Supporting Information

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Intercalating Gold Nanoparticles As Universal Labels For DNA Detection

Maryam Mehrabi and Robert Wilson*

Department of Chemistry, Liverpool University, Liverpool L69 7ZD, UK.
Fax: +44 (0)151 794 3588; E-mail: R.Wilson@liv.ac.uk

The following methods describes how to prepare GNPs conjugated to a known number of intercalators

Characterization of GNPs

The GNPs used in this work were obtained from a commercial source. Each batch is prepared from a known mass of gold (in gold (III) chloride) and characterized by TEM. If the amount of gold used to prepare the particles and the mean diameter is known, the number of particles in a given volume of can be calculated using a value of 1.7×10^{-2} cubic nanometers for the volume occupied by one atom of gold. The particles used in our work were supplied by a commercial source with a data sheet that gave: 1) the mean diameter of the particles (10.0 nm; CV% < 10) and, 2) the absorbance of these particles at a known number of particles per ml (10nm GNPs at 5.7×10^{12} particles per ml have an absorbance of 0.8 at 520 nm). In the remainder of this protocol the number of particles per ml multiplied by 1000 and divided by Avogadro's number (6.02×10^{23}) will be referred to as the GNP concentration.

Synthesis of functionalized dextrans (see also note 1 below).

The method for synthesizing dextrans functionalized with psoralen and pyridyldithiopropionyl (PDP) groups is described in the paper. Briefly, a high molecular weight aminodextran (MW 70 kDa) is reacted with: 1) Succinimidyl-[4-(psoralen-8-yloxy)]-butyrate (SPB), and 2) N-succinimidyl 3-(2-pyridyldithio) propionic acid (SPDP); both of these compounds are N-hydroxysuccinimide esters that form amide bonds when they react with primary amines. The products of these reactions were dialyzed against water to remove any small molecules that were not covalently attached to the dextran. The dextran concentration before dialysis was 43 µM in 3 ml, and the volume after dialysis was 4.68 ml; therefore the dextran concentration after dialysis was ~27.5 µM. Figure I shows the structure of a dextran functionalized with psoralen and PDP.

Characterization of the functionalized dextran

The absorbance spectrum of a 1:10 dilution of a dialyzed solution containing dextran functionalized with psoralen and PDP is shown in Figure II. When dithiothreitol (DTT) was added to this solution the disulfide bonds in PDP were ruptured and pyridine-2-thione was released leading to an increase in absorbance of 0.11 at 343 nm. Pyridine-2-thione has an extinction coefficient of 8.08×10^3 at 343 nm and therefore the concentration of PDP in this solution was 13.2 µM (1). Psoralen has an extinction coefficient of $1 \times 10^4\ M^{-1}cm^{-1}$ at 300 nm (2), but the absorbance spectrum shown in Figure II must be corrected for any contribution due to PDP. PDP has an extinction coefficient of 1.46×10^3 at 300 nm and therefore in this example it contributes only 0.02 to the total absorbance of 0.38 at 300 nm. Therefore the concentration of psoralen in this solution is calculated to be 38 µM.

Titration of functionalized dextran against a fixed number of GNPs

The minimum amount of dextran required to stabilize the particles was found by adding different amounts of functionalized dextran to a fixed amount of GNPs followed by a salt solution. On contact with the particles, the disulfide bonds in PDP rupture and anchor the dextrans to the gold by a plurality of dative covalent bonds. In the absence of enough added dextran the solution changed colour from red to purple when the salt solution was added due to flocculation of the particles. Flocculated particles eventually precipitate, but to facilitate the next step they were removed by passing the solutions through a 0.2 µm microbiological filter. A plot of the absorbance at 520 nm of the filtered solutions against the amount of functionalized dextran added is shown in Figure II. The arrow in Figure II marks the point at which just enough dextran has been added to prevent any decrease in absorbance due to flocculation. If particles mixed with this amount of dextran are purified by centrifugal precipitation their performance in biological assays is identical to particles that have not been purified. This suggests that there are no free competing functionalized dextrans in solution and implies that all of the functionalized dextrans that have been added are attached to the particles. The concentration of psoralen at the point marked by the arrow is 276 nM. Because the concentration of GNPs (5.03 nM) is also known from the absorbance at 520 nm, the mean number of psoralen molecules per particle is: 276 / 5.03 = 55

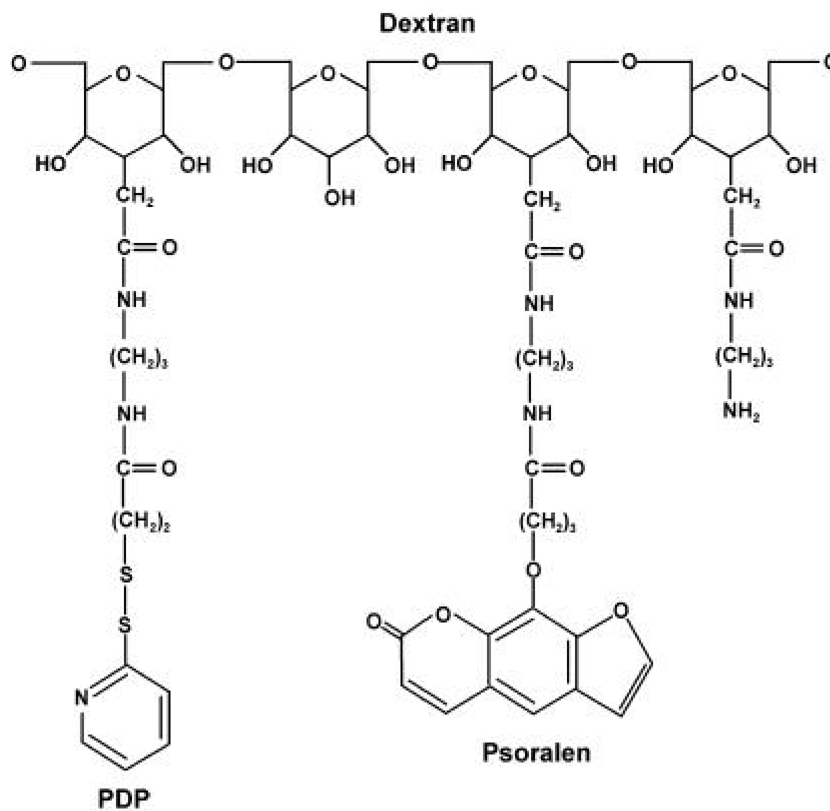

Figure I Structure of aminodextran functionalized with protected disulfide bonds (PDP) and the intercalator psoralen. The mean number of psoralen and PDP functionalities per 70kDa molecule of dextran was 13.8 and 4.8 respectively.

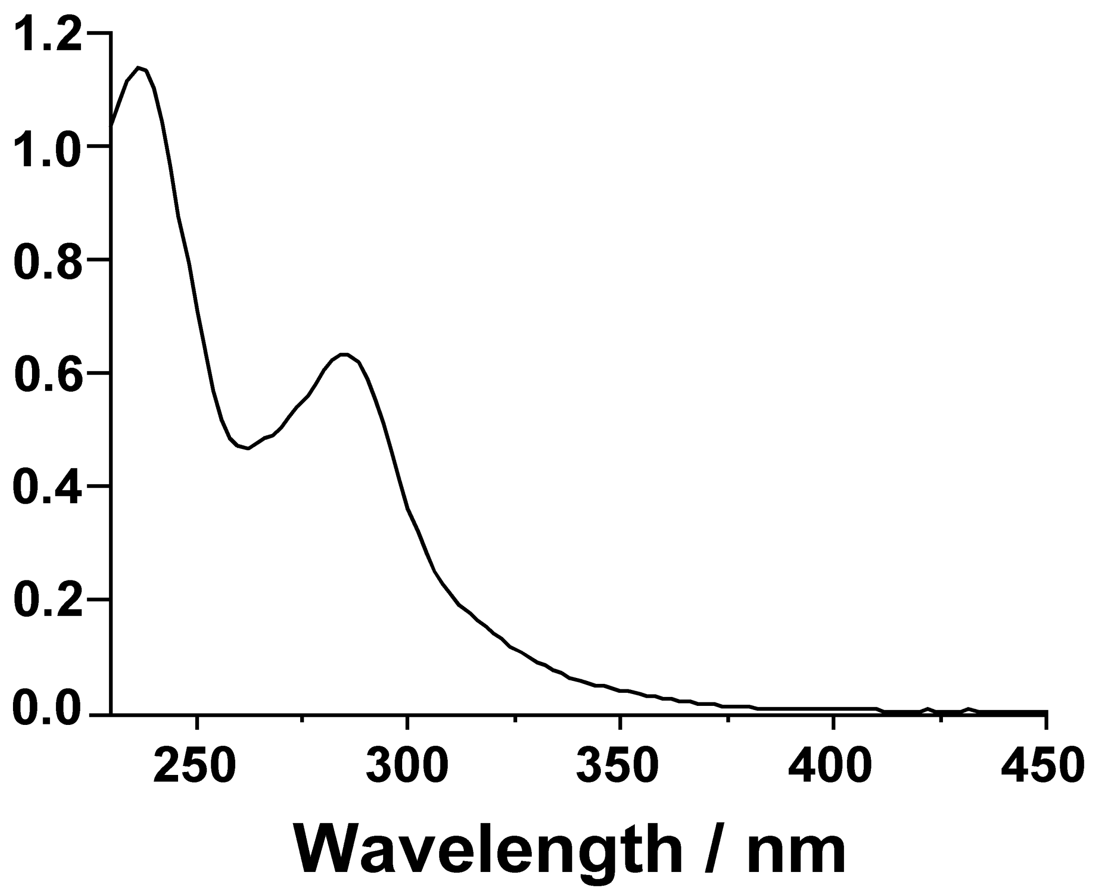

Figure II Absorbance spectrum of dialyzed solution containing dextran functionalized with psoralen and PDP

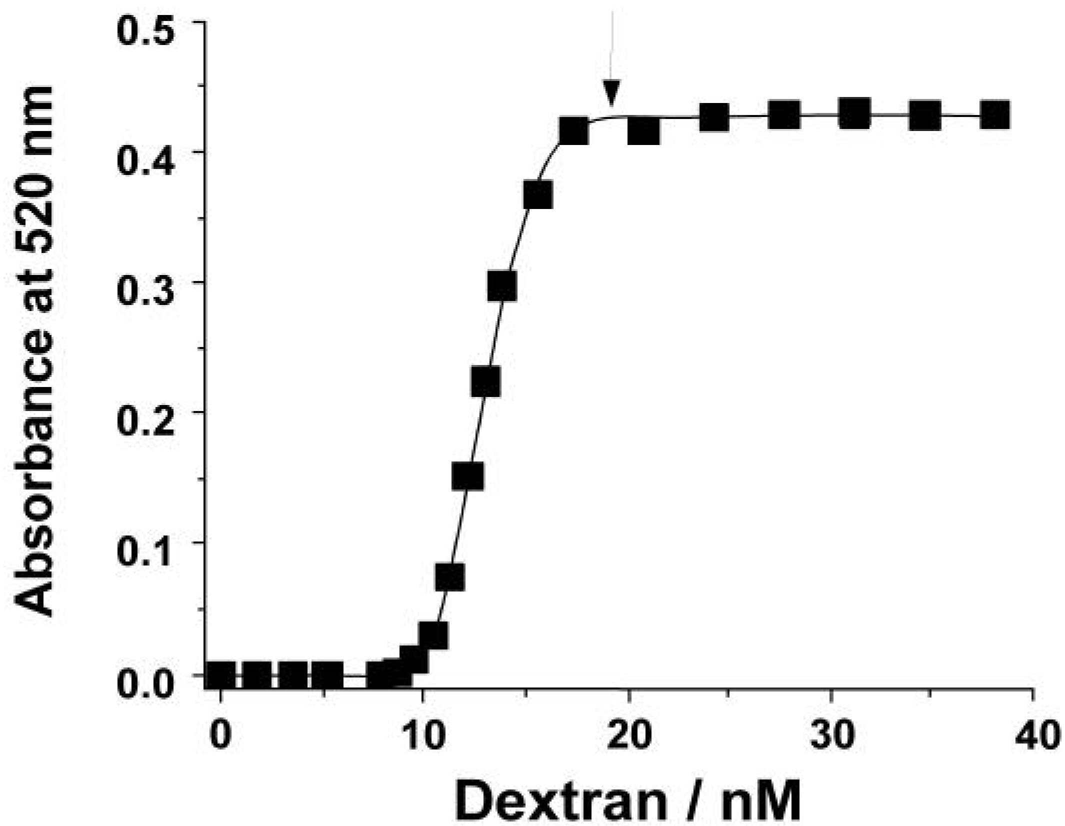

Figure III Graph of showing how the absorbance at 520 nm of filtered 10 nm GNPs solutions increases as more functionalized dextran is added. The arrow indicates the point at which just enough dextran has been added to prevent any decrease in absorbance due to salt-induced flocculation of the particles.

Microsphere assays

The protocol for performing the microsphere assays is shown schematically in Figure IV, and a diagram of the in-house multiwell plate with dimensions is shown in Figure V.

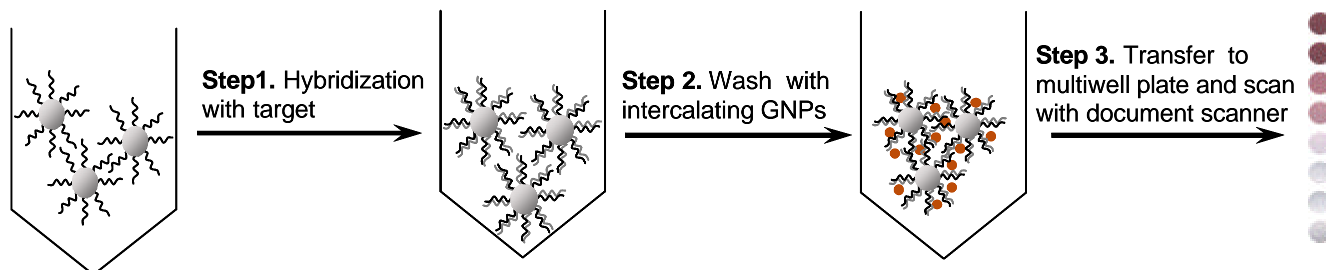

Figure IV Schematic diagram of microsphere-based assay for nucleic acid hybridization products with intercalating GNPs as the label. In **Step 1** the microspheres coated with single stranded capture probes are incubated with the target sequence, and in **Step 2** double stranded hybridisation products are labelled with intercalating GNPs. After labelling (**Step 3**), the microspheres are transferred to an in-house multiwell plate and imaged with an ordinary documents scanner.

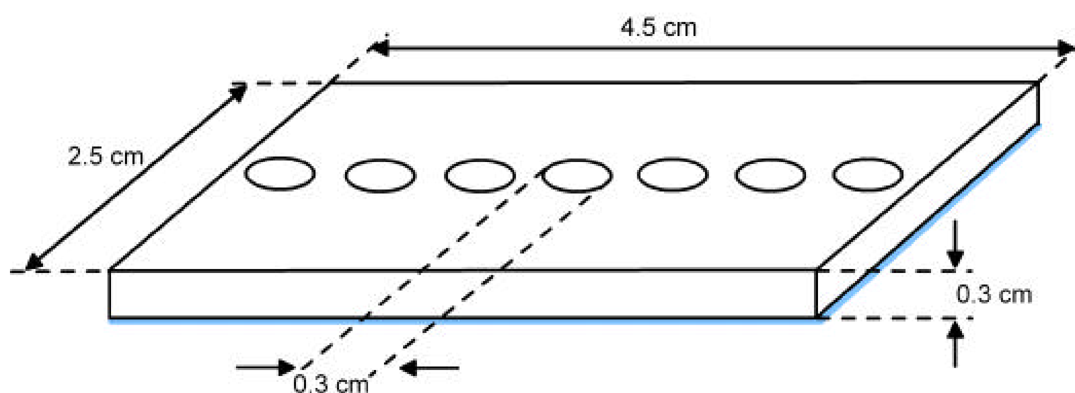

Figure V The multiwell plate was made by drilling a line of 0.3 cm diameter holes in 0.3 cm thick sheet of white PVC. The underside of the plate was sealed with white tape to create the wells; the position of the tape is shown as a blue line in the diagram. Each well is just large enough to hold one aliquot of microspheres suspended in 21 µl of water. After transferring microspheres to the plate the uperside of the wells are sealed with transparent tape. The plate is then inverted (transparent tape facing down) on a document scanner for imaging.

Note for supporting information

1. The amount of psoralen attached to the dextran is varied by changing the amount of SPB used to functionalize the aminodextran.

References for supporting information

1. J. Carlsson, H. Drevin, R. Axén, *Biochem. J.* **1978**, 173, 723-737.
2. S. S. Sastry, B. M. Ross, A. Parraga, *J. Biol. Chem.* **1997**, 272 (6), 3715-3723.