Supplementary Material (ESI) for Chemical Communications

One Step Visual Detection Of PCR Products With Gold Nanoparticles And A Nucleic Acid Lateral Flow (NALF) Device

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Electronic Supplementary Information

Materials

Aminodextran (MW 70 kDa, 16 primary amines per molecule) was from Molecular Probes, Eugene, OR. Succinimidyl 4-hydrazinonicotinate acetone hydrazone (SANH; Solulink, San Diego, CA). 3-(2-pyridyldithio)propionic acid N-hydroxysuccinimide ester (SPDP), bovine serum albumin (BSA), dithiothreitol (DTT) were from Sigma. Thiopropyl Sepharose 4B was from Amersham Biosciences. PCR master mix (BioMix) and agarose were from Bioline, London, UK. Ethidium bromide was from Continental Laboratory Products, San Diego, CA. Oligonucleotides with a 5' terminal aldehyde modification were from Trilink Biotechnologies, San Diego, CA. All other oligonucleotides were from Operon, Cologne, Germany. PBS: 15 mM sodium phosphate, 0.15 M NaCl, pH 7.4. Running buffer: PBS containing 1 mM ethylenediaminetetraacetic acid (EDTA). MES buffer: 0.2 M 2-(N-morpholino)ethanesulphonic acid, 0.3 M NaCl, pH 5. Bicarbonate solution: 0.1 M sodium hydrogen carbonate, pH 8.3. TBE: 45 mM Tris-borate, 1 mM EDTA, pH 8.3. Gold nanoparticles (GNPs) (10, 20, 40, 60, 80, 100 and 150 nm) were prepared by BBInternational, Cardiff, UK. The diameters of the particles were determined using a ZetaPlus analyser (Brookhaven Instruments, Worcestershire, UK) and by transmission electron microscopy (TEM) using a Phillips 410 operating at 80 kV or a Phillips CM12 operating at 100 kV. TEM samples were prepared by placing a drop of GNP solution on a 200 mesh nickel grid (Agar Scientific) and allowing it to dry in air. PCR was carried out with an MJR thermal cycler from MJ Research Inc, MA.

Methods

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 are 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 with a data sheet that gave: 1) the mean diameter of the particles (CV% < 10) and, 2) the absorbance of these particles at a known number of particles per ml (for example, the 10 nm particles had 5.7×10^{12} particles per ml and an absorbance of 0.8 at 520 nm). The concentration of GNPs was determined by multiplying the number of particles per ml by 1000 and then dividing by Avogadro's number (6.02×10^{23}).

Oligonucleotide concentrations

Oligonucleotides were supplied with a molar extinction coefficient at 260 nm. This value was used to determine the concentrations of stock solutions before dilution to the required concentration.

Functionalization of aminodextran and covalent attachment of aldehyde terminated oligonucleotide

Aromatic hydrazide functionalities were introduced into aminodextran by adding 300 µl of 60 mM SANH in dry DMF, dropwise with stirring to 15 mg of aminodextran in 2.4 ml of PBS (Figure S1A). After stirring for 2 hours, protected disulfide groups (3-(2-pyridyldithio)propionyl; PDP) were introduced by adding 300 µl of 60 mM SPDP in dry DMF in the same way. After stirring overnight, unreacted SANH and SPDP were removed by dialyzing the solution against 4 x 1 litre of distilled water at 4 °C for a total of 48 hours. The concentration of protected disulfide groups in the dialyzed solution was determined according to the method of Carlsson *et al.*[1] A 2-fold excess (relative to the original amine content of the dextran) of aromatic aldehyde terminated oligonucleotide (5'CHO-TCT GCT GCC TGC TTG TCT GCG TTC T) in MES was added to the functionalized dextran solution and slow-tilt rotated at 4 °C for 24 hours.

Purification of oligonucleotide-functionalized dextrans

Unreacted oligonucleotides were removed from functionalized dextrans by covalent chromatography [2] on Thiopropyl Sepharose (Figure S1B). Protected disulfide groups in the dextran were reduced to unprotected thiol (-SH) groups by adding 100 µl of DTT in 1 M sodium bicarbonate to 1 ml of oligonucleotide-functionalized dextran and slow-tilt rotating the solution at room temperature for 1 hour. At the end of this time DTT was then removed by gel exclusion on Sephadex G-25. The eluted dextrans were immediately

mixed with 25-fold excess (relative to the original protected disulfide content of the dextran) of washed Thiopropyl Sepharose 4B gel and slow-tilt rotated at room temperature for 1 hour. Unreacted oligonucleotides were removed from the functionalized dextrans by washing the gel with running buffer. Bound oligonucleotide-functionalized dextrans were released from the gel with 10 mM DTT in bicarbonate solution and DTT was removed from the released dextrans by dialyzing against 3 x 2 litres of distilled water for 48 hours. For conjugation to GNPs (Figure S1C), the minimum amount of oligonucleotide-functionalized dextran required to prevent salt-induced flocculation of GNPs was determined as described in previous methods.[2,3] To purify and concentrate the GNPs to the required OD, GNPs were centrifugally precipitated for 10 minutes and then resuspended in PBS containing 1 mg ml^{-1} BSA and 0.5 % Tween 20**.**

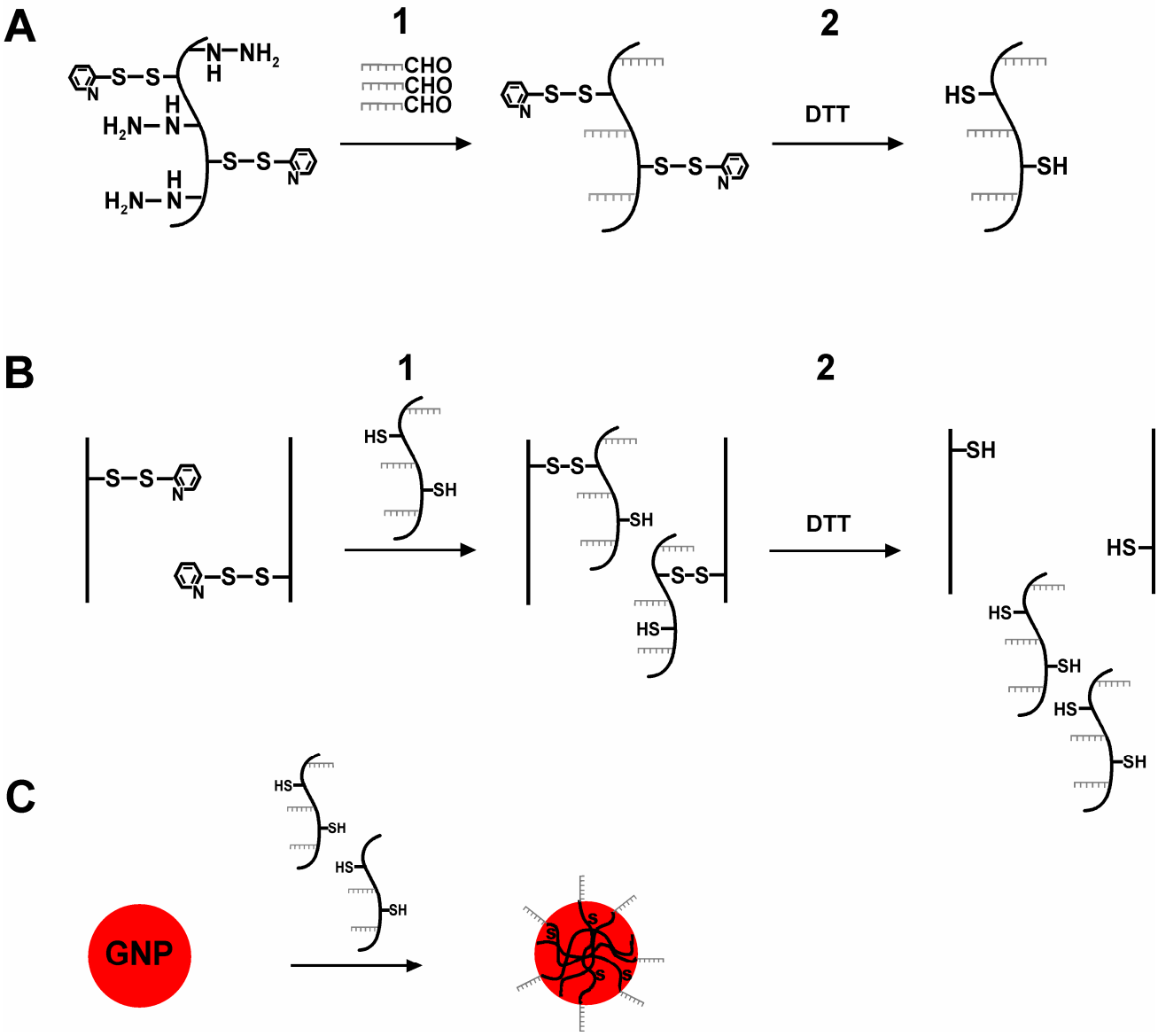

Figure S1: Preparation of reporter oligonucleotide-GNP conjugates. **A)** In step **1** aldehyde-terminated oligonucleotides are covalently attached to dextrans functionalised with aromatic hydrazides and protected disulphide bonds, and in step **2** disulphide

bonds in dextran are reduced to thiols (-SH) with DTT. **B)** In step **1** reduced dextrans are extracted by covalent attachment to Sepharose functionalised with disulphide bonds, and in step **2** purified dextrans are eluted with DTT. **C)** Purified dextrans are conjugated to GNPs via a plurality of dative covalent bonds as previously reported.

Lateral flow detection of DNA

Lateral flow devices were prepared by striping tailed capture oligonucleotides onto pre-assembled, pre-blocked Hi-Flow plus nitrocellulose membrane (Millipore, UK) using a Kinematic Matrix 1600 (Kinematic Automation, CA) and cut into 40 mm x 3 mm strips using a Kinematic Matrix 2360 automatic cutter. For detection of the 32 base target sequence (5'-GGGACTGACGATTCGGGTGATATCCAGAACGCAGACAAGCAGGCA) chromatographic devices were inserted into 50 µl of a freshly prepared 1:1 mixture of the target sequence and 2 OD oligonucleotide functionalized GNPs (both in PBS) and developed for 15 minutes. PCR products were detected in the same way except that PCR products were diluted 1:1 with GNPs in PBS. Images of all lateral flow strips were acquired with an office document scanner and then imported into iGrafx Image 1.0 (Bournemouth, UK) and converted to greyscale. The depth of colour was determined on a scale of 0 – 255 by activating the "view → information" option and pointing the mouse cursor at the area to be interrogated.

PCR

The template sequence (5'-AGAGTTTGATCCTGGCTCAGTCTGCTGCCTGCTTGT CTGCGTTCTGGATATCACCCGATTAGA TACCCTGGTAGTCC), comprised a 32 mer sequence (underlined) nested between two 20 mer sequences. The 32 mer sequence was the same as the reporter oligonucleotide linked to the capture oligonucleotide, the 20 mer sequence at the 5' end was the same as the forward primer (5'-AGAGTTTGATCCTGGCTCAG) and the 20 mer sequence at the 3'end was the complementary to the reverse primer (5'-GGACTACCAGGGTATCTAAT). The PCR mixture comprised 25 µl of master mix, 20 pmol of reverse and 100 pmol of forward primer. The PCR protocol was an initial denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 °C for 45 s, and completed with a final extension at 72 °C for 2 min. The product was analyzed by electrophoresis on 2.5% agarose with TBE containing 0.5 µg/ml ethidium bromide as the buffer.

Supplementary Information Results

Figure S2 shows images of nucleic acid lateral flow devices that have been developed with different diameter GNPs in the range 10 – 150 nm and Figure S3 shows how the amount of colour developed by these devices depends on the amount of nucleic acid target sequence.

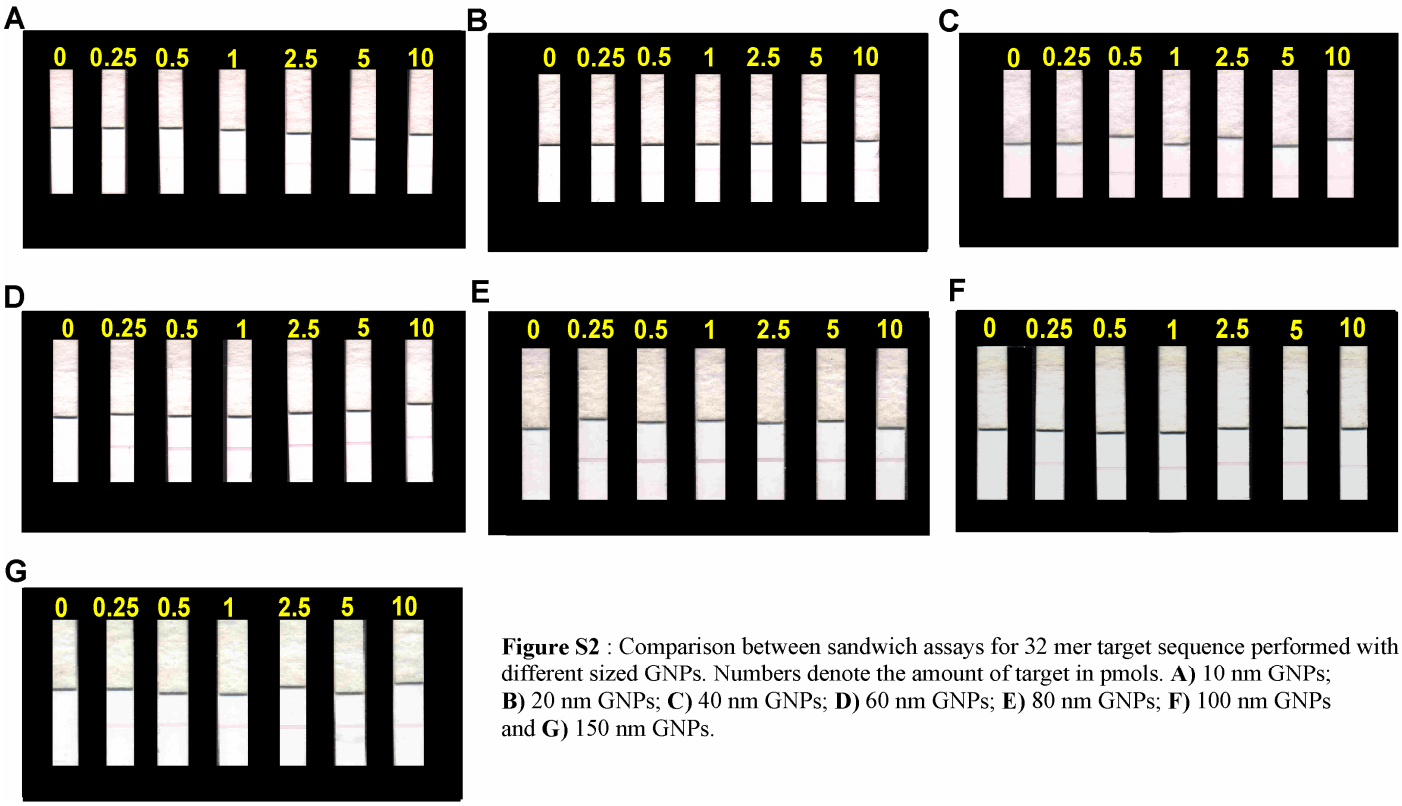

Figure S2 : Comparison between sandwich assays for 32 mer target sequence performed with different sized GNPs. Numbers denote the amount of target in pmols. **A)** 10 nm GNPs; **B)** 20 nm GNPs; **C)** 40 nm GNPs; **D)** 60 nm GNPs; **E)** 80 nm GNPs; **F)** 100 nm GNPs and **G)** 150 nm GNPs.

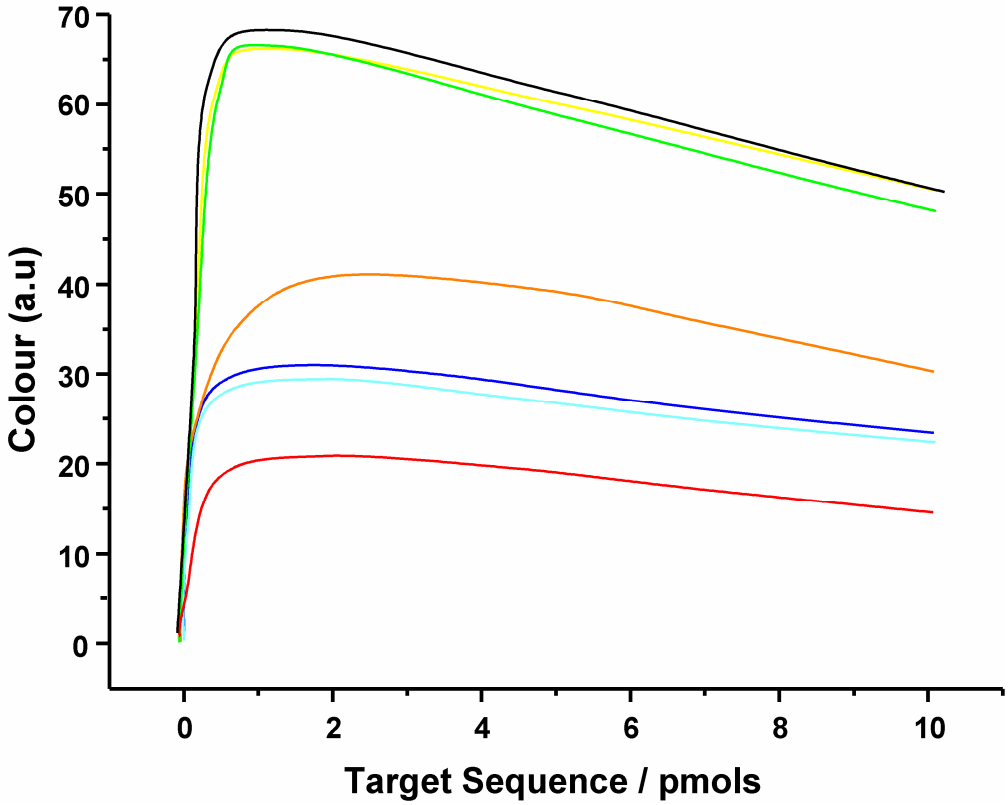

Figure S3: Graph showing the effect of GNP diameter on the amount of colour developed at the test-line of nucleic acid lateral flow devices. Key: Red= 10 nm; Light blue= 20 nm; Dark blue= 40 nm; Yellow= 60 nm; Black= 80 nm; Green= 100 nm; Orange= 150 nm.

Supplementary Discussion On The Effect Of Gold Nanoparticle Diameter On Sensitivity

Our results show that the sensitivity of lateral flow devices can be enhanced by using 80 nm diameter GNPs instead of the more commonly used 40 nm particles. The reason for this is not obvious, because the total absorbing power of GNPs (at 520 nm) on every lateral flow device was the same. This means that if all the 10 nm GNPs on a device were bound to the test line then the amount of colour developed would be the same when if all the 80 nm particles on a device were bound. The explanation for why more colour is developed by 80 nm GNPs depends on the number of particles on the device rather than their total absorbing power. Figure 4C in the paper shows that there are eight times as many 40 nm GNPs as there are 80 nm GNPs in the same volume of suspension, even though the absorbance at 520 nm is the same.

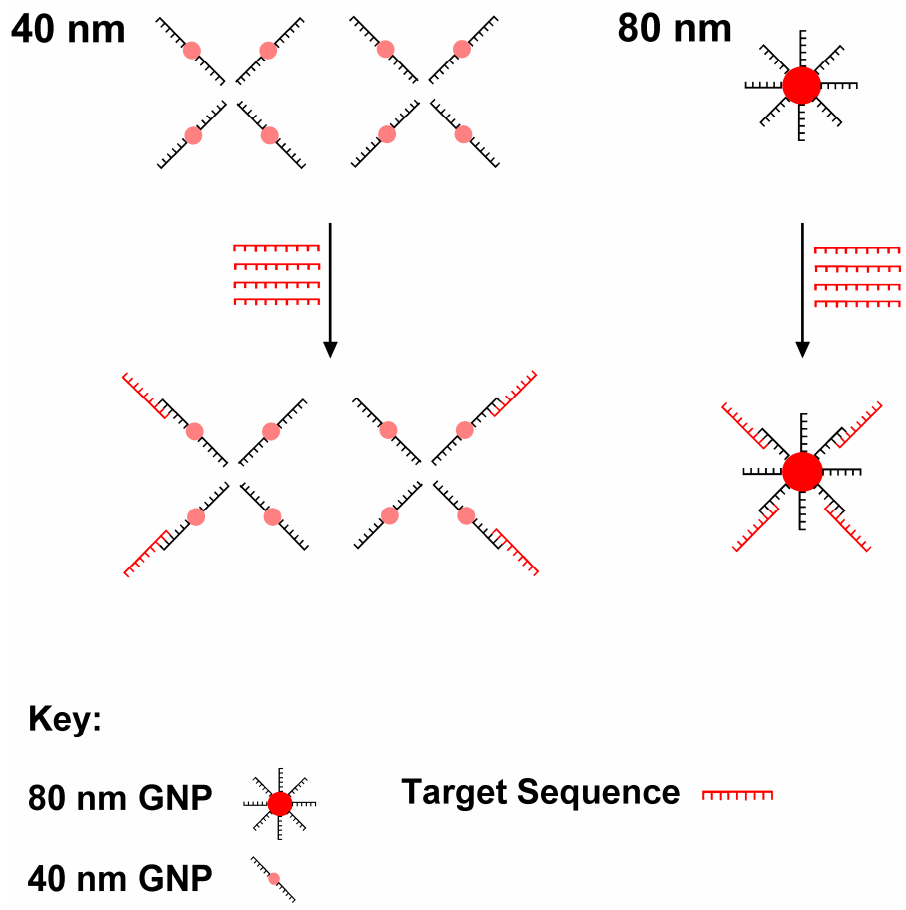

Figure S4: There are eight times as many 40 nm GNPs as 80 nm particles in a suspension that has the same volume and the same absorbance. Therefore, when they are mixed with the same number of target sequences, each 80 nm particle is hybridized to a mean of 8 times as many target sequences as each 40 nm particle.

Figure S4 shows schematically what happens when the same numbers of target oligonucleotides are mixed with suspensions of 40 and 80 nm GNPs that have the same volume and the same absorbance. Under well-mixed conditions the target sequences are evenly distributed among the reporter probes conjugated to the particles. Each 80 nm particle is hybridised to a mean of eight times as many target sequences as each 40 nm particle. Figure S5 shows what happens when these particles migrate through the test line of a lateral flow device. Even if it is assumed that any particle hybridised to a target sequence will also hybridise to a capture probe some 40 nm particles migrate through the test line without binding. In practice, the probability that a given particle will bind is related to the number of target sequences hybridised to its surface. This can be understood by thinking of each particle as a ball coated with Velcro hooks rolling across a test line composed of some adhesive material such as wool: a ball with only one hook has a low probability of binding, but a ball coated with many hooks has a high probability of binding. This additional effect explains why the amount of colour developed by 40 nm particles is less than half of the colour developed by 80 nm particles as shown in Figure S3.

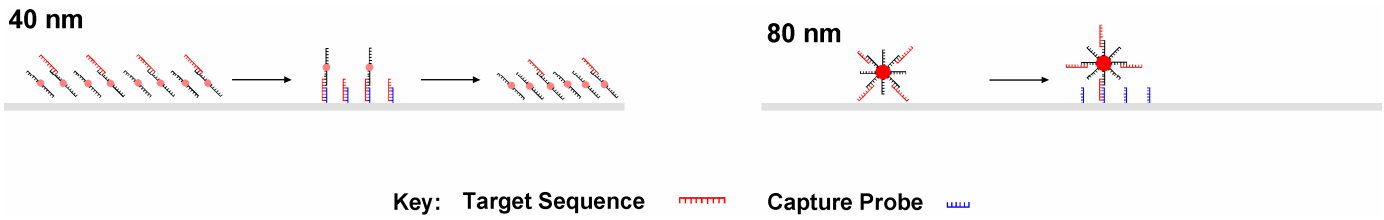

Figure S5: All 40 nm particles, even those hybridized to target sequences, have a lower probability of binding to capture probes than 80 nm particles. Therefore the latter have a higher probability of contributing to colour development at the test line.

If the increase in absorbance and scattering that accompanies the increase in diameter of GNPs was the only factor that affected colour development then it would go on increasing beyond 80 nm, but the increase in diameter is also accompanied by movement of the spectrum to longer wavelengths that are not visible to the human eye. The reason why GNPs are widely used as labels in lateral flow devices is because they are very efficient at preventing reflected light from being transmitted to the observer. Nitrocellulose and other materials used in lateral flow devices are white in colour, and therefore they reflect visible light at all wavelengths. When GNPs bind to the test line of a lateral flow device they prevent some of this light from being reflected as shown in Figure S6A. The overlaid spectra in Figure S6B show that even though 150 nm particles have a higher extinction maximum than 80 nm particles they are less efficient at absorbing visible light. The result is that the sensitivity of lateral flow devices developed with GNPs, and interpreted by the unaided eye, reaches a maximum when the particles have a diameter of around 80 nm, and then decreases as the diameter is further increased.

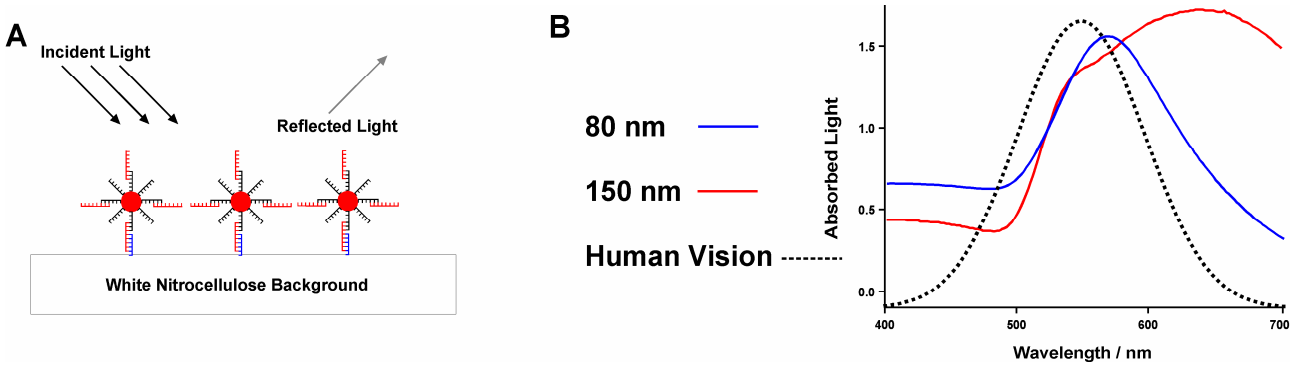

Figure S6: A) One reason why GNPs are widely used as labels in lateral flow devices is because they are very effective at preventing visible light from being reflected by white membrane materials into the eye of the user. **B)** 80 nm GNPs are more effective than 150 nm particles at preventing visible light being reflected from a white background because their spectrum coincides more closely with the wavelengths that are seen by the human eye.

Supplementary Information References

1. Carlsson, J., Drevin, H., Axen, R. *Biochem. J.,* (1978), **173**, 723-737.
2. Brocklehurst, K., Carlsson, J., Kierstan, M.P.J., Crook, E.M. *Biochem. J.*, (1973), **133**, 573-584.
3. Wilson, R., Chen, Y., Aveyard, J., *Chem. Commun.* (2004), 1156-1157.
4. Chen, Y., Aveyard, J., Wilson, R., *Chem. Commun.*, (2004), 2804-2805.