



Silver and magnetic nanoparticles for sensitive DNA detection by SERS†

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This paper describes the first report of the combination of functionalised silver nanoparticles and silver-coated magnetic nanoparticles in a stable sandwich assay for DNA detection using SERS, providing robust multi-target recognition.

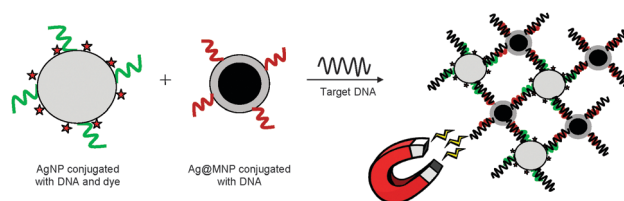
Rapid, accurate and sensitive detection of specific sequences of DNA is important for diagnosis of bacterial or viral infection and the determination of genetic mutations. The most commonly used methods involve PCR-amplification of the sample, followed by fluorescence-based detection.^{1,2} This has some inherent limitations, such as limited sensitivity and the production of broad, overlapping bands, which severely limits the multiplexing capabilities of this technique.^{3,4} In recent years, surface enhanced Raman scattering (SERS) has been widely investigated as an alternative detection method^{5,6} due to its high sensitivity and the presence of narrow, distinct spectral bands that make it amenable for the detection of several targets simultaneously.⁶

To exploit SERS for DNA detection using DNA-functionalised nanoparticles (NPs), Graham *et al.*⁷ and then Qian *et al.*⁸ separately demonstrated the novel combination of DNA-directed NP assembly with SERS analysis in solution using functionalised AgNPs and AuNPs, respectively, highlighting how a biological recognition event can result in a change from a 'SERS off' to 'SERS on' state. In this case a Raman active reporter molecule is used as a marker for the presence of target DNA.

DNA-functionalised magnetic NPs (MNPs) have also been incorporated into molecular diagnostic assays to allow simple, efficient separation of target DNA from the sample matrix. They can be used in a single probe system to extract target DNA from a biological medium, with subsequent detection by methods such as electrochemical⁹ or PCR-ELISA.¹⁰ Alternatively, the MNPs can be incorporated within a dual DNA-probe sandwich

assay alongside Au or AgNPs, combining target separation with the plasmonic properties of the NP, allowing sensitive DNA detection by methods such as extinction spectroscopy¹¹ or SERS.^{12,13} The assays developed to date have generally used polymer,¹³ silica¹² or Au-coated MNPs,¹¹ although Lin *et al.* recently described an assay that incorporated a very thin Ag shell around the MNP core.¹⁴ AuNPs are generally more stable than Ag, however AgNPs give a better ratio of scattering to absorption,¹⁵ thereby typically giving stronger SERS enhancements.

We describe a novel multi-target molecular diagnostic assay combining positive target recognition with magnetic capture to allow sample concentration and clean-up, with highly sensitive detection provided by SERS. The assay incorporates two probes, with the DNA sequence on each probe complementary to a section of the target DNA (Scheme 1). Probe 1 consists of a citrate-reduced AgNP conjugated to a 12-base DNA sequence and a Raman reporter; probe 2 consists of a Ag-coated MNP (Ag@MNP) conjugated to a different 12-base DNA sequence. The MNPs are composed of maghemite ($\gamma\text{-Fe}_2\text{O}_3$) prepared using a co-precipitation method, and are coated with a shell of Ag using glucose reduction. NP characterisation data is provided in Fig. S1 and S2 (ESI†). In the presence of a 24-base target sequence, hybridisation between the probes and target leads to a controlled aggregation of the NPs. This places the Raman reporter in areas of high electric field intensity, giving rise to a strong enhancement of the Raman signal; hence the reporter molecule acts as a marker for the presence of target DNA. Using a simple flow cell, the hybridised NP network is



Scheme 1 Schematic representation of assay showing controlled NP assembly in presence of target DNA.

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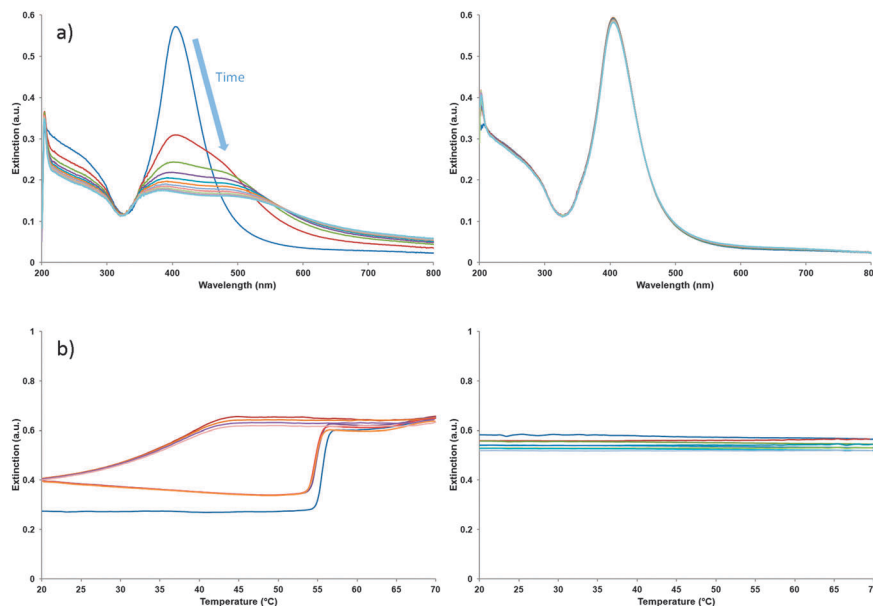


Fig. 1 Changes in extinction spectra for samples of KRU-P1 and KRU-P2 probes with 5 nM target (left) or non-complementary sequence (right) in 0.3 M PBS, showing (a) spectra collected every 10 min for 100 min; and (b) changes in extinction at 405 nm for samples subjected to 4 heat-cool cycles (20–70–20 °C).

concentrated using a magnet and the immobilised target washed to remove interfering background material, followed by direct laser interrogation of the magnetic plug. By conjugating the Raman reporter to the AgNP probe only, and combined with the wash step to remove any un-hybridised AgNP probe, there should be no reporter present within the immobilised magnetic network unless target DNA is present. This should result in much lower background reporter levels when compared to solution-based systems in which the reporter-labelled probes remain in suspension throughout SERS analysis.

Data relating to two different 24-base synthetic target sequences are presented here, with each target specific to a different *Candida* fungal species. *Candida* species are a common cause of nosocomial bloodstream infections and, while *Candida albicans* is present in the majority of the population, overgrowth of the fungus can lead to problems in immunocompromised individuals. Reliable and sensitive detection of these fungal species in clinical samples is therefore important. The first target was specific for *C. krusei* and used 4-mercaptopyridine (MP) as a reporter (probes designated KRU-P1 and KRU-P2). The second target was specific for *C. albicans* and used malachite green isothiocyanate (MG) as a reporter (ALB-P1 and ALB-P2).

Reaction kinetics studies were carried out using extinction spectroscopy to determine the extent of aggregation within a sample following addition of either target or non-complementary DNA to the two probes (Fig. 1a). In the presence of target DNA there was a reduction in absorbance of the main peak and the appearance of a second peak at a longer wavelength; this was due to individual NPs being brought into close proximity resulting in a change in surface plasmon resonance (SPR), indicating successful DNA-mediated NP assembly. Previous studies have also shown this effect in a AgNP–AgNP sandwich assay.¹⁶ In contrast, addition of a non-complementary sequence caused no SPR changes, indicating

that no hybridisation or non-specific aggregation occurred, and highlighting the stability of both probes in the hybridisation buffer.

Since the probe-target hybridisation is reversible, heating samples beyond their melting temperature (T_m) will cause the NP conjugates to once again be free in suspension. This leads to distinctive changes in the SPR of the NPs, as shown in Fig. 1b where mixtures of probes and either target or non-complementary DNA were subjected to four repeated heating and cooling cycles. For the target sample, the T_m value can be clearly seen as a sharp increase in the extinction value at 405 nm during heating of the sample, and both the extinction and T_m are little changed following repeated cycles. In contrast, no change in extinction at 405 nm is observed for the non-complementary sample. This data also shows that both probes are stable at elevated temperatures.

In the presence of target DNA, the Raman reporter experiences an enhancement in the electric field intensity of its immediate environment due to the induced NP aggregation.¹⁷ This is illustrated in Fig. 2a, which shows the change in intensity of the peak at 1179 cm^{-1} (in-plane aromatic C–H bending vibration¹⁸) for MG over a 60 min period when a mixture of ALB-P1 and ALB-P2 conjugates were mixed with either target or non-complementary DNA. These are suspension-based SERS measurements, carried out in cuvettes without the application of a magnet. A rapid increase in SERS intensity is observed for the 5 nM target sample, which plateaus after ~30 min (for a 220 μL sample containing 1.1 pmol target DNA). For the non-complementary DNA sample, no increase in SERS intensity was observed throughout the sampling period. The increase in signal intensity for the target vs. non-complementary sample is ~4-fold. When the target concentration was reduced to 1 nM (219 fmol), there was no discrimination between target and non-complementary DNA sequences. Following these measurements, the same samples were pumped around a closed flow-cell loop with a magnet fixed underneath, allowing collection of a magnetic plug of the

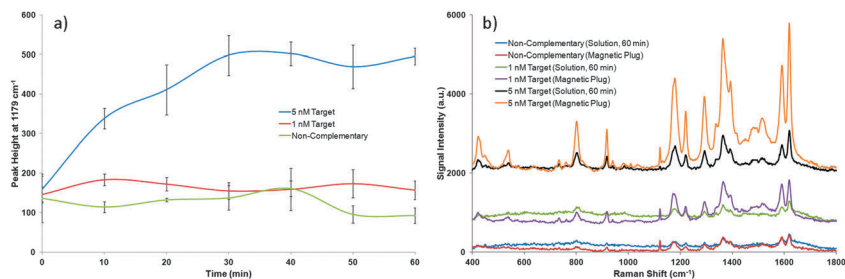


Fig. 2 Differences in MG signal intensity for samples containing either target or non-complementary DNA showing (a) change in peak height at 1179 cm^{-1} over 60 min for solution-based samples; and (b) comparison of the SERS spectra for solution-based samples (after 60 min) vs. the magnetic plug of the same samples.

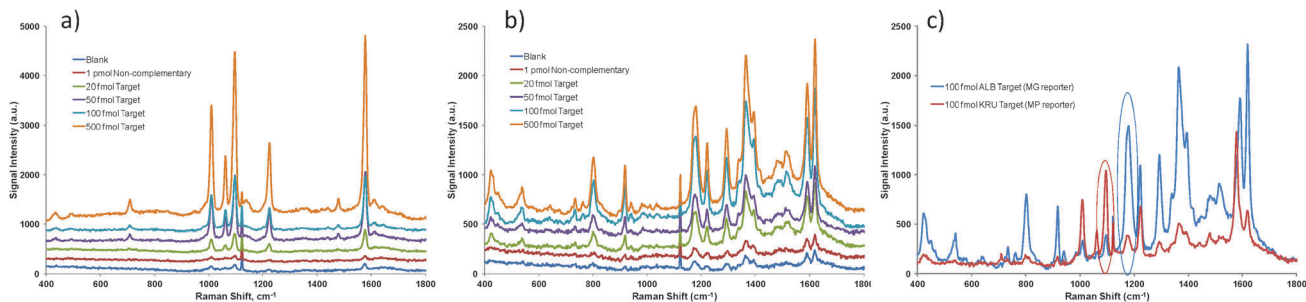


Fig. 3 Comparison of SERS spectra showing changes in signal intensity for (a) MP measured for a range of *C. krusei* target concentrations; (b) MG measured for a range of *C. albicans* target concentrations; and (c) samples containing 100 fmol of either *C. albicans* or *C. krusei* target DNA (NP probes relating to both targets are present in both samples). Note, the sharp peak at $\sim 1125\text{ cm}^{-1}$ comes from the glass capillary tube.

assemblies, which were then washed and SERS measurements taken. The results (Fig. 2b) illustrate the strong signal enhancement that was achieved when SERS analysis was carried out on the concentrated magnetic plug compared to analysis of the sample in suspension, with a greater than 3-fold signal enhancement for both the 1 nM and 5 nM target concentrations. For the magnetic plug measurements, a greater than 10-fold signal enhancement was obtained for the 5 nM target vs. non-complementary sample.

Fig. 3(a and b) shows the changes in SERS intensity of the Raman reporter for magnetic plug measurements of samples containing a range of concentrations of *C. krusei* or *C. albicans* target. In each case, there was a non-linear increase in signal intensity with increasing target concentration. While a low Raman reporter signal was observed in both blank samples (*i.e.* probes and buffer only) and non-complementary DNA samples, good discrimination in signal intensity was observed for just 20 fmol of target DNA for both target sequences. This is more than a 10 times improvement in detection limit compared with suspension-based measurements.

The multiplexing potential of SERS-based assays has been one of the driving factors in a significant number of studies dedicated to this technique in recent years.¹⁹ In order to assess the functionality of the current assay in a duplex format, a new Ag@MNP-P2 conjugate was prepared containing an equal ratio of P2_{alb} and P2_{kru} oligonucleotides, designated ALB/KRU-P2. Although not essential, having both probe sequences present on the same NP helps to minimise the collection of excess MNPs that are not involved in any hybridisation event. Fig. 3c shows the results for samples containing ALB-P1, KRU-P1 and

ALB/KRU-P2 probes, along with 100 fmol of either *C. krusei* or *C. albicans* target DNA. In the presence of *C. albicans* target, a strong SERS signal from MG was obtained, while in the presence of *C. krusei* target, a strong SERS signal from MP was obtained (the peak at 1096 cm^{-1} relates to the in-plane ring-breathing mode, coupled to the C-S stretching mode of MP²⁰). While a low background level of reporter was observed in both samples, the presence of each of the target sequences can be clearly distinguished when present in this duplex format.

In conclusion, a stable assay with potential for molecular diagnostics has been developed combining the high sensitivity of SERS detection with the benefits of magnetic manipulation. Sensitive detection of two different synthetic DNA sequences was demonstrated, both individually and in a duplex format. Some issues exist regarding background SERS levels of the Raman reporter which are observed in non-target samples, however clear discrimination is obtained at low target concentrations in both formats. This research represents the first combination of DNA-functionalised AgNPs and Ag@MNPs in a sandwich assay for DNA detection by SERS. The robust molecular recognition provided demonstrates the potential for further development of the assay to allow target DNA detection within a complex clinical sample matrix.

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