Supplementary Material (ESI) for Chemical Communications

Supporting Information

A Reliable Method For Attaching Biological Molecules To Layer-by-Layer Self-Assemblies

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Materials

Paramagnetic polystyrene microspheres functionalized with epoxy groups were from Dynal-Invitrogen; they had a mean diameter of 4.5 μm (C.V. ~5%) and were supplied at a concentration of ~4 x 10^8 microspheres per ml (30 mg ml^{-1}). Poly(sodium 4-styrenesulfonate) (PSS; MW 70kDa) and aminoproply triethoxysilane (APTES) were from Aldrich. Cyanine-5 *N*-succinimidy ester (Cy5-NHS) was GE healthcare. Branched poly(ethyleneimine) (PEI; MW 750 kDa), Sephadex-G25, 2-iminothiolane (Traut's reagent; HCl salt), succinimidyldithiopropionate *N*-succinimidy ester (SPDP), biotinamidocaproic acid *N*-succinimidy ester (biotin-NHS), monoclonal anti-Cy-5 antibodies, dithiothreitol (DTT), gelatin (from cold water fish skin) and streptavidin were from Sigma. Aminodextran (MW 70 kDa, 16 primary amines per molecule) was from Molecular Probes, Eugene, OR. Silica nanoparticles (Ludox® TM-40; SiNPs) were the kind gift of W.R. Grace & Co, Columbia, MA. Biotinylated nucleic acid probe sequences and Cy-5 labelled target sequences were from Operon, Cologne, Germany; solutions of known concentrations were prepared using the extinction coefficients supplied by Operon. Other reagents were of Analar (or equivalent grade) or higher, and all solutions for LBL self-assembly were prepared in HPLC grade water from Aldrich.

Equipment

Magnetic precipitation of Dynal microspheres was carried out using an MPC-S sample concentrator (Dynal-Invitrogen) and (slow-tilt) rotation of microspheres was carried out on a MX2 sample mixer (Dynal-Invitrogen). Fluorescence images of microspheres were acquired with a Leica DMBL fluorescence microscope equipped with a SPOT 2 camera system (SPOT Diagnostic Instruments, inc, Sterling Heights, MI) and custom filters matched to the emission peaks of Cy-5 and the photoluminescent nanoparticles (PNPs). Cy-5 was excited at 660nm and the PNPs were excited at 400 nm. The image acquisition time was 250 ms. All images were acquired with a 100x oil-immersion objective lens at a total magnification of 1000x. Samples for transmission electron microscopy (TEM) were prepared by adding a drop of the microsphere suspension in methanol to Agar 100 epoxy resin (Agar Scientific) and allowing it to polymerise overnight at 60^{o}C. 70nm sections of the polymerised resin were cut with an ultramicrotome and viewed with a Tecnal Spirit transmission electron microscope at an accelerating voltage of 100kV. Zeta potential measurements were made on microspheres washed and resuspended in HPLC grade water with a Zeta Plus zeta potential analyzer supplied by Brookhaven Instruments Ltd., Redditch, UK.

Assembly of LBL base

In **Step 1** 0.5 ml of 0.5 M NaCl containing 100 mg ml^{-1} PEI was mixed 1:1 (v/v) with a vortexed suspension containing 6 mg of washed epoxy functionalized microspheres in 0.5 ml of 0.5 M NaCl, and rotated overnight at room temperature. At the end of this time the microspheres were washed and sonicated with 1) 4 x 1ml of 0.5 M NaCl; 2) 4x 1ml of 0.1 M sodium borate solution; 3) 4 x 1 ml of 0.1 M sodium acetate pH 4.5; 4) 6 x 1 ml of 0.5 M NaCl. In **Step 2** 100 μg aliquots of these microspheres were resuspended in 0.5 ml of 0.5 M NaCl and mixed 1:1 with a pH 6.0 solution containing 1mg ml^{-1} PSS and 0.5 M NaCl. After rotating for 15 minutes the microspheres were magnetically precipitated and washed with 4 x 1 ml of 0.5 M NaCl. In **Step 3** the microspheres were resuspended in 0.5 ml of 0.5 M NaCl and mixed 1:1 with a pH 8.0 solution containing 1mg ml^{-1} PEI and 0.5 M NaCl and rotated for 15 minutes. At the end of this time they were magnetically precipitated and washed with 4 x 1 ml of 0.5 M NaCl. Additional layers (a total of ten [five bilayers] in this work) were assembled by repeating steps 2 and 3 as required.

Assembly of silica nanoparticles

In **Step 1** 100 μg microspheres with an outer layer of PEI were suspended in 0.5 ml of 0.5 M NaCl and mixed 1:1 with HPLC grade water containing an excess of SiNPs; the pH of this solution was 9.5. They were then rotated for 15

minutes, and magnetically precipitated and washed with 4 x 1 ml of 0.5 M NaCl. In **Step 2** the microspheres were rotated for 15 minutes in 1ml of PEI solution, and then magnetically precipitated and washed with 4 x 1 ml of 0.5 M NaCl. Step 1 was repeated three times and Step 2 was repeated twice, giving microspheres with outer shell of SiNP/PEI/SiNP/PEI/SiNP; mean zeta potentials for these layer were respectively: -43.44 mV; +37.77 mV; -42.39 mV; +36.59 mV; -41.74.

PDP functionalized streptavidin

SPDP (0.154 mg) in 39 µl of DMF was added dropwise to a stirred solution of 7.7 mg of streptavidin in 1.54 ml of PBS (15 mM sodium phosphate, pH 7.4, 0.15 M NaCl). After stirring for 1 hour the PDP-streptavidin (PDP = pyridyldithiopropionyl) was purified by dialysis at 4°C against 3 x 250 ml of PBS. The PDP concentration of the dialyzed solution was determined at 343 nm after reduction with DTT (ε = 8.08 x 10^3 M^{-1} cm^{-1}), and the protein concentration was determined from the absorbance at 280 nm (0.1% = 3.4) corrected for PDP. Results showed that the molar ratio of PDP to protein was 3.1:1.

Coating the microspheres with streptavidin

Microspheres with an outer shell of SiNPs (100 µg) were washed with 4 x 1 ml of ethanol and then rotated overnight in 1 ml of a mixture of ethanol/H_2O/APTES (95:3:2 v/v). At the end of this time the microspheres were washed with 4 x 1ml of ethanol (the mean zeta potential in water at this stage was +61.13 mV) and resuspended in 0.5 ml of bicarbonate solution (0.1 M $NaHCO_3$, pH 8.6). The microspheres were then added to 0.5 ml bicarbonate solution containing 1 mg of Traut's reagent and rotated for 30 minutes. At the end of this time the microspheres were washed with 4 x 1 ml of bicarbonate solution (the zeta potential at this stage was +84.43 mV) and resuspended in 1 ml of PBS containing of 0.5 mg of PDP-streptavidin. After rotating overnight the microspheres were washed with 4 x 1ml of PBS (the mean zeta potential at this stage was –27.13 mV) and rotated in 1 ml of blocking solution (PBS containing 10 mg ml^{-1} gelatin) for 1 hour. The microspheres were then washed with 4 x 1ml of PBS and stored at 4°C.

Fluorescence detection of nucleic acids

Streptavidin-coated microspheres (6µg) were rotated with a biotinylated target sequence (5'-(biotin)-GAGTGAGGTTAATAACCTCATTCAT) in 1ml of assay buffer (PBS containing 0.05% (v/v) Tween-20 and 1 mg ml^{-1} gelatin) for 15 minutes. At the end of this time the microspheres were magnetically precipitated and washed with 2 x 1 ml of PBS-Tween (PBS containing 0.05% (v/v) Tween-20) and then rotated with an excess (100 picomoles) of a complementary sequence labelled with Cy-5 (5'-Cy5-GGAAGGGAGTAAAGTTAAT). After rotating for 15 minutes the microspheres were magnetically precipitated and washed with 3 x 1 ml of PBS-Tween and then imaged with the epifluorescence microscope**.**

Biotinylation of antibodies

Methods for the biotinylation of antibodies and other molecules are described in reference 1. The method used by us to biotinylate anti-Cy5 antibodies was as follows: 0.1 mg of biotin-NHS in 250 µl of dry DMF was added dropwise with stirring to 1mg of antibodies in 5 ml of PBS. After stirring for 2 hours the antibodies were purified by dialysis against 3 x 250 ml of PBS at 4°C.

Synthesis of biotinylated dextrans linked to low molecular weight molecules

This method is based on one previously reported in reference 2: Cy5-NHS and biotin-NHS were dissolved in dry DMSO to final concentrations of 10 and 1 mg ml^{-1}, respectively. This solution (0.2 ml) was added, dropwise with stirring, during a 15-min period, to 10 mg of aminodextran dissolved in 5 ml of bicarbonate solution. After standing overnight at 4 °C in darkness, the solution was loaded onto a column packed with Sephadex G-25 and eluted with PBS.

References

1. G.T. Hermanson, *Bioconjugate Techniques* 1996, Academic Press, San Diego.

2. R. Wilson, C. Clavering, A. Hutchinson, *J. Electroanal. Chem.* 2003, 577, 109-118.