

EXPERIMENTAL

Chemicals and Instrumentation

Oligonucleotide sequences used for probes and targets were purchased from ADT Technologies. Distilled water was used for all chemical reactions. A Varian Cary 300Bio UV-Visible Spectrophotometer with Temperature Controller attached was used for all extinction measurements, with Cary software used for data analysis. For SERS measurements, the instrument used was an inVia Raman Microscope by Renishaw, fitted with a 514 nm Flexible Laser Solutions Modu-Laser and a Leica DMLM microscope platform. All Raman data interpretation was carried out using GRAMS AI software. The dynamic light scattering instrument used was a Zetasizer Nano series by Malvern.

Synthesis of Silver Nanoparticles

AgNP synthesis was carried out according to a modified Lee and Meisel method.¹ 500 mL water was added to a round bottom flask and heated to 45 °C using a Bunsen burner, then AgNO₃ (90 mg in 10 mL water) was added and heated rapidly to boiling with vigorous stirring. Sodium citrate was then added (100 mg in 10 mL water) and the solution kept boiling for 30 min with vigorous stirring, then allowed to cool to room temperature.

Synthesis of Magnetic Nanoparticles and Ag@MNPs

Maghemite (γ -Fe₂O₃) MNPs were prepared by co-precipitation, following a method used by Kumar et al²: FeCl₂·4H₂O (1.98 g), FeCl₃·6H₂O (5.335 g) and concentrated HCl (821 μ L) were added together and made up to 25 mL with distilled water. NaOH (15.058 g) was added to a round-bottom flask along with 250 mL distilled water and heated to 50 °C on a heating mantle. The acidified iron salt solution was then added drop-wise with vigorous stirring and a black precipitate formed immediately. Stirring was continued for a further 20 min at 50 °C, then the solution left to settle and cool. The black precipitate was washed twice with distilled water and once with 0.1 M HNO₃, then a further 125 mL of 0.1 M HNO₃ added and the solution heated to 95 °C in a round bottom flask, with constant stirring, for 40 min. The resultant reddish-brown solution was centrifuged in triplicate and resuspended in distilled water; this produced the stock maghemite MNPs.

Ag@MNPs were prepared using a glucose-reduction method, adapted from that used by Mandal et al³: 1 mL stock MNPs was added to a screw-cap glass vial along with glucose (0.25 g), water (4 mL), and AgNO₃ (1%, 1.5 mL). The mixture was sonicated for 10 min and then heated at 90 °C for 90 min while rotating. The resultant Ag@MNPs were centrifuged three times and redispersed in sodium citrate (5 mM, 6 mL). Note that magnetic collection of a solution of the synthesised Ag@MNPs resulted in a clear supernatant, indicating that no non-magnetic silver nanoparticles were formed during the silver nitrate reduction.

Conjugation of AgNPs and Ag@MNPs

AgNP-P1 conjugates were typically made according to the procedure developed by Zhang *et al*⁴: AgNPs (608 pM, 0.5 mL) and probe 1 oligonucleotide sequence (e.g. 59.8 μ M, 10.2 μ L, 2000:1 ratio) were added to an Eppendorf tube, followed by 2 aliquots of 500 mM citrate HCl buffer (10 μ L each, 10 min gap between additions). The sample was left for 40 min then

HEPES buffer (500 mM, 30 μ L) and NaCl (2M, 104 μ L) were added, the sample was left for a further 45 min then centrifuged and resuspended in phosphate buffer (10 mM, 0.5 mL). The Raman reporter was then added at, typically, a 1000:1 ratio (e.g. 10 μ M, 30.4 μ L), left overnight then the conjugate triple-centrifuged and stored in phosphate buffer (10 mM, 0.5 mL).

Oligonucleotide Probe and Target Sequences

For the *C. krusei* conjugates, the oligonucleotide sequences used were as follows (where (HEG)₃ denotes three hexaethylene glycol units, and SH denotes a thiol group):

P1: SH-(HEG)₃-GGCGAACCAGGA

P2: SH-(HEG)₃-CGATTACTTTGA

Target: TCAAAGTAATCGTCCTGGTTCGCC

Non-complementary: CGAGTTGACGTTAAGATCCGTATT

For the *C. albicans* conjugates, the sequences used are:

P1: SH-(HEG)₃-TGGGTCTTGTA

P2: SH-(HEG)₃-TTGGAATGAGTA

Target: TACTCATTCCAATTACAAGACCCA

Non-complementary: CGAGTTGACGTTAAGATCCGTATT

SERS analysis

Samples were prepared according to the following procedure: samples were made up to a total volume of 10 μ L in 0.3 M PBS buffer, containing AgNP-P1, Ag@MNP-P2, target or non-complementary sequence, and buffer. Samples were left to hybridise for one hour at room temperature then transferred to a glass capillary tube with a rare earth magnet fixed underneath. A magnetic plug was allowed to collect for a period of 20 min, with occasional mixing of the solution back and forth, then PBS (0.3 M, 1 mL) was pumped through the capillary, using a length of silicon tubing attached to the end of the capillary and fixed to a peristaltic pump.

Samples were analysed through the capillary tube using x20 magnification lens and with a manually-adjusted laser focal point. 5 x 1 sec accumulations were obtained at 100% laser power (unfocused laser power at sample = 22.5 mW), 514 nm laser, and 3 replicates were obtained for each sample type. For the data shown in Figure 3, the spectra are the average of 3 replicate measurements obtained from 2 different samples of the same type (e.g. same target concentration). Error bars represent \pm one standard deviation.

Characterisation of Nanoparticles and Probes

The MNPs were coated in silver to allow them to be linked directly with thiol-terminated oligonucleotides, to ensure stability of the NP-oligonucleotide conjugates in phosphate buffer and salt solutions, and to benefit from the strong Raman enhancement provided by AgNPs. The glucose-reduction method used to coat the MNPs, modified from that used by Mandal *et al*³, was chosen to maximise the silver coverage on the MNPs and allow optimal stability of the Ag@MNPs before and after conjugation with oligonucleotides. Figures S1 and S2 compare some properties of both the citrate-reduced AgNPs and glucose-reduced Ag@MNPs, and their respective oligonucleotide-conjugates. The extinction spectra for the

Ag@MNP conjugate (Fig S2a) shows a strong surface plasmon band at ~ 405 nm, very similar to that of the AgNP conjugate (Fig S1a), highlighting the significant Ag coverage on the MNP core. Likewise, the SEM measurements indicate that both types of nanoparticle are of a similar size, calculated as 31 ± 10 nm for the AgNPs and 25 ± 11 nm for the Ag@MNPs. Conjugation of the Ag@MNPs was carried out following the same procedure as for AgNP conjugation, and both sets of probes were stable for several months following preparation when stored in 10 mM phosphate buffer.

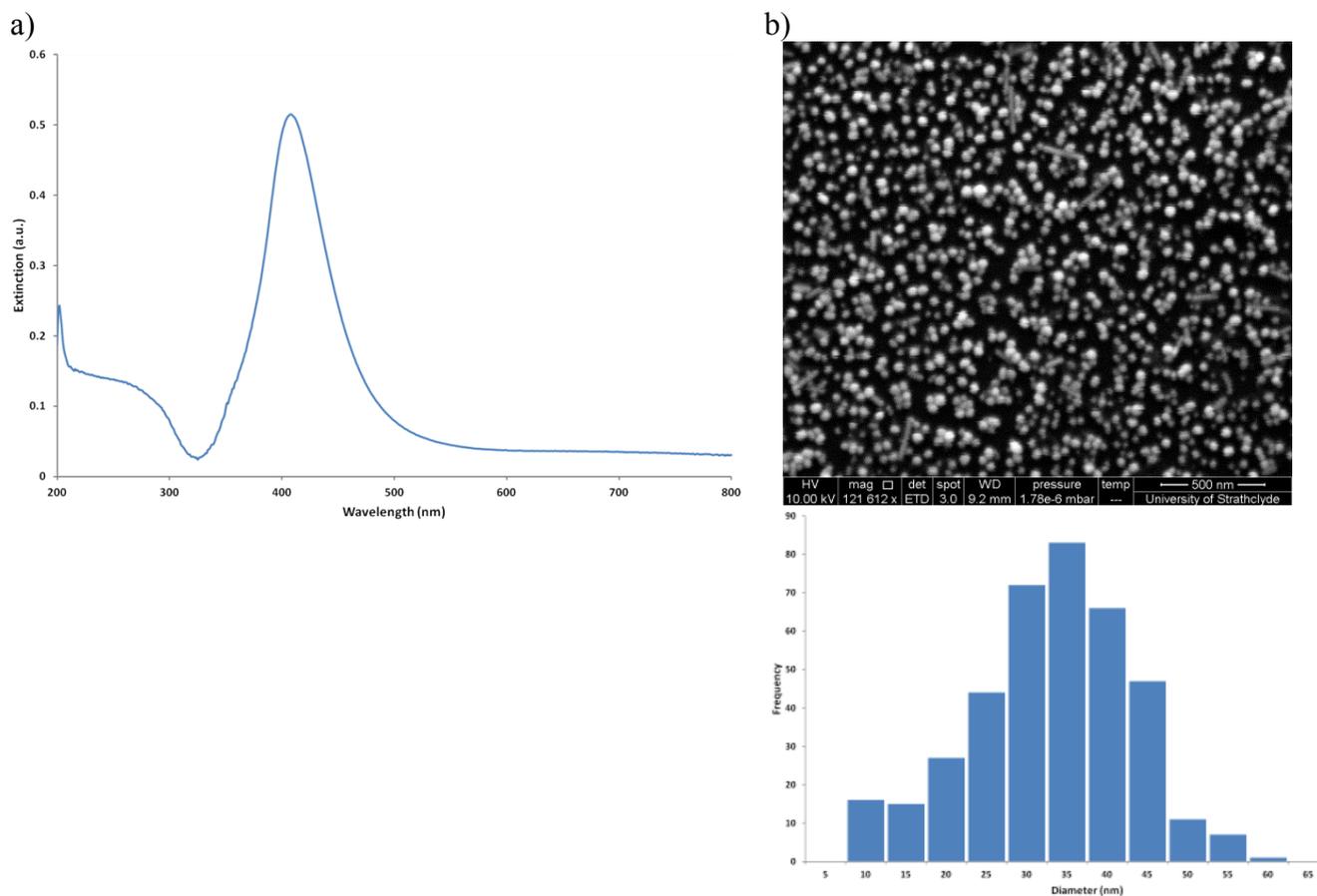


FIGURE S1: Properties of AgNPs showing: a) extinction spectrum of a KRU-P1 conjugate with λ_{\max} at 408 nm; b) SEM image of citrate-reduced AgNPs (top) and size distribution of AgNPs as calculated from this (bottom)

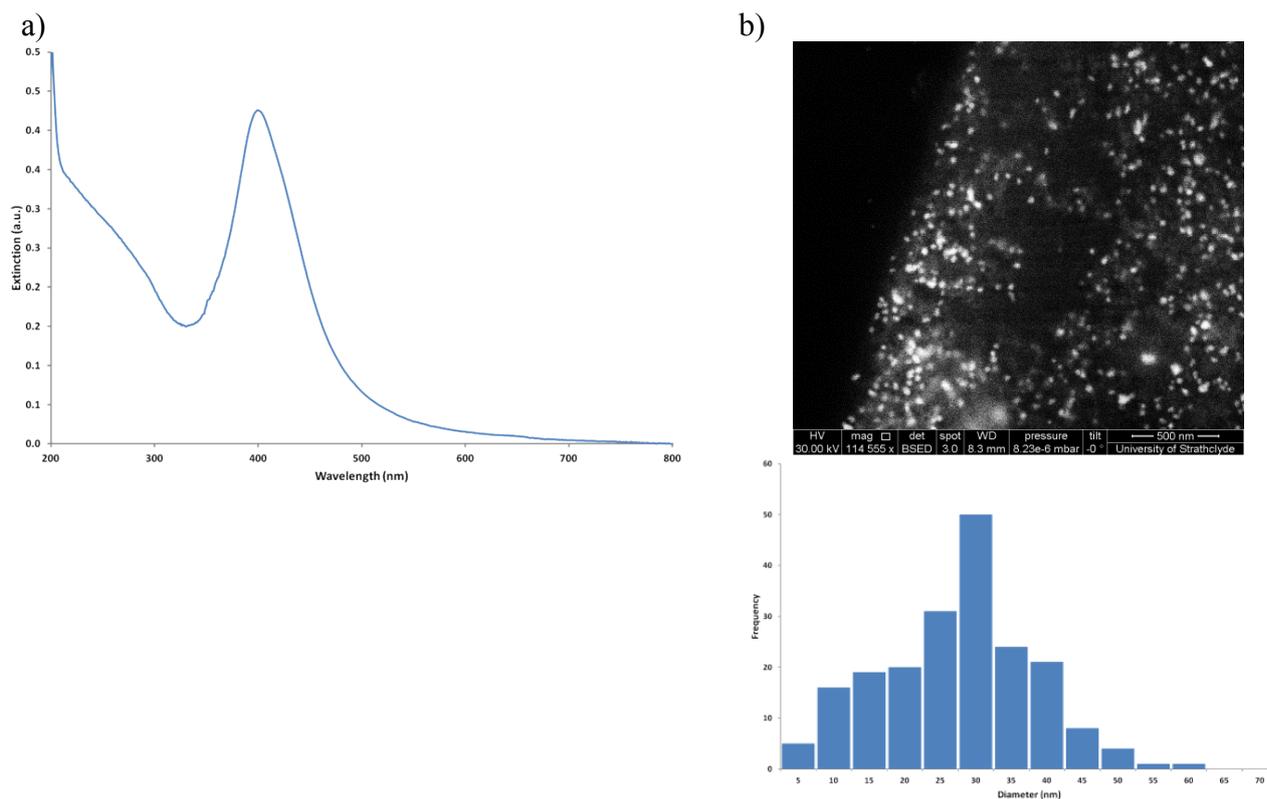


FIGURE S2: Properties of Ag@MNPs showing: a) extinction spectrum of a KRU-P2 conjugate with λ_{\max} at 399 nm; b) SEM image of glucose-reduced Ag@MNPs (top) and size distribution of Ag@MNPs as calculated from this (bottom)

Figure S3 shows the change in particle size, as measured by dynamic light scattering (DLS), for a sample of conjugates and target or non-complementary DNA. The samples consist of a solution containing 5 pM of each of the KRU-P1 and KRU-P2 conjugates and 5 nM of either target or non-complementary DNA, in 0.3 M PBS buffer. Measurements were taken immediately prior to DNA addition, then at 20 minute intervals for a total of 2 hours following this. The results show an increase in particle size from 69 nm before target addition to 426 nm 2 hours after addition of target DNA, with no increase in size observed for the non-complementary DNA sample. Note that the particle sizes measured by DLS are larger than the true values since DLS measures the hydrodynamic diameter of particles.

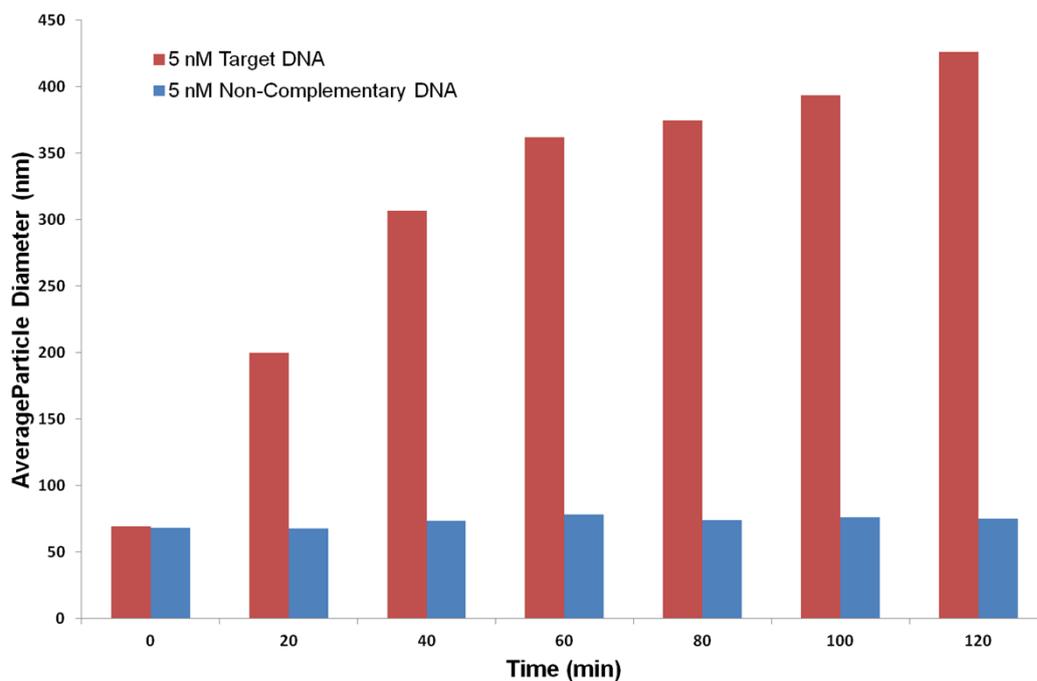


FIGURE S3: Changes in average hydrodynamic diameter over time for a sample of KRU-P1 and KRU-P2 conjugates following addition of either target or non-complementary DNA.

REFERENCES

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- (2) Kumar, G.; Rangarajan, N.; Sonia, B.; Deepika, P.; Rohman, N.; Narayana, C. *Bulletin of Materials Science* **2011**, *34*, 207.
- (3) Mandal, M.; Kundu, S.; Ghosh, S.; Panigrahi, S.; Sau, T.; Yusuf, S.; Pal, T. *Journal of Colloid and Interface Science* **2005**, *286*, 187.
- (4) Zhang, X.; Servos, M. R.; Liu, J. *Chemical Communications* **2012**, *48*, 10114.