

DNA Detection using Enzymatic Signal Production and SERS

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Supplementary Information

Experimental

Reagents and Equipment: Oligonucleotides were purchased from ATDBio Ltd. on 1 μmol scale with HPLC purification. λ -Exonuclease was purchased from New England Biolabs (UK) Ltd along with exonuclease buffer (10 $\times$). Streptavidin coated magnetic beads were purchased from New England Biolabs (UK) and were washed immediately prior to use. Chemicals for the preparation of silver colloid were purchased from Aldrich.

Silver Colloid Preparation

500 mL of distilled water was heated to 45 $^{\circ}\text{C}$ at which point silver nitrate (90 mg, 0.5 mmol) pre-dissolved in 10 mL of water was added. The solution was rapidly heated to 98 $^{\circ}\text{C}$ and trisodium citrate (10 mL, 1 % (w/v)) was added. The solution was carefully maintained at 98 $^{\circ}\text{C}$ (± 0.5 $^{\circ}\text{C}$) for 1 h 30 min before being allowed to cool to room temperature. Vigorous stirring was continued throughout. $\lambda_{\text{max}} = 401$ nm.

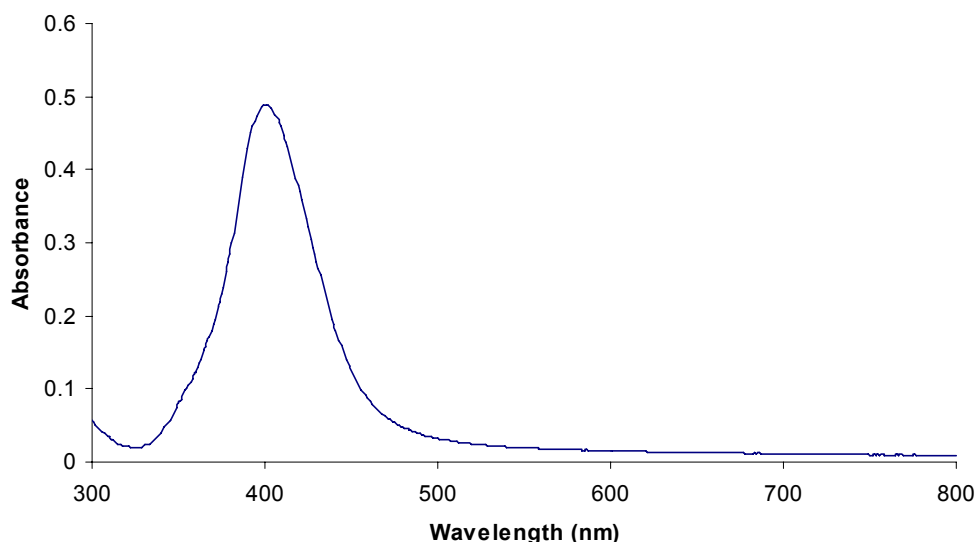


Figure S1: UV-vis spectrum of silver colloid.

Exo-SERS Assay

An aliquot of each oligonucleotide probe reporter and capture (10 μL , 1 μM) was added to a PCR tube and incubated in 0.3 M PBS (70 μL) with an aliquot of target oligonucleotide (10 μL , X^* μM). The temperature was raised to 90 $^{\circ}\text{C}$ for 5 min and cooled to 20 $^{\circ}\text{C}$ for 15 min. Streptavidin beads were prepared by washing in 0.3 M PBS $\times 3$ and then 20 μL was added directly to the cooled hybridised solutions. The samples were left to incubate at room temperature with the beads for 10 min. The beads were separated from the supernatant by the application of a magnet to the side of the tube and the supernatant removed to waste. This process was repeated three times with increasing volumes of 0.3 M PBS. Finally, the beads were re-suspended in 30 μL of exonuclease buffer ($\times 1$) and 2 μL of λ -exonuclease was added. The suspensions were incubated at 37 $^{\circ}\text{C}$ for 1 h before being cooled to 4 $^{\circ}\text{C}$.

In a disposable microcuvette silver "citrate reduced" colloid (150 μL , 0.3 nM) was diluted by the addition of 250 μL of distilled water. To this, 30 μL of the assay mixture was added having been separated from the magnetic beads, followed by an aqueous solution of spermine hydrochloride (20 μL , 0.1 M). The SERS spectra

were recorded within 5 min of these additions on a Renishaw Probe system with an excitation wavelength of 532 nm (1×5 s, 35 mW, 0.5 NA, $50 \times$ obj). The spectra were levelled and set to zero using Grams software.

* Where X ranges from 0.6 to 0.01 μ M. For the duplex target experiments PCR product of *C. trachomatis* and *C. glabrata* were added at a concentration of 0.1 μ M. The PCR products were supplied “as prepared” with no purification.

5 replicate analyses of 5 replicate samples at each concentration were analysed.

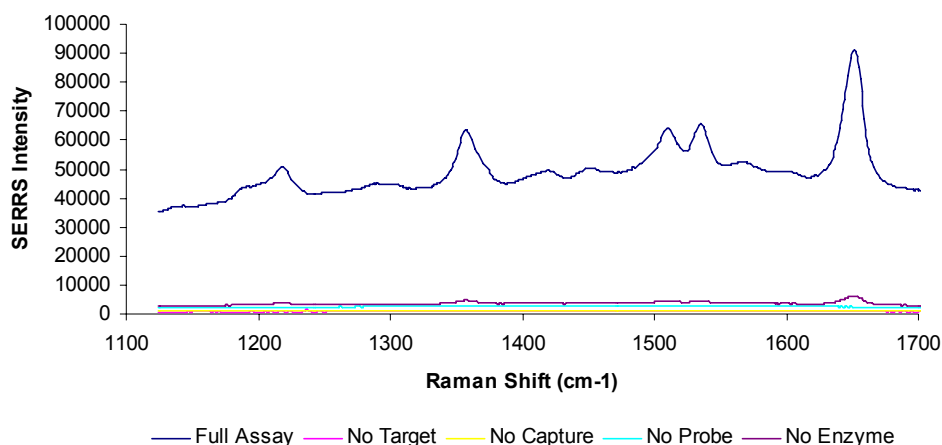


Figure S2: Uncorrected, averaged, SERRS spectra from exo-SERRS assay.

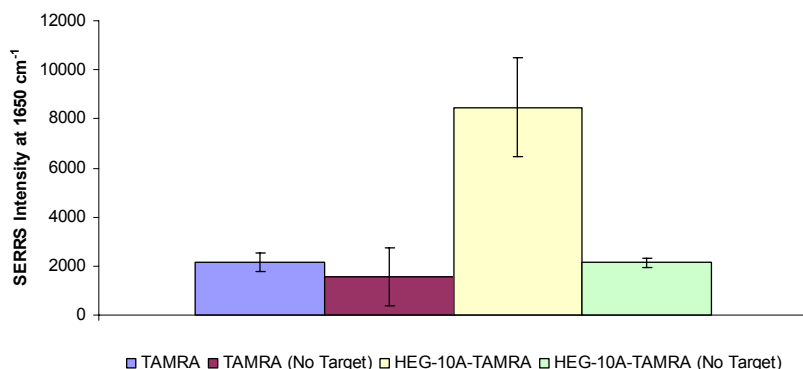


Figure S3

The comparison of two “reporter” probes:

P-GCT*GCG ACA GGG ACT:

P-GCT GCG ACA GGG ACT-HEG-10A-TAMRA

TAMRA probe (where * is the site of TAMRA)

HEG-10A-TAMRA probe

Exo-SERS experimental protocol as described above except duplex left to incubate with TurboBeads for 1 h and sample incubated with enzyme for 2 h. The sample, separated from the TurboBeads, was added (30 μ L) to “citrate reduced” silver (as described above) (50 μ L) diluted by 50 μ L of distilled water with the addition of an aqueous solution of spermine hydrochloride (20 μ L, 0.1 M). The spectra were recorded from 200-2000 cm^{-1} , 10 s, 1 accumulation. The SERS spectra were recorded within 5 min of the final addition. The SERS spectra were recorded using an excitation wavelength of 514.5 nm: A Leica DM/LM microscope equipped with an Olympus 20×0.4 long-working-distance objective was used to collect 180° backscattered light from a standard microtiter plate. The spectrometer system was a Renishaw inVia (Gloucestershire, UK) with an Ar⁺ Spectra Physics laser as the excitation source. The spectra were recorded from 200-2000 cm^{-1} , 10 s, 1 accumulation. Each spectra was recorded five times.

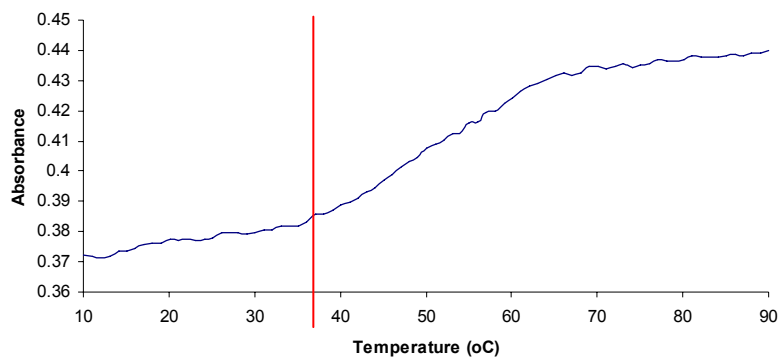


Figure S4

An aliquot of target (20 μ L, 10 μ M), reporter (10 μ L, 20 μ M) and capture (20 μ L, 10 μ M) were added to a microcuvette in exonuclease buffer $\times 1$. The temperature was cycled between 10 $^{\circ}$ C and 90 $^{\circ}$ C at a rate of 1 $^{\circ}$ C per minute and the absorbance monitored on a Cary 300 Bio UV-vis spectrophotometer fitted with a 6×6 cell changer and Peltier temperature controller at 260 nm. The temperature of the enzyme digestion, 37 $^{\circ}$ C, is represented by the red line.