Supplementary Information

Experimental Section

Gold(III) chloride hydrate (99.9999% purity), trisodium citrate, magnesium sulphate, tribasic sodium phosphate and Tris-EDTA buffer (pH 7.4 (Fluka) were purchased from Sigma-Aldrich. Aqueous solutions were prepared using doubly distilled, deionised (DDI) water from a Barnstead NANOpure Diamond™ system. All glassware was washed with aqua regia followed by extensive rinsing with water prior to use. All DNA oligomers, thiolated and unthiolated, used in this study were purchased from Eurogentec Ltd. (Belgium) and underwent sePOP (Selective Precipitation Optimized Process) desalting purification by the vendor. The thiolated DNA sequences were dissolved in Tris-EDTA (TE) buffer and were used immediately after they were dissolved to avoid the formation of disulfide bonds.

The citrate reduced Au nanoparticles (AuNP) were prepared using a variation of Frens' citrate reduction.[1] A solution of $HAuCl_4$ was brought to a boil (50 ml of 0.1% w/v in DDI water) and 5.6 ml of 1% w/v solution of trisodium citrate rapidly added to the boiling solution, after 1 minute the solution was then left to cool to room temperature.[2] The colloid was further diluted in 10^{-4} M trisodium citrate (typically 1 ml colloid plus 1ml of citrate and 1 ml of water) and then centrifuged only until the particles were concentrated at the bottom of the tube (typically this required 60 minutes at 2500 rpm but varied between different colloid batches). The supernatant was then removed and replaced with fresh 10^{-4} M trisodium citrate solution. Typically this preparation technique yielded colloids where ~75% of the particles were *ca.*51 nm, determined by using a Zetasizer Nano ZS system (Malvern Instruments). For SERS measurements, 50µl of colloidal solution were mixed with 50µl of analyte solution and 25µl of aggregating agent. The aggregating agent was normally 0.1 M $MgSO_4$. For the data shown on Figure 2(a) and (b) the samples were allowed to sit for 12 hours and then 0.1 M $MgSO_4$ was added to aggregate the colloid since immediate $MgSO_4$ did not cause aggregation. For the data in Figures 2(c) and (d) the colloids were aggregated with 1M Na_3PO_4 which was more rapid since it could be used immediately after DNA addition.

Preparation of MB- functionalized AuNP

AuNP were functionalised with Molecular Beacon (MB) DNA probes that were thiolated in the 5' end. For this experiments AuNP were diluted by a factor of two using DDI water to reduce the tendency to aggregate during the NaCl salting process. 300 µl of diluted AuNP were mixed with 300 µl of 20 µM thiolated molecular beacon (MB). After ~ 16 h the colloids

were brought to 0.05 M NaCl by drop-wise addition of 2M NaCl and allowed to stand for 6−8 h, they were next salted to 0.1 M and allowed to age for another 6−8 h, they were then salted to 0.2 M and after standing for 6−8 h were finally salted to 0.3 M NaCl. To remove excess thiolated-MB, the colloids were centrifuged for 15 min at 4000 rpm. Following removal of the supernatant, the oily precipitate was then washed with 0.3 M NaCl-10 mM TE buffer solution. Hybridization studies were carried out by adding 300 µl of 20 mM DNA targets to the solutions of the MB-functionalized AuNP. The solution of MB functionalized-Au nanoparticles containing the DNA target was then heated to ~70 °C (above the melting point) for 5−10 min, and allowed to cool at room temperature. The complementary sequence (C-1) was 5'-ACA-AAT-AAA-TTA-AAG-CAT-AAA-AGG-AGA-CCT -3' and the non-complementary (NC-1) was 5'-GAC-TGC-GAC-CAA-CCT-AGC-CTG-CTA-TGA-TGT). The MB- functionalised Au nanoparticles which had been heated gave good SERS spectra without any need for additional aggregating agent. For SERS measurements on the blank samples before heating (Figure 4(a)), 50 µL of the cooled colloidal solution was mixed with 25 µL of 1M spermine tetrahydrochloride, which efficiently aggregated the colloid.

Instrumental Details

The SERS spectra were recorded on an Avalon Instrument RamanStation R1. This instrument uses a 785 nm diode laser and an echelle spectrograph. The laser power was 100 mW and spectra were typically recorded with an exposure time of 2x60s in 96 well polypropylene microtitre plates which were lined with aluminium foil to prevent detection of background polymer signals.

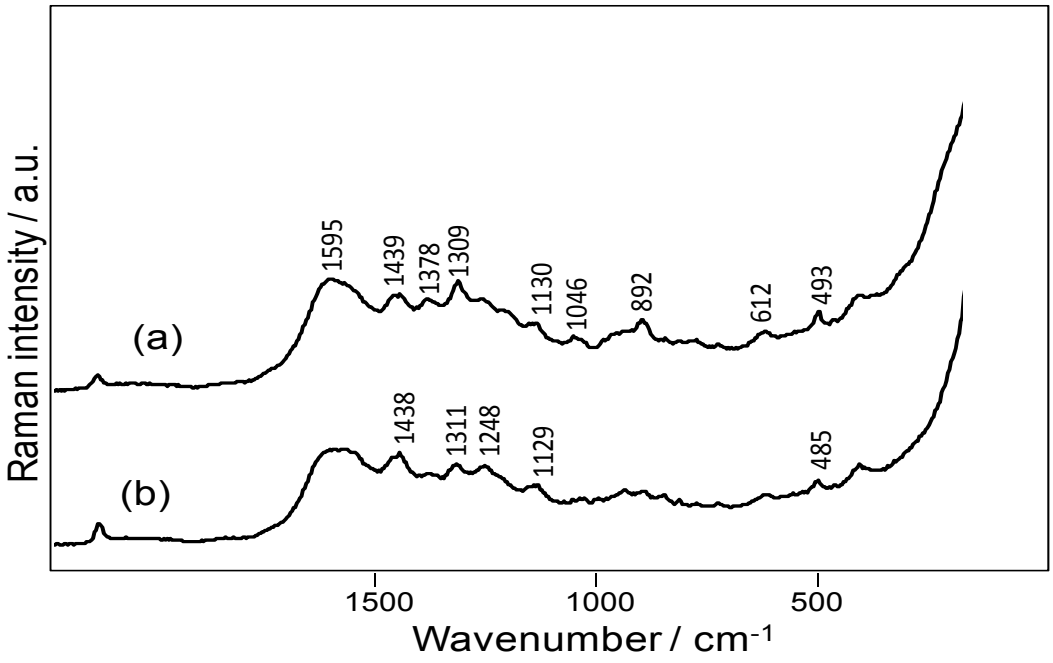

Fig. S1: SERS spectra of AuNP mixed with TE buffer and aggregated with (a) 1 M Na_3PO_4 and (b) 0.1 M $MgSO_4$.

1 G. Frens, *Nat.-Phys. Sci.,* 1973, **241,** 20.
2 R. McCourt Maighread R. PhD Thesis, Queen's University Belfast, 2010