved efficiency in applications ranging from medicine to pharmaceuticals to materials science.

**Keywords:** Surface functionalization, Aminosilane, nanoparticles, DNA hybridization, Tri-phasic reverse emulsion, magnetic bio-separation

## 1 INTRODUCTION

Engineering atomic and molecular nanostructures on flat surfaces is extensively reported [1,2] whereas that in the case of nanoparticles in suspension is not fully understood as the process can be difficult to control due to the aggregation of nanoparticles in suspension, solvation of nanoparticles and an uncontrolled reaction of reactant molecules in the suspension. Surface functionalization of flat surfaces by aminosilane is a controlled process due to the elimination of water from the surface by a simple drying step [3-6] whereas in suspension phase they are difficult to control due to a series of consecutive reactions of aminosilane molecules in water to form polymeric species (see reaction scheme 1). Under heterogeneous conditions e.g. in the presence of a solid support, the reactive monomers could react with native surface -OH groups to form a monolayer of -NH<sub>2</sub> functionalities or they can undergo self polymerization before they condense onto the surface. The oligomers or polymers can further react on the surfaces to form -NH<sub>2</sub> functionalized multilayer. The

rate constants ( $k_1, k_2, k_3, k_4, k_4', k_5$ ) of these various reactions control the functionalization of solid surfaces. In order to produce a uniform monolayer of -NH<sub>2</sub> functionalized surfaces, ideally  $k_1$  should be equal to  $k_4$  and other rate constants ( $k_2, k_3, k_4'$  and  $k_5$ ) should be zero.



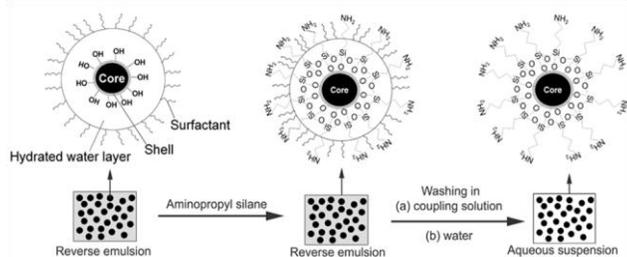
**Scheme 1** Hydrolysis and condensation reaction of aminosilane molecules in water under homogeneous and heterogeneous (in the presence of solid surfaces) condition

Moreover, the flat surfaces have only two dimensions so that the silane molecules only interact through the z-axis onto the surfaces. Bein and co-workers [4] reported that 3-aminopropyl triethoxy silane (APTS) molecules formed a monolayer (surface amine density of  $5.3 \times 10^{14}$  silanes/cm<sup>2</sup>; thickness of 7Å) on Quartz surfaces under gas phase reaction. Atomic layer deposition (ALD) technique under gas phase is also reported [7, 8] to be a controlled process where the water molecules were excluded from the reaction. Gas phase deposition by ALD technique or surface engineering of flat surfaces by controlled water condition is well studied; however, surface engineering of hydrophilic nanoparticles in suspension using aminosilanes is not fully studied.

Surface functionalization using aminosilane is reported to be an essential step for the chemical conjugation of bio-molecules. Applications in nano and nanobiotechnology [9, 10] rely on surface functionalization step using aminosilane. However, no one has ever reported the mechanism of formation of aminosilane layers on the nanoparticles surfaces and its importance in improving the

efficiency in such applications. Sen *et al* have reported [11] the difficulty for the functionalization of nanoparticles in suspension using aminosilane. Herein, we report that tri-phasic reverse emulsion (TPRE) approach can be used as a generalized strategy for surface engineering of hydrophilic nanoparticles in suspension for various applications such as highly efficient hybrid capture of bio-molecules.

In the actual process, amino molecules were added to a tri-phasic (nanoparticles-surface water-organic solvent) reverse emulsion of hydrated magnetic nanoparticles in an organic solvent (toluene), in the presence of a common biocompatible non-ionic surfactant, (Triton X100). The bulk water was magnetically separated from a core-shell silica magnetite suspension and the hydrated nanoparticles were dispersed in toluene in the presence of Triton X100. As aminosilane is soluble but do not hydrolyse or self condense in toluene, hence, it will remain unreacted in the continuous toluene phase. Aminosilane can only hydrolyse and subsequently condense onto the surfaces of the nanoparticles where there is water present in the system. In the present system this water is present only as adsorbed water on the surfaces of the nanoparticles. This permits the aminosilane to react in a controlled fashion and form an ordered uniform layer of aminosilicate (Scheme 2). In this approach therefore the process that degrade the quality of the nanoparticles and the functionalisation i.e. aggregation of nanoparticles and self condensation of APTS monomers to oligo/polymers are eliminated.



**Scheme 2** Surface engineering of core-shell nanoparticles in suspension using TPRE approach

## 2 EXPERIMENTAL

### 2.1 TPRE approach of surface engineering of nanoparticles

150mg of either core-shell silica-magnetite nanoparticles (I) and diamagnetic silica spheres (II) were collected either magnetic separation or centrifugation. 30ml of toluene and 5gm of triton X100 were added and the mixture shaken to form a tri-phasic reverse emulsion. APTS was added to the emulsion to a final concentration of 2% (w/v) and allowed to react in a 100mL glass reactor fitted with condenser at 50°C in an oil bath for 5 hrs with stirring. The amount of surface water was controlled by washing stepwise with water miscible solvent (dry tetrahydrofuran). The suspension was washed with coupling solution (0.8% v/v glacial acetic acid in dry methanol) three times and

stored at RT in the same solution. Surface amine densities were determined by colorimetric assay using 4-nitrobenzaldehyde [12]. The total amine densities were determined by combustion (CHN) analysis.

### 2.2 Covalent coupling of single stranded oligonucleotides (5'-NH<sub>2</sub> dC<sub>6</sub> dT<sub>25</sub>) to nanoparticles

(1×)SSC and (13×)SSC buffers were prepared by diluting a stock solution of (20×)SSC buffer (175.3g NaCl, 88.2g sodium citrate, 1L H<sub>2</sub>O, pH 7.4) with distilled, deionized water, adjusted to pH 7.4 and autoclaved before use. Glutaraldehyde solutions were prepared immediately before use. 2mg of aminosilane nanoparticles were washed (×3) with 1ml of coupling buffer (1×SSC buffer, pH 7.3) for 2 minutes at 18°C. After removal of the supernatant, 0.5ml of a 5% v/v glutaraldehyde solution in coupling buffer were added and the suspension incubated for 3 hours with end-over-end rotation at 18°C. The material was subsequently washed (×3) with 1ml coupling buffer to remove excess glutaraldehyde. 1ml of a 3.3μM solution of 5'-amine modified oligo-dT<sub>25</sub> were added and the mixture left incubating overnight whilst shaking. The oligo-modified nanoparticles were then washed once with coupling buffer and placed in 0.8ml of NaBH<sub>3</sub>CN solution (0.03% w/v in coupling buffer) for 30 minutes at 18°C. After this the material was washed (×3) with 0.8ml of coupling buffer and finally resuspended in 200 μl of the same.

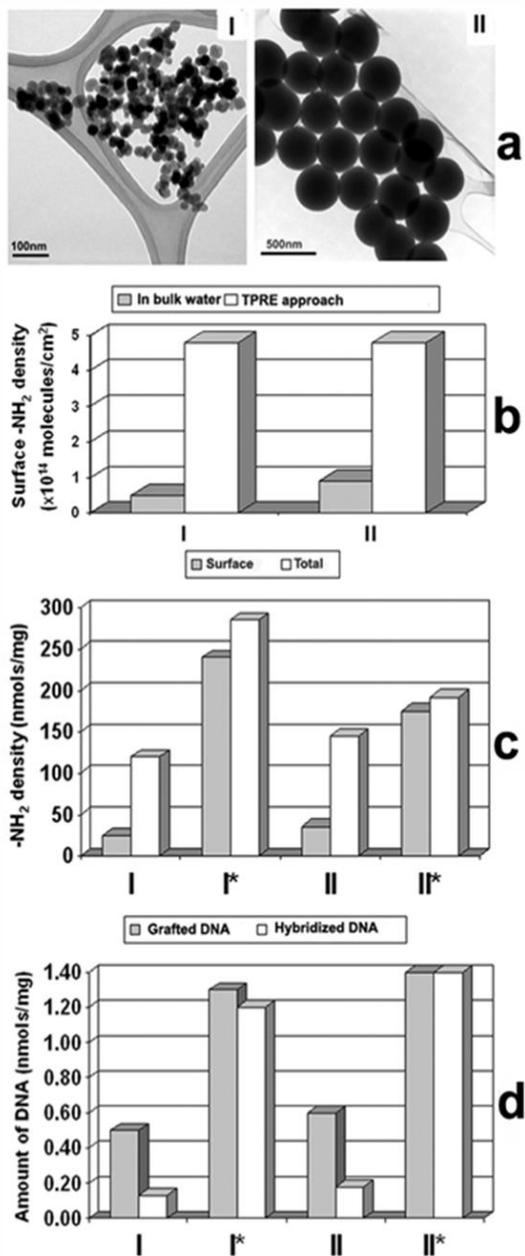
### 2.3 DNA hybrid capture experiments (model assay)

1mg of oligo-dT<sub>25</sub> modified nanoparticles was washed twice with 0.5ml of water and resuspended in and heated to 80°C for 4 minutes. 1ml of a 1.5μM solution of 5'-fluorescein modified oligo-dA<sub>25</sub> in (13×)SSC /0.05%BSA was added to the particles and the suspension incubated with gentle shaking for 30 minutes at 18°C. The supernatant was removed and kept for analysis. After washing (×3) with 1ml of 13×SSC, 200μl of water was added to the particles and the suspension heated to 85°C for 4 minutes to disassociate the annealed/hybridized oligo-dA<sub>25</sub> sequences. The supernatant was removed and analyzed by fluorescence spectrophotometer (excitation 460nm and emission 515nm).

## 3 RESULTS AND DISCUSSION

Transmission electron microscopy (TEM) showed both shell-core silica-magnetite (I) and model silica (II) materials were spherical in morphology (figure 1a). The diameter of shell-core silica-magnetite was measured to be around 40nm whereas the diameter of model silica nanoparticles was measured to be around 400nm. The

silica nanoparticles (II) were used as a diamagnetic analog of superparamagnetic core shell silica-magnetite.



**Figure 1** (a) TEM images of core-shell silica-magnetite (I) and silica spheres (II). (b) Surface amine density of amine functionalized nanoparticles of shell-core silica-magnetite (I) and spherical silica (II) measured by colorimetric assay. (c) Surface to total amine density of nanoparticles (I, II), \* indicate TPRE approach. (d) Amount of oligonucleotide (5' NH<sub>2</sub>-dC<sub>6</sub>-dT<sub>25</sub>) grafted on nanoparticles (I, II) and the hybridization efficiency of fluorescent labeled complementary oligonucleotide (dA<sub>25</sub>).

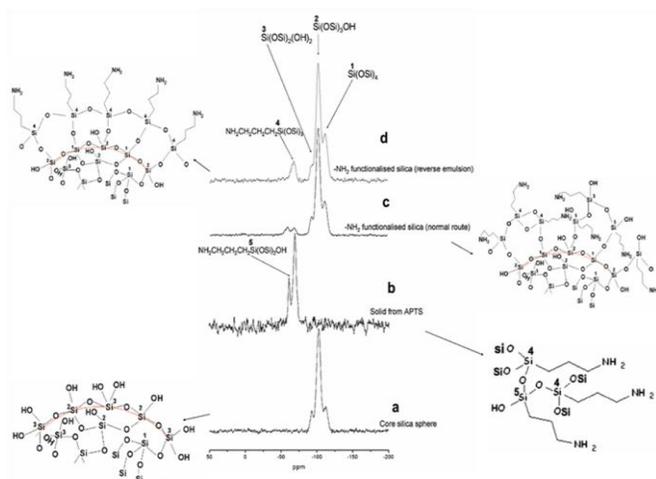
The Brunauer, Emmett, Teller (BET) surface area of materials I and II were measured to be 30 and 22m<sup>2</sup>/g. Both shell-core silica-magnetite (I) and model spherical silica (II) behaved similarly in Salmon sperm DNA binding and elution experiment (data not shown) indicating that both surfaces were identical in nature hence pure silica nanoparticles could be used as model nanoparticles.

Functionalized nanoparticles (I and II) by TPRE approach exhibited a high surface amine density (4.8x10<sup>14</sup> molecules/cm<sup>2</sup>) compared to nanoparticles functionalized in water (Fig. 1b). A high value (>80%) of surface to total amine density was observed in materials functionalized by TPRE approach compared to functionalization in water (20%). The high value of surface to total amine density by TPRE approach is a direct proof of a controlled surface engineering and the surface amine density values (4.8 x10<sup>14</sup>/cm<sup>2</sup>) were close to the surface amine density of a monolayer (5.3x10<sup>14</sup>/cm<sup>2</sup>) on flat surfaces [4].

The orientation of the surface amine groups (shown in scheme 2) was tested by applying them in biology (Figure 2c and 2d) by attaching oligonucleotide (5' NH<sub>2</sub>-dC<sub>6</sub>-dT<sub>25</sub>) and their efficiency in capturing fluorescent labeled complementary target oligonucleotide (dA<sub>25</sub>) by hybridization mechanism. Surface engineered nanoparticles prepared by TPRE approach exhibited a high value (85%) of 5' NH<sub>2</sub>-dC<sub>6</sub>-dT<sub>25</sub> attachment efficiency with respect to the initial concentration of 5' NH<sub>2</sub>-dC<sub>6</sub>-dT<sub>25</sub> during the reaction whereas materials functionalized by bulk water phase exhibited only 43% attachment of 5' NH<sub>2</sub>-dC<sub>6</sub>-dT<sub>25</sub>. The hybrid capture efficiency of oligonucleotide (5' NH<sub>2</sub>-dC<sub>6</sub>-dT<sub>25</sub>) attached bio-nanoparticles in a solution of complementary fluorescence labeled oligonucleotide (dA<sub>25</sub>) was observed to be very different in TPRE approach compared to nanoparticles functionalized in water. TPRE approach provided up to 100% capture of oligonucleotide (dA<sub>25</sub>) by hybridization mechanism compared to 30% capture in water phase. This high performance of hybridization efficiency is due to the proper orientation of grafted oligonucleotide (5' NH<sub>2</sub>-dC<sub>6</sub>-dT<sub>25</sub>) to the surface of the nanoparticles so that it is available to capture the complementary oligonucleotide (dA<sub>25</sub>). These results suggest that the proper orientation of surface -NH<sub>2</sub> groups is very important and is achieved by TPRE approach. Maxwell *et al* [13] reported that the orientation of grafted bio-molecules in gold nanoparticles surfaces is related to the performance and detection of target bio-molecules for the applications in biosensors.

<sup>29</sup>Si CPMAS solid state NMR spectra (Figure 2) of surface engineered diamagnetic silica core materials and polymerized silica obtained from APTS indicate the presence of various silicon environments due to two distinct chemical shift regions: -90 to -110ppm {Q Si sites {Si\*(OSi)<sub>n</sub>(OH)<sub>4-n</sub>, n can have values from 1 to 3} and -50 to -70ppm {T Si sites {H<sub>2</sub>NCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Si\*(OSi)<sub>n</sub>(OH)<sub>3-n</sub> with n can have values from 1 to 3}. Core silica sphere was observed to have three different silicon environments

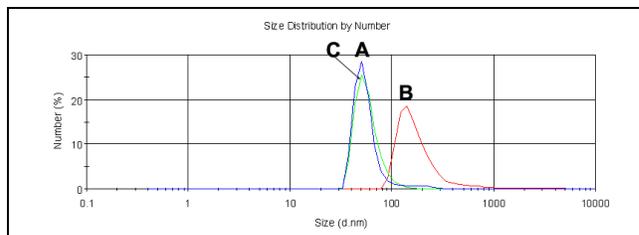
(Figure 2; types 1, 2, 3) only in Q Si-sites and self polymerized APTS have two different silicon environments (Figure 2; types 4, 5) only in T Si-sites. The surface engineered silica nanoparticles exhibited both Q and T Si sites. TPRE approach provided only one type of T site (type 4) whereas functionalization in bulk water provided two types of T Si-sites (types 4 and 5). The absence of type 5 Si-site on surfaces support the model structure (shown in scheme 2) proposed in TPRE approach. The absence of other types of bonding such as amino end of APTS to surface silica (N-Si) as previously reported by ALD technique [7,8]. Vanblaaderen *et al* [14] reported the one step synthesis of amino-functionalized monodispersed colloidal organo-silica spheres using APTS but the surface amine groups were observed to be various types  $\{H_2NCH_2CH_2CH_2Si^*(OSi)_n(OH)_{3-n}$  with  $n$  values from 1 to 3} similar to uncontrolled functionalization in bulk water phase. It is also observed that the relative amount of type 3 to type 1 Si-species decreased after surface functionalization. The change is very prominent in the case of surface engineered nanoparticles prepared by TPRE approach and this is due to the simultaneous conversion of surface Si-species from type 3 to 2 to 1.



**Figure 2**  $^{29}Si$  CPMAS NMR spectra of (a) Diamagnetic core silica sphere; (b) Polymerized APTS; (c) Amine functionalized silica sphere engineered in bulk water; (d) Amine functionalized silica sphere engineered by TPRE approach.

The organization of amino silane molecules around the nanoparticles could also have an effect on the resultant particle sizes. The surface engineered magnetic nanoparticles by TPRE approach exhibited no change in particle sizes (see Figure 3) whereas average particle sizes shifted from 50nm to 145nm when surface engineering of nanoparticles was carried out in water. In the case of TPRE approach, the density ( $4.8 \times 10^{14}$  molecules/cm<sup>2</sup>) and an ordered orientation of surface functional groups around the nanoparticles supports the formation of silane monolayer (7Å i.e. 0.7nm thickness) identical to the flat surfaces

measured by ellipsometry and therefore there is no change in particle size. Surface functionalization of nanoparticles in water exhibited an increase (ca. 100nm) in size and this may due to the presence of polymerized layer of silane or an interparticle connectivity through polymerized amino silane molecules as presented in scheme 2.



**Figure 3** Particle size distribution of magnetic nanoparticles (I) before (A) and after surface engineering in water (B) and by TPRE approach (C)

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