

Electronic supplementary information

Surface activation of nanoparticles in suspension by a novel strategy: Tri-phasic reverse emulsion for highly efficient DNA hybridisation†

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Methods

TPRE approach of surface engineering of nanoparticles. 150mg of either core-shell silica-magnetite nanoparticles (I) or diamagnetic silica spheres (II) were collecting 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 50⁰C in an oil bath for 5 hrs with stirring. The suspension was washed with coupling solution three times and stored at RT in the same solution. Prior to use the activated materials were washed with de-ionized water.

Surface amine analysis by colorimetric method. 5mg of nanoparticles were placed in a 1.5ml Eppendorf tube and washed (×4) with 1ml coupling solution (0.8% [v/v] glacial acetic acid in dry methanol). Subsequently, 1ml of 4-nitrobenzaldehyde solution (7mg in 10ml coupling solution) was added to the particles and the suspension allowed to react for 3h with gentle end-over-end rotation. After removal of the supernatant and washing (×4 in 1ml coupling solution), 1ml of hydrolysis solution (75ml H₂O, 75ml MeOH and 0.2ml glacial acetic acid) was added to the particles and the tube shaken for a further hour. The supernatant was then removed from the particles and its absorbance measured at 282nm.

Covalent coupling of single stranded oligonucleotides (5'-NH₂ dC₆ dT₂₅) 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 H₂O, pH 7.4) with distilled, deionised water, adjusted to pH 7.4 and autoclaved before use. Glutaraldehyde solutions were prepared immediately before use. 2mg of aminosilanised 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-dT₂₅ 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 NaBH₃CN 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. In the case of *Salmonella enterica* (SP), 16SrRNAGramm+ and 16SrRNAGramm- oligo-modified nanoparticles, material was washed three times with 0.5ml blocking solution (1xPBS, 40mM glycine, 0.5% bovine serum albumin [BSA]) and finally resuspended in 1xPBS with 0.1% BSA to 5 mg/ml and stored at +4°C until used in hybrid capture experiments.

DNA hybrid capture experiments (model assay). 1mg of oligo-dT₂₅ 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-dA₂₅ 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-dA₂₅ sequences. The supernatant was removed and kept for fluorescence analysis.

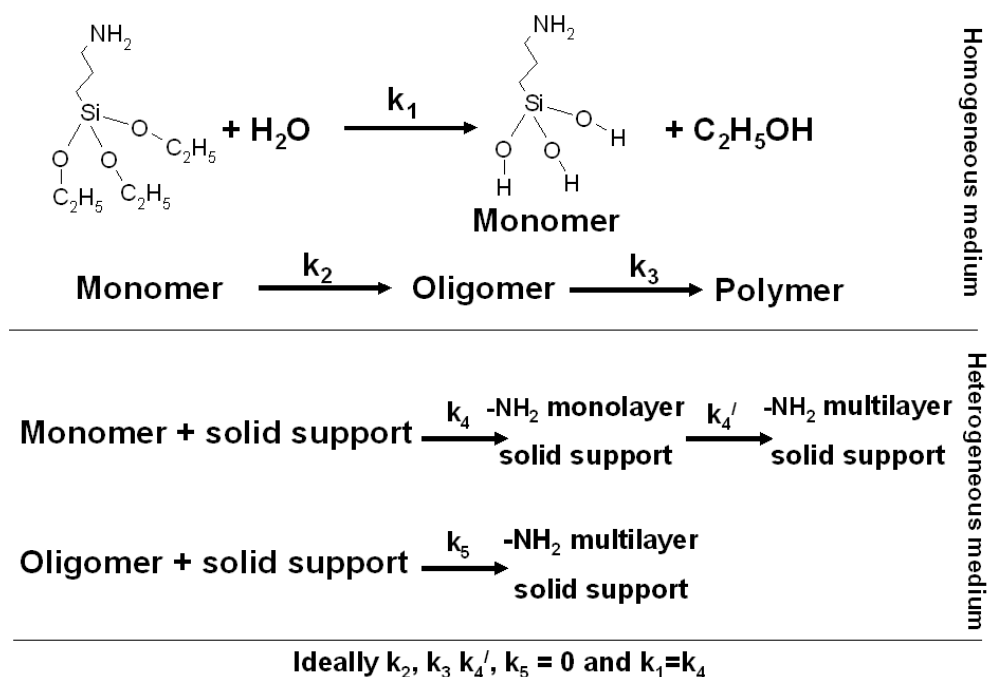


Fig. S1 Hydrolysis and condensation reactions of aminosilane molecule

The pH of an aminosilane solution in water is around 12 is due to the protonation of the -NH_2 groups of APTS and at this pH, APTS subsequently reacts vigorously with water *via* many consecutive reactions. APTS is hydrolyzed by water to form silicate monomers with the release of ethanol. These monomers subsequently react to form oligomers and polymers. 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_2 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_2 functionalized multilayer. The rate constants ($k_1, k_2, k_3, k_4, k_4', k_5$) of these various reactions control the nature of the functionalization of solid surfaces. In order to produce a uniform monolayer of -NH_2 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 ideally be zero.

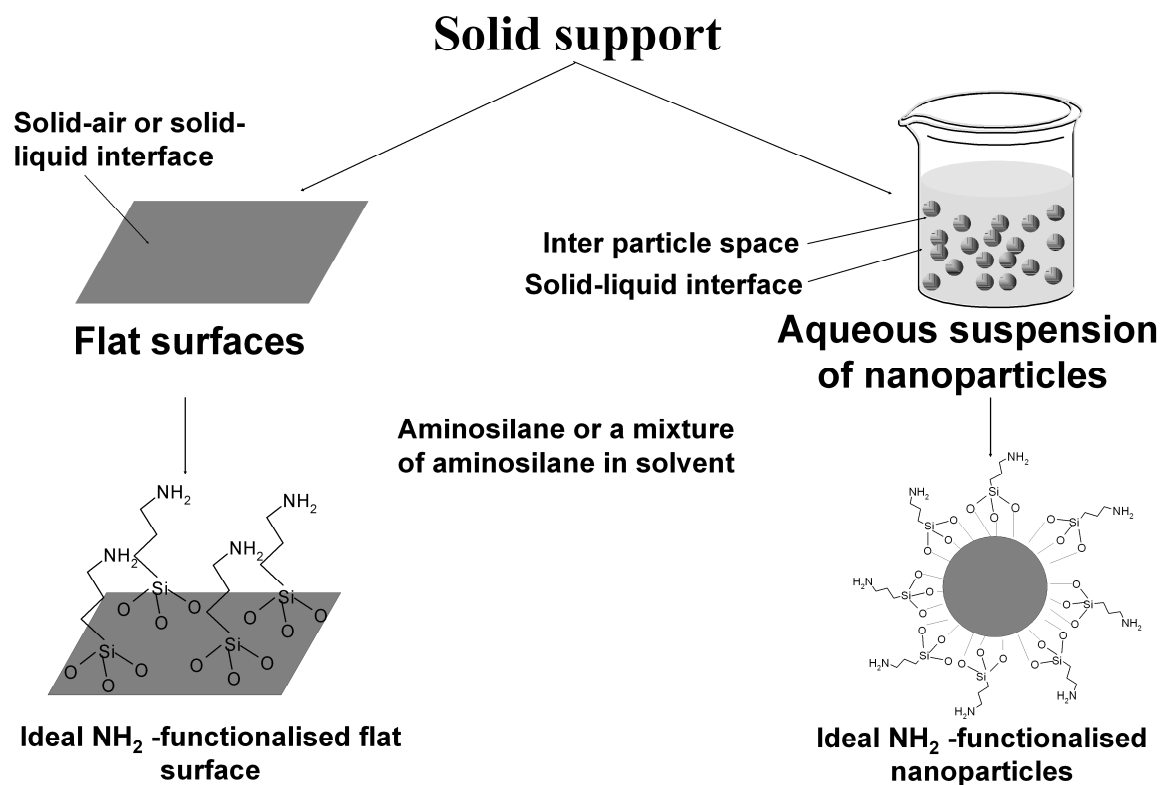


Fig. S2 Nanoengineering of flat and nanoparticles surfaces

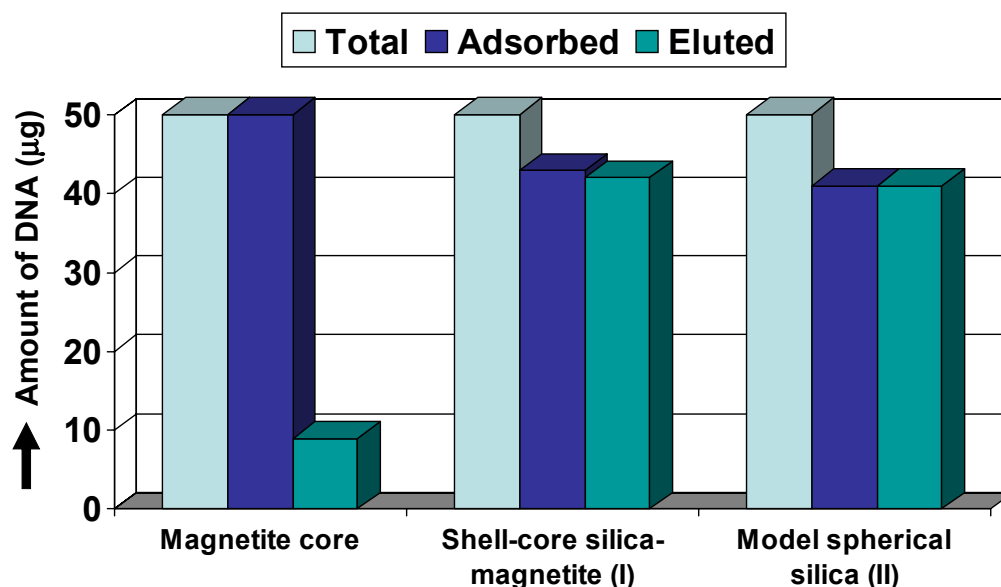


Fig. S3 Binding and elution of Salmon sperm DNA to and from various nanoparticles

DNA binding and elution: 50µl of sheared salmon sperm DNA (concentration: 1mgmL⁻¹) was added in 350µl of TEN buffer (100ml Tris base-HCl of pH 8, 50mM EDTA of pH 8, 500mM NaCl) and 400µl of polyethylene glycol (20% PEG, MW 8000) in 4M NaCl to 1g of various nanoparticles such as core magnetite and superparamagnetic silica-magnetite nanoparticles (I) and model spherical silica (II). The mixture was incubated with gentle agitation for 5 minutes at room temperature before the magnetite or silica-magnetite (I) was immobilized using a magnetic stand (Promega Corp. Madison, USA) and spherical silica (II) by centrifugation at 2000rpm for 30 sec. The supernatant measured for DNA content by UV spectrophotometry (Spectronic Unicam 500UV, Cambridge, UK) read at OD 260. All experimental values were compared to a standard curve constructed previously (data not shown). Nanoparticles were then washed with 1ml 70% v/v aqueous ethanol with gentle agitation for 5 minutes at 25⁰C. After re-immobilization of the particles using the magnetic stand or centrifugation the aqueous ethanol was removed and the particles resuspended in 200µl of sterile deionized water and left to incubate for 5 minutes at RT. The supernatant containing the eluted DNA was subsequently removed using a micropipette after magnetic separation or centrifugation. This process was repeated and the elutents measured for their DNA content spectrophotometrically.

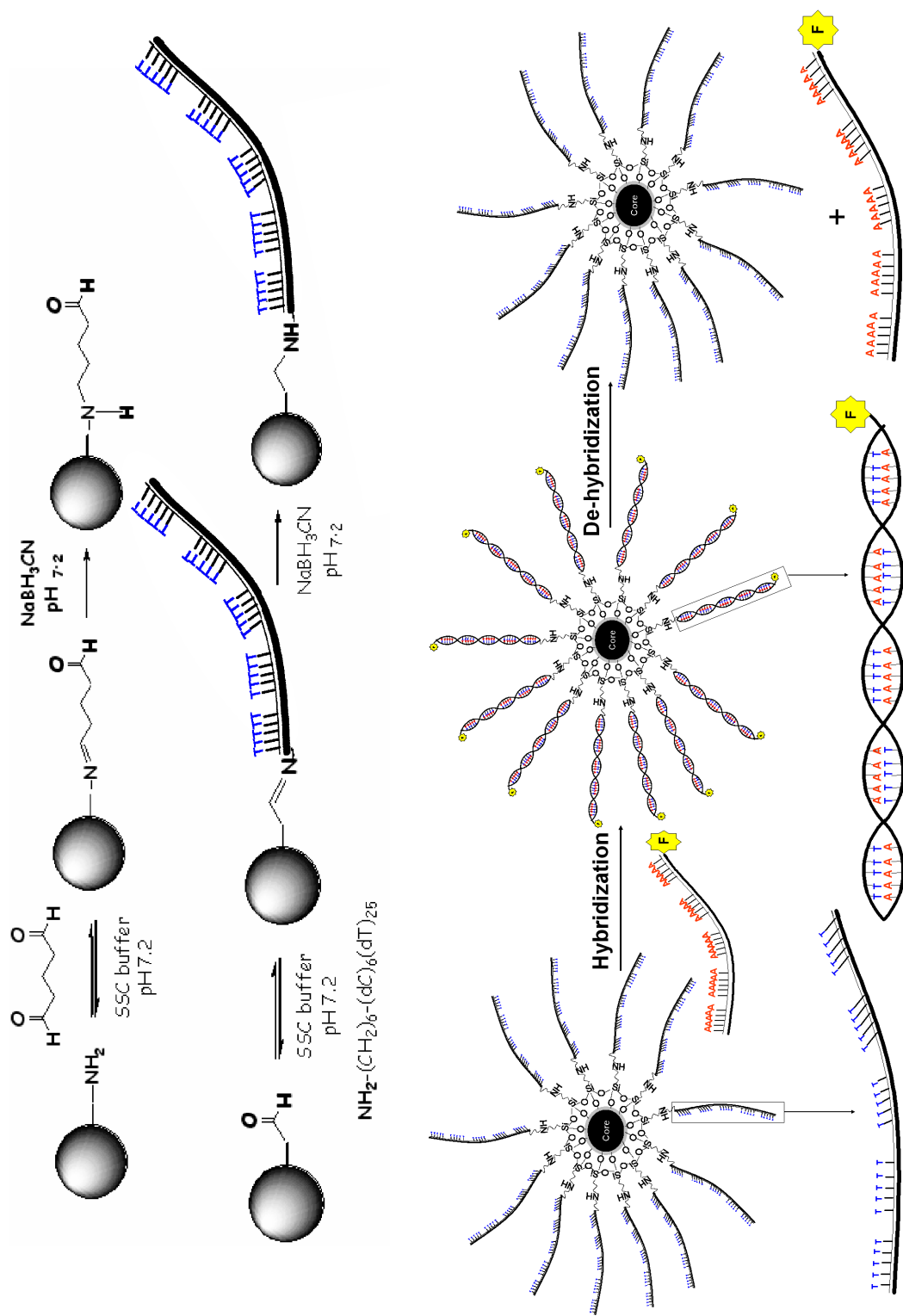


Fig. S4 Grafting of DNA (dT_{25}) on amine functionalized nanoparticles and the mechanism of hybridization/dehybridization of complementary DNA (dA_{25}) for magnetic bio-separations.

Table S1

Nano- particle	Particle size (nm)	surface area (BET) (m ² /g)	Water content (wt%)	Surface -NH ₂ density colorimetric (nmols/mg)	Surface -NH ₂ density colorimetric (molecules/cm ²)	Total -NH ₂ density combustion (nmols/mg)	dT ₂₅ surface density (nmols/mg)	5F dA ₂₅ hybridized (nmols/mg)	5F dA ₂₅ nonspecific adsorption (nmols/mg)	% hybridization efficiency
I	Av. 40	30	99 [*]	25	0.5×10 ¹⁴	120	0.5	0.13	0	26
			0.06 ^d	240	4.8×10 ¹⁴	285	1.3	1.2	0.1	92
			99 [*]	36	1×10 ¹⁴	145	0.6	0.18	0	30
			0.23 ^a	100	2.7×10 ¹⁴	116	1.3	1.2	0.1	92
II	Av. 400	22	0.1 ^b	114	3.1×10 ¹⁴	135	1.4	1.4	0	100
			0.06 ^c	173	4.7×10 ¹⁴	196	1.4	1.4	0	100
			0.06 ^d	175	4.8×10 ¹⁴	192	1.4	1.4	0	100

* indicate the surface activation of nanoparticles in bulk water

a, b, c and d indicate the number of washings (0, 1, 2 and 3) with dry tetrahydrofuran.