Supplementary Material (ESI) for Chemical Communications

Supporting Information

Synthesis of biotinylated PDP dextrans

A 2.5 mg ml^{-1} solution of biotinamidocaproate N-hydroxysuccinimide ester (Sigma) in dry DMSO was used to prepare a 25 mg ml^{-1} solution of 3-(2-pyridyldithio) propionic acid N-hydroxysuccinimide ester (SPDP; Sigma). This solution (0.16 ml) was reacted with 4 ml of 2 mg ml^{-1} aminodextran (MW 2000 kDa; 351 primary amines per molecule; Helix Research, Springfield, OR) in 0.1 M sodium bicarbonate solution, for 2 hours at room temperature. At the end of this time the solution was dialyzed against water at 4°C. The final dextran concentration was calculated from the original dextran concentration and the increase in volume during dialysis. The corresponding PDP and biotin concentrations were determined with DTT and HABA respectively. Figure 1 shows the UV/vis spectrum of biotin substituted PDP dextran after dialysis. The peaks at 234 and 280 nm are due to PDP; biotin does not make a significant contribution to the spectrum and therefore it was determined by the displacement of HABA from avidin.

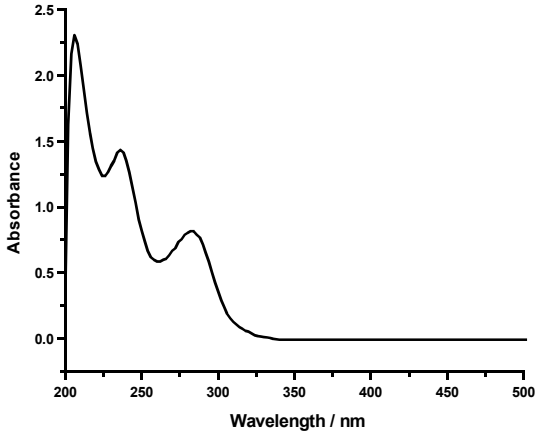

Figure 1 UV/vis spectrum of biotinylated PDP dextran.

Structure of PDP dextran

During dialysis, the dextran solution increased from a volume of 3.82 ml^{-1} to 5.53 ml^{-1}. Therefore the final dextran concentration was 1.38 mg ml^{-1}, or 0.69 μM assuming a MW of 2000 kDa. An aliquot of this solution was diluted 1:4 and assayed for PDP. The concentration of PDP corrected for dilution was 0.23 mM. A second aliquot of solution was assayed for biotin. The concentration of biotin was 10.4 μM. Therefore there were 336 PDP groups and 15 biotins per molecule of 2000 kDa dextran.

Conjugation of biotinylated PDP dextrans to 15 nm gold nanoparticles

Gold NPs of known diameter and number per ml were supplied by BBI International, Cardiff, UK; full details are available on their web site. The 15nm particles had a concentration of 1.4×10^{12} particles per ml. Different amounts of biotinylated PDP were added to a fixed number of GNPs. When high molecular weight PDP dextrans are conjugated to 15 nm GNPs intermediate dextran concentrations produce stable blue colloids; TEM images show that these blue colloids contain clusters of GNPs. Intermediate concentrations of low molecular weight dextrans do not produce stable blue colloids as shown in Figure 2. After diluting 1:3 with 3xPBS (45 mM sodium phosphate, 0.45 M NaCl, pH 7.5) the solutions were passed through a 0.2 μm polyethersulfone (PES) filter (Millipore) and the UV/vis absorbance spectra of the filtrates were recorded. The minimum number of PDP dextran required to prevent any flocculation is taken as the minimum amount that prevents any decrease in absorbance of the filtrate at 520 nm.

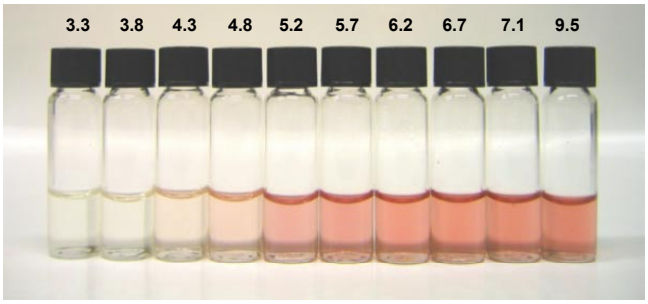

Figure 2 Filtered solutions of GNPs conjugated to 70kDa PDP dextrans. Numerical values indicate the mean number of dextran molecules per particle.

Microbead Assays

Different amounts of biotinylated gold nanoparticles were slow tilt rotated with 200 µg of streptavidin-coated microbeads (0.56 µm diameter; Bangs Laboratories, Fishers, IN) in PBS (containing 1 mg ml^{-1}BSA and 0.5 % Tween-20) for 10 minutes at room temperature. At the end of this time the beads were spun down at 300 g for 15 minutes in PBS containing 0.5 % Tween-20 (twice) and at 9000 g in water (once). The final precipitate was evaporated to dryness in a vacuum centrifuge, resuspended in 25µl of water, and imaged with a document scanner in an in-house multiwell plate. Control experiments were carried out with the same numbers of gold particles conjugated to PDP dextran that was not biotinylated.

PCR protocol

Figure 3 shows the results of microbead assays carried out on GNP conjugates that had been subjected to the PCR protocol. Control experiments were carried out with the same number of gold particles conjugated to PDP dextran that was not biotinylated.

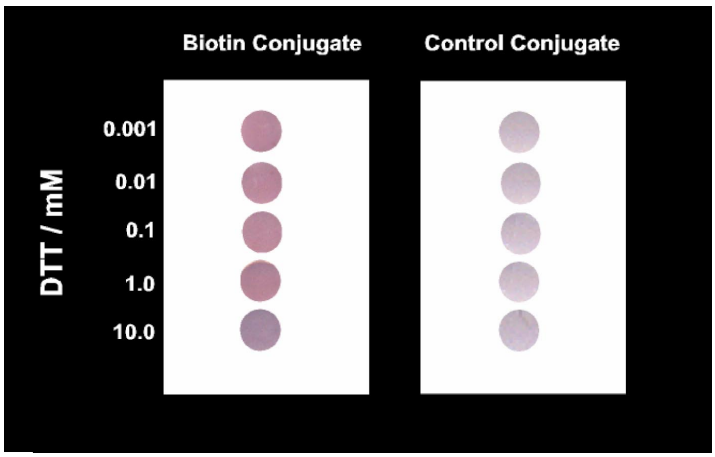

Figure 3 Effect of PCR protocol on affinity of GNP conjugates for streptavidin-coated microbeads.

Hand-In-Glove

The effect of adding increasing amounts of 2000 KDa PDP dextran to a fixed amount of GNPs is shown in Figure 4. In the absence of PDP dextran, or at low ratios of dextran to particles (0.2 dextrans per particle), a black precipitate is formed, which can be removed by passing the solution through a 0.2 μm filter; the filtrates do not contain any particles. As the ratio of dextrans to particles is increased (0.6 dextrans per particle) stable blue solutions that do pass through a 0.2 μm filter are produced. TEM images show that these solutions contain clusters of GNPs. The size of the clusters decreases as the ratio of dextran to particles increases (0.7 and 0.8 dextrans per particle). At the minimum ratio of dextran to particles that prevents any decrease in absorbance (~1 dextran per particle) the solution is monodisperse. The minimum ratio required to prevent any decrease in absorbance depends on the MW of the dextran and square of the particle diameter; the latter is proportional to the surface are of the particles. These observations are consistent with the idea that the minimum ratio of dextran does not produce clusters because it is the lowest amount of dextran required to completely coat the surface of all the particles.

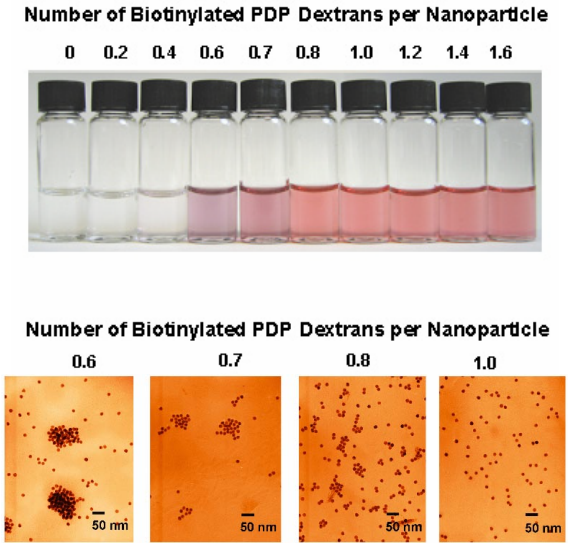

Figure 4 Effect of dextran to particle ratio on dispersity.

One Molecule Per Particle

How do we know that there is only one dextran molecule conjugated to each particle? What the paper actually says is that the minimum number of 2000 kDa dextran molecules required to prevent any flocculation of 15 nm gold nanoparticles corresponds to a mean of 1.05 molecules per particle. This does not mean that one molecule of dextran is conjugated to each particle because not all dextrans molecules or particles are exactly the same size. The values of 2000 kDa and 15 nm refer to the peak molecular weight of the dextran and the mean diameter of the particles respectively. This is why we refer to mean rather than absolute numbers of dextran molecules per particle. When we conjugate PDP dextrans to gold nanoparticles we add a known amount of dextran to a known number of particles, and therefore there is certainly a known ratio of dextran molecules to particles in the solution, but how do we know that all these dextrans are actually conjugated to the particles? If there was unconjugated dextran in the solution then the biotin molecules linked to it would decrease the sensitivity of our microbead assays. To investigate this we carried out microbead assays with biotinylated gold nanoparticles that had been washed by centrifugal precipitation and resuspension. This did not lead to any increase in sensitivity and therefore we conclude that all the dextrans are conjugated to the particles.