Porous functionalised silica particles: a potential platform for biomolecular screening

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Received (in Cambridge, UK) 4th October 2004, Accepted 6th December 2004
First published as an Advance Article on the web 17th January 2005
DOI: 10.1039/b415376f

Novel, porous, functionalised silica particles have been developed with controlled morphology, which promote covalent attachment of fluorescent dyes which can act as an optical barcode.

Colloidal particles are increasingly being used as supports for biological screening.[1,2] One of the key issues facing colloid based assays is finding supports which are suitable for withstanding the synthesis of the biomolecule as well as identifying the biomolecule during the screening process. A number of technologies have been developed to optically code the particles so the biomolecules can be identified during the screening process,[1] but to date, none of these encoding techniques has been demonstrated as suitable for the synthesis of combinatorial libraries.

We outline here a novel class of functionalised silica particle which is encodable with fluorescent dyes, is robust and is functionalised so biomolecules (primarily DNA and peptides) may be coupled and synthesised onto the surface of the particles.

The most common method for synthesising colloidal silica particles is the Stöber process,[3] which involves hydrolysis and condensation of tetraethoxysilane (TEOS) in a solution of ethanol, water and ammonia. Fluorescent dyes can be incorporated into the particles during synthesis through the addition of 3-aminopropyl trimethoxysilane (APS) which has been coupled to an isothiocyanate modified dye.[4] However, these particles are limited to a size of less than 3 μm[3] and the dye must be incorporated during the synthesis of the particle. A new synthesis procedure for producing fully functionalised particles 1–100 μm in diameter, into which multiple fluorescent dyes can be covalently incorporated post-synthesis, has been developed.

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These novel particles are synthesised from the functionalised silane monomer, 3-mercaptopropyl trimethoxysilane (MPS) (Fig. 1).[5] Initially, the MPS monomer is added to an acidic solution where it forms an emulsion upon stirring. The MPS is insoluble in the solution and over a period of approximately two hours, the emulsion gradually breaks down to give a clear solution. This is attributed to the hydrolysis of the monomer, forming the more soluble silica species (shown in Fig. 1), and methanol as a by-product.

As in the Stöber process, a condensation reaction occurs after the hydrolysis of the silane monomer. In the process described here, the thiol functionalised monomer has three reactive sites for condensation, rather than four. As the condensation reaction proceeds, short polymer chains are formed. These short polymer chains are not soluble in the aqueous phase; however, being liquid, they form an 'oil-in-water' emulsion. Stable, solid particles are formed by adding ammonia (a cross-linking catalyst)[6] to the emulsion after approximately 24 hours.

As shown by brightfield optical microscopy (Fig. 2), dark regions immediately appear within the emulsion droplets upon

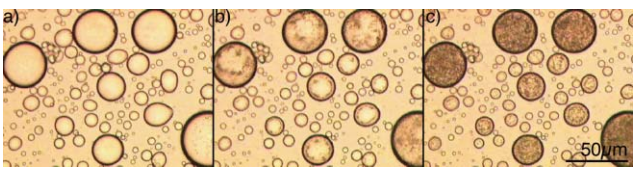

Fig. 2 *Emulsion condensation.* a) Emulsion droplets before the addition of ammonia. b) Immediately after the addition of ammonia, water is produced as a by-product of the condensation reaction. The water coalesces within the emulsion droplets, forming a double emulsion, seen as the dark regions within the droplets. c) Particles after cross-linking. The water droplets within the particles template large pores, visible under SEM Fig. 3).

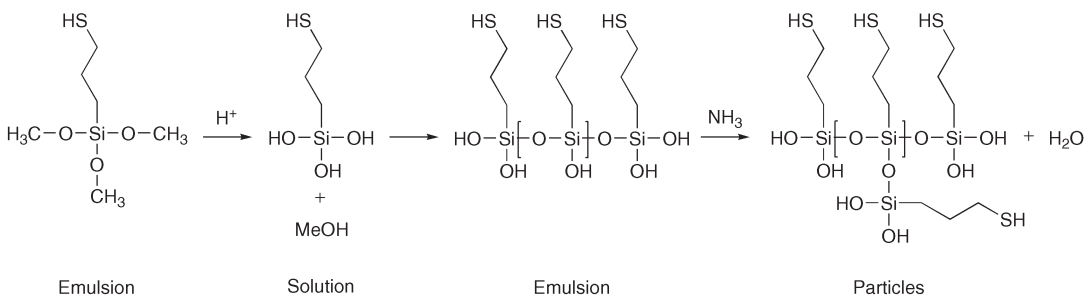

Fig. 1 *Condensation mechanism.* Hydrolysis and condensation of 3-mercaptopropyl trimethoxysilane (MPS). MPS is hydrolysed using hydrochloric acid (HCl) to form reactive silanol groups. The silanol groups slowly condense to form short, linear chains which form an emulsion. The emulsion droplets are cross-linked through the addition of ammonia (NH_3), forming stable particles.

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addition of the catalyst. These dark regions, which are confined to the oil droplet, are swirled around until the cross-linking process is complete (within 10 seconds) and solid particles are formed. We believe that when the cross-linking catalyst in the aqueous phase reacts with the outer surface of the emulsion droplet, cross-linking between the short polymer chains at the surface of the droplet occurs. A product of the cross-linking reaction is water, which is not soluble within the hydrophobic emulsion droplet. The water produced from the condensation reaction coalesces to form fine droplets of water within the emulsion droplets, forming a double emulsion. These water droplets appear as dark regions when observed using optical microscopy (Fig. 2), and appear to rapidly swirl around until cross-linking is complete.

It is proposed that, as the short polymers at the surface of the silica emulsion droplets condense, short polymers from the centre of the particle migrate to the surface, to combat the concentration gradient which occurs. Upon completion of the cross-linking process, the water regions have templated out pores within the solid particles (Fig. 3).

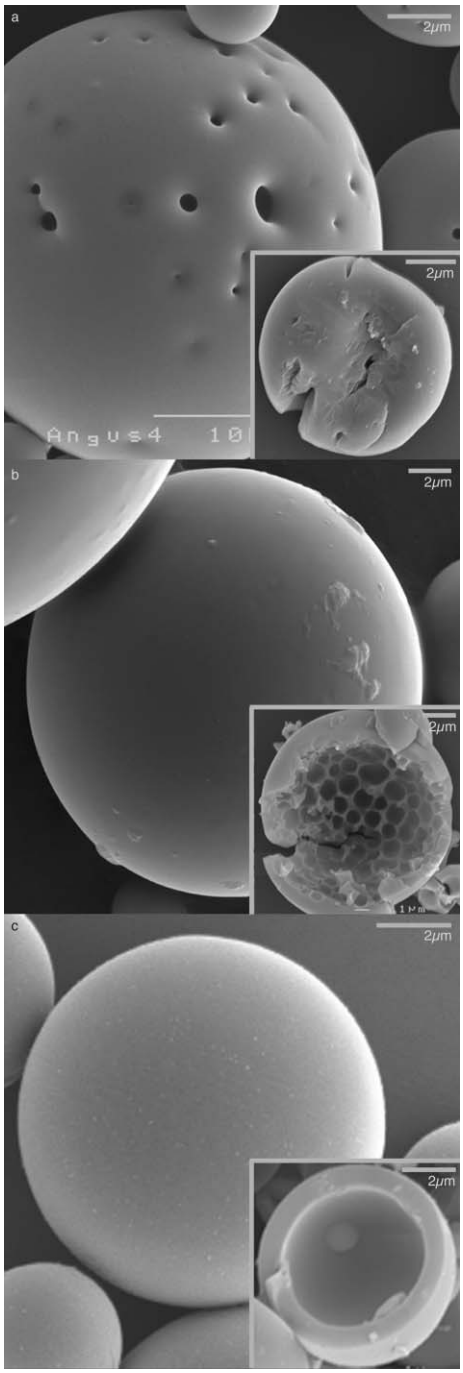

Fig. 3 *Scanning electron microscopy images of cross-linked functionalised silica particles.* a) Particles synthesized with a low (0.14 M) ammonia concentration show large surface pores and a crushed particle (inset) shows the particles have a rubbery texture. b) Particle synthesized with the optimal ammonia concentration (2.7 M) and a crushed particle (inset). c) Hollow particles formed by cross-linking with high ammonia concentration (6.7 M), and a crushed particle (inset).

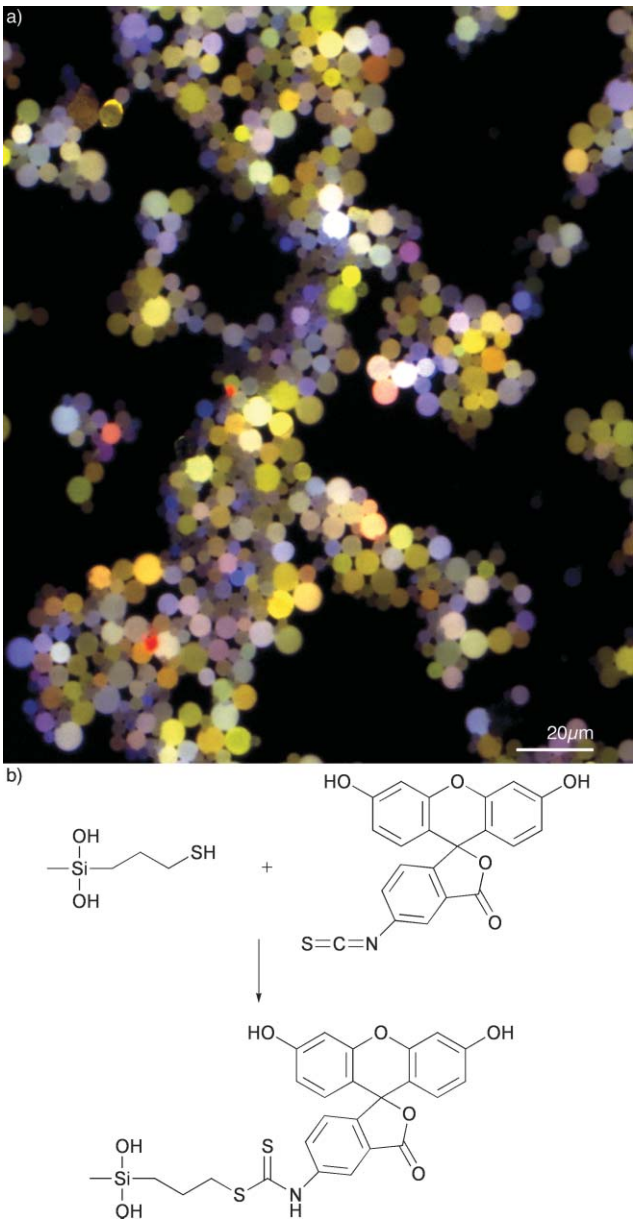

Fig. 4 *Optically diverse fluorescent particles.* a) Fluorescence microscopy image of particles possessing a diverse range of optical signatures synthesised in a split-and-mix process using three fluorescent dyes (AlexaFluor 350, Oregon Green and TAMRA). b) Chemical reaction of fluorescein isothiocyanate with the thiol groups in the particle.

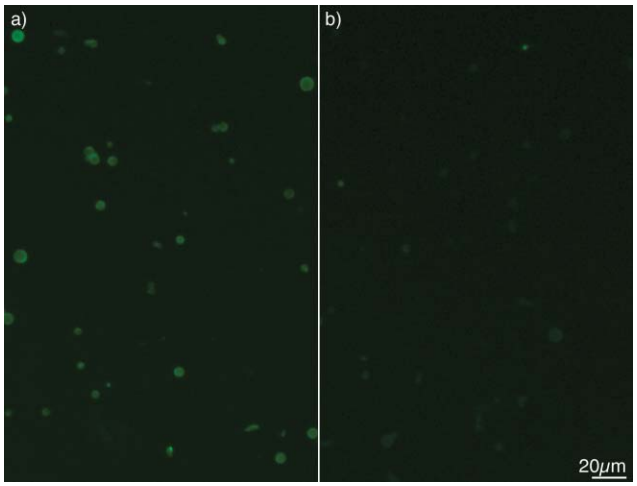

Fig. 5 *DNA Hybridisation.* Fluorescence microscopy shows that: a) the hybridisation of a fluorescent target on a particle coupled with a complementary DNA sequence, is much higher than: b) the hybridisation of the fluorescent target to a mismatched sequence.

Ammonia concentration significantly affects the cross-linking rate and the size of pores within the particles. Fig. 3 shows the morphological changes which occurred upon cross-linking with different ammonia concentrations. The use of low catalyst concentrations (0.14 M) typically results in poorly cross-linked particles with large pores on the outer surface (Fig. 3a). The cross-linking of the emulsion droplet is slow due to the low availability of catalyst. The slow rate of cross-linking allows the water formed within the emulsion droplet to migrate to the outer surface and escape into the aqueous reaction mixture, before the outer surface has completely solidified. Thus, large pores were evident on the outer surface of the cross-linked particle.

When the catalyst concentration was increased, the outer surface of the emulsion droplet appeared to solidify more quickly, forming particles with a smooth surface (Fig. 3b) indicating that the rate of cross-linking had increased. The particles formed at the highest concentration of catalyst (6.7 M) were hollow. When a small amount of force was applied to the particles, the particle fractured cleanly around the circumference of the particle, revealing an outer shell approximately 1 μm thick (measured by SEM, Fig. 3c). Because each particle has a unique pore distribution, this translates to a unique optical signature as measured by light (Mie) scattering and multiplexed fluorescence analysis.

Thiol functional groups are distributed throughout the particles and dyes were covalently attached to the thiol groups by exposing the particles to solutions of isothiocyanate and succinimidyl ester functionalised dyes dissolved in solvent (*e.g.* ethanol).[7] The resultant fluorescence intensity of each particle was dependant on the dye concentration in the solvent. By incorporating dyes into the particles in a combinatorial split-and-mix manner (*i.e.*, using a variety of dyes and dye concentrations over a number of split-and-mix cycles), particles displaying a diverse range of optical signatures were produced (Fig. 4).

To illustrate that the particles could be used for biomolecular screening, two different fluorescently labelled DNA sequences were coupled to the surface of the particles.[8] The first sequence, completely complementary to a fluorescent target (Fig. 5a), showed a high level of fluorescence, whereas the second sequence, a mismatched sequence (Fig. 5b), showed significantly lower fluorescence.

We have outlined here a new class of functionalised silica particles with controlled morphology. The functionalised, porous nature of the particle means that it is possible to covalently incorporate fluorescent dyes into the particle. The combination of several dyes in a single particle acts like a barcode to identify biomolecules subsequently synthesised onto the particles.

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5 Particle synthesis: 800 μl of 0.1 M HCl was added to 80 ml of water to give a pH of approximately 3 and 10 ml of MPS (3-mercaptopropyl trimethoxysilane, Lancaster Chemicals, UK) was added. The solution was stirred at 2000 rpm for 24 hours. The cross-linking catalyst (NH_3, 25% solution) was added in varying amounts to 100 ml of water and MPS polymer emulsion was slowly added. Immediately the MPS reaction vessel was rinsed with ~75 ml of ethanol, which was added to the ammonia–MPS solution. After the addition of the ethanol the mixture was centrifuged at 2000 g and the pellet was resuspended in 10 ml of ethanol using ultrasonication.
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7 Dye coupling: four concentrations of Alexa Fluor 350 carboxylic acid, succinimidyl ester (A350) Oregon Green 488 carboxylic acid, succinimidyl ester (Oregon Green) and 5-carboxytetramethylrhodamine, succinimidyl ester (TAMRA) (all sourced from Molecular Probes, Eugene, Oregon, USA) were made up to 100 mM in spectroscopy grade ethanol. Split-and-mix dye incorporation was performed by splitting a sample of ~500 000 beads into 4 reaction vessels and adding 50 μl of dye solution to each mixture (0, 5, 10 and 20 mM) respectively. The dye was allowed to react with the beads for 60 min after which the dye was washed out and the reaction vessels combined. The process was then repeated for each subsequent dye.
8 Amine modified DNA was coupled to carboxylic acid functionalized particles using standard EDC coupling[9] (carboxylic acid functionality was achieved by first coupling APS to the particles to form amine groups,[10] followed by modification to carboxylic acid groups using adipic acid). A complementary sequence 5′ NH_2-TACAGGCCTCAC-GTTACCTG 3′ and mismatched DNA sequence 5′ NH_2-CAGGTAACGTGAGGCCTGTT 3′ were used. The hybridization was performed by adding 10 μl of a 5 μM solution of 5′ FITC-CAGGTAACGTGAGGCCTGTT 3′ to the particles in pH7 phosphate buffered saline. The hybridization was performed at 40 °C for 1 hour and after hybridization, the particles were washed three times at room temperature to remove excess fluorescent target.
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