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Retracted article: Surface activation of nanoparticulates in suspension by a novel strategy: tri-phasic reverse emulsion for highly efficient DNA hybridisation†

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We, the named authors, hereby wholly retract this Chemical Communication. Signed: Tapas Sen and Ian James Bruce, Canterbury, UK, February 2009. Retraction endorsed by Sarah Thomas, Editor. Retraction published 27 February 2009

Engineering nano and microparticles using colloidal templating[1–4] or *via* bi-phasic reverse emulsion routes[5–7] is well established in the context of micro/nanotechnology whereas surface engineering of nanoparticles are beginning to explore for applications in biology such as; drug delivery, gene targeting, tumor therapy, medical diagnostics and magnetic bio-separations.[8–11] Usually the nano/microparticles involved are surface functionalised/grafted with a ligand useful for a particular application and attachment of the ligand commonly requires that the particle possesses surface functional groups to which the ligand can be conjugated. Surface density and orientation of activated groups are of utmost importance in the context of subsequent grafting of ligands to the nanoparticles ' surface and consequently their applications.

Engineering of atomic and molecular nanostructures on flat surfaces is well understood[12,13] whereas that in the case of nanoparticles in suspension is little known as this is a complex process due to the aggregation of nanoparticles and an uncontrolled reaction in solution phase. Surface functionalisation of flat surfaces by aminosilane is a controlled process due to the ready elimination of water from the surface by a simple drying step[14–17] whereas in suspension phase they are difficult to control due to a series of consecutive reactions of aminosilane molecules in bulk water phase (see Fig. S1 in ESI†). Moreover, the flat surfaces have only two dimensions so that the silane molecules only interacts perpendicular to the surfaces (see Fig. S2 in ESI†). Bein and co-workers[15] reported that aminopropyl triethoxysilane molecules formed a monolayer (surface amine density of 5.3×10^{14} silanes cm^{-2}; thickness of 7 Å) on quartz surfaces under gas phase reaction whereas in suspension the silane molecules always form multilayers. Recently atomic layer deposition (ALD) technique under gas phase is reported[18,19] to be a controlled process where the water molecules were excluded from the reaction. The ALD technique has limitation for the application in suspension and the resultant surface bonding species are randomly oriented on the surfaces which lacked applications in the area of nanobiotechnology. So far formation of monolayers on nanoparticles surfaces in suspension is not reported and it remains as a challenge in the applications in nano/nanobiotechnology.

Various authors[20–23] have attempted the surface functionalisation of nanoparticles in suspension using aminopropyl triethoxysilane (APTS) in organic, aqueous or in organic/aqueous solvent mixtures. It is possible to achieve higher total amine values for silanised materials than those represented by monolayers *e.g.* when using dendrimers [24] or APTS in water[20–23] but these values include internal $–NH_2$ groups not present at the materials surface. It is the surface amine that is important in ligand grafting and applications both in terms of its density and organisation.

Herein, we report the surface functionalisation of individual nanoparticles by a strict control of the water layer surrounding the nanoparticles *via* tri-phasic reverse emulsion (TPRE). This approach produces an optimum density of surface amine groups with an ordered orientation on the nanoparticles surfaces. Consequently the nanoparticles exhibited a

high value of DNA grafting and up to 100% hybridisation efficiency with 1 : 1 stoichiometry of grafted DNA to hybridised DNA in a model assay.

In the actual process, APTS 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 (see Movie S1 in ESI†). As APTS is soluble but does not hydrolyse or self condense in toluene it will remain unreacted in the continuous toluene phase. APTS 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 APTS to react in a controlled fashion and form an ordered uniform layer of aminosilicate (Fig. 1). The amount of surface water was controlled by washing stepwise with water miscible solvent (dry tetrahydrofuran; see ESI,† Table S1). 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.

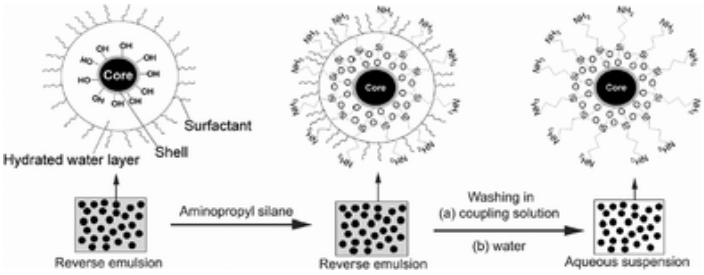

Fig. 1 A schematic diagram of TPRE approach of surface functionalisation.

Solid-state nuclear magnetic resonance (NMR) is a powerful technique to prove the molecular structure (Fig. 1) of surface groups presents on nanoparticles . However, NMR has the limitation in obtaining this information with a superparamagnetic core; we have therefore used a diamagnetic silica core as a model system and used the similar approach for surface engineering.

Transmission electron microscopy (TEM) shows both shell–core silica-magnetite (I)[25] and model silica (II)[26] materials are spherical in morphology (Fig. 2(a)). The diameter of shell–core silica-magnetite is around 40 nm and it is a composite of core magnetite covered with a very thin layer of silica shell whereas the diameter of model silica is around 400 nm. The Brunauer–Emmett–Teller (BET) surface area of materials I and II were measured to be 30 and 22 $m^2\ g^{-1}$. The surface of shell–core silica-magnetite (I) and model spherical silica (II) were characterised by testing the performance of DNA binding and elution to and from the surfaces (see Fig. S3 in ESI†) and compared the value with pure magnetite (Fe–O) surfaces. The high binding value of DNA and high recovery of adsorbed DNA to and from silica-magnetite (I) and model spherical silica (II) confirmed that the surfaces of both materials are similar and they are different from pure magnetite surfaces. This is a characteristic property of two different surfaces as we previously reported.[25]

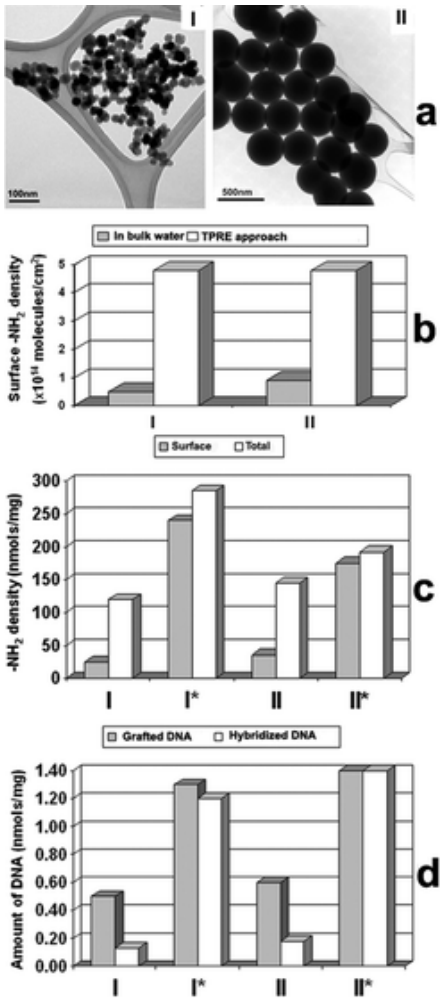

Fig. 2 (a) TEM images of core–shell silica-magnetite (I) and silica spheres (II). (b) Surface amine density of amine functionalised nanoparticles of shell–core silica-magnetite (I) and spherical silica (II) measured by colorimetric assay. (c) Surface to total amine density of nanoparticles (I, II), * indicate TPRE approach. (d) Amount of DNA (dT_{25}) grafted on nanoparticles (I, II) and the hybridisation efficiency of complementary DNA (dA_{25}) for bio-separations.

Functionalised nanoparticles (I and II) by TPRE approach exhibited a high surface amine density (4.8×10^{14} molecules cm^{-2}) measured by standard colorimetric assay[27] compared to nanoparticles functionalised in bulk water phase (Fig. 2(b)). However the total amine density in nanomaterials was measured by elemental analysis was high in both cases and a comparison of surface to bulk amine density is presented in Fig. 2(c). A high value (>80%) of surface to total amine density was observed in materials functionalised by TPRE approach compared to functionalisation in bulk water phase (20%). The high value of surface to total amine density by TPRE approach is a direct proof of a controlled surface functionalisation and the values are close to the surface amine density of a monolayer (5.3×10^{14} cm^{-2}) on flat surfaces.[15,16]

The orientation of the surface amine groups (shown in Fig. 1) were tested by applying them in biology (Fig. 2(d)) by grafting a single strand DNA (dT_{25}) and their efficiency in capturing complementary target DNA (dA_{25}) by hybridisation mechanism (see Fig. S4 in ESI†). Surface functionalised nanoparticles prepared by TPRE approach exhibited a high grafting (85%) efficiency with respect to the initial concentration of DNA (dT_{25}) during the reaction whereas materials functionalised in bulk water phase exhibited only 43% grafting. The capture efficiency of DNA (dT_{25}) grafted nanoparticles in a solution of complementary fluorescence labeled DNA (dA_{25}) was observed to be very different in TPRE approach compared to nanoparticles functionalised in bulk water phase. TPRE approach provided up to 100% capture of DNA (dA_{25}) by hybridisation mechanism compared to 30% capture in bulk water phase without any non-specific binding of DNA (dA_{25}). This high performance of hybridisation efficiency may well be due to the proper orientation of grafted DNA (dT_{25})

to the surface of the nanoparticles so that it is available to capture the complementary DNA (dA_{25}). Maxwell *et al.*[28] reported that the orientation of grafted biomolecules in gold nanoparticles surfaces is related to the performance and detection of target biomolecules for the applications in biosensors.

^{29}Si CPMAS solid-state NMR spectra (Fig. 3) 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 −110 ppm {Q Si sites $\{Si^*(OSi)_n(OH)_{4-n}$, n can have values from 1 to 3} and −50 to −70 ppm {T Si sites $\{H_2NCH_2CH_2CH_2Si^*(OSi)_n(OH)_{3-n}$ with n can have values from 1 to 3}. Core silica sphere was observed to have three different silicon environments (Fig. 3; types 1, 2, 3) only in Q Si-sites and self polymerized APTS have two different silicon environments (Fig. 3; types 4, 5) only in T Si-sites.[29] The surface engineered silica nanoparticles exhibited both Q and T Si sites. TPRE approach provided only one type of T site (type 4) whereas functionalisation 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 Fig. 1) 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.[18,19] Vanblaaderen *et al.*[29] reported the one-step synthesis of amino-functionalised 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 functionalisation in bulk water phase. It is also observed that the relative amount of type 3 to type 1 Si-species decreased after surface functionalisation. 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.

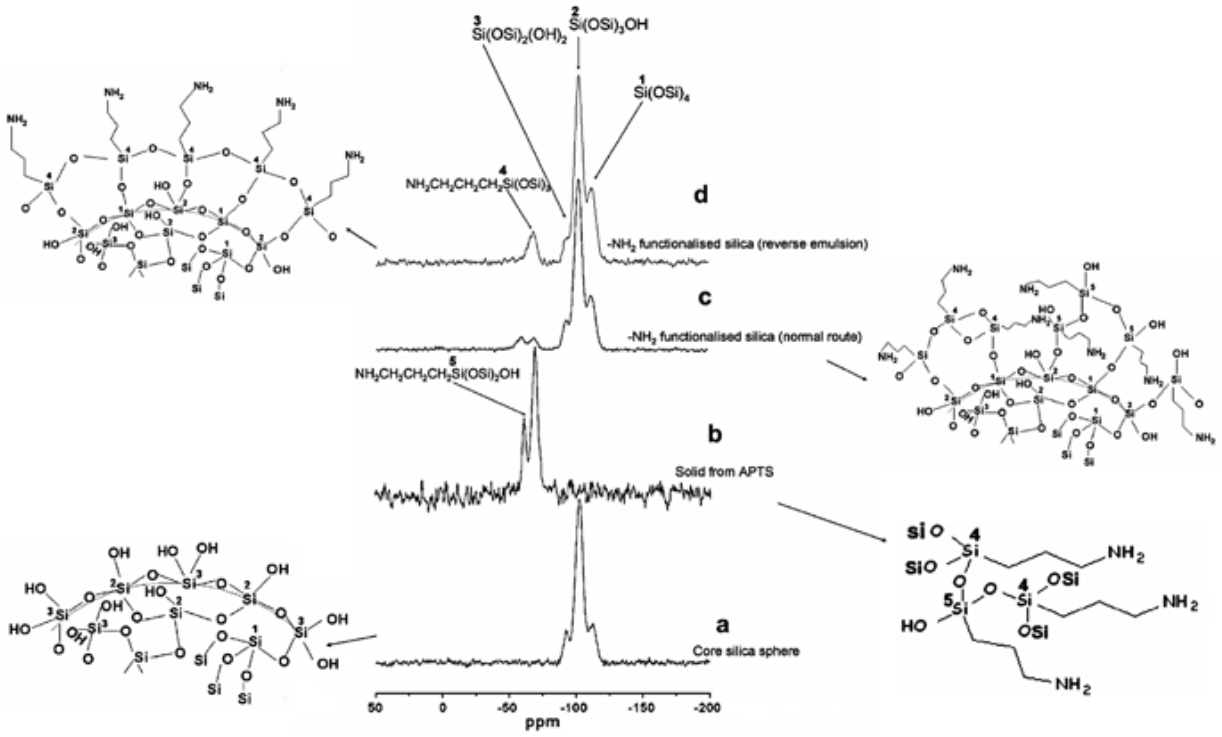

Fig. 3 ^{29}Si CPMAS NMR spectra of (a) diamagnetic core silica sphere; (b) polymerized APTS; (c) amine-functionalised silica sphere engineered in bulk water; (d) amine-functionalised silica sphere engineered by TPRE approach.

In conclusion, it has been demonstrated that the TPRE approach, when applied to produce surface engineered nanoparticles , provides a uniform layer of functional amine groups on the surface of nanoparticles . Consequently materials exhibited a high value of DNA grafting and the high performance in capturing DNA in magnetic bio-separations.

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Footnote

† Electronic supplementary information (ESI) available: Detailed experimental protocol for surface functionalisation, grafting single stranded DNA , hybrid capture of complementary oligonucleotide , Fig. S1–S4, Table S1 and Video file (Movie S1) showing triphasic reversion emulsion strategy. See DOI: 10.1039/b817354k