Formation of SERS active nanoparticle assemblies via specific carbohydrate-protein interactions

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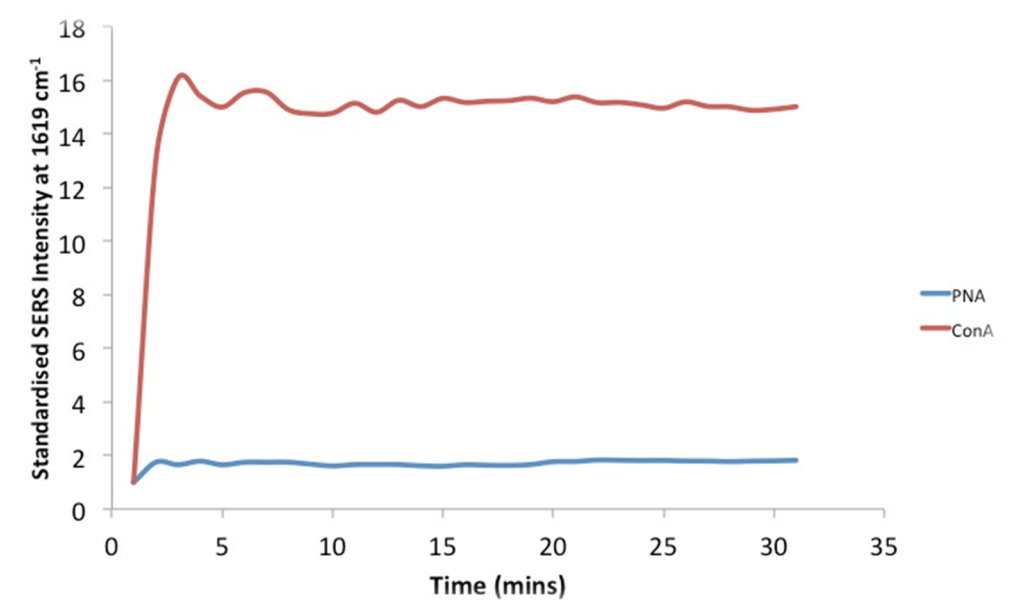

Figure S1: Comparison of SERS enhancement obtained when 10 nM solutions of ConA and PNA added to solutions of lactose functionalised nanoparticles by monitoring the intensity at 1619 cm^{-1} using 514.5 nm laser excitation.

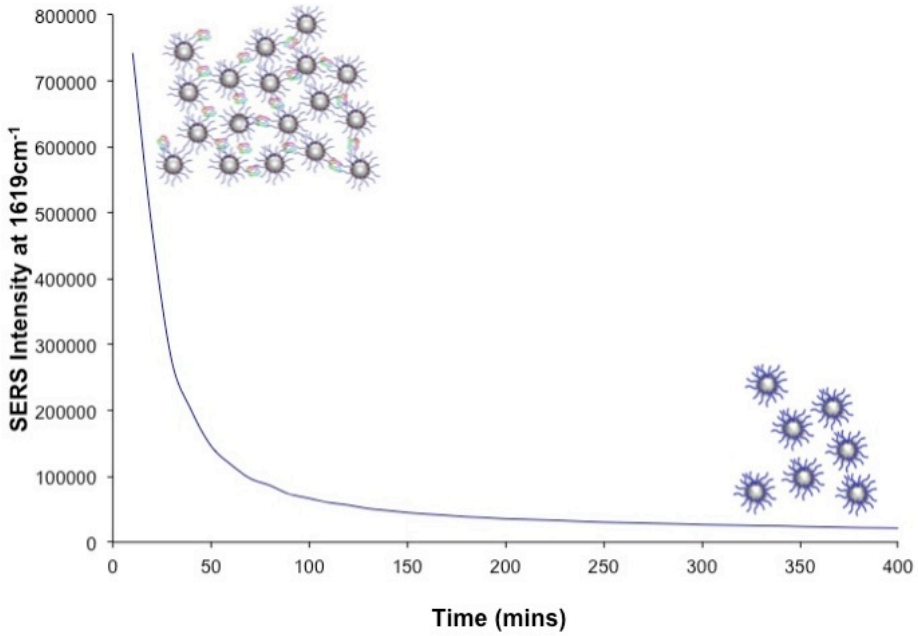

Figure S2: SERS intensity of peak 1619 cm^{-1} over 6 hours following the addition of 10 nM solution of mannose at 514.5 nM laser excitation with 1s accumulation

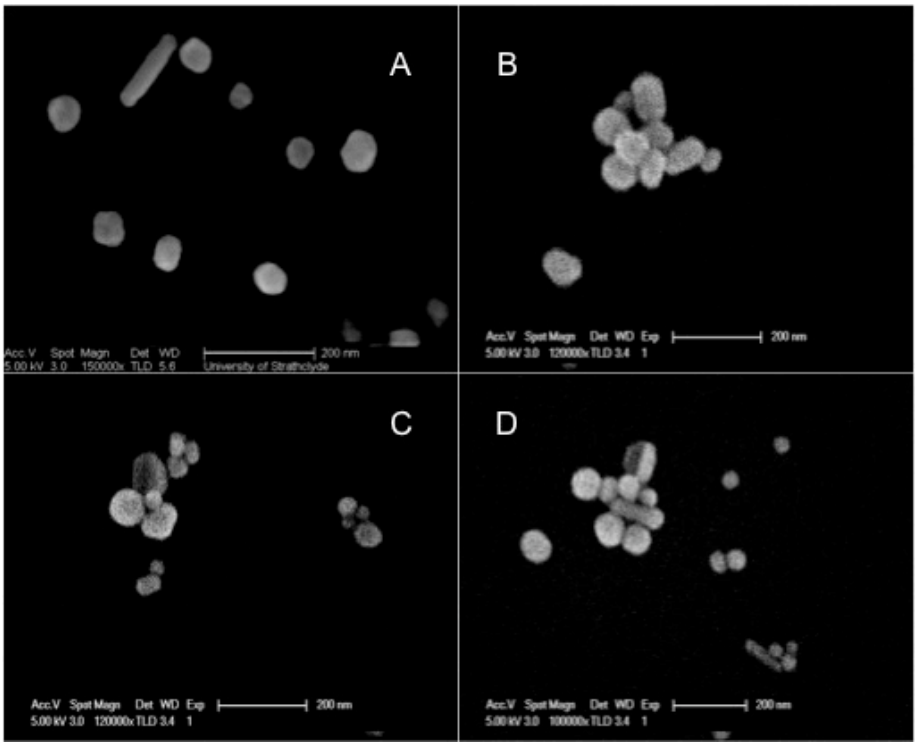

Figure S3: Comparison of SEM images of templated aggregate assembly with various concentrations of ConA (A) No ConA present (B) 10 µM ConA (C) 5 µM ConA and (D) 40 pM ConA

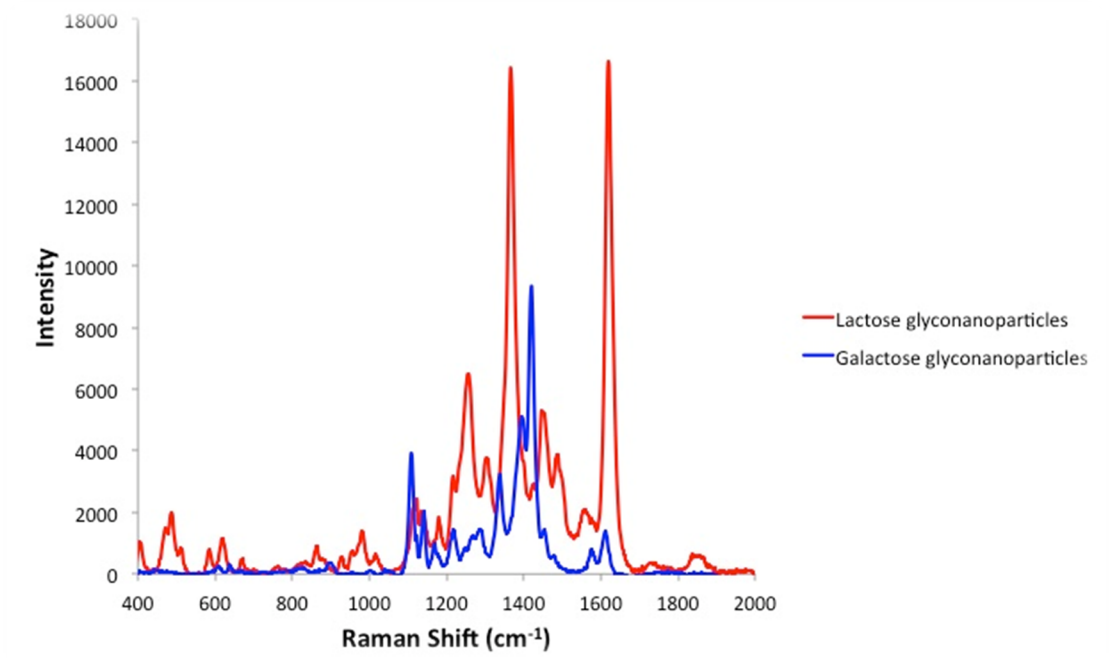

Figure S4: Individual SERS spectra of lactose and galactose funcationalised AgNPs at 514.5 nM laser excitation with 1s accumulation.

Experimental section

Reagents: All reagents used were analytical grade and purchased from Sigma Aldrich (UK) unless otherwise stated.

Colloidal preparation. Colloidal silver suspensions were prepared by a modified Lee and Meisel method.[29] A sample of silver nitrate (90 mg) was dissolved in 10 mL of distilled water and added to 480 mL of distilled water and both heated and stirred. Upon vigorous boiling sodium citrate (100 mg dissolved in 10 mL of distilled water) was immediately added and after a few minutes a colour change occurred. The solution was then boiled for a further 15 minutes before leaving to cool for 2 hours whilst maintaining stirring.

Lactose ligand synthesis. 3-mercaptopropionic acid (3-MPA) (43.58ul, 0.5 mM, 1eq), 4-dimethylaminopyridine (DMAP) (89mg, 0.75 mM, 1.5 eq) and lactose (339.2 mg, 1mM, 2eq) were added to 50 mL of dimethylformamide (DMF) at 0°C and stirred for 5 minutes. The sample was then brought to room temperature before N,N'-Diisopropylcarbodiimide (DCC) (154.5 mg, 1.5 mM, 3eq) was added and the solution was stirred at room temperature for 3 hours. The DMF was removed *in vacuo* at high pressure. The resulting urea by product was then removed by washing with the minimum volume of distilled water required to dissolve the desired product. The solution was then filtered and the distilled water removed in vacuo to yield a yellow crystalline solid. Yield 55.1%.

^{1}H NMR (400 MHz, DMSO) δ 8.11 (dd, *J* = 5.0, 1.5 Hz, 1H), 6.60 (dd, *J* = 5.0, 1.5 Hz, 1H), 4.90 (d, *J* = 3.6 Hz, 1H), 4.33 (d, *J* = 7.8 Hz, 1H), 4.20 (t, *J* = 7.8 Hz, 1H), 3.71 (dt, *J* = 11.2, 9.8 Hz, 1H), 3.66 – 3.22 (m, 13H), 3.18 (dd, *J* = 8.6, 4.5 Hz, 1H), 2.94 (s, 2H), 2.51 (dd, *J* = 3.4, 1.7 Hz, 3H), 2.31 (s, 1H).

^{13}C NMR (400 MHz, DMSO) δ148.81, 106.67, 103.79, 81.30,80.97,75.46,74.97,73.22,70.54 ,68.10,60.34,38.62
MS: Found m/z 431.3, M+H+, *Melting Point:* 152-154°C

Galactose ligand synthesis. 3-mercaptopropionic acid (3-MPA) (43.58ul, 0.5 mM, 1eq), 4-dimethylaminopyridine (DMAP) (90mg, 0.75 mM, 1.5 eq) and galactose (180.3 mg, 1mM, 2eq) were added to 50 mL of dimethylformamide (DMF) at 0°C and stirred for 5 minutes. The sample was then brought to room temperature before N,N'-Diisopropylcarbodiimide (DCC) (156.0 mg, 1.5 mM, 3eq) was added and the solution was stirred at room temperature for 3 hours. The DMF was removed *in vacuo* at high pressure. The resulting urea by product was then removed by washing with the minimum volume of distilled water required to dissolve the desired product. The solution was then filtered and the distilled water removed *in vacuo* to yield a pale orange oil. Yield 44.3%.

^{1}H NMR (400 MHz, DMSO) δ 8.14 (dd, *J* = 6.6, 1.5 Hz,1H), 6.64 (dd, *J* = 6.8, 1.7 Hz 1H), 4.95(d, *J* = 3.4 Hz, 1H), 3.88-3.17(m,6H), 2.91(t, *J* = 6.5 Hz , 2H),2.61(t, *J* = 6.8 Hz, 2H)

^{13}C NMR (400 MHz, DMSO) δ148.88, 106.67, 74.98, 72.09, 70.51, 70.33, 68.25, 62.86, 60.57, 38.58
MS: Found m/z 267.2, M+H+

Sample preparation. The short thiol linker and benzotriazole dye were added to a stirring solution of silver nanoparticles to achieve a final concentration of 1 x10^{-6} M. The samples were mixed for a further 30 minutes before being purified. Purification was completed by centrifugation at 6000 rpm (2820 g) for 20 minutes for 2 cycles whilst washing the nanoparticles with 10 mM tris buffer pH 7.5 in between cycles. The purified modified nanoparticles were resuspended in tris buffer pH 7.5 and stored at 4°C until needed.

UV-Visible spectroscopy measurements. Samples were prepared by a 1:10 dilution of the nanoparticles in Tris buffer pH 7.5 and measured in a quartz cuvette. Measurements were made between 200-800nm unless otherwise stated.

SERS measurements. SERS was recorded using a using a Renishaw InVia Raman Microscope with an argon ion laser with an excitation frequency of 514.5 nm as the source of radiation. The laser was focused onto a sample using 20x long working distance (LWD) objective lens. Detection was achieved using a charge coupled device (CCD) detector and spectra were recorded in the range 0 – 3000 cm^{-1}. All samples were analysed using 100% laser power and an accumulation time of 1 second with 10 accumulations made in total. Control spectra were obtained by analysing the above components without any lectin present. After analysis all spectra were baseline corrected using liner baseline correction performed by GRAMS/AI software.