

## **Supporting Information**

### **Experimental Methods**

#### ***Instrumentation***

Zeta potentials and sizes of colloidal nanoparticles were recorded using a Malvern Zetasizer Nano ZS dynamic light scattering system, equipped with a 633nm laser. They were further characterized using an Agilent 8453 diode array UV-Vis spectrometer. Raman spectra were acquired using an Avalon R2 Ramanstation, 785nm diode laser delivering 100mW at the sample, Echelle spectrograph and thermoelectrically cooled CCD. SERS samples were prepared by adding 20uL of analyte solution to 200uL colloid in a 96 well plate, and aggregating with 20uL of suitable aggregating agent, as discussed in the main text. Accumulation times in all spectra are 30s.

#### ***Materials***

All chemicals used, except sodium hydroxide, were purchased from Sigma Aldrich. Sodium hydroxide was purchased from Riedel-de Haën. All chemicals were used as received without further purification. All solutions were prepared from distilled, deionized (DDI) water resistivity 18.2MΩ, from a Branstead Nanopure Diamond system.

#### ***Thiocholine Bromide Preparation<sup>1</sup>***

S-Acetylthiocholine bromide (490mg,  $2 \times 10^{-3}$  mol) was dissolved in 10mL of nitrogen purged H<sub>2</sub>O. HBr<sub>(aq)</sub> (5mL, 48% by weight) was added to this and the mixture was heated at 80°C for 30 minutes. The mixture was aspirated to remove most of the acid and water, then diethyl ether (~10mL) was added and shaken. This was aspirated once more, and the solid washed in absolute ethanol. Diethyl ether was added to the product and aspirated to leave a white powder. This was dissolved in hot ethanol (15mL) and precipitated with diethyl ether (10mL). The white precipitate was filtered allowed to dry, and a yield of 209mg (52%) was recorded. Melting point 190-192°C. <sup>1</sup>H NMR (300 MHz; D<sub>2</sub>O; TMS): 2.77-2.83 (m, 2H, CH<sub>2</sub>S), 3.00 (s, 9H, N(CH<sub>3</sub>)<sub>3</sub>), 3.37-3.43 (m, 2H, CH<sub>2</sub>N).

#### ***Colloid Preparation***

HRSC was synthesized following the method published by Leopold and Lendl.<sup>2</sup> 5mL of NaOH (0.1M) was added to 5mL of aqueous hydroxylamine hydrochloride (6mM), then the whole mixture added to 90mL of aqueous AgNO<sub>3</sub> (0.1mM) with stirring. The colloid formed spontaneously and was left stirring for ~20 minutes before use. The nanoparticles had a mean diameter of 73.4nm, and a zeta potential of -49.5mV.

CRSC was synthesized according to Lee and Meisel's method.<sup>3</sup> AgNO<sub>3</sub> (45mg) was dissolved in 250mL and heated until boiling. When boiling temperature had been reached, 5mL of 1% sodium citrate solution was added dropwise with stirring. The mixture was allowed to boil for 90 minutes, and was allowed to cool to room temperature. Any water lost

from evaporation was added at this stage. These nanoparticles had a mean diameter of 70.7nm, and a zeta potential of -48.6mV.

### ***Modification of Colloids with Thiocholine***

Both colloids were modelled as spheres of uniform diameter and the total surface area of the colloid was calculated based on the mass of silver per sample and mean nanoparticle diameters found above. The amount of thiocholine required was approximated by dividing the total surface area of the colloidal sample by the surface area of one mercaptopropionic acid molecule, calculated by Spégel *et al* to be  $20.2 \text{ \AA}^2$ .<sup>4</sup>

HRSC was modified directly without further treatment; 6mL of  $1 \times 10^{-4} \text{M}$  thiocholine solution (approximately a two-fold excess) was stirred vigorously at room temperature in a conical flask, while adding 50mL of HRSC dropwise over several minutes to produce the positive colloids HRSC+.

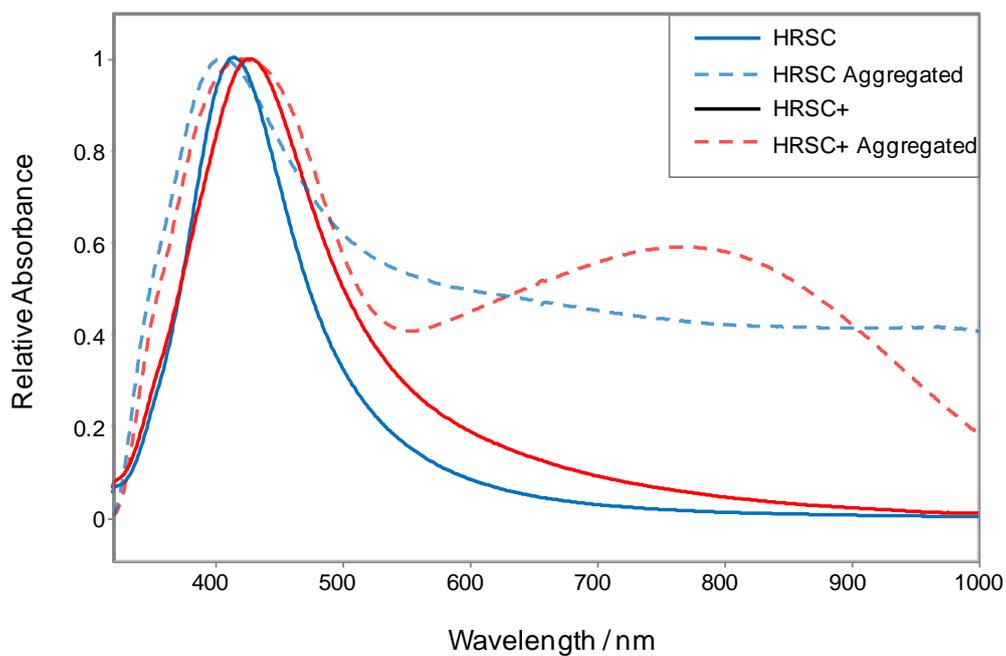
CRSC could not be modified directly, but was first washed by adding 2% (v/v) 0.01M NaCl then centrifuging at 3000rpm for 30 minutes. The pale yellow supernatant was removed and the nanoparticles were resuspended in DDI water up to the original volume. This process was repeated once more, at which point no citrate peaks were visible on in the SERS spectrum of the colloid, having been replaced by chloride. 30mL of this washed colloid was added to 3mL of  $1 \times 10^{-4} \text{M}$  thiocholine solution dropwise with vigorous stirring to produce the positive colloids CRSC+.

The positive colloids, HRSC+ and CRSC+, were washed by centrifugation at 3000rpm for 30 minutes in polyethyleneimine (PEI) coated centrifuge tubes, and resuspension in DDI water. The tubes were coated by dissolving 1g of PEI in 50mL of hot water, and adding to the plastic tubes to be coated. These were vigorously shaken for 15 minutes and left to cool. Once at room temperature, the tubes were rinsed thoroughly with cold water.

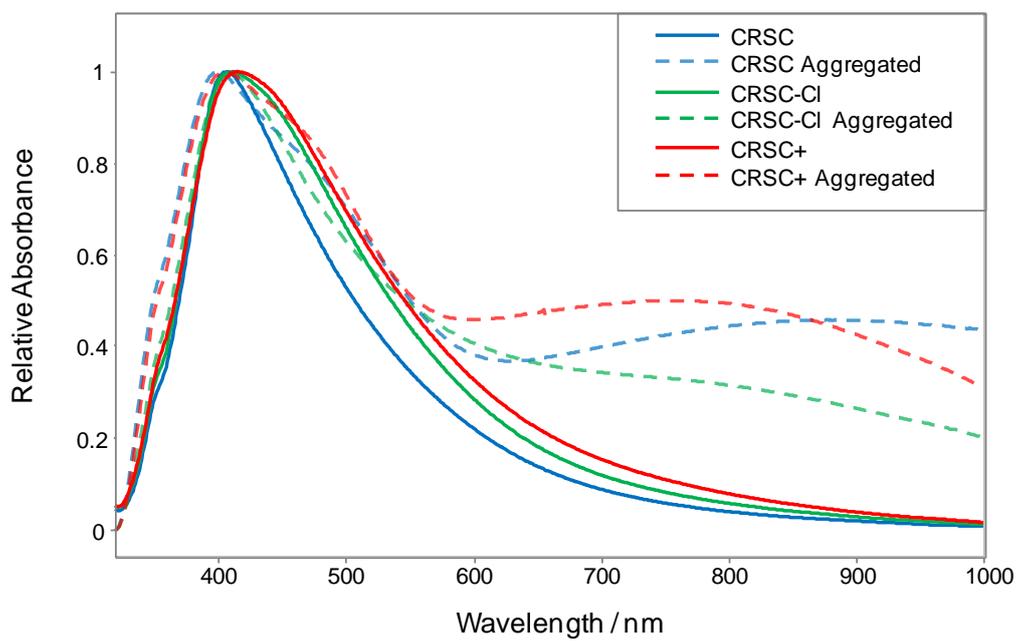
### **Positive Colloid Characterization**

#### ***UV-Vis***

Colloids were dilute 2:1 in DDI H<sub>2</sub>O and the UV-Vis absorbance spectra taken between 300 and 1000nm. Figure S1 shows the UV-Vis of HRSC and HRSC+. The dashed lines represent the spectra produced when 200 $\mu$ L of 1M NaCl was added to 2mL colloid. It is clear that HRSC+ is very similar to HRSC, with a very slight red-shift, and that the colloid is still unaggregated until activated with NaCl. Figure S2 shows CRSC, CRSC washed with NaCl (CRSC-Cl) and CRSC+. Again, it is clear that CRSC+ remains unaggregated, and that adsorption of thiocholine to the surface results in no aggregation.



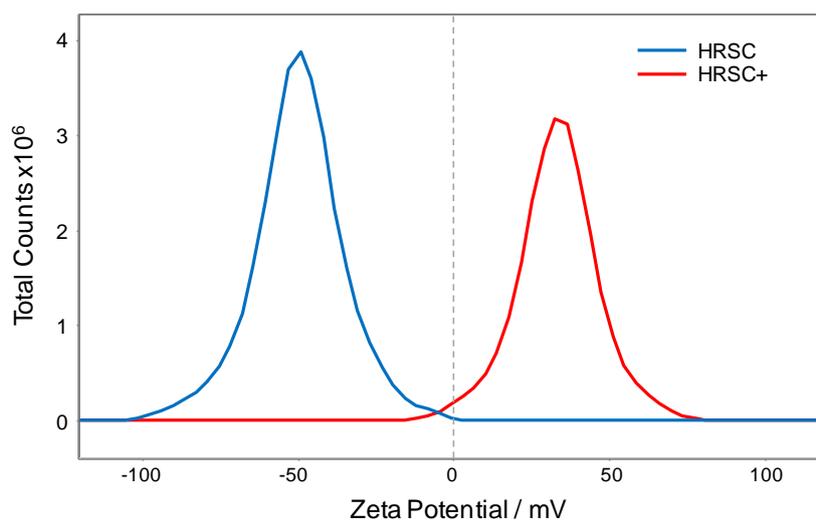
**Figure S1.** UV-Vis spectra of HRSC, HRSC+ and the same colloids after addition of 1M NaCl.



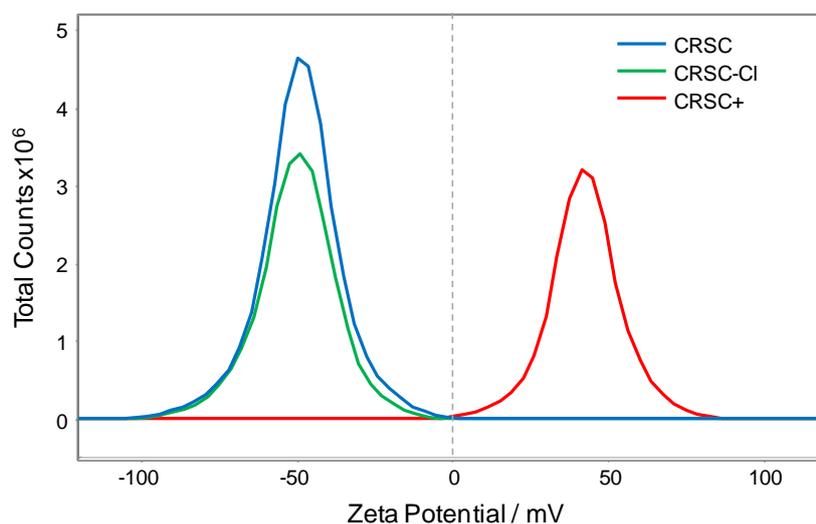
**Figure S2.** UV-Vis spectra of CRSC, CRSC-Cl and CRSC+, and their spectra after addition of 1M NaCl

### Zeta Potentials

Zeta potential distributions, as recorded by laser Doppler electrophoresis on a Malvern Zetasizer Nano ZS, of HRSC and HRSC+ are shown in Figure S3. Distributions of CRSC, CRSC-Cl and CRSC+ are shown in Figure S4. It is clear that HRSC+ and CRSC+ are positively charged, in stark contrast to the original colloids. The difference in number of counts is believed to be a consequence of the number of particles in each sample.



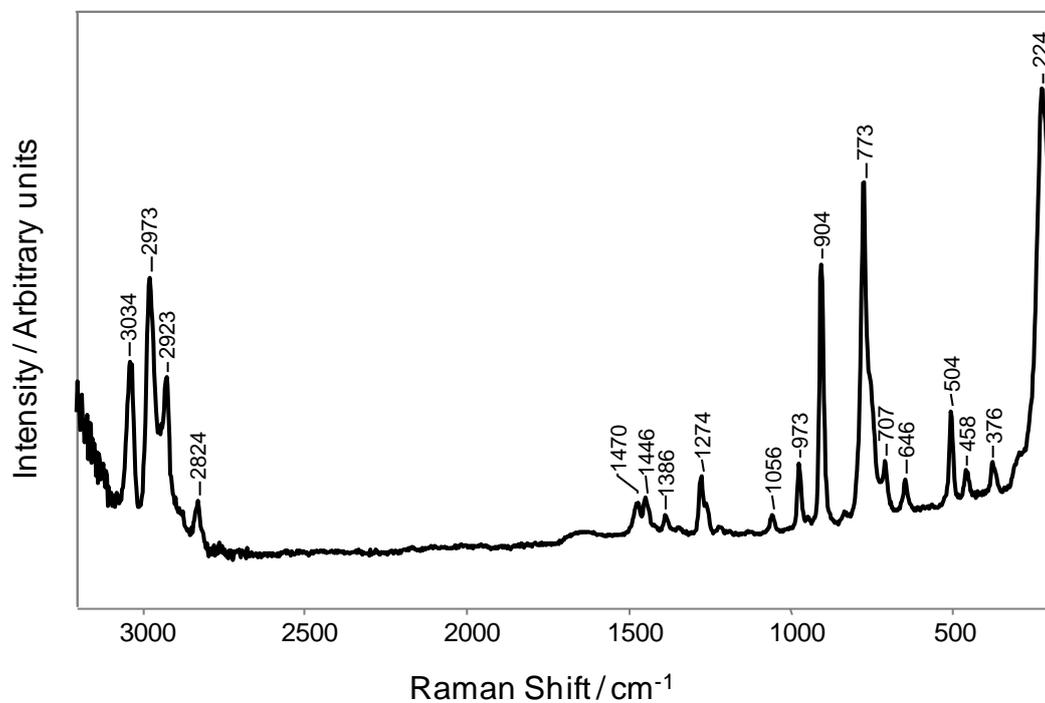
**Figure S3.** Zeta potential distributions of HRSC (blue) and HRSC+ (red).



**Figure S4.** Zeta potential distributions of CRSC (blue), CRSC-Cl (green) and CRSC+ (red).

### *Raman Characterisation*

The SERS spectrum of CRSC+ is shown in Figure S5, with all major peaks shown. The peak positions of thiocholine on HRSC+ were identical to that of CRSC+, and so Figure S5 is representative of both. The main peak positions and their assignments are given in Table S1.



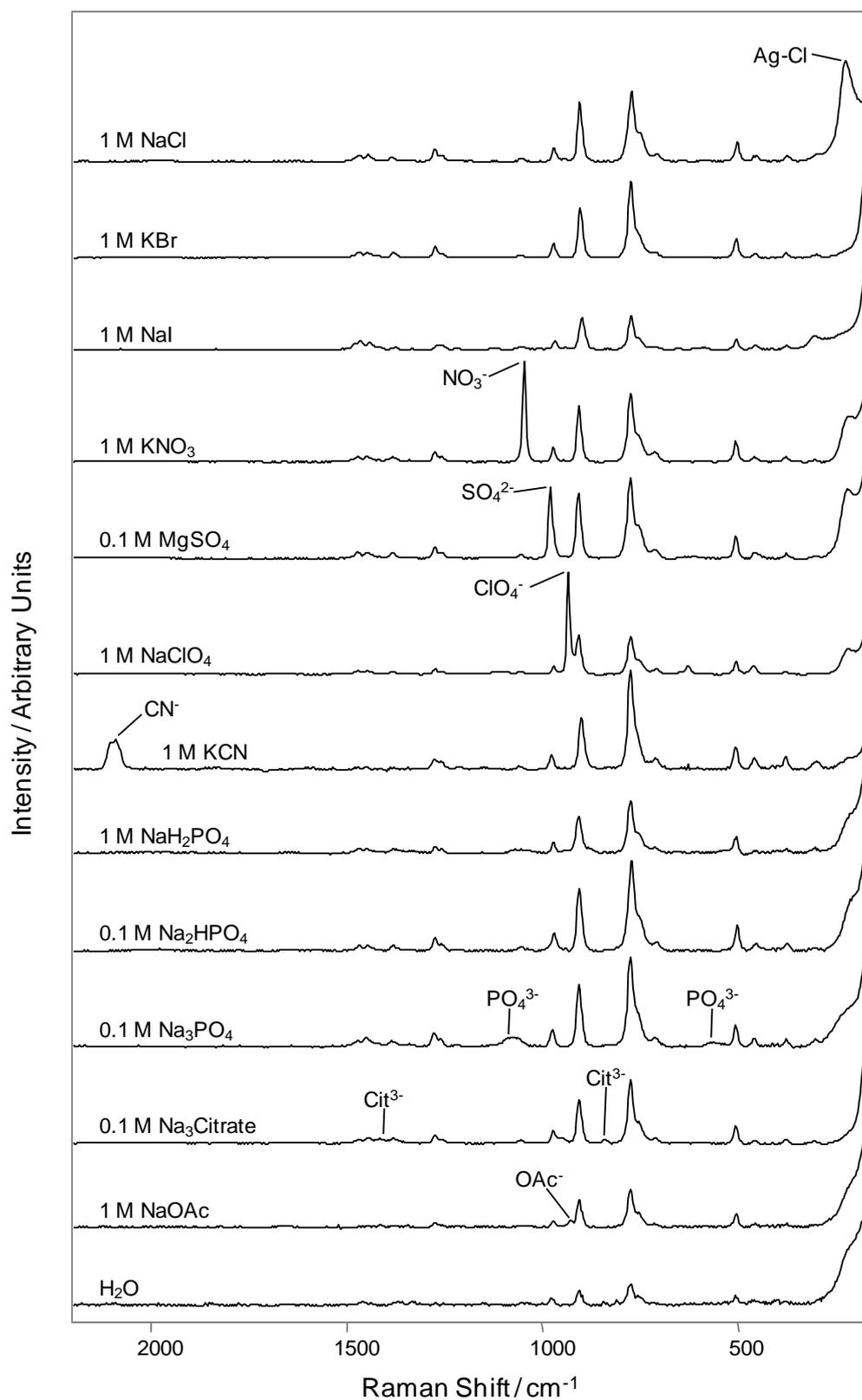
**Figure S5.** SERS spectrum of CRSC+, taken by adding 20 $\mu$ L of 1M NaCl to 200 $\mu$ L of colloid.

Raman Shift (cm <sup>-1</sup> )	Assignment
224	$\nu(\text{Ag-Cl})$
376	
458	
504	
646	$\nu(\text{S-C-C})_{\Gamma}$
707	$\nu(\text{S-C-C})_{\text{G}}$
773	$\nu(\text{S-C-C-N})$
904	$\nu(\text{S-C-C-N})$
973	
1056	
1274	
1386	
1446	
1470	
2824	$\nu(\text{C-H})$
2923	$\nu(\text{C-H})$
2973	$\nu(\text{C-H})$
3034	$\nu(\text{C-H})$

**Table S1.** List of Raman bands and appropriate assignments for CRSC+.

### *Aggregating Agent Comparison*

Several ionic salts were tested as aggregating agents and, in most cases, the peaks attributable to TC were unaffected. However, peaks attributable to each aggregating agent appeared in the spectrum due to adsorption of the anion of the salt, either to the TC monolayer or to the silver surface itself. Figure S6 shows the results of aggregating HRSC+ with a range of ionic salts; CRSC+ gave similar results. Table S2 shows the exact position of the extra bands found.



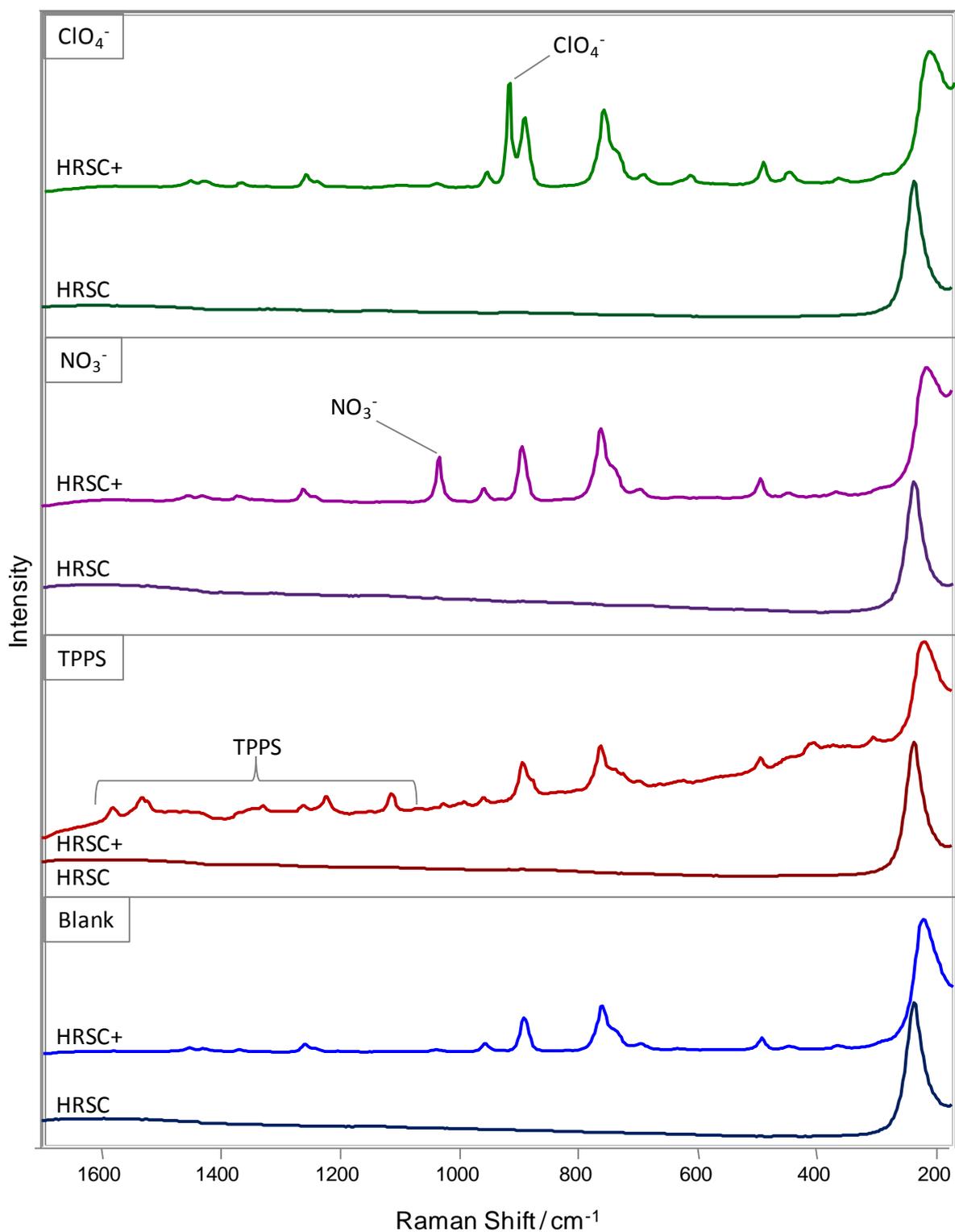
**Figure S6.** SERS spectra of 200  $\mu\text{L}$  HRSC+ aggregated with 20  $\mu\text{L}$  of various ionic salts or H<sub>2</sub>O. Bands not attributed to TC have been highlighted, and spectra are scaled and shifted for clarity. Note: NaAc represents sodium acetate.

Aggregating Agent	Concentration of Added Salt	Additional Bands (cm <sup>-1</sup> )
NaCl	1 M	226
KBr	1 M	150(sh)
NaI	1 M	114(sh)
KNO <sub>3</sub>	1 M	1046
MgSO <sub>4</sub>	0.1 M	980
NaClO <sub>4</sub>	1 M	934
KCN	1 M	2092, 2104
NaH <sub>2</sub> PO <sub>4</sub>	1 M	-
Na <sub>2</sub> HPO <sub>4</sub>	0.1 M	1070
Na <sub>3</sub> PO <sub>4</sub>	0.1 M	562, 1076
Na <sub>3</sub> Citrate	0.1 M	835, 1414
NaAc	1 M	928

**Table S2.** Additional SERS bands found when aggregating with various ionic salts. (sh) denotes a shoulder, rather than a full peak.

### Performance with Selected Analytes

Several anionic analytes were added to HRSC+ and HRSC, and their comparison compared. Figure S7 shows spectra of three analytes, NaClO<sub>4</sub>, NH<sub>4</sub>NO<sub>3</sub> and tetraphenylporphine tetrasulfonic acid (TPPS). In each case, bands from the analyte can be seen when the positive colloid is used, but not when the negative colloid is used. These examples clearly show that positively charged colloids can have distinct advantages over negatively charged ones when detecting anionic analytes. The lowest observable concentrations for NaClO<sub>4</sub> and NH<sub>4</sub>NO<sub>3</sub> when using the aggregating agents, NaCl, MgSO<sub>4</sub> and NaOAc are shown in Table S3.



**Figure S7.** SERS spectra  $8.3 \times 10^{-2}$  M  $\text{NaClO}_4$ ,  $8.3 \times 10^{-2}$  M  $\text{NH}_4\text{NO}_3$  and  $8.3 \times 10^{-7}$  M TPPS adsorbed on HRSC+ and HRSC. A water blank of each colloid is provided for reference. For  $\text{NaClO}_4$  and  $\text{NH}_4\text{NO}_3$  samples, the salt was concentrated enough to aggregate the colloid without a further agent.  $\text{NaCl}$  was used to aggregate TPPS sample and water blank.

Aggregating Agent	Lowest Observable Concentration (mol dm <sup>-3</sup> )	
	NaClO <sub>4</sub>	NH <sub>4</sub> NO <sub>3</sub>
1 M NaCl	6.7x10 <sup>-5</sup>	6.7x10 <sup>-4</sup>
0.01 M MgSO <sub>4</sub>	6.7x10 <sup>-5</sup>	5x10 <sup>-4</sup>
1 M NaAc	5.0x10 <sup>-5</sup>	3.3x10 <sup>-4</sup>

**Table S3.** Lowest observable concentrations of NaClO<sub>4</sub> and NH<sub>4</sub>NO<sub>3</sub> for three aggregating agents.

### References

- 1 R. A. Moss, G. O. Bizzigotti, and G. W. Huang, *Journal of the American Chemical Society*, 1980, **102**, 754.
- 2 N. Leopold and B. Lendl, *Journal of Physical Chemistry B*, 2003, **107**, 5723.
- 3 P. C. Lee and D. Meisel, *Journal of Physical Chemistry*, 1982, **86**, 3391.
- 4 C. Spiegel, A. Heiskanen, J. Acklid, A. Wolff, R. Taboryski, J. Emneus, and T. Ruzgas, *Electroanalysis*, 2007, **19**, 263.