Supporting Information

Synthesis of PDP-dextran-oligo

A 25 mg ml^{-1} solution of C6-succinimidyl 4-hydrazinonicotinate acetone hydrazone (C6-SANH; Solulink, San Diego, CA) 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} 70 kDa aminodextran[1] (22 primary amines per molecule of dextran; Molecular Probes, Eugene, Or) in PBS, for 2 hours at room temperature. The dextran was purified on Sephadex G-25 with 0.1 M sodium acetate buffer (pH 4.5) as the eluent. For other reports describing the synthesis of PDP dextrans functionalized with bio-specific probes see references 2 and 3. An excess of oligonucleotide with a 5'-terminal aldehyde group (Solulink) was reacted with hydrazone functionalized PDP-dextran in acetate buffer, overnight at $4^{o}C$. Then unreacted oligonucleotide was removed with a 30 kDa MWCO centrifugal concentrator (Millipore); the washing solution was ice-cold HPLC grade water. The UV/vis spectrum of the oligonucleotide functionalized PDP dextran (PDP-dextran-oligo) is shown in Figure 1A. The peak at 360 nm corresponds to hydrazone bonds between the oligonucleotide and the dextran ($\varepsilon_{360} = 1.8 \times 10^4$). Most of the absorbance at 280 nm is due to oligonucleotides ($\varepsilon_{260} = 1.63 \times 10^5$), but separate determination with dithiothreitol (DTT)[4] showed that the remainder (13%) was due to PDP. The structure of the PDP-dextran-oligo is shown in Figure 1B.

Gold NP titration

Variable amounts of PDP-dextran-oligo were mixed with 0.75 ml of 9.3 nm GNPs (7 x 10^{12} particles per ml; BBInternational, Cardiff, UK) and then the solution was diluted 1:3 with pH 7.4 buffer solution to give final concentrations of 15 mM phosphate and 0.15 M

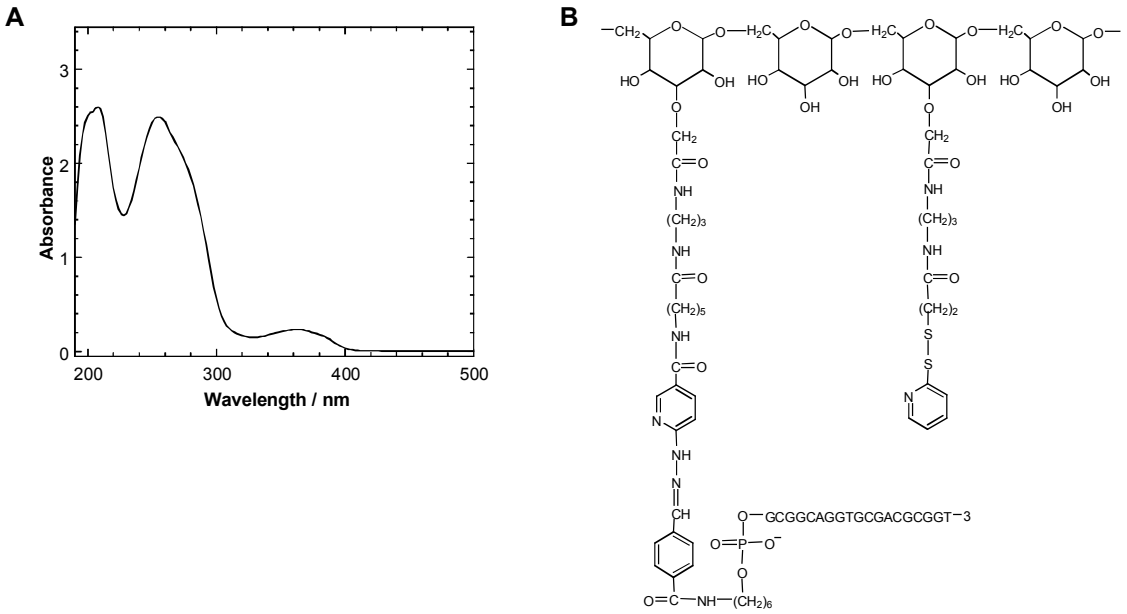

Figure 1 A) UV/vis spectrum of PDP-dextran-oligo; **B)** Structure of PDP-dextran-oligo. One 70 kDa dextran molecule is a mainly linear polymer (there is some branching) of 431 anhydroglucose monomers, of which a mean of 17 were derivatized with PDP and with 4 with oligonucleotides.

NaCl. The solutions were passed through a 0.2 µm PES filter (Millipore) and the UV/vis absorbance spectra of the filtrates were recorded. The absorbance at 520 nm was plotted against the ratio of oligonucleotides per particle in the solution prior to filtration. The minimum amount of PDP-dextran-oligo required to prevent flocculation was taken as the minimum amount that did not result in a decrease in absorbance at 520 nm. This corresponded to a mean of 29 oligonucleotides per particle.

Silver NP titration

Variable amounts of PDP-dextran-oligo were mixed with 0.75 ml of 20 nm Silver NPs (7 x 10^{10} particles per ml; BBInternational) and then the solution was diluted 1:3 with pH

7.4 buffer solution to give final concentrations of 15 mM phosphate, 0.15 M NaCl. The particles were not filtered because (unlike gold) there was no spectral interference between dextran-coated and flocculated particles.

DNA detection with gold nanoparticles

The target oligonucleotide was captured on microbeads by incubating (slow-tilt rotating) 200 μg aliquots of streptavidin-coated beads (560 nm diameter, Bangs Laboratories, Fishers, IN) with different amounts of 5'-terminal biotinylated oligonucleotide. Based on the manufacturers data sheet each aliquot of beads could bind a maximum of 218 pmols of biotinylated oligonucleotide. The amount of oligonucleotide incubated with the beads was always less than the maximum binding capacity of the beads. This incubation step is identical to the procedure used to isolate target sequences from polymerase chain reaction products prior to quantification.[5,6] The sequence of the target oligonucleotide was complementary to the sequence conjugated to the gold nanoparticles. Any unbound oligonucleotide was removed from the beads by centrifugal precipitation at 9000 g in PBS, containing 0.05% Tween-20, four times. The beads were then slow-tilt rotated with excess oligo-GNP conjugate in hybridization solution (10 mM sodium citrate, pH 7.0, containing 0.5 M NaCl, 1 mg ml^{-1} BSA, 1 mg ml^{-1} glucose and 0.5% Tween-20) for 1 hour at room temperature. Then the beads were washed for 15 minutes at 300 g in PBS (three times) and at 9000 g in water (once). The final precipitates were evaporated to dryness in a vacuum centrifuge, resuspended by sonicating in 21 μl of water, and then imaged in an in-house multiwell plate with a document scanner. Numerical values given in Figures 3B and 4B of the paper are based on the assumption that the amount of

biotinylated oligonucleotide captured by the beads was the same as the amount of oligonucleotide that was incubated with the beads. This assumption is based on the high association constant of the biotin streptavidin binding reaction, but is should be understood that that the numerical values given in these figures refer to the maximum amount of target oligonucleotide bound to the beads. Figure 2 shows TEM images of oligo-GNP conjugate hybridized to beads loaded with 200 pmol of the complementary oligonucleotide. Less than 10 % of the bead surface is covered with GNP conjugates, which suggests that the dynamic range of the microbead assay could be extended for at least one more order of magnitude.

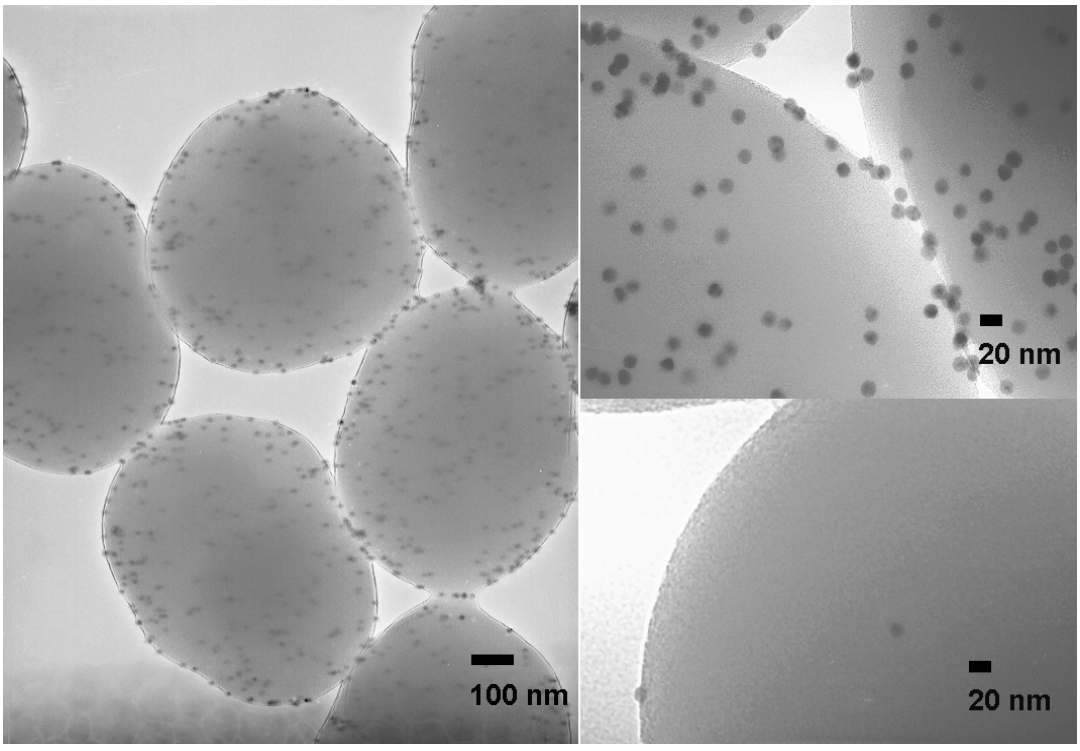

Figure 2 TEM images of oligo-GNP conjugate hybridized to beads loaded with 200 pmol of target oligonucleotide (left and upper right) and no target control bead (lower right).

Mismatch assays

DNA hybridization is not an all-or-nothing process. Under the right conditions sequences with less than 100% homology can form duplexes. In order to distinguish between complementary and mismatch sequences it is necessary to hybridize under high stringency conditions. In this work we increased the stringency of the hybridization buffer by adding formamide and decreasing the ionic strength of the hybridization buffer. Microbeads were coated with the target by slow-tilt rotating the beads with an excess of the biotinylated target (mismatch) oligonucleotide. The beads were then washed to remove unbound target oligonucleotide as described above and slow-tilt rotated with an excess of oligo-nanoparticle conjugate. The hybridization solution was 10 mM sodium citrate, pH 7.0, containing 30 mM NaCl and 70 % (v/v) formamide. It was not possible to separate beads from unbound conjugate by centrifugal precipitation in this solution and therefore a 0.22 μm PTFE centrifugal filter (Millipore) was used. The beads were imaged on the filter after washing away unhybridized GNPs with 0.5% aqueous Tween-20.

DNA detection with silver nanoparticles

The method for silver nanoparticles was the same as for gold, except that the particles could not be separated from the beads by centrifugal washing, and therefore 0.22 μm PTFE filters were used as in the mismatch assays.

A Known Number Of Oligonucleotides Per Particle

We use gold and silver nanoparticles of known size and concentration supplied by BBI. The mean diameter of these nanoparticles has been determined by dynamic light scattering and the number of particles per ml is based on the OD at 520 nm. The concentration of oligonucleotides in the PDP-dextran-oligo solution is known from calculations based on the UV/vis spectra shown in Figure 1. A known volume of particles is mixed with the minimum volume of PDP-dextran-oligo that prevents flocculation in PBS. After mixing the particle concentration and the oligonucleotide concentration are both known, and therefore the mean number of oligonucleotides per particle in the solution can easily be calculated, but are all the oligonucleotides conjugated to the particles? We use this solution for microbead assay without further purification. If there were unconjugated oligonucleotides in the solution they would decrease the sensitivity of these assays. To investigate this we carried out microbead assays with oligonucleotide-functionalized nanoparticles that had been washed by centrifugal precipitation. Purification by this method would remove any unconjugated oligonucleotides, but it does not lead to an increase in sensitivity, and therefore we conclude that all the oligonucleotides are conjugated to the particles.

References

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