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Gold and silver nanoparticles functionalized with known numbers of oligonucleotides per particle for DNA detection†

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The biospecificity of gold and silver nanoparticles, functionalized with known numbers of oligonucleotides, is demonstrated in colorimetric microbead assays for complementary and mismatch sequences.

The convergence of nanotechnology and biology is expected to produce major advances in diagnostics, therapeutics and materials science,[1] but the range of molecules that have been conjugated to nanoparticles is still limited. Recently we have reported a new type of conjugation method in which the entire surface of the NP conjugate is synthesized prior to conjugation.[2] The advantage of this approach is that the surface can be purified and characterized at high concentration in the absence of interference from the particles themselves. The surface is then conjugated to the particles by a process of self-assembly, in which the number of surface molecules conjugated to each particle depends on the relationship between MW of the surface molecules and the diameter of the particles. High stability is possible because the surface molecules are conjugated to the particles by multiple bonds between sulfur and gold.[2] In the previous report we showed how this method could be used to conjugate low MW organic molecules to GNPs, but the NHS ester chemistry used in this work is less suitable for hydrophilic molecules such as oligonucleotides and proteins. In this communication we show how this conjugation method can be extended to hydrophilic molecules using hydrazine/carbonyl chemistry. We also show that the method can be used to conjugate molecules to silver NPs.

The conjugation method is shown in Fig. 1. In the first step of the method molecules of 70 kDa aminodextran (Molecular Probes, Eugene, OR) were functionalized with hydrazone and disulfide groups by reacting them with C6-succinimidyl 4-hydrazinonicotinate acetone hydrazone (C6-SANH; Solulink, San Diego, CA) and 3-(2-pyridyldithio)propionic acid *N*-hydroxysuccinimide ester (Sigma) respectively, followed by purification on a gel-exclusion column. In the second step of the method the hydrazone functionalized PDP (pyridyldithiopropionyl) dextran was reacted with an excess of oligonucleotide terminating in an aromatic aldehyde group (Solulink). Unreacted oligonucleotide was removed with a centrifugal concentrator. In the third step of the method the oligonucleotides were conjugated to 9.3 nm citrate-stabilized GNPs of known concentration (5.7×10^{12} particles per ml; BBInternational, Cardiff, UK) by mixing them with the minimum amount of PDP-dextran-oligo required to prevent flocculation in the presence of PBS. The PDP-dextran-oligo is bound to the particles by bonds between sulfur and gold that form when the disulfide bonds in PDP are ruptured on contact with gold.[3] In the absence of enough PDP-dextran-oligo the solutions changed colour from red to purple when PBS (15 mM sodium phosphate, 0.15 M NaCl, pH 7.5) was added, due to flocculation of the particles. Flocculated particles were selectively removed by passing the solution through a 0.2 μm filter. A plot of filtrate absorbance at 520 nm against the amount of oligonucleotide in PDP-dextran-oligo added is shown in Fig. 2. The minimum amount of oligonucleotide in the PDP-dextran-oligo required to prevent any decrease in absorbance due to flocculation corresponds to a mean of 29 oligonucleotides per particle. Once the minimum amount of PDP dextran required to prevent flocculation has been determined larger volumes of conjugate can be prepared by mixing equivalent ratios of dextran and particles. GNPs functionalized with this minimum amount of PDP-dextran-oligo were used without further purification in the following assays.

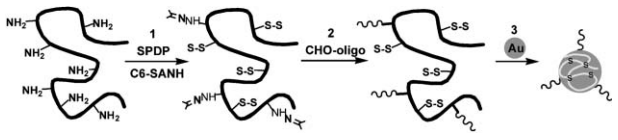

Fig. 1 The three-step conjugation method.

The immediate goal of our work is to prepare oligonucleotide functionalized nanoparticles that can be used for microbead and microarray analysis. For this purpose it is important that the oligo-GNP conjugate is able to hybridize specifically with the complementary oligonucleotide. To investigate this we carried out a microbead assay of the kind used to capture and quantify PCR products.[4] Different amounts of target oligonucleotide were captured on white streptavidin-coated microbeads, and then the washed beads were incubated with excess oligo-GNP conjugate (Fig. 3A). After one hour the beads were washed, concentrated and transferred to an in-house multiwell plate where they were imaged with a document scanner. The results (Fig. 3B) show that less than 500 fmol of the complementary oligonucleotide can be distinguished with the unaided eye. We also investigated the specificity of the GNP conjugate for base mismatch sequences. Although two-base mismatch sequences could be distinguished without difficulty, we were unable to distinguish single base mismatch-sequences even under stringent hybridisation conditions (Fig. 3B).

Recently there has been considerable interest in the use of silver nanoparticles as enhancers for surface enhanced Raman scattering (SERS).[5] This enhancement arises from the intense localized fields

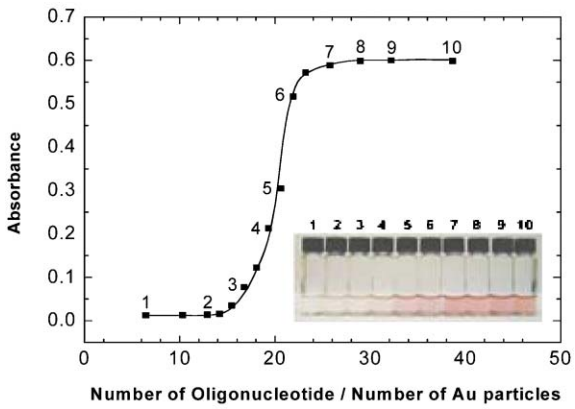

Fig. 2 Absorbance at 520 nm of filtered oligo-GNP solutions showing how the minimum amount of PDP-dextran-oligo required to prevent any decrease in absorbance corresponds to 29 oligonucleotides per nanoparticle. Inset: image of filtered solutions; numbers correspond to numbered points on the graph.

† Electronic supplementary information (ESI) available: experimental section. See http://www.rsc.org/suppdata/cc/b4/b411181h/

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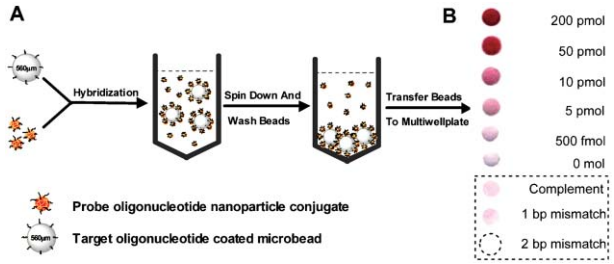

Fig. 3 (A) Schematic diagram of microbead assays. (B) Image of washed and filtered microbeads showing the effect of target oligonucleotide loading and sequence on colour. The oligonucleotide sequences were 5′ → 3′: GNP conjugate: CHO-GCGGCAGGTGCGACGCGGT; complement: biotin-ACCGCGTCGCACCTGCCGC; one base-mismatch: biotin-ACCGCGTCG*G*ACCTGCCGC; two base-mismatch: biotin–ACCG*G*GTCG*G*ACCTGCCGC.

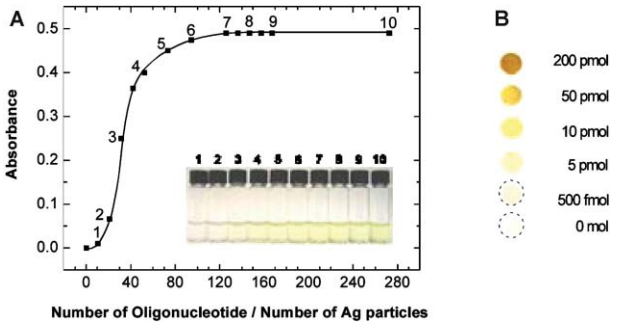

Fig. 4 (A) Absorbance at 408 nm of oligonucleotide silver nanoparticle solutions showing how the minimum amount of PDP-dextran-oligo required to prevent any decrease in absorbance corresponds to oligonucleotides per nanoparticle. Inset: image of unfiltered solutions; numbers correspond to numbered points on the graph. (B) Image of filtered microbeads showing the effect of oligonucleotide loading on colour.

that are associated with surface plasmon resonance in these particles. It allows low numbers of biological binding events to be detected when these result in an accumulation of Raman active molecules at the surface of the particles. Most of the work on silver nanoparticles has been carried out with non-specifically adsorbed molecules, which limits the range of materials that can be studied. To investigate whether our method could be used to conjugate molecules to silver we mixed different amounts of PDP-dextran-oligo with a fixed volume of 20 nm silver nanoparticles of known concentration (7×10^{10} particles per ml; BBInternational, Cardiff, UK). In the absence of enough PDP-dextran-oligo the solutions became colourless when PBS was added as shown in Fig. 4A. A plot of absorbance at 408 nm against the amount of PDP-dextran-oligo added showed that the minimum amount of PDP-dextran-oligo required to prevent any decrease in absorbance corresponded to a mean of 126 oligonucleotides per particle. Microbead assays with particles conjugated to this amount of dextran showed that as little as 500 fmol of complementary oligonucleotide could be detected with the unaided eye, as shown in Fig. 4B. These results demonstrate that oligonucleotides conjugated to silver nanoparticles with PDP-dextrans are suitable for use in biomolecular assays.

In summary we have shown how PDP-dextrans can be used to conjugate oligonucleotides to gold and silver nanoparticles for biomolecular assays. The oligonucleotides were first linked to PDP-dextrans using hydrazine/carbonyl chemistry. The advantages of this chemistry are that both functional groups are stable in aqueous solution for many weeks and the reaction between them can be performed under a variety of conditions that are compatible with biological molecules. We have also used this chemistry to conjugate proteins (antibodies) to gold nanoparticles after activating them with succinimidyl 4-formylbenzoate (Solulink). This suggests that our method can be used to conjugate a broad range of molecules to metal nanoparticles. A particular advantage of the method is that it allows a known number of one or more molecules to be conjugated to the same particle. In the examples described here we have demonstrated how known numbers of oligonucleotides can be conjugated to gold and silver nanoparticles. Previous work has shown that an ability to modulate the number of molecules conjugated to nanoparticles is important when optimising the sensitivity of biomolecular binding assays.[6] We expect that this advantage, along with the ability to conjugate well-defined ratios of two or more molecules (such as an oligonucleotide and a Raman active dye) to the same particle, will have important applications in the new generation of surface enhanced resonance Raman scattering (SERRS) based assays.

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Notes and references

1 C. M. Niemeyer, *Angew. Chem., Int. Ed.*, 2001, **40**, 4128.
2 R. Wilson, Y. Chen and J. Aveyard, *Chem. Commun.*, 2004, 1156.
3 H. B. Lu, C. T. Campbell and D. G. Castner, *Langmuir*, 2000, **16**, 1711.
4 (*a*) C. S. Martin, L. Butler and I. Bronstein, *BioTechniques*, 1995, **18**, 908; (*b*) A. C. Syvanen, M. Bengstrom, J. Tenhunen and H. Soderlund, *Nucleic Acids Res.*, 1990, **16**, 11327.
5 (*a*) K. Faulds, W. E. Smith and D. Graham, *Anal. Chem.*, 2004, **76**, 412; (*b*) D. Graham, W. E. Smith, A. M. T. Linacre, C. H. Munro, N. D. Watson and P. C. White, *Anal. Chem.*, 1997, **69**, 4703.
6 (*a*) K. Okano, S. Takahashi, K. Yasuda, D. Tokinaga, K. Imai and M. Koga, *Anal. Biochem.*, 1992, **202**, 120; (*b*) S. Kubitschko, J. Spinke, T. Bruckner, S. Pohl and N. Oranth, *Anal. Biochem.*, 1997, **253**, 112; (*c*) M. Hall, I. Kazakova and Y. M. Yao, *Anal. Biochem.*, 1999, **272**, 165; (*d*) T. Soukka, H. Harma, J. Paukkunen and T. Lovgren, *Anal. Chem.*, 2001, **73**, 2254.