Electronic Supplementary Information

Stabilized Gold Nanorod–Dye Conjugates with Controlled Resonance Coupling create Bright Surface–Enhanced Resonance Raman NanoTags

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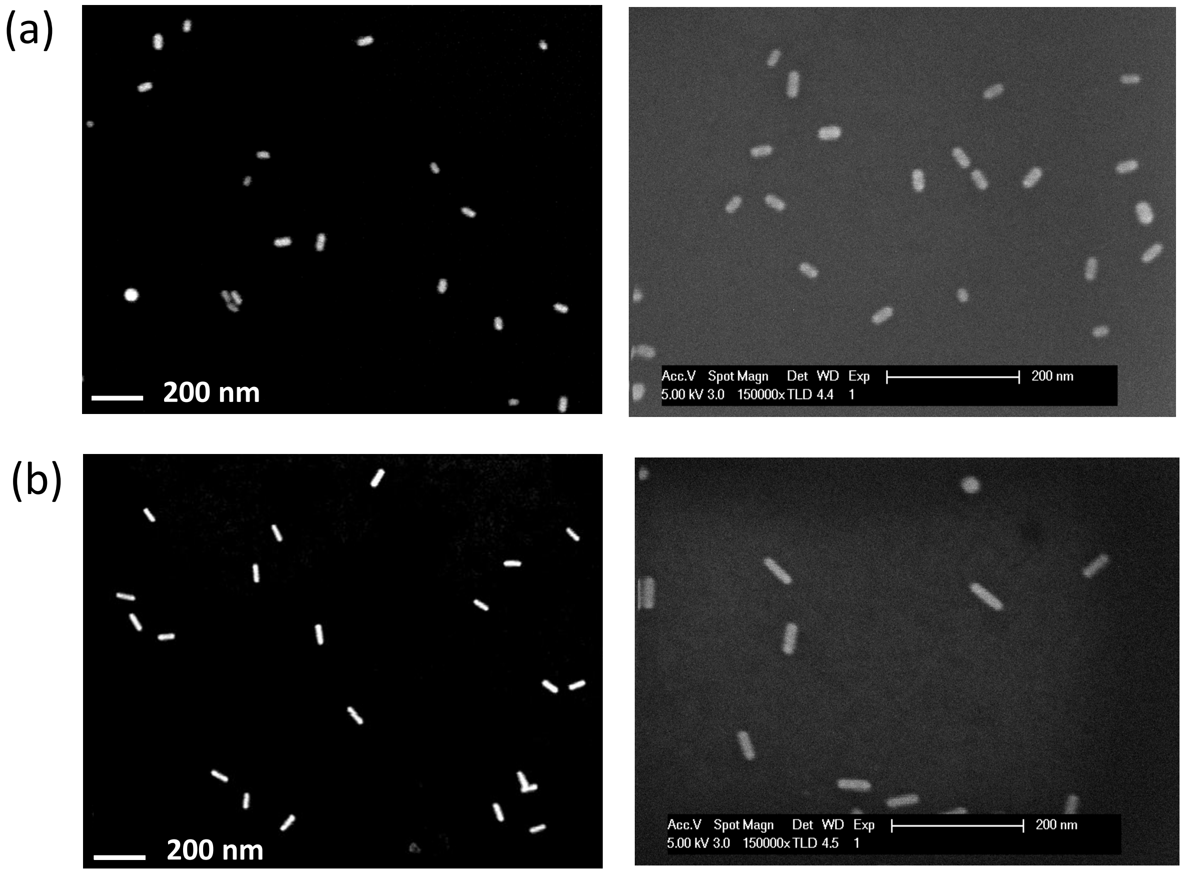

Figure S1. Representative SEM images of PSS wrapped nanorod-dye conjugates prepared using (a) 618 nm λ_{max} nanrod and (b) 770 nm λ_{max} nanorod stock solutions.

SEM images were used to calculate the average size and to confirm that adsorption of the dyes and subsequent PSS wrapping did not lead to significant aggregation.

Substrate preparation

Si wafer substrates (5 x 5 mm; Ted Pella Inc.) were first rinsed sequentially with methanol and distilled 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 PDDAC in 1 mM NaCl for 30 mins to create a positively charged surface. Each wafer was washed with distilled water and dried under a stream of N_2 before 30 μl of nanorod sample was dropped onto the wafer and stored in a humidity chamber for a further 30 mins to prevent the droplet drying, before rinsing with water and N_2 drying. The deposition methodology was designed to ensure that the sample distribution was representative of the bulk solution rather than by drying-induced aggregation.

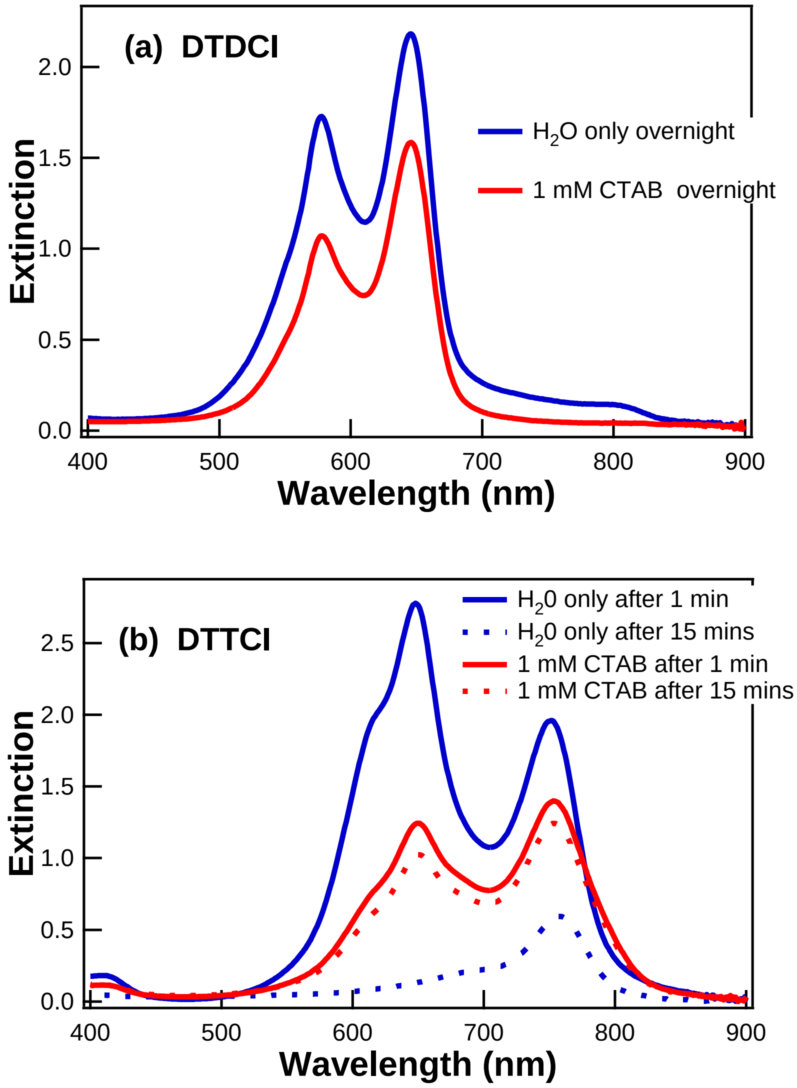

Figure S2. UV-vis spectroscopy measurements of 2 μM (a) DTDCI and (b) DTTCI dye solutions prepared in both water only and in 1 mM CTAB solution. In both cases there is a significant difference in the dye spectral properties as a function of time comparing with and without the presence of CTAB micelles.

Fluorescence quenching

Demonstration that both DTTCI and DTDCI quickly adsorb onto the nanorod surface where the fluorescence signal is quenched thus providing evidence of both dyes having a high affinity for the CTAB bilayer. All measurements in Fig. S3 below were performed in the presence of a fixed bulk CTAB concentration of 1 mM.

Figure S3 (a). Fluorescence spectra obtained for a 1 μM DTTCI solution and for a second solution containing 1 μM DTTCI and 1 nM gold nanorods (λmax = 770 nm). Measurements taken 1 min and 30 mins after mixing are shown and show very quick loss in signal. **(b)** An identical 1 μM DTTCI solution in 1 mM CTAB monitored both immediately and 2 hours after preparation. The excitation wavelength used was fixed at 750 nm.

Figure S3 (c) shows the same experiment carried out instead with DTDCI using the same NR stock solution. The fluorescence is quenched more slowly with measurements taken at 1 min, 30 mins and 18 hours after preparation. **(d)** Very little change in signal is observed after 2 hours for 1 μM DTDCI only in CTAB solution. The excitation wavelength used was fixed at 615 nm.

The difference between the two dyes is attributed to DTDCI being more hydrophilic than DTTCI and having a lower affinity for the CTAB bilayer. Differences in dye aggregation behaviour will also occur as a result of different solubility.

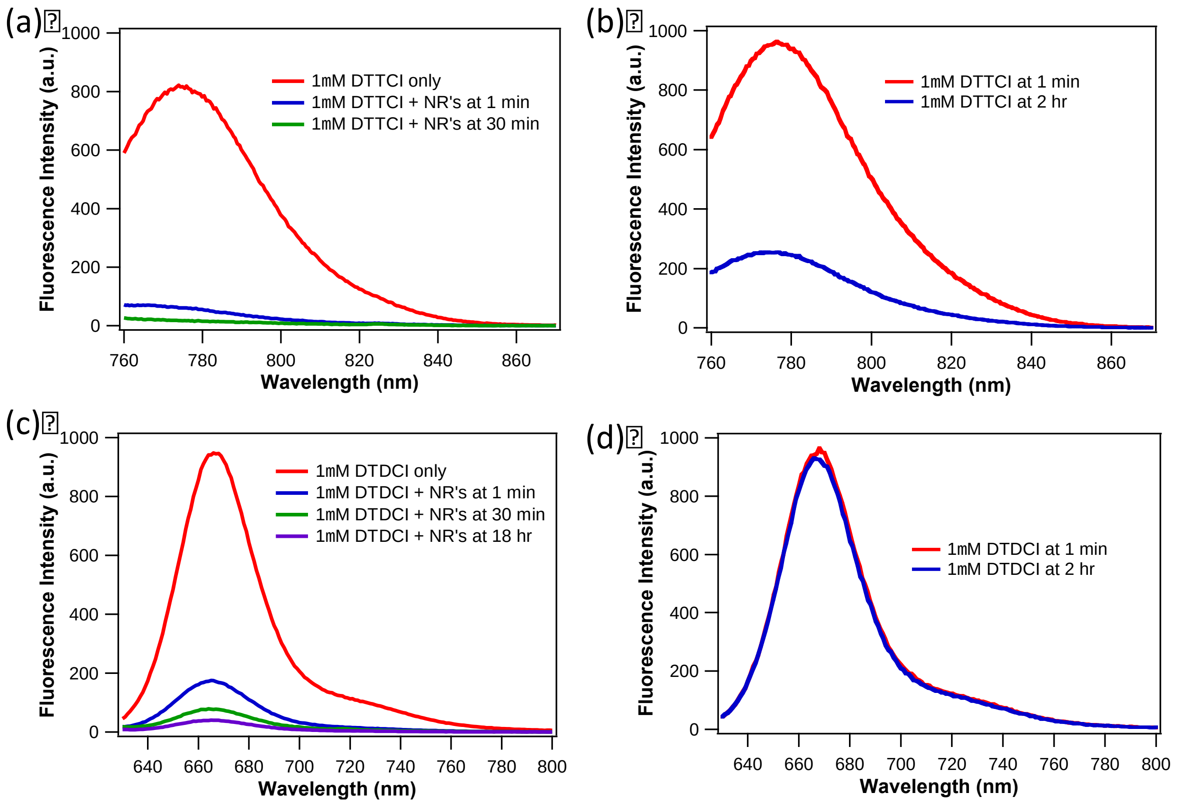

Figure S3. See description above.

Additional SERRS data

Figure S4. Representative SERRS profiles of nanorod (λmax = 770 nm) – DTDCI conjugates prepared using bulk dye concentrations ranging from 0.5-50 µM at a fixed nanorod concentration of ~1 nM. Laser excitation wavelength = 785 nm. The spectra have been vertically offset to allow comparison.

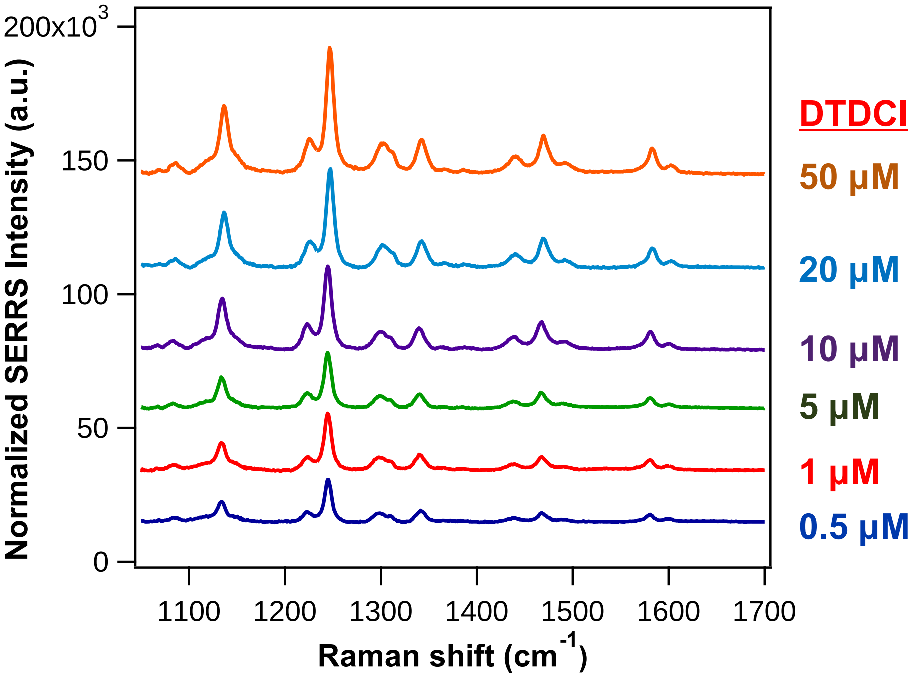

Figure S4. See description above.

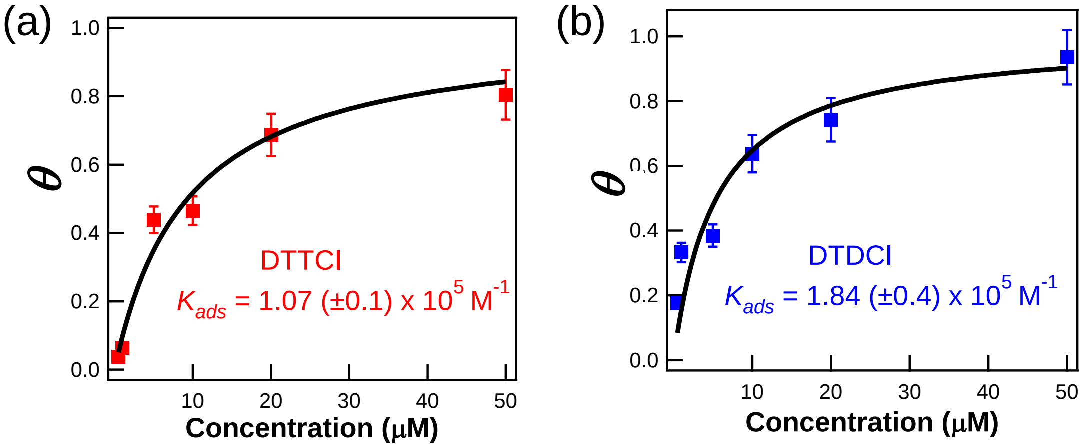

Figure S5. Plot of relative fractional surface coverage of (a) DTTCI and (b) DTDCI for a fixed concentration of NR's (λ_{max} = 770 nm). Plots obtained by subtracting the intensity minimum at 1195 (± 5) cm^{-1} from the maximum intensity at (a) 1241 (± 2) cm^{-1} and (b) 1245 (± 2) cm^{-1} followed by applying a Langmuir fit analysis.

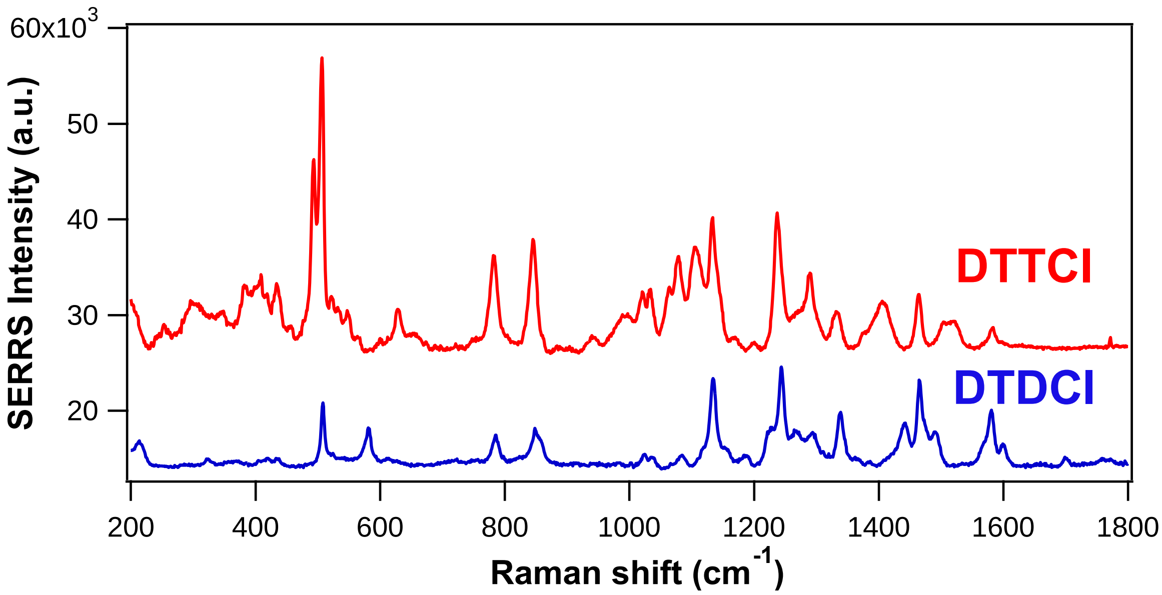

Figure S6. Representative extended SERRS spectra of conjugates prepared using 5 μM DTTCI and 20 μM DTDCI dyes plus nanorods (λ_{max} = 618 nm) and then PSS-wrapped. Laser excitation wavelength = 785 nm. The spectra have been vertically offset to allow comparison.

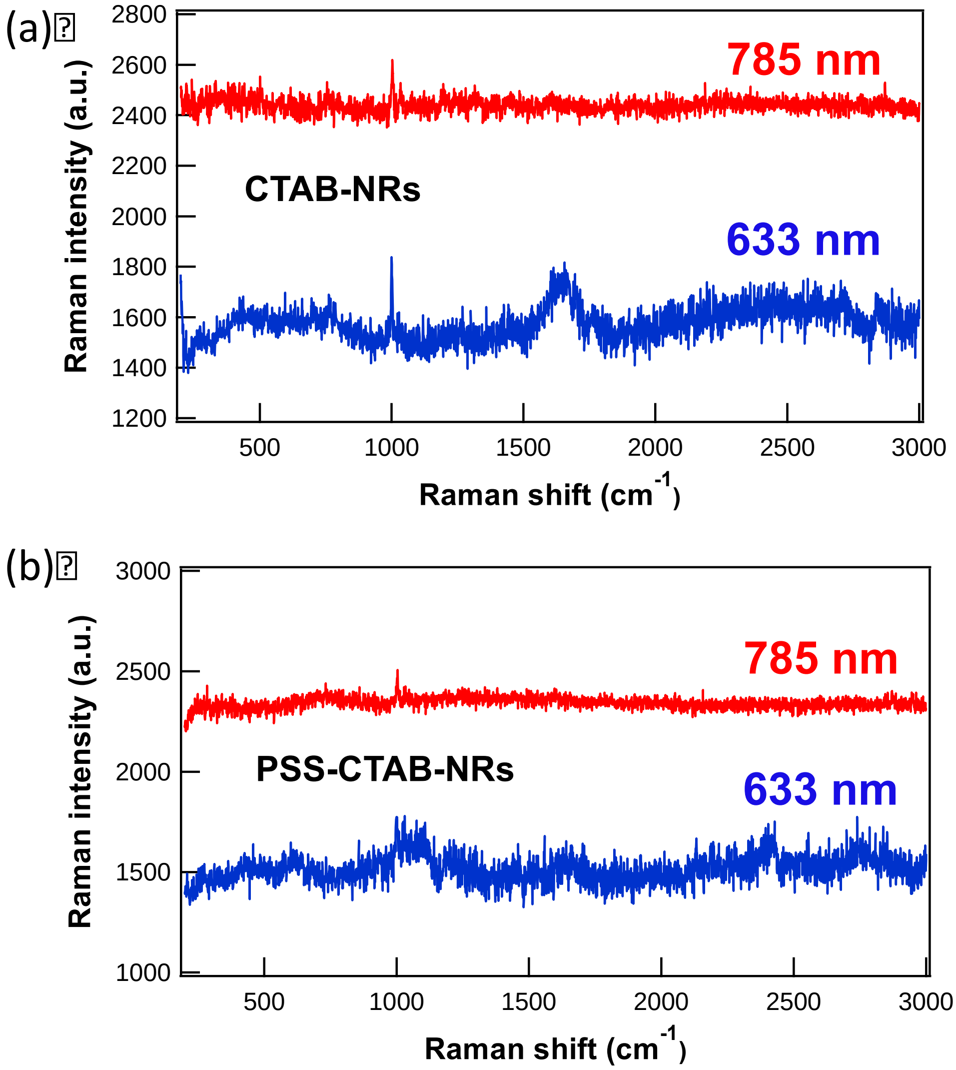

Figure S7. Control Raman measurements acquired in the absence of dye molecules for (a) stock solution NR's in 1 mM CTAB and (b) PSS wrapped nanorods. In each case the laser powers and integration times (10 s) and also rod concentrations were maintained at the same values used for the acquisition of the SERRS spectra shown in the main article. The rod λ_{max} was 770 nm.

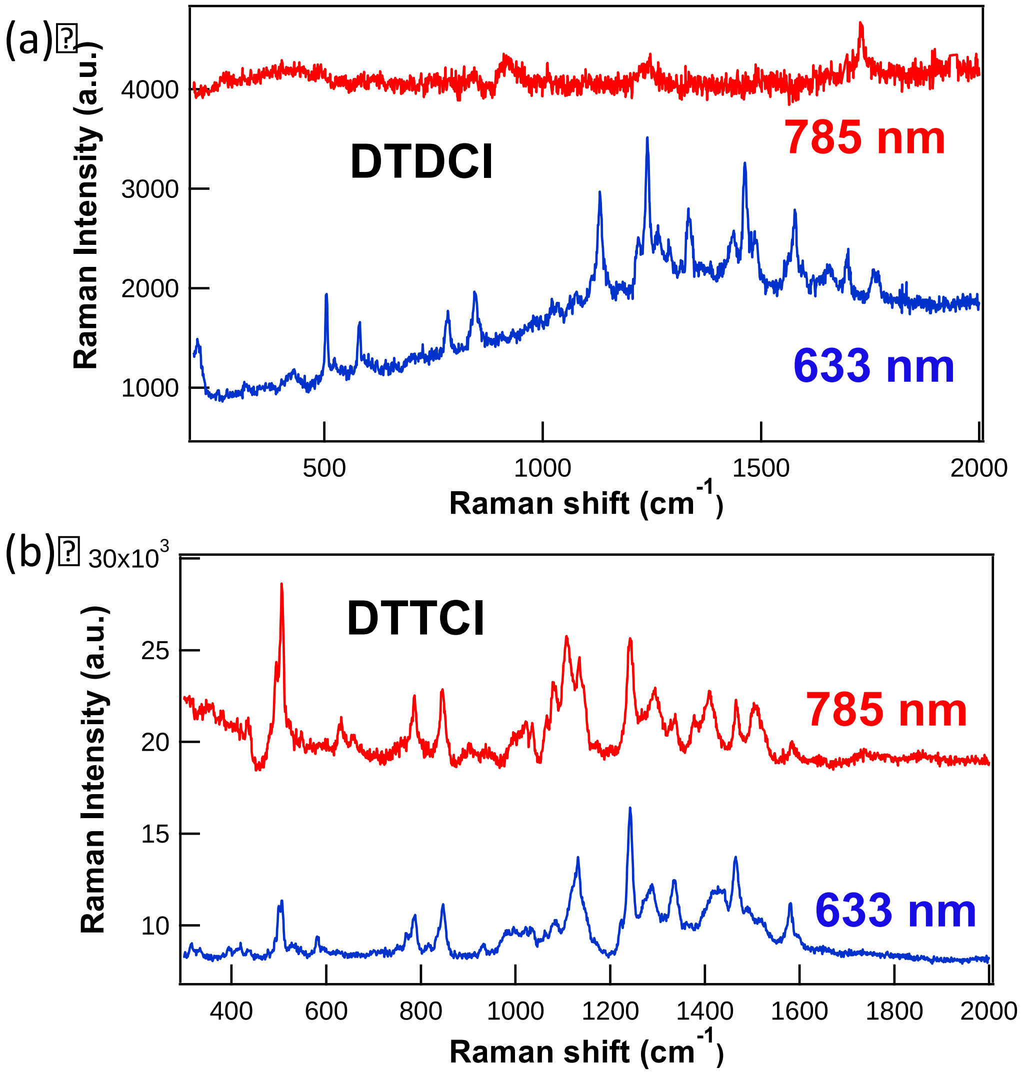

Figure S8. Control Raman measurements acquired for (a) DTDCI and (b) DTTCI dyes deposited (by simple solution evaporation) on a planar gold film. The acquisition conditions used are as follows: DTDCI at 785 nm laser power 60 mW, 60 s acquisition time. DTTCI at 785 nm excitation, laser power 1.2 mW, 10 s acquisition time. DTDCI and DTTCI at 633 nm excitation, laser power 1.0 mW, 30 s acquisition time.

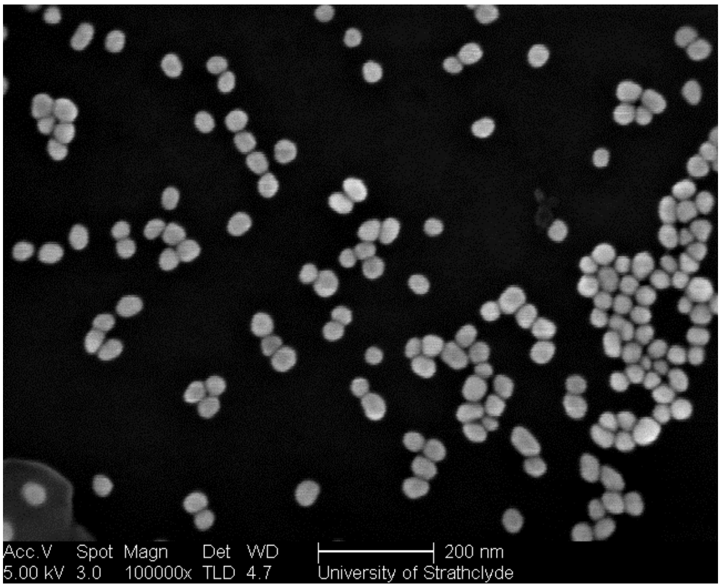

Figure S9. Representative SEM image of quasi-spherical gold nanoparticles prepared using trisodium citrate reducing agent.

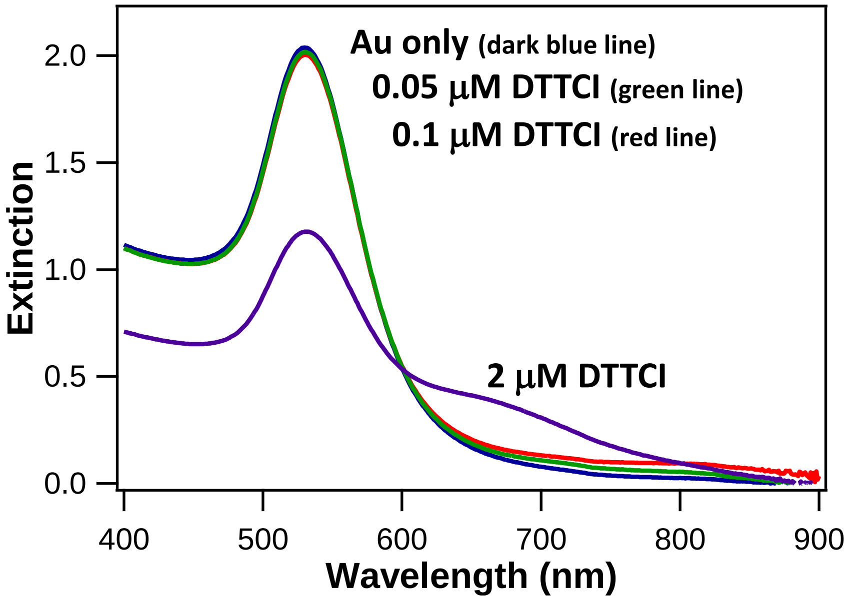

Figure S10. UV-vis spectra of quasi-spherical Au nanoparticle stock solution both before and ~ 1 min after the addition of 0.05 μM, 0.1 μM and 2 μM DTTCI.

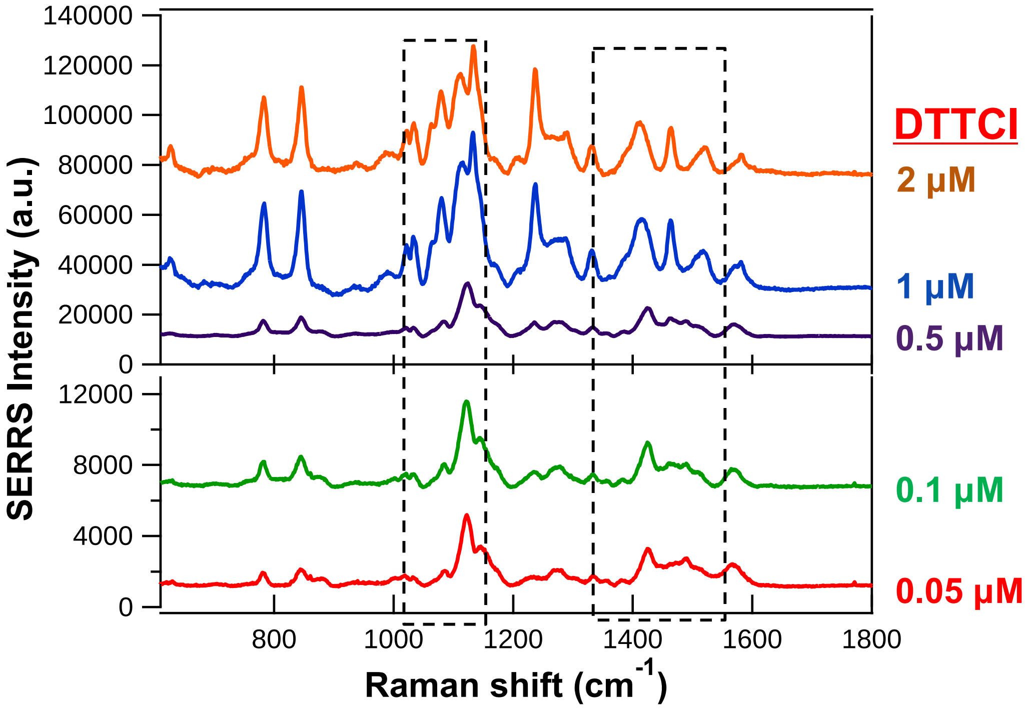

Figure S11. Baseline corrected SERRS profiles obtained at 785 nm excitation for Au spheres-DTTCI conjugates, with dye concentrations ranging from 0.05-2 μM.

The above SERRS spectra were obtained for quasi-spherical Au particles at DTTCI concentrations ranging from 0.05-2 μM. These samples do not contain the CTAB bilayer, and the dye is instead adsorbed directly onto the nanoparticle surface. The profiles obtained for samples containing 1-2 μM DTTCI are comparable, and the peak profiles are the same as those obtained for dye-rod conjugates, however, at dye concentrations below 1 μM, the spectrum profile changes. The boxed areas highlight regions of significant change. For the NR-dye samples, no dependence on the spectral profile as a function of dye concentration was observed, even at very low dye concentrations.

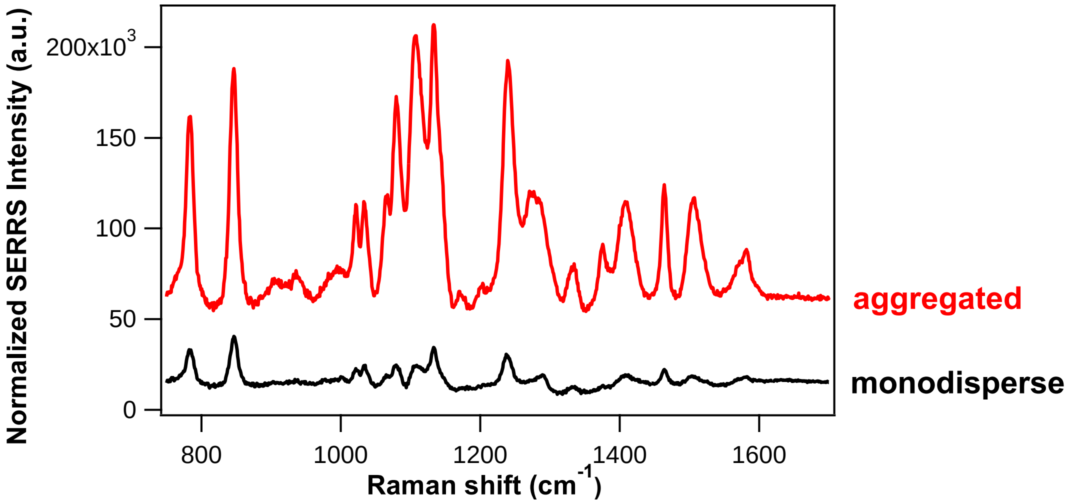

Figure S12. Baseline corrected SERRS profiles obtained at 785 nm excitation for PSS-wrapped nanorod (λ_{max} = 770 nm) – DTTCI (1 µM) conjugates comparing before and after a random aggregation induced by the addition of citrate to the colloidal suspension.

Calculation of dσ/dΩ:

Raman cross section values for cyclohexane liquid at 802 cm^{-1} have been reported at the following wavelengths [1]:

Laser wavelength (nm)	**407**	**488**	**514.5**	**647**
σ_R (cm^2 sr^{-1} molecule^{-1}) × 10^{-30}	**17.6**	**9.06**	**5.2**	**2.1**

No values for σ_R could be found in the literature at 633 and 785 nm excitation wavelengths, therefore fitting the above tabulated entries assuming a 1/λ^4 trend in the differential cross-section produced estimated values of 2.28 × 10^{-30} and 0.97 × 10^{-30} cm^2 sr^{-1} for 633 and 785 nm respectively.

As described previously in the literature[2, 3], the differential Raman cross section (dσ_R/dΩ) for a single cross-scattering object can be defined as:

$$\left(\frac{d\sigma_R}{d\Omega}\right)_{Label} = \frac{I_{Label}}{C_{Label}}\ \frac{C_{Standard}}{I_{Standard}}\ \left(\frac{d\sigma_R}{d\Omega}\right)_{Standard}$$

Where C$_{Label}$ is the concentration of the nanorod-dye conjugates, I_{Label} is the SERS intensity of the sample, $I_{Standard}$ is the Raman signal of the cyclohexane liquid with known concentration (C$_{Standard}$) and known absolute cross section. Both the sample and standard measurements were performed under identical conditions.

The values for the SERS Intensities were obtained by performing a Lorentzian fit of the Raman peaks to obtain the area of the 802 cm^{-1} cyclohexane peak and the area of the highest intensity peak for the nanorod-dye conjugates (1241 cm^{-1} for DTTCI and 1245 cm^{-1} for DTDCI) which were fitted with a polynomial baseline.

Using values of $C_{Standard}$ = 9.26 M, C_{Label} = 1.11 nM (DTTCI), C_{Label} = 1.36 nM (DTDCI) the following estimates were obtained:

$$\left(\frac{d\sigma_R}{d\Omega}\right)_{Label} = 1.4 \times 10^{-20}\, cm^2\, sr^{-1}$$

$$for\ DTDCI\ nanorod\ (\lambda max = 618\, nm)\ conjugates\ prepared\ at\ a\ dye\ concentration$$

$$of\ 50\, \mu M\ and\ laser\ excitation\ of\ 633\, nm$$

$$\left(\frac{d\sigma_R}{d\Omega}\right)_{Label} = 3.9 \times 10^{-20}\, cm^2\, sr^{-1}$$

$$for\ DTTCI\ nanorod\ (\lambda max = 770\, nm)\ conjugates\ prepared\ at\ a\ dye\ concentration$$

$$of\ 50\, \mu M\ and\ laser\ excitation\ of\ 785\, nm$$

References

1. R. L. McCreery, in *Handbook of Vibrational Spectroscopy*, John Wiley & Sons, Ltd2006.
2. E. C. Le Ru, E. Blackie, M. Meyer and P. G. Etchegoin, *Journal of Physical Chemistry C*, 2007, **111**, 13794-13803.
3. V. Amendola and M. Meneghetti, *Advanced Functional Materials*, 2012, **22**, 353-360.