Electronic Supplementary Material (ESI) for Chemical Communications

Positively charged silver nanoparticles and their effect on Surface-enhanced Raman scattering of dye-labelled oligonucleotides

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Supplementary information

Experimental procedures

Buffers

Phosphate buffered saline (PBS) was obtained from Oxoid Limited (Basingstoke, Hampshire, England). One tablet was dissolved in 100 mL Milli Q water, afterwards the solution was sterilised in an autoclave for 15 minutes at 121°C.

Materials

All materials were obtained from Sigma Aldrich unless stated otherwise. Oligonucleotide sequences 5'→3' 5-carboxyfluorescein-TGCTTGAAATGTCTGTATGGGATGC, GCATCCCATACAGACATTTCAAGCA, and Cy5-T*CT*CT*CT*CT*CT*CGCGTCATCGTATACACAGGAGCAG, (T* is 5-propargylamine-2'-deoxyuridine) were HPLC purified and obtained from ATD Bio (Southampton) and dissolved in DEPC treated water (Bioline).

Glassware preparation for positively charged nanoparticles preparation and storage

To prevent attachment of positively charged nanoparticles to glass surfaces, vials used for particle preparation and storage were coated with Polyethyleneimine (PEI).[1] Briefly, prior to use all glassware was cleaned with chromic acid overnight and extensively rinsed with milli-Q water and dried in N_2 flow. The glassware was then filled with 0.2% w/w PEI aqueous solution and incubated for 2h. Vials were then rinsed once with milli-Q water and dried with N_2 flow.

Preparation of positively charged silver nanoparticles (Ag-positive)

To 10 mL aqueous $AgNO_3$ (1 mM) 0.1 M spermine hydrochloride was added (5 µL) and the mixture was degassed for 30 min under N_2 flow, protected from light. Subsequently, under vigorous stirring, 25 µL of aqueous $NaBH_4$ (0.1 M) was quickly added to the solution and stirred for 20 min. Particle formation was immediately observed as the colour of the dispersion turned bright yellow. Formation of the particles was verified by UV-visible spectroscopy observing a typical plasmon resonance peak in the 390-400 nm wavelength range.
Following this preparation method, Silver nanoparticles (AgNPs) stabilised with branched PEI (bPEI), spermidine, and ethylenedioxydiethylamine were prepared.

Poly-*L*-Lysine stabilised silver nanoparticles (Ag-PLL)

Au-Ag core/shell nanoparticles of 45 nm diameter were prepared following the two-step particle seeded growth method.[2] Briefly, 40 µmoles of $AgNO_3$ were added to 125 mL of milli-Q water and heated to boiling, protected from light. Subsequently, 5 mL of gold nanoparticles (14 nm gold seeds) were added, followed by addition of 1% w/w aqueous trisodium citrate (22 µmol). The mixture was refluxed for 30 min under vigorous stirring. Subsequently, to further stabilize the particles, 5 mL of 1% w/w aqueous trisodium citrate was added and further refluxed for 1h. The particles were filtered through a 0.45 µm Millipore® filter and further centrifuged (500·g, 10 min) to remove any large aggregates before further functionalization with thioctic acid.
Citrate stabilised Au-Ag nanoparticles were further functionalized with thioctic acid in a ligand exchange reaction. Typically, to 10 mL nanoparticle dispersion, methanolic solution of thioctic acid (2.5 µL, 40 mM) was added and left stirring for 3 h protected from light. The excess ligand was removed by centrifugation (1073·g, 15 min) and redispersion in milli-Q water.
The attachment of Poly-*L*-lysine was achieved by EDC/sulfo-NHS coupling reaction. Briefly, 600 µL of the nanoparticle dispersion was diluted in 10 mL of milli-Q water and 1.5 µL of an aqueous (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride) (EDC) 200 mM solution was added along with 50 mM aqueous N-hydroxysulfosuccinimide (sulfo-NHS) in 1:1 molar ratio and left for 30 min protected from light in order to activate the COOH functionality. Subsequently the sample was divided into smaller aliquots and to each aliquot containing 0.8 mL of activated COOH 0.05% w/w aqeous solution of PLL was added (200 µL) and left overnight. Particles were then purified by centrifugation (2415·g, 20 min) and redispersed in milli-Q water.

CTAB stabilised silver nanoparticles (Ag-CTAB)

Ag-CTAB were prepared according to literature reference.[3] Briefly, two solutions were prepared: A) 20 mL of an aqueous solution containing 2 mM $AgNO_3$, 0.4 M NH_4OH, 0.5 mM CTAB and B) 20 mL of an aqueous solution containing 8 mM $NaBH_4$ and 0.5 mM CTAB. The solutions A and B were then cooled for 10 min in an ice bath. Subsequently under vigorous stirring solution B was added dropwise to solution A in an ice bath. After 2 min the solution turned yellow indicating the formation of the Ag-CTAB nanoparticles and left stirring for further 3h protected from light. Subsequently the particle dispersion was brought to boil rapidly, refluxed for 10 min and then cooled to room temperature.

Silver citrate nanoparticles (Ag-Citrate)

All glassware was cleaned with aqua regia and thoroughly rinsed with distilled water. Ag-Citrate nanoparticles were prepared according to the Lee & Meisel procedure.[4] A clean three necked round bottom flask was filled with 500 mL of Milli Q water. The solution was heated to 45 °C with a Bunsen burner under continuous stirring with a glass stirrer. Then silver nitrate (90 mg dissolved in 10 mL of Milli Q) was added. The solution was heater further to 98 °C. Tri-sodium citrate (100 mg in 10 mL Milli Q) was added and the solution was kept at 98 °C for 90 minutes and afterwards allowed to cool to room temperature.

Characterisation of Ag-nanoparticles

UV-Visible (UV-Vis) spectroscopy

UV-Vis spectroscopy was carried out on a Varian Cary 300 BIO spectrophotometer using 1 cm path quartz cells. Nanoparticle concentrations were calculated by Beers law using the extinction coefficient for silver nanoparticles of $1.85 \cdot 10^{10}$ M^{-1} cm^{-1}, derived from literature.[5]

Dynamic light scattering (DLS)

The hydrodynamic radius of the nanoparticles were measured by DLS on a Malvern HPPS particle sizer. Fifteen replicate measurements were taken from each sample and the average value reported. A standard sample consisting of commercially available Nanosphere™ Size Standards (Thermo Scientific Fremont CA.) 40 nm polymer microspheres in water were used as a standard before each set of measurements.

Scanning electron microscopy (SEM)

For the particle sample depositions silicon wafers (Agar Scientific) were cleaned with methanol and oxygen plasma (Diener electronic femto oxygen plasma cleaner, 72 cm^3/min gas flow) to produce a negatively charged surface for better adhesion of the cationic particles. Imaging was carried out on a Sirion 200 Schottky field-emission electron microscope (FEI) operating at an accelerating voltage of 5 kV. Image analysis was carried out using Image J, v1.43u.

ζ-potential

The ζ (Zeta) potential measurements were carried out using a Malvern 2000 Zetasizer, using the default method protocol and a minimum sample volume of 3 ml. Before each measurement a standard solution of -68.0 ± 6.8 mV was measured.

Surface enhanced Raman spectroscopy (SERS)

Samples were prepared with 90 µL of the nanoparticles and 10 µL of the DNA solution in water, these samples were prepared in triplicate in PMMA micro-cuvettes and analysed using an Avalon probe system Ramanstation R3 optical fibre with a 532 nm diode laser excitation. Typical integration times were 1 second and 3 accumulations. Data analysis was carried out using the xanthene ring C-C stretch[6, 7] of the fluorescent dye 5-Carboxyfluorescein (5-FAM) at 1632 cm^{-1} in the spectrum.

Laser intensity measurements

The laser intensity measurements were carried out using a Thorlabs PM100D laser intensity meter equipped with a Thorlabs S130C sensor. The laser intensity at the sample was ~ 24 mW.

PEI "leakage" from coated vial

Glass vials coated with PEI were tested for leakage by analysing the washing and comparing it to a reference sample containing PEI. Significant SERS signals were obtained from the reference PEI sample and no PEI peaks were obtained from the washing sample (Fig.S1) indicating stable coating of the glass vials.

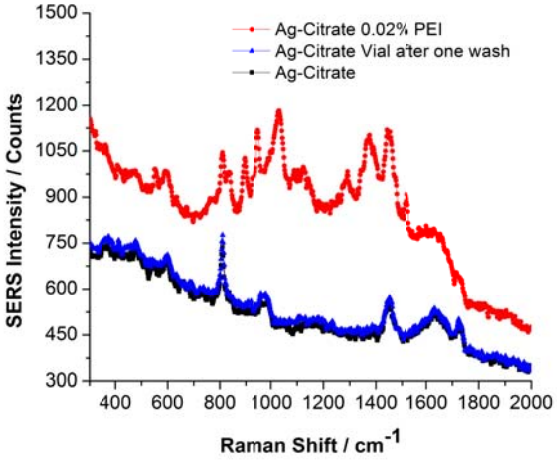


Fig.S1 Investigation of PEI leakage from the vial by SERS. A 532 nm laser excitation of approximately 24 mW at the sample was used (three accumulations of 1 second).

Nanoparticle SERS background

The background SERS signal of the positively charged silver nanoparticles.

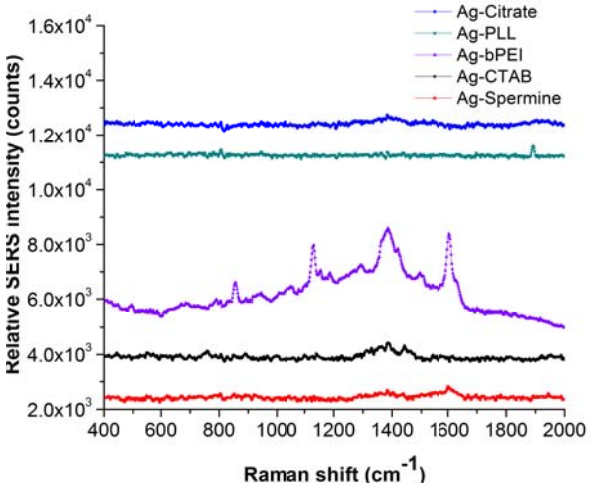


Fig. S2 SERS background of the different types of positively charged silver nanoparticles. SERS measurements were three accumulations for 10 seconds using a 532 nm laser excitation of approximately 24 mW at the sample. The spermine coated nanoparticles showed as little background as the poly-*L*-lysine stabilised nanoparticles, the bPEI stabilised nanoparticles showed higher background possibly due to partial aggregation.

Ag-Spermine SERS background

A SERS spectrum was taken of aggregated and un-aggregated Ag-Spermine particles to obtain the background signal of Ag-spermine nanoparticles (Fig. S3).

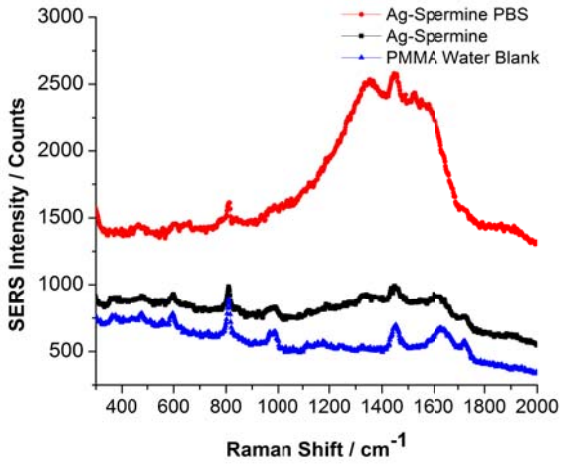


Fig.S3 Investigation of background signal from Ag-Spermine nanoparticles un-aggregated and aggregated. A 532 nm laser excitation of approximately 24 mW at the sample was used (three accumulations of 1 second).

Characterisation of the silver nanoparticles

Scanning Electron Micrographs (SEM)

SEM images of the Spermine and bPEI coated nanoparticles

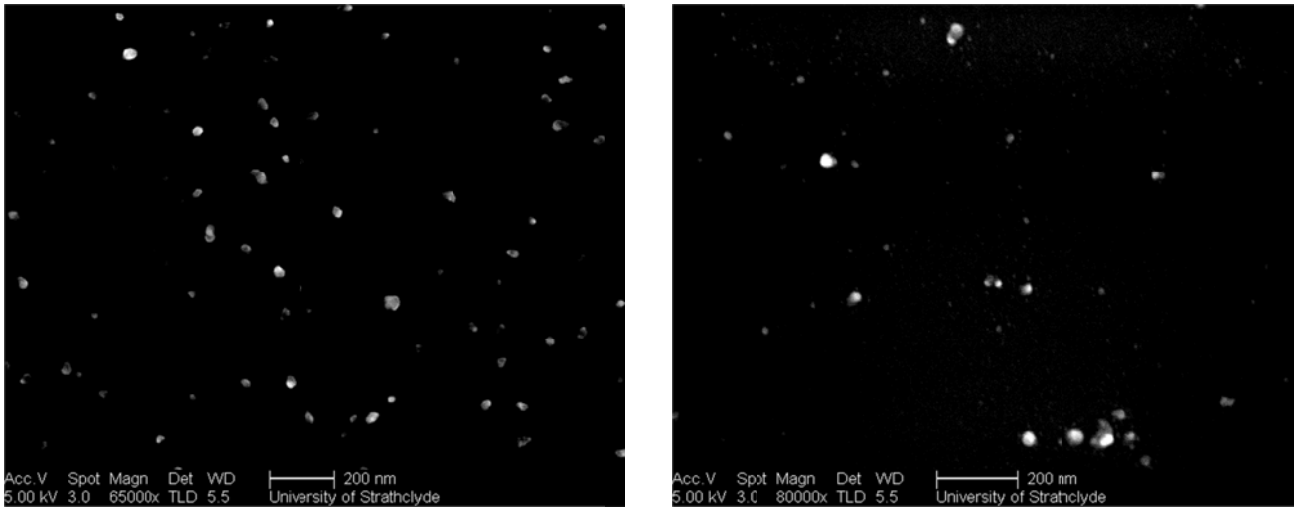


Fig. S4 SEM image of Spermine coated silver nanoparticles (left), and bPEI coated nanoparticles (Right).

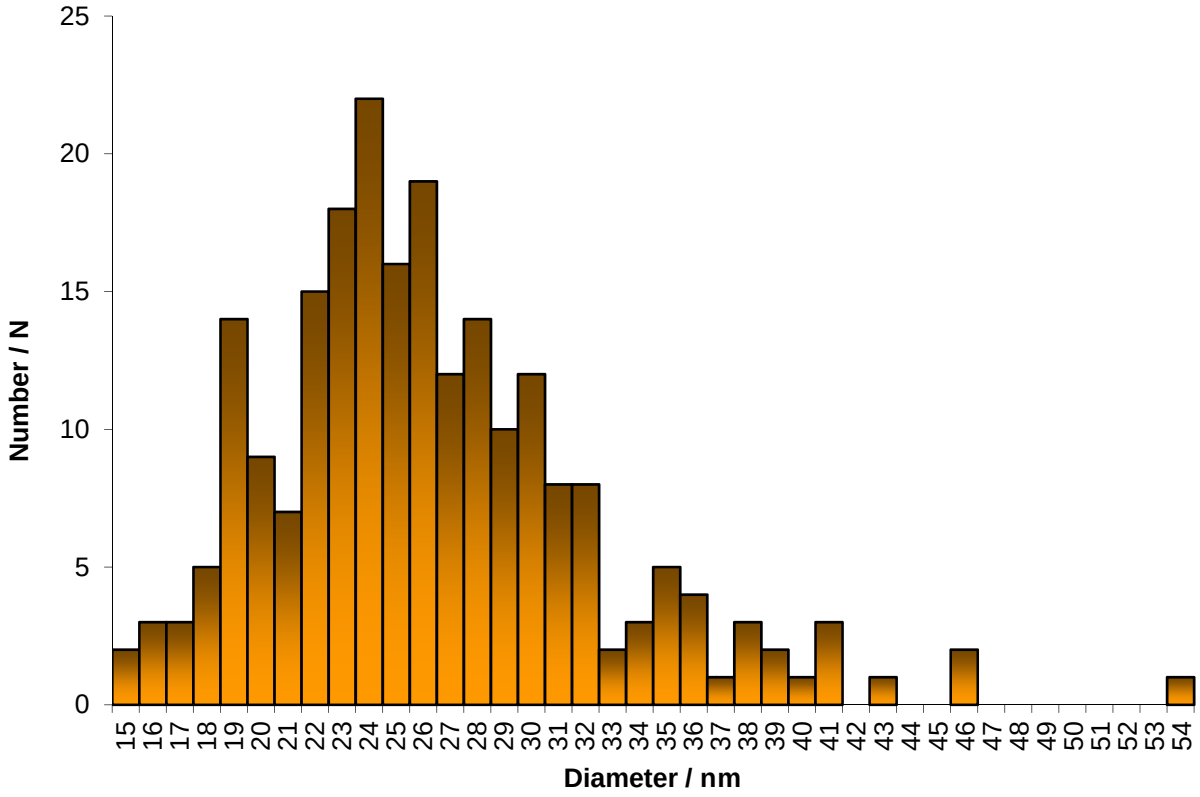


Fig. S5 Ag-Spermine nanoparticle distribution determined by SEM (N=225). The average size was 26.8 ± 6 nm (1 standard deviation).

UV-Vis absorbance

UV-Vis spectra obtained from positively charged silver nanoparticles previously reported in the literature are presented in Fig S3

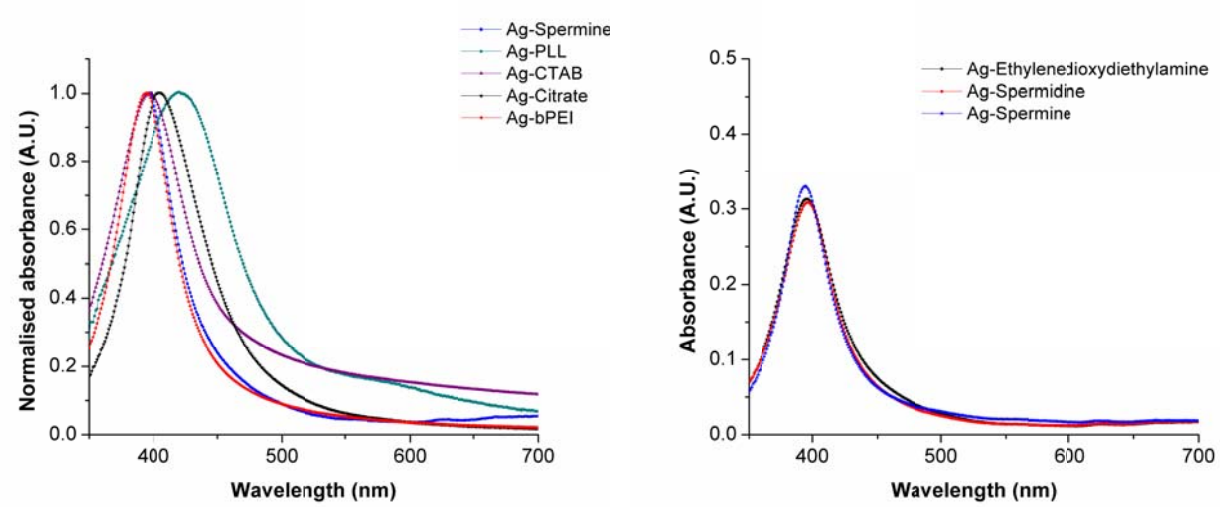


Fig.S6 UV-Vis traces of silver nanoparticles coated with different ligands (left). The obtained λ max value for Ag-Spermine was 395 nm, for Ag-PLL λ max was 420 nm, for Ag-CTAB λ max was 397 nm, for Ag-Citrate λ max was 405 nm, and for Ag-bPEI λ max was 394 nm. UV-Vis spectra obtained from the similar length ligand silver nanoparticles are presented (right). Samples were ten times diluted in water prior to analysis. The observed λ max value for Ag-Ethylenedioxydiethylamine was 395 nm, for Ag-Spermidine λ max was 397 nm, for Ag-Spermine λ max was 395 nm.

Hydrodynamic radius

The hydrodynamic radius of the nanoparticles was obtained with dynamic light scattering (DLS) measurements. Table S1 positively charged silver nanoparticles found in the literature, as well as the novel silver nanoparticles stabilised with similar length ligands; spermine, spermidine, ethylenedioxydiethylamine.

Table S1 Hydrodynamic radius of the silver nanoparticles used

	Mean Count Rate $kcps^{-1}$	Peak 1 $d.nm^{-1}$
Ag-Citrate	231.1	63
Ag-bPEI	440.7	75.7
Ag-CTAB	153	76.3
Ag-PLL	368	54.5
Ag-Ethylenedioxydiethylamine	338.5	45.1
Ag-Spermidine	386.3	47.4
Ag-Spermine	209.7	55.4

Note: However, we are comparing different systems where the nanoparticle sizes are different (25-45 nm) as well as several other variables that cannot be carefully controlled (surface chemistry, aggregation dynamics, interparticle distances at the generated hot-spots). Therefore we decided to carry out the experiment at a fixed laser line and nanoparticle concentration. Since Ag-spermine belongs to the group of the smallest nanoparticles (Fig. S6) of the nanoparticles investigated, by fixing the nanoparticle concentration we chose a rather conservative approach to evaluate their sensing ability as compared to bigger nanoparticles (i.e. the metal surface available in the Ag-spermine samples is lower than that for bigger particles).

ζ-potential

The zeta potential measurements showing the nanoparticles surface charge (Table S2).

Table S2 Zeta potential measurements

	Zeta (mV)	Error ±
Ag-Citrate	-37.7	± 2.0
Ag-bPEI	+42.0	± 7.04
Ag-CTAB	+18.0	± 1.9
Ag-PLL	+37.0	± 0.8
Ag-Ethylenedioxydiethylamine	+42.4	± 11.4
Ag-Spermidine	+44.2	± 13.3
Ag-Spermine	+46.7	± 7.97

Surface plasmon shift of the Ag- Spermine nanoparticles after addition of DNA over time

The surface plasmon red shift of the Ag-Spermine nanoparticles after addition of DNA in the concentration range of 0 - 10 nM was measured every 10 minutes over a time period of 120 min. Samples contained 40 µL of Ag-spermine nanoparticles, 320 µL water, and 40 µL of DNA solution.

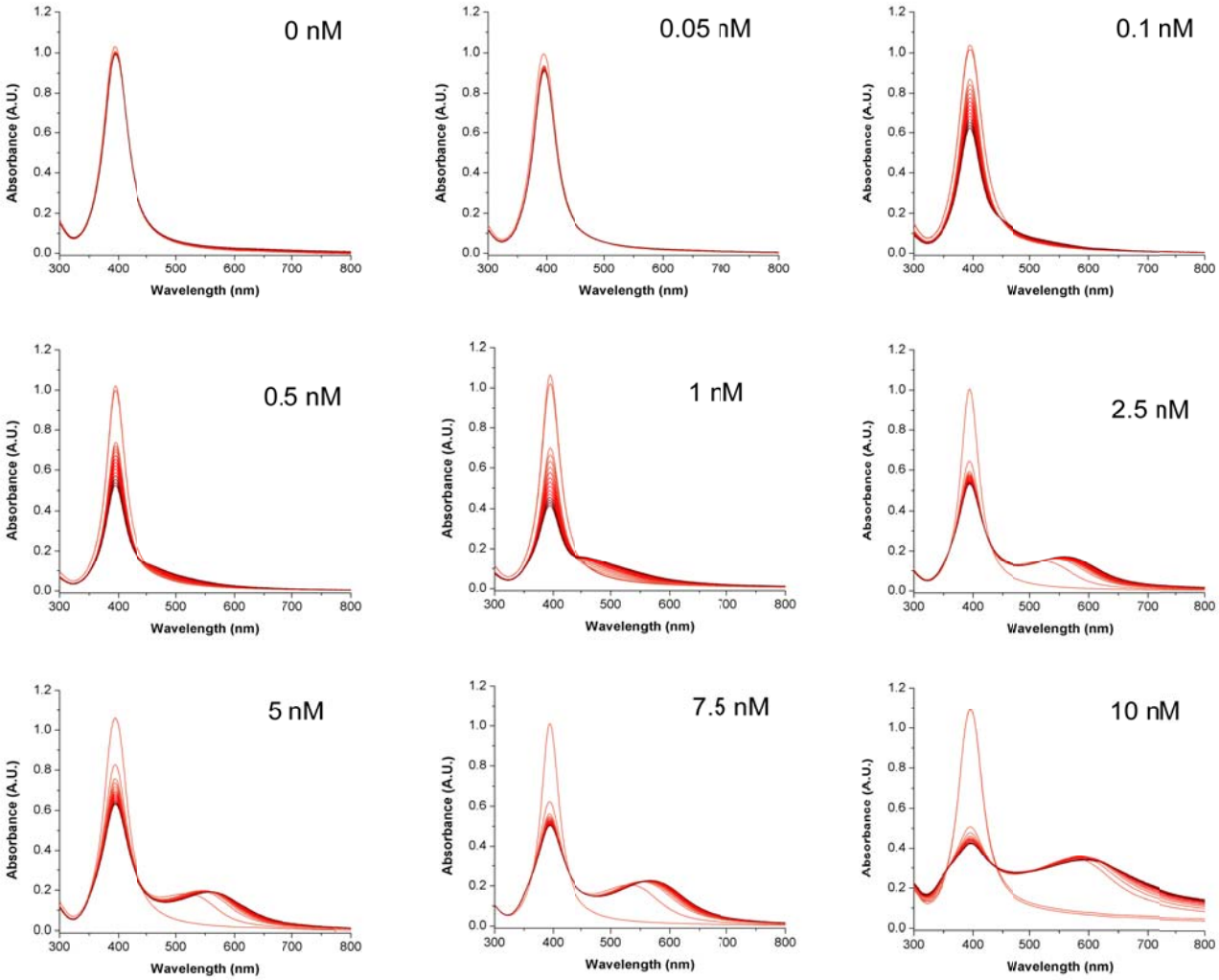


Fig. S7 The surface plasmon red shift of the Ag-Spermine nanoparticles after addition of DNA in the concentration range of 0-10 nM (from left to right and from top to bottom) was measured every 10 minutes over a time period of 120 min

Ag- Spermine nanoparticle size after addition of DNA over time

The Ag-Spermine nanoparticle size without (red circles) and with (blue triangles) 1 nM DNA present in the sample measured over a time period of 120 minutes. Samples contained 120 µL of Ag-spermine nanoparticles, 960 µL water, and 120 µL of 10 nM DNA solution or water in case of the control sample. The average Ag-Spermine nanoparticle size increased after the addition of DNA and reached the plateau phase after 30 minutes which corresponds to the presented UV-Vis and SERS data.

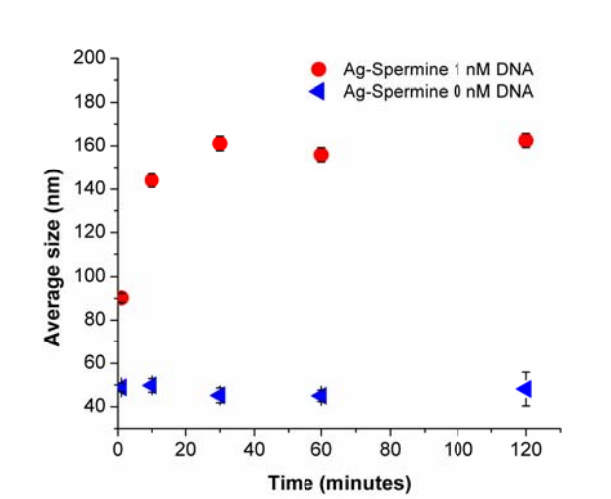


Fig.S8 Average nanoparticle size without (red circles) and with (blue triangles) 1 nM DNA present in the sample measured over a time period of 120 minutes. Error bars represent ± one standard deviation of three measurements.

Surface enhanced fluorescence by Ag-CTAB

Surface enhanced fluorescence (SEF) of FAM observed in the Ag-CTAB experiments.

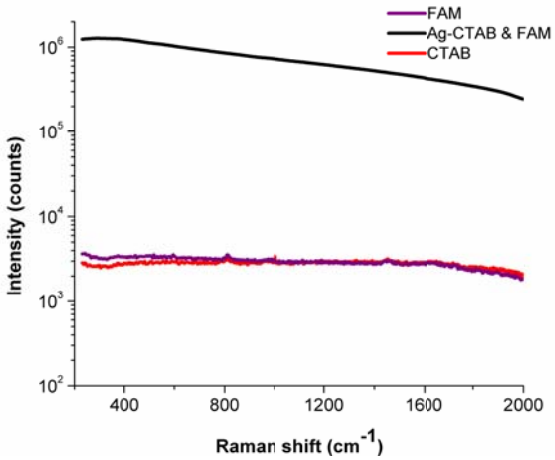


Fig. S9 SEF of FAM on Ag-CTAB nanoparticles (black line), FAM fluorescence (purple line), Ag-CTAB signal (red line). A 532 nm laser excitation of approximately 24 mW at the sample was used (three accumulations of 1 second).

Double stranded DNA detection

Double stranded DNA was made by adding 5 µL of FAM labelled DNA (400 nM) to 5 µL of its complementary DNA (400 nM) in 10 µL PBS (140 mM Sodium Chloride, 5.11 mM Potassium, 8.1 mM Na_2HPO_4,and 1.47 mM KH_2PO_4 pH 7.4) and in case of single stranded DNA 5 µL water was used. These samples were hybridised in a thermo-cycler (Agilent Stratagene MX3005P) by 95°C for 5 minutes and cooling to room temperature by 2 degrees per second. Afterwards these samples were transferred to PMMA microcuvettes and 180 µL of Ag-Spermine nanoparticles was added followed by SERS analysis. Detection of double and single stranded DNA was compared and intensities were found to be very similar (Fig. S10).

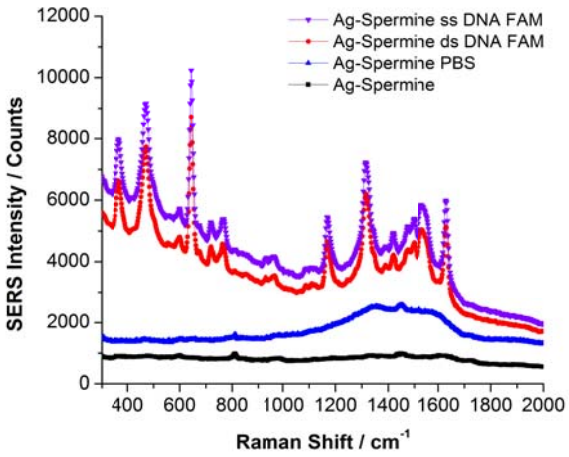


Fig. S10 Detection of dye labelled double and single stranded DNA. A 532 nm laser excitation of approximately 24 mW at the sample was used (three accumulations of 1 second).

A scanning kinetics SERS experiment measuring FAM labelled double and single stranded DNA over time showed similar SERS intensities for double and single stranded DNA with overall slightly higher intensities for double stranded DNA.

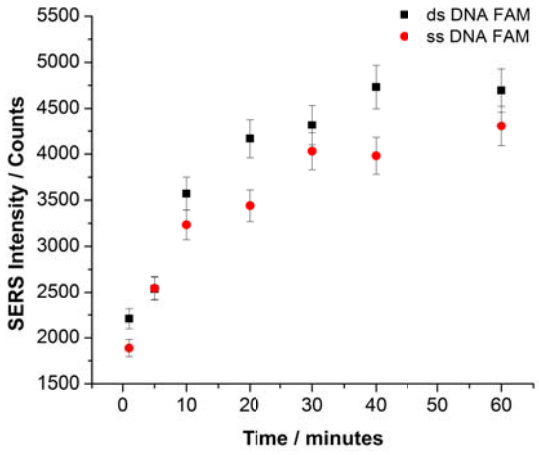


Fig. S11 SERS intensity of the FAM 1632 cm^{-1} peak measured over time for double and single stranded DNA. A 532 nm laser excitation of approximately 24 mW at the sample was used (three accumulations of 1 second). Error bars represent ± one standard deviation of three separate samples.

Positively charged dye labelled DNA detection

DNA labelled with a positively charged dye (Cy5) and negatively charged dye (5'FAM) was detected by SERS using Ag-Spermine nanoparticles (Fig. S12).

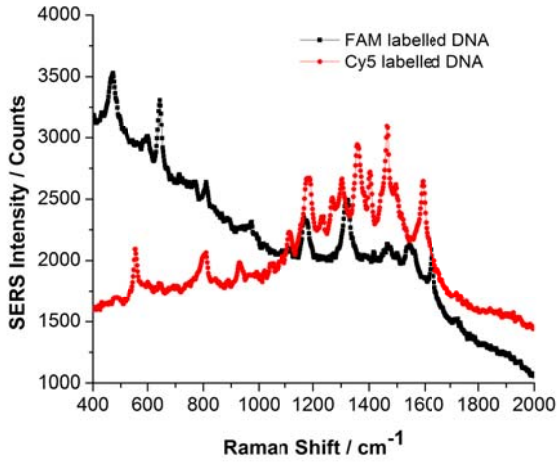
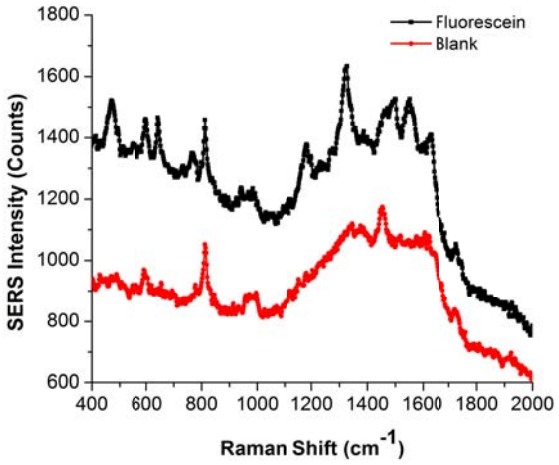


Fig. S 12 Detection of positively charged dye (Cy5) labelled DNA and negatively charged dye (5'FAM) labelled DNA by SERS. A 532 nm laser excitation of approximately 24 mW at the sample was used (three accumulations of 1 second).

Direct SERS detection of negatively charged dye Fluorescein

Negatively charged dye fluorescein (20 µL, 100 nM) was added to Ag-Spermine nanoparticles (180 µL) and detected directly by SERS.



X

Fig.S13 Direct detection of a negatively charged dye (Fluorescein) by SERS. A 532 nm laser excitation of approximately 24 mW at the sample was used (three accumulations of 1 second).

Ligand structures of the similar length ligand

The chemical structure of the similar length ligands for SERS experiments; spermine, spermidine, and ethylenedioxydiethylamine are shown in Fig S13 these ligands differ with respect to their positive charge, 4+, 3+ and 2+ respectively.

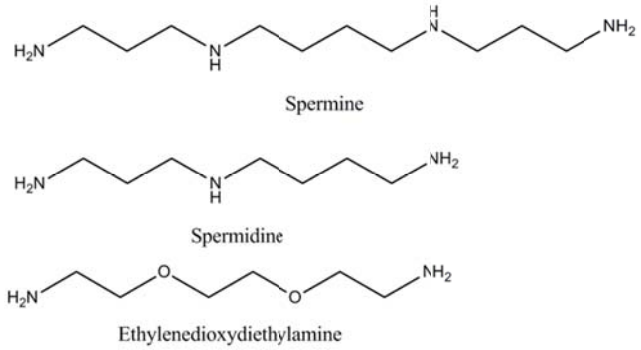


Fig. S14 Chemical structures of the ligands used for nanoparticle stabilisation.

References

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