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One molecule per particle method for functionalising nanoparticles†

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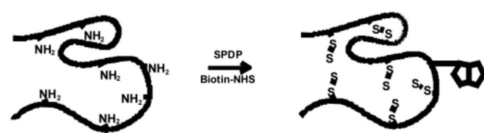
A mean of one biotinylated dextran molecule per particle is conjugated to 15 nm gold nanoparticles, by a process of self-assembly, which depends on the relationship between dextran molecular weight and particle size.

During the last few years the range of molecules that have been conjugated to gold and semiconductor nanoparticles has been extended by synthesizing thiolated derivatives, and conjugating them to the particles by dative covalent bonds.[1,2] Monothiolated oligonucleotides in particular have been conjugated to nanoparticles (NPs) and used in programmed self-assembly, hybridization assays, microarray analysis, enhanced surface plasmon resonance assays, dual labelled probes, and rapid test devices. Although monothiolated molecules are relatively easy to conjugate to nanoparticles they are also known to dissociate, especially at high temperature and in the presence of competing molecules such as mercaptoethanol.[3] Monothiolated oligonucleotides are readily available, but thiolated derivatives of most other probe molecules, such as haptens, are not. By contrast a wide range of molecules that can be covalently attached to primary amines have been synthesized and many of these are available commercially. In this paper we describe a method for conjugating an amine reactive derivative of biotin to a gold NP (GNP), by first linking it to a high molecular weight dextran polymer, and then conjugating the polymer to the particle by a plurality of dative covalent bonds. The number of polymers per particle is controlled by the relationship between the molecular size of the polymers and the surface area of the particles. This relationship allows a known number of biotin molecules to be conjugated to each particle. The specificity of the functionalized particles, and their ability to tolerate high temperature in the presence of competing thiols, is demonstrated in reactions with streptavidin-coated microbeads.

The method for functionalizing nanoparticles is shown schematically in Fig. 1. In the first step 2000 kDa aminodextran was functionalized with pyridyldithio propionate (PDP) and biotin by reacting it with the NHS (*N*-hydroxysuccinimide) esters of 3-(2-pyridyldithio) propionic acid (SPDP) and biotinamidocaproic acid (both from Sigma). The amounts of PDP and biotin in the purified product were determined with dithiothreitol (DTT)[4] and 4′-hydroxyazobenzene-2-carboxylic acid (HABA)[5] respectively. The final dextran concentration was calculated from the original concentration, and the volume of solution before and after purification. This allowed the mean numbers of PDP and biotin per dextran molecule to be calculated. The proposed structure of the biotinylated PDP dextran based on the results of these calculations is shown in Fig. 2.

Dextrans are long flexible polymers of D-glucose. Although there is some branching, molecules with MW of 2000 kDa have micrometer dimensions when extended. In the second step of the method the minimum amount of biotinylated PDP dextran required to prevent flocculation of 15 nm citrate-stabilized GNPs in the presence of PBS, (see ESI† for details), was conjugated to the particles. The dextran is bound to the particles by a plurality of dative covalent bonds that form when the disulfide bonds in PDP are broken on contact with the gold. Unlike certain other conjugation methods, which take more than two days,[1] this step is complete in a few seconds. The minimum amount of dextran required to stabilize the particles was found by adding different amounts of PDP dextran to a fixed amount of 15 nm citrate stabilized GNPs, followed by PBS. In the absence of sufficient dextran the solution changed color from red to purple due to flocculation of the GNPs. Flocculated particles were removed by passing the solution through a 0.2 μm PES filter. An image of the filtered particles and a plot of filtrate absorbance against the amount of dextran added is shown in Fig. 3. The minimum amount of dextran required to prevent flocculation corresponded to a mean of 1.05 dextran molecules per 15 nm particle. To our knowledge this is the first time that the similarity in size between biological molecules and nanoscale particles has been exploited in this way. Our results have also shown that for a given MW of PDP dextran there is a linear relationship between the minimum number of molecules required to prevent flocculation and the square of the particle diameter. This dependence on the square of the diameter suggests that the surface of a particle is enveloped by the dextran like a hand inside a glove. GNPs functionalized with this minimum

1) Functionalisation



2) Self-Assembly

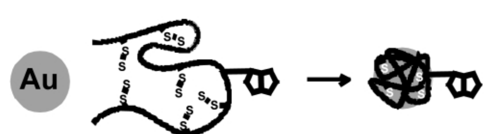


Fig. 1 The two-step conjugation method.

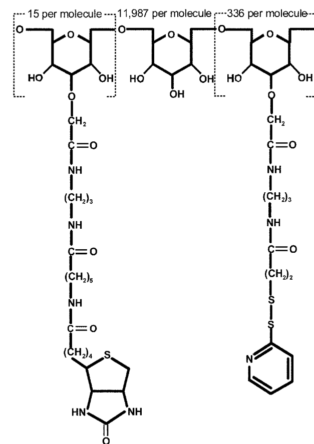


Fig. 2 Structure of 2000 kDa biotinylated PDP dextran.

† Electronic supplementary information (ESI) available: experimental details. See http://www.rsc.org/suppdata/cc/b4/b402786h/

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"hand-in-glove" amount of dextran were used without further purification in the following experiments.

One aim of our work is to prepare nanoparticle conjugates that can be used in biomolecular assays. For this purpose it is important that probe molecules conjugated to the particles are able to react specifically with the corresponding binding molecule. The interaction of biotin (vitamin H) with avidin or streptavidin is one of strongest not-covalent affinities known. The strength of this interaction has led to its widespread use for specific targeting applications in biomolecular assays and self-assembly. To investigate the targeting properties of our particles we mixed different amounts with a fixed amount of white streptavidin-coated microbeads. Control experiments were carried out by incubating the beads with particles that were functionalized with PDP dextran, but not biotin. After 10 minutes the beads were washed, concentrated, and transferred to an in-house multiwell plate where they were imaged with a document scanner; the results are shown in Fig. 4. The pink color of the beads increased as the amount of biotin GNP conjugate increased, but the control beads remained white. This result shows that the functionalized particles bind specifically to streptavidin.

It is well known that monothiolated molecules conjugated to GNPs can dissociate, especially at high temperature in the presence of competing thiols such as mercaptoethanol and DTT.[3] These conditions are encountered during the polymerase chain reaction (PCR) and up until now there are no reported examples of functionalized GNPs that are stable under these conditions.[6] This has severely limited the range of applications for which GNPs are useful compared with otherwise inferior labels such as fluorescent dyes. The bond energy for molecules conjugated to GNPs by single bond between sulfur and gold is in the order of -30 to -40 kJ mol^{-1},[7] which corresponds to equilibrium constants (K_a values) of 0.2–10.4 $\times 10^6$ M^{-1}. For comparison the K_a value for the biotin avidin interaction is 7.6×10^{14} M^{-1}. Clearly a situation in which a molecule is more tightly bound to the target molecule than to the particle is unsatisfactory. The stability of functionalized nanoparticle can be increased by conjugating probe molecules to the particles by more than one bond.[8] In the absence of intramolecular effects the K_a value for a molecule conjugated by n identical bonds is equal to $(K_a)^n$. The method described in this paper takes advantage of this effect by conjugating molecules to GNPs by a plurality of dative covalent bonds. To investigate their stability we exposed them to the conditions that are encountered during a typical PCR protocol. The buffer (30 mM tricine, pH 8.4, 2 mM MgCl) contained DTT up to a maximum concentration of 10 mM. The functionalized particles were heated to 94.5 °C for 1 minute, and then for 35 cycles of 94.5 °C (30 seconds), 37 °C (30 seconds) 72 °C (30 seconds), and finally for 10 minutes at 72 °C; the total time for the PCR protocol was 132 minutes. After allowing the solutions to cool, microbead assays were carried out as described above; a decrease in the number of biotins per particle would result in a decrease in the number of particles bound to the beads. Results showed that the particles were stable at DTT concentrations up to 1mM. At a DTT concentration of 10 mM the solution became slightly blue, indicating that some flocculation had occurred, but the particles still bound specifically to the beads. Biotinylated particles maintained at room temperature remained red and bound to the beads without loss of color at all the DTT concentrations that were studied. We expect that even more stable conjugates could be prepared by increasing the number of PDP groups per molecule of dextran.

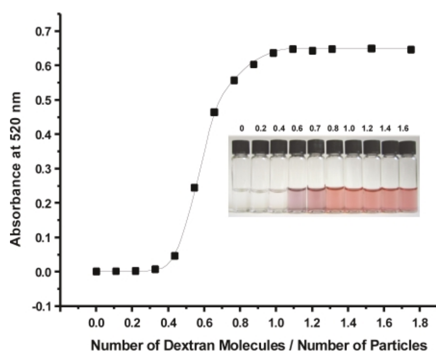

Fig. 3 Absorbance of filtrates at 520 nm showing how a minimum of 1.05 biotinylated PDP dextrans are required to prevent any decrease in absorbance. Inset: filtered GNPs; numerical values indicate number of dextrans per particle.

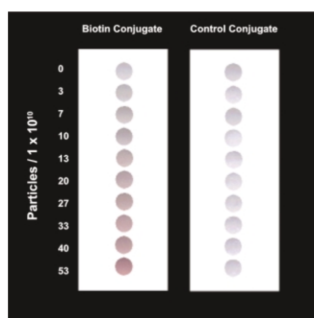

Fig. 4 Image of multiwell plate showing how colour of beads changed when they were incubated with increasing amounts of GNP conjugate.

In addition to biotin, we have used the method described here to conjugate a range of other molecules, including haptens, antibodies and oligonucleotides, to GNPs and semiconductor quantum dots. The method differs from other nanoparticle conjugation methods because the entire surface layer of the functionalized particle is synthesized prior to conjugation. This allows the surface layer to be purified and characterized at high concentration in the absence of interference from the particles themselves. The surface layer is then conjugated to the particles by a process of self-assembly, in which the number of polymer molecules per particle is determined by the relationship between polymer and particle size. Because the numbers of probe molecules per molecule of polymer and the number of polymer molecules per particle are both known, the number of probe molecules per particle is also known. Previous work has shown that the number of probe molecules per nanoparticle is important in optimizing the sensitivity of biomolecular assays, and at present we are using our method to optimize the sensitivity of several nanoparticle-based bioassays. We have also used it to prepare nanoparticles functionalized with known ratios of more than one different molecule per particle, which we expect to be useful in other areas of nanotechnology such as programmed self-assembly.

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