Supplementary Information

Turning up the Lights - Fabrication of Brighter SERRS Nanotags

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Silver colloid preparation

90 mg of silver nitrate was added to 500 mls of water at 45°C, this was rapidly heated to boiling, whereupon 10 mls of 1% sodium citrate was added and the temperature reduced to 98°C for 90 minutes. After this time heating was removed and the solution allowed to cool to room temperature.

Nanotag formation

110 μl of 2 x 10^{-6} M Nile blue solution was added to 500 μl of silver colloid followed by 500 μl of 20 mM KCl. This solution was allowed to stabilise over 48 hours before encapsulation. 2.2 μl of 0.77 μM thiol-PEG-5000 was added to each 1.11 ml mixture and shaken overnight. The nanotag solution was washed by centrifuging the solution at 7000 rpm for 20 minutes and re-suspending the pellet by sonication with 1 ml deionised water. This gave a working colloid concentration of 9 x 10^{-11} M for all subsequent analysis.

Control experiments were carried out as above but with the addition of water rather than 20 mM KCl.

Analysis

SERRS spectra were recorded on a Leica DM/LM microscope equipped with an Olympus 20x/0.4 long-working distance objective to collect 180° backscattered light from a micro-cuvette. The spectrometer system was a Renishaw Ramascope System 2000 with the 632.8 nm line of a helium-neon laser as the excitation source. The unfocussed power output was measured to be approximately 4.8 mW at the sample. Dielectric edge filters were used to reject the Rayleigh scattered light. Spectra were accumulated for either 60 or 10 seconds and five replicates were conducted, the spectra were normalised to a silicon standard.

UV-visible spectra were recorded on a Cary 300 Bio UV-visible spectrophotometer using 1 cm path length cells. UV-Vis studies were carried out with 300 μl aliquots of the colloid or 600 μl of the colloid, dye and salt mixture, diluted to 2000 μl with water, all UV-Vis spectra were normalised.

SEM investigations were carried out by preparing 3-aminopropyltrimethoxysilane (3-APTMS) coated silicon wafers. Wafers were cleaned with methanol before being immersed in a 10% v/v solution of 3-APTMS in methanol for nine hours. After this time the wafers were removed and rinsed with copious amounts of methanol and air dried. 10 μl of sample were deposited on individual wafers and allowed to rest for 1 minute, the solution was then removed, the area marked and the wafer washed with water before imaging. Imaging was carried out on a Sirion 200 Schottky field-emission electron microscope (FEI) operating at an accelerating voltage of 5 kV. The samples did not require additional metallic coating before imaging.
SEM sample deposition

Figure S1, shows representative images of a partially aggregated colloid mixture which had been left on 3-APTMS modified silicon wafers for different lengths of time. After four minutes larger aggregates were observed in the sample area, these became more prominent at eight minutes. However, only small aggregates were observed at one and two minutes indicating the absence of additional surface induced aggregation.

![SEM images](image)

**Fig. S1.** SEM images of small cluster enriched colloid solutions deposited on 3-APTMS functionalised silicon wafers for a) one minute, b) two minutes, c) four minutes and d) eight minutes. The scale bars are 500 nm (a and b) and 1 μm (c and d) respectively.

Stability

Table S1 details the $\lambda_{\text{max}}$ and absorbance of a control colloid solution and one with the addition of 20 mM KCl added to cause partial aggregation.

<table>
<thead>
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<th>Sample</th>
<th>Control</th>
<th>20 mM KCl</th>
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<tr>
<td>Time / hour</td>
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</tbody>
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Table S1. $\lambda_{\text{max}}$ and absorbance of control and 20 mM KCl added colloid solutions.

The fluctuations in the values observed for the 20 mM KCl added colloid solution were similar to those observed for the control sample which had additional water rather than electrolyte.

Figure S2. shows representative SERRS signal of Nile blue over a time range of 15 days. The signal was observed to develop over the first two hours after mixing, after which the signal was stable for the remainder of the 15 day period that was monitored.

Figure S3 and S4 show representative SERRS spectra of 200 nM Nile blue, at various stages during encapsulation, in a control and a partially aggregated colloid solution respectively. The SERRS response was observed to decrease after PEG and washing steps for the control sample, however it then increased to a value greater than observed before encapsulation. It is suggested that this was due to the re-suspension of aggregates that had formed during the washing stage.

The reverse was observed in the partially aggregated sample, the response increased slightly after PEG addition and washing, but it returned to a value similar to that before encapsulation. SEM monitoring of the encapsulated mixtures indicated that there was no significant changes in the populations of the cluster groups within the partially aggregated sample.
Fig. S3. Representative SERRS spectra recorded from a control colloid solution; a – before PEG, b – after PEG, c – after wash, d – after 72 hours, e – after 170 hours. Spectra were accumulated for 10 seconds.

Fig. S4. Representative SERRS spectra recorded from a partially aggregated colloid solution; a – before PEG, b – after PEG, c – after wash, d – after 72 hours, e – after 170 hours. Spectra were accumulated for 10 seconds.

Figure S5 shows the UV-Vis data for the prepared dimer enriched nanotags as a function of additional NaCl salt concentration. There was a stepped decrease in the absorbance value. However, the relative decrease in absorbance was only slight when compared to a non-encapsulated control experiment, Figure S6. Note the reduction in the absorbance value and the broadening of the plasmon peak after 2 M NaCl had been added to the non-encapsulated colloid solution, this was a clear indication of
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aggregation of the colloid. Such a significant peak broadening was absent from the nanotag results.

![UV-Vis spectra](image)

**Fig. S5.** UV-Vis spectra of dimer enriched nanotag solution as a function of added NaCl. Sample volumes were kept consistent and the spectra normalised.

![UV-Vis spectra](image)

**Fig. S6.** UV-Vis spectra of a non-encapsulated silver colloid solution recorded with no additional salt and 2 M NaCl.

References
