

Supplementary Information (ESI)

Controlled side-by-side assembly of gold nanorods and dye molecules into polymer-wrapped SERRS-active clusters

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Experimental details

Chemicals

All chemicals including poly-(sodium 4-styrenesulfonate) $M_w = \sim 70,000$ (PSS), cetyltriethylammonium bromide (CTAB) and 3,3'-diethylthiatricarbocyanine iodide (DTTCI) were purchased from Sigma-Aldrich and used as received. All solutions were prepared using Millipore deionized water.

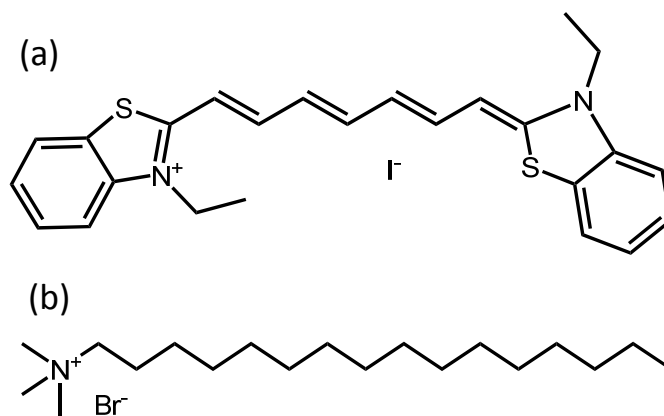


Fig. S1. Chemical structures of (a) DTTCI and (b) CTAB.

Nanorod Synthesis

The typical seed-mediated nanorod synthesis described in the literature is for a 10 ml total reaction volume.¹⁻³ In order to gain increased control over the final aspect ratios obtained, and to avoid the complication of batch-to-batch variations, the synthesis was scaled up to a 1 L

reaction volume. Control of the nanorod aspect ratio was obtained by varying both the concentration of silver nitrate and the volume of seed solution added to the growth solution. The same stock solution was used for all the data presented here with the separate stages for seed formation and nanorod growth described below. Prior to synthesis, all glassware was soaked in aqua regia for a period of at least 30 mins and then rinsed thoroughly in doubly-distilled water.

Preparation of Seed Solution: A CTAB solution (5 ml, 0.2 M) was mixed with HAuCl_4 (5 ml, 0.01 μM) under stirring, to which freshly prepared, ice-cold NaBH_4 (0.6 ml, 0.01 M) was added, producing a brownish-yellow solution. This solution was kept at 27-30 °C and used immediately following preparation.

Growth Solution (large scale synthesis): The following procedure was used to prepare nanorods with a target longitudinal plasmon resonance of 800 nm. First, AgNO_3 (20 ml, 4.0 mM) was added to CTAB (400 mL, 0.2 M). Next, HAuCl_4 (400 mL, 1.0 mM) was introduced and the solution gently mixed by inversion. Ascorbic acid (5.6 mL, 0.8 M) was then added resulting in the growth solution changing from dark yellow to colourless. Freshly prepared seed solution (1.05 ml) was added to the growth solution and the reaction allowed to proceed for 48 h in a water bath maintained at 27-30°C. The solution was centrifuged at 8500 rpm for 45 min and resuspended in 1 mM CTAB at half the original reaction volume i.e. 500 ml. This last step was repeated a further 3 times and the rod stock solution stored until use.

Nanorod aggregation (Figure 1)

300 μL of sodium citrate tribasic dihydrate (1.75 mM) was initially added to 2 mL of stock nanorod solution, and subsequently monitored by UV-vis extinction spectroscopy over a period of 24 h. The spectra displayed were acquired at 0 min, 1 min, 1 hour and 24 hours. A slightly different citrate:rod ratio was used for the clusters prepared in Figure 2.

Cluster preparation

Four different samples were prepared and characterised using UV-Vis spectroscopy, SEM and SERRS. Each of the samples were stabilised with a PSS layer prior to analysis. Furthermore, all samples were prepared using the same starting volume of stock nanorod solution with each cluster resuspended in the same final volume to allow direct comparison of UV-Vis and SERRS intensities. The bulk concentration of the nanorod stock solution was

estimated at 1 nM based on an extinction coefficient of $1.02 \times 10^9 \text{ M}^{-1} \text{ cm}^{-1}$. All samples were PSS coated with additional key sample details described below:

A: rod stock solution

B: rod stock solution + DTTCI

C: rod stock solution + DTTCI + citrate (low-level aggregation)

D: rod stock solution + DTTCI + citrate (medium-level aggregation)

Sample A. A PSS solution (1.2 ml, 10 mg/ml 5 mM NaCl) was added dropwise to 6 ml of nanorod stock solution and stirred vigorously for 10 mins. The PSS-coated sample was then centrifuged at 6500 rpm for 15 mins and resuspended in 6 ml of MilliQ water.

Sample B. DTTCI/CTAB mixed layers were prepared by adding 0.6 ml of 10 μM DTTCI solution to 6 ml of rod stock solution and leaving overnight. PSS coating was then performed as described for sample A.

Sample C. Low-level aggregation (i.e. a blue shift in the longitudinal resonance of ~ 10 nm) was achieved by firstly preparing a 6.6 ml nanorod-DTTCI solution as described for Sample B and allowed to equilibrate overnight. Aggregation was then achieved by adding 0.540 ml of 1.75 mM sodium citrate solution to the nanorod-DTTCI solution. After 30 mins, a 10 nm shift was observed (verified by UV-Vis) and the sample was PSS coated. Here, PSS (1.2 ml, 10 mg/ml 5 mM NaCl) was added dropwise to the nanorod-DTTCI mixture and gently swirled for 10 mins rather than stirred to prevent further aggregation. The PSS-coated sample was centrifuged at 6500 rpm for 15 min and resuspended in 6 ml of MilliQ water.

Sample D. Medium-level aggregation (i.e. a blue shift in the longitudinal resonance of 54 nm) was achieved by firstly preparing a nanorod-DTTCI solution as described above for Sample B. Following equilibration overnight, aggregation was achieved by the addition of 0.660 ml of 1.75 mM sodium citrate solution. After 1 hour, the cluster was stabilised via PSS coating as described for Sample C. Repeats of the sample D preparation using the same stock solution showed the final resonance peak position to vary by less than 10 nm across all samples.

SERRS measurements

All SER measurements were performed using a Renishaw *InVia* Raman inverted microscope system (Renishaw, Wotton-under-Edge, UK), equipped with a 20x long working distance objective. Two excitation sources were used at 632.8 nm and 785 nm along with a 1200 gr/mm grating. Samples were analysed using transparent bottom micro-titre plates with 200 μL of the nanorod sample solution placed in each well. Spectra were acquired from 100 to 2000 cm^{-1} , and a collection time of 10s per spectral acquisition was used. Cyclohexane was used to optimise the signal collection as well as provide an intensity reference. Three aliquots of each sample, all in a fresh well, were measured and compared. Spectra were processed and background corrected using Grams/AI software (version 7.0).

Fluorescence measurements

Characterisation of DTTCI and its adsorption to form dye-CTAB bilayers was performed using a Varian Cary Eclipse Fluorescence Spectrophotometer. Fluorescence spectra were acquired at an excitation wavelength of 750 nm with the emission and excitation slits set to 5 nm. For the measurements shown in Fig. S2, the nanorod stock solution was first diluted 10-fold in water. Next, 220 μL of 10 μM DTTCI was added to 200 μL of the diluted nanorod stock solution and 1.8 ml MilliQ water. This corresponds to a dye molecule to nanorod ratio of $\sim 10,000 : 1$. When undiluted rod stock solution is used (resulting in a $\sim 1000 : 1$ ratio) the fluorescence signal decreases out of the measurement's dynamic range even more rapidly (data not shown). For dye-only measurements, 220 μL of 10 μM DTTCI was added to 2 ml of MilliQ water maintaining a bulk dye concentration of 1 μM in all experiments.

Scanning Electron Microscopy (SEM)

SEM images were obtained using an FEI Sirion 200 ultra-high resolution Schottky field emission scanning electron microscope with FEI software. Si wafer substrates (5 x 5 mm; Ted Pella Inc.) were first rinsed sequentially in methanol and water and dried in a stream of N_2 . Next, the Si wafers were treated in an O_2 plasma cleaner for approximately 60 s and then immediately covered with a ~ 10 mg/ml solution of poly(diallyldimethylammonium chloride) in 1 mM NaCl for ~ 30 mins to create a positively charged surface. Each wafer was washed with water and dried under a stream of N_2 before ~ 50 μl of nanorod sample was dropped onto the wafer and stored in a humidity chamber for a further 30 mins to prevent droplet drying before removing the sample solution, water rinsing and N_2 drying. The deposition

methodology was designed to ensure that the cluster distribution was representative of the bulk solution by avoiding drying-induced aggregation.

Supporting Data

DTTCI adsorption onto nanorods

The data in Fig. S2 below demonstrates that DTTCI rapidly adsorbs onto the nanorod surface where it is quenched providing evidence that the dye has a high affinity for the covering CTAB bilayer.

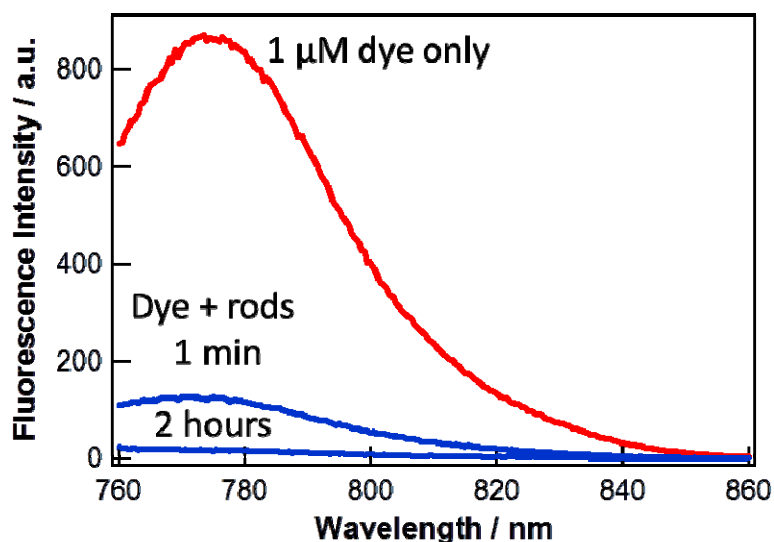


Fig. S2 Fluorescence spectra acquired for a 1 μM DTTCI solution and for a second solution containing both 1 μM DTTCI and ~ 0.1 nM gold nanorods at 1 min and 2 hours after preparing. The excitation wavelength used was fixed at 750 nm.

Cluster stabilisation

PSS wrapping immediately halts the nanorod assembly process as demonstrated in Fig. S3 where UV-Vis spectra of sample **D** are compared before and after the subsequent addition of PSS and centrifugation and colloid resuspension in water. In addition, the PSS wrapped clusters were found to remain stable for a minimum of 2 months at room temperature (see Fig. S4 below).

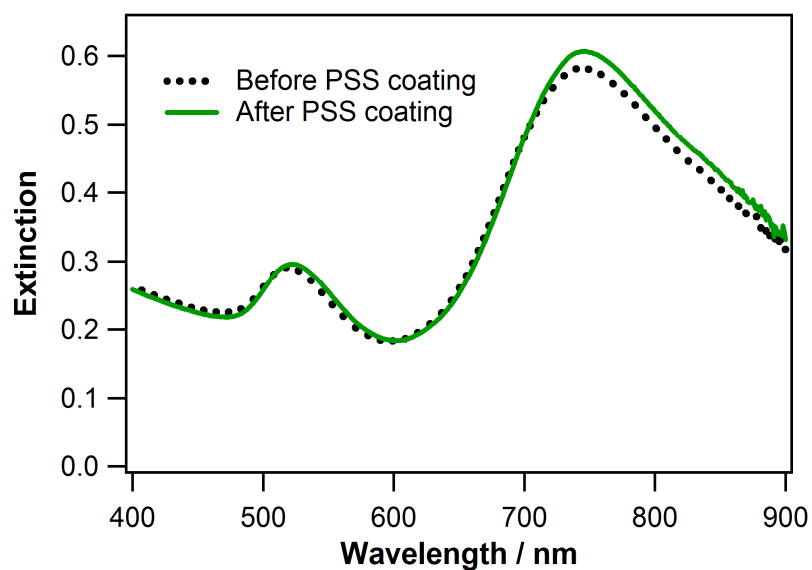


Fig. S3 UV-vis spectra of a solution of nanorod clusters (sample D) acquired before and after the PSS coating step.

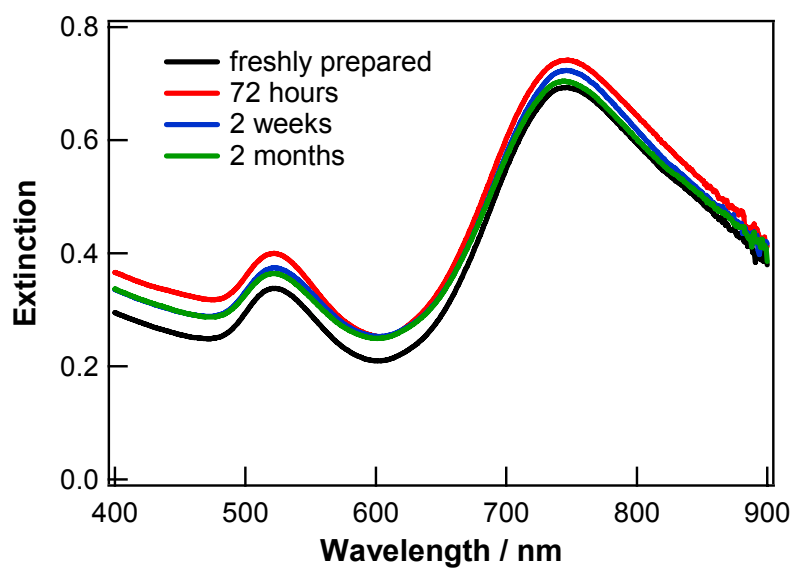


Fig. S4 UV-vis spectra of a solution of PSS stabilised nanorod clusters (sample D) acquired at various time intervals.

Scanning Electron Microscopy (SEM)

Representative images of samples **B**, **C** and **D** are shown below:

Sample B (PSS coating + DTTCl but no citrate-induced aggregation)

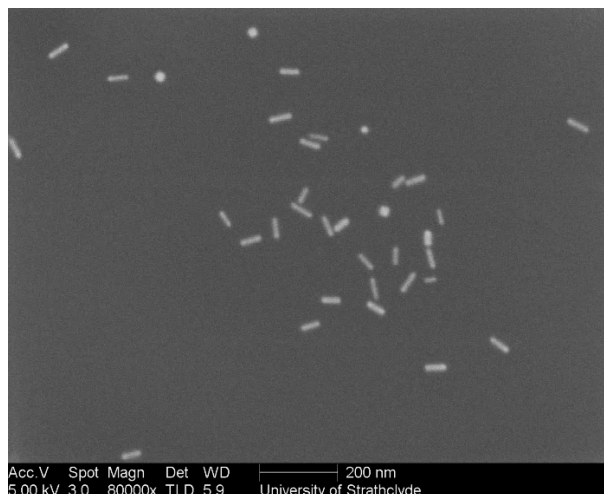


Fig. S5 Representative SEM image of Sample B indicating the absence of aggregated rod nanostructures.

Sample C

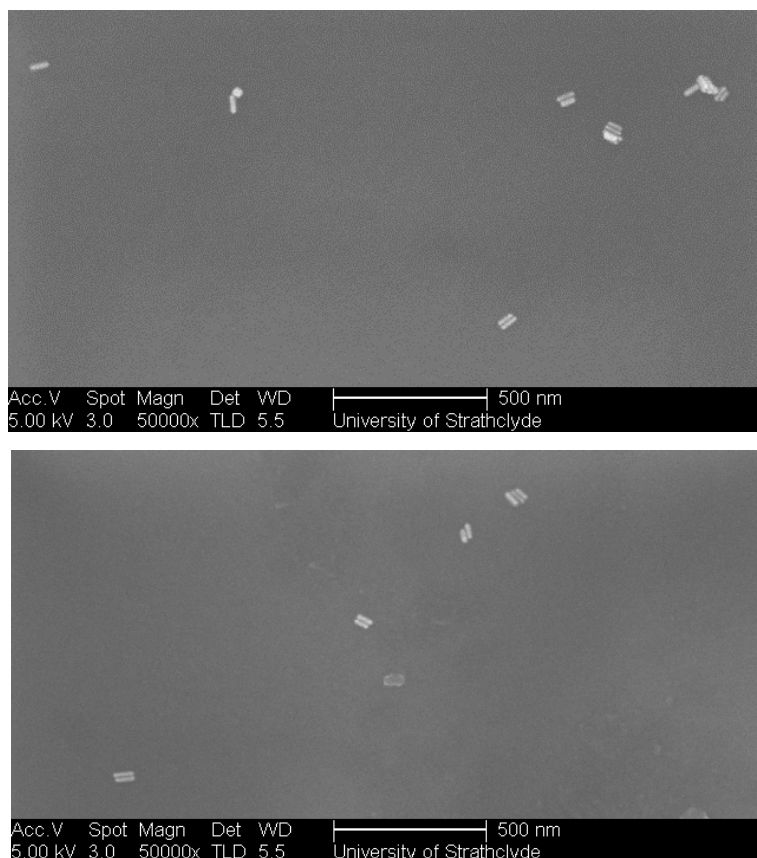


Fig. S6 Representative SEM images of Sample C showing a low surface density of immobilized clusters typically 2-5 rods in size along with a small percentage of single rods.

Sample D

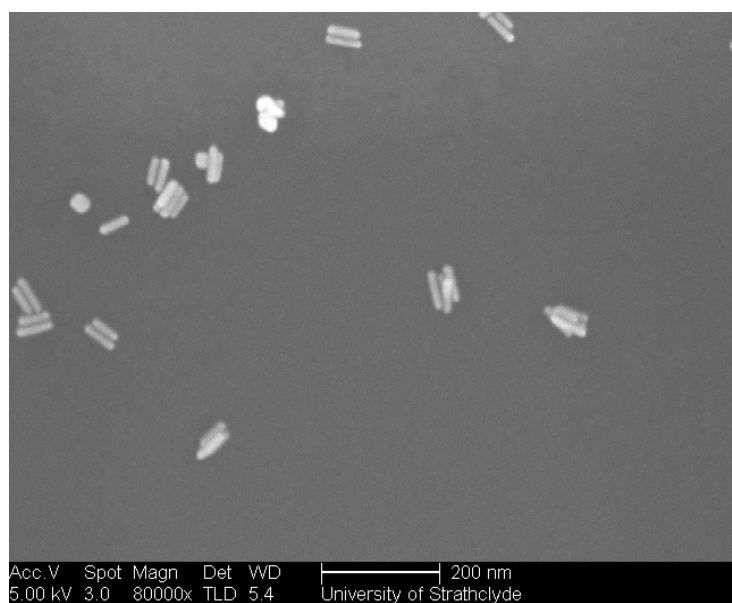


Fig. S7 Additional SEM image of Sample D providing further evidence for the assembly of rods into small clusters.

References

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- (2) Nikoobakht, B., El-Sayed, M.A. *Chem. Mater.* 2003, **15**, 1957.
- (3) Khanal, B. P., Zubarev, E.R. *Angew. Chem. Int. Ed.* 2007, **46**, 2195.