

## **Supporting Information**

### **Anchoring Molecular Chromophores to Colloidal Gold Nanocrystals: Surface-Enhanced Raman Evidence for Strong Electronic Coupling and Irreversible Structural Locking**

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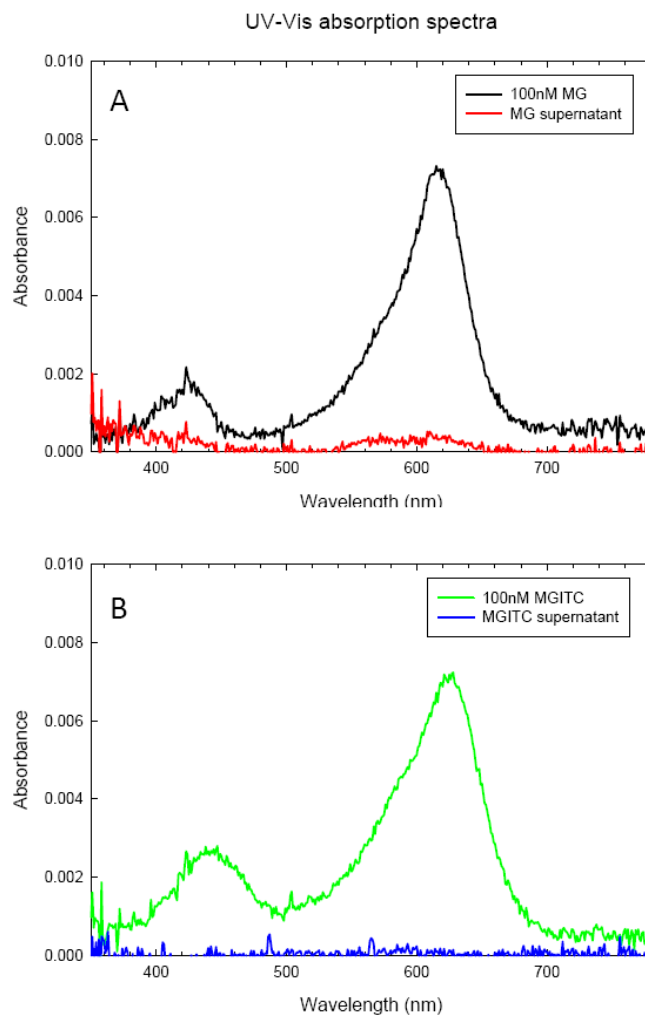
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**Materials.** Ultrapure water ( $18\text{ M}\Omega\text{ cm}^{-1}$ ) was used throughout the work. The following chemicals were obtained from commercial sources and were used without further purification: 60-nm citrate-stabilized gold particles at a concentration of  $2.6\times 10^{10}$  particles per milliliter (Ted Pella Inc., Redding, CA); malachite green isothiocyanate (MGITC) dye (Invitrogen Corporation, Carlsbad, CA). All other reagents were obtained from Sigma-Aldrich (St Louis, MO) at the highest purity available.

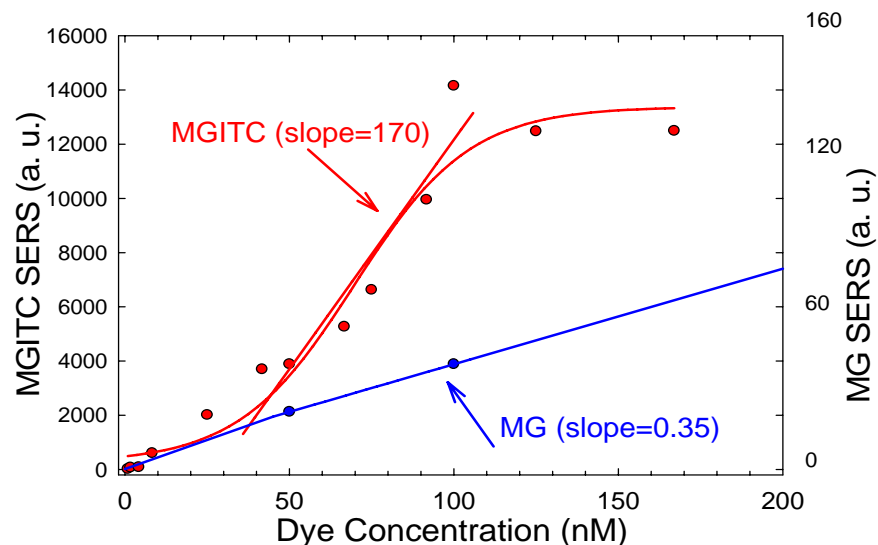
**Experimental procedures.** Stock MGITC and MG solutions (reporter dyes) were freshly prepared in DMSO and dilutions were made with pure water in a range from 5 nM to 5 mM for different measurement settings. Freshly prepared aqueous solutions of Raman reporter dyes were slowly added under rapid mixing with Au nanoparticles to allow adsorption of the reporter dye to the gold surface. Typically, the dye adsorption onto the gold nanoparticle surface was rapid (equilibration time less than 10 min). In this study, the Au-reporter solution was incubated at room temperature from 30 min to 2 hrs to provide sufficient time for equilibration. After incubation, excess dye was removed by two runs of centrifugation at 2,000 G for 20 min. The pellet was then re-dispersed in H<sub>2</sub>O. For sequential pH switching experiments, after each SERS measurement, the sample was centrifuged to remove any desorbed dye and then re-dispersed into corresponding pH solution (pH = 13 or 2). The final concentration of Au nanoparticles were kept at 14 pM for all SERS measurements.

**Characterization and Spectroscopy.** UV-Vis absorption spectra were recorded on a Varian (Cary100) spectrometer using quartz cuvettes. Transmission electron micrographs (TEM) were taken by using a Hitachi H7500 high-magnification electron microscope. The nanoparticle sample (5  $\mu\text{L}$ ) was dropped onto copper 200 mesh grids that were pre-treated with UV light to reduce static electricity. After 30 min, the solvent was wicked away with a filter paper. Dynamic light scattering data were obtained by using a Brookhaven 90Plus particle size analyzer instrument. Each sample was measured three times consecutively. SERS spectra were recorded on a compact Raman system using 633 nm (3 mW) or 785 nm (40 mW) laser excitation (Advantage Raman Series, DeltaNu, Laramie, WO). SERS intensities were normalized to the Raman spectra of cyclohexane and polystyrene to correct for variations in optical alignment and instrument response. The spectral resolution was approximately  $5\text{ cm}^{-1}$  for the Advantage Raman systems.

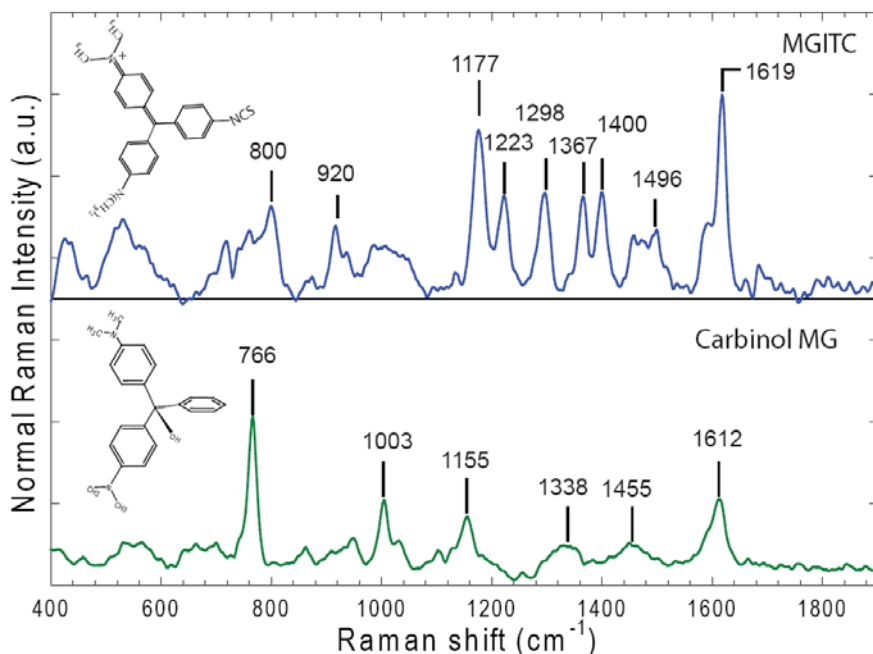
## Supporting Figures:



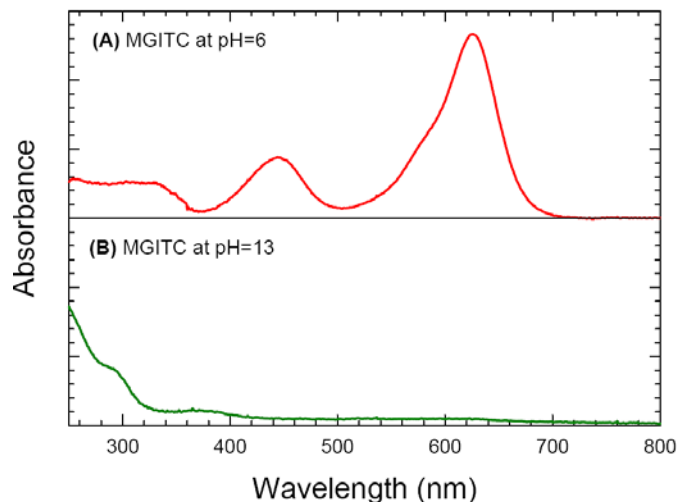
**Supporting Figure S1. UV-Vis absorption spectra showing quantitative adsorption of (A) MG and (B) MGITC on colloidal gold nanocrystals.** Briefly, 100 nM MG or MGITC solution was added to Au colloids (14 pM). After 2hrs incubation, SERS nanoparticles were centrifuged at 2,000 G for 20min. The supernatants were collected to measure absorption spectra (**red** for MG and **blue** for MGITC, respectively). Comparing the peak intensity at ~620 nm between the stock solution and supernatant, over 95% of MG or MGITC was adsorbed to the surface of Au nanocrystals. Three independent measurements for each dye showed reproducible data. For display clarity, only one set of the data are shown here.



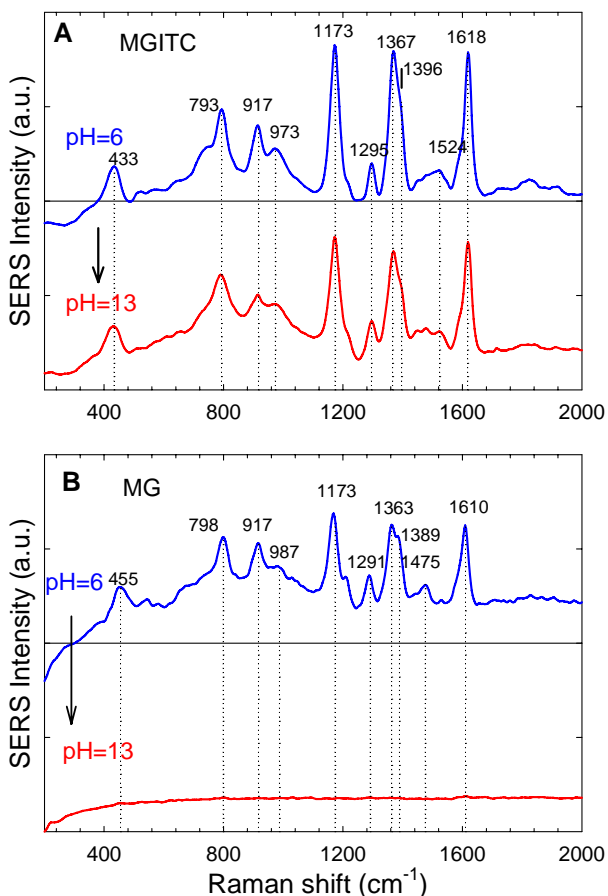
**Supporting Figure S2. SERS intensity as a function of dye concentration.** Note the intensity scales for MGITC (left) and for MG (right) are different by 100-fold. The MGITC data showed a linearly fitted slope of 170, while the MG data showed a slope of 0.35. The ratio of these slope values is approximately 500. See the main text for discussion.



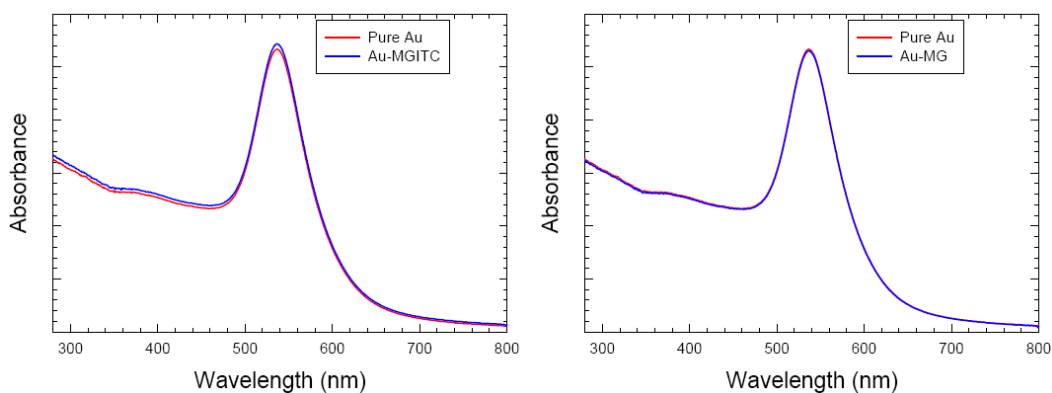
**Supporting Figure S3. Normal Raman spectra of MGITC (upper) and Carbinol MG (lower) in powder form.** To avoid sample heating, Raman spectra were acquired with 0.005 % of MGITC and 5 % of Carbinol MG (by weight) in potassium chloride (KCl) at 785 nm excitation. Laser power = 40 mW; integration time = 5 s. Note: carbinol MG is the basic form of MG. Due to its insolubility in aqueous solution and extremely low Raman cross section, we have to acquire normal Raman spectra using solid form to demonstrate the spectroscopic signatures are indeed different.



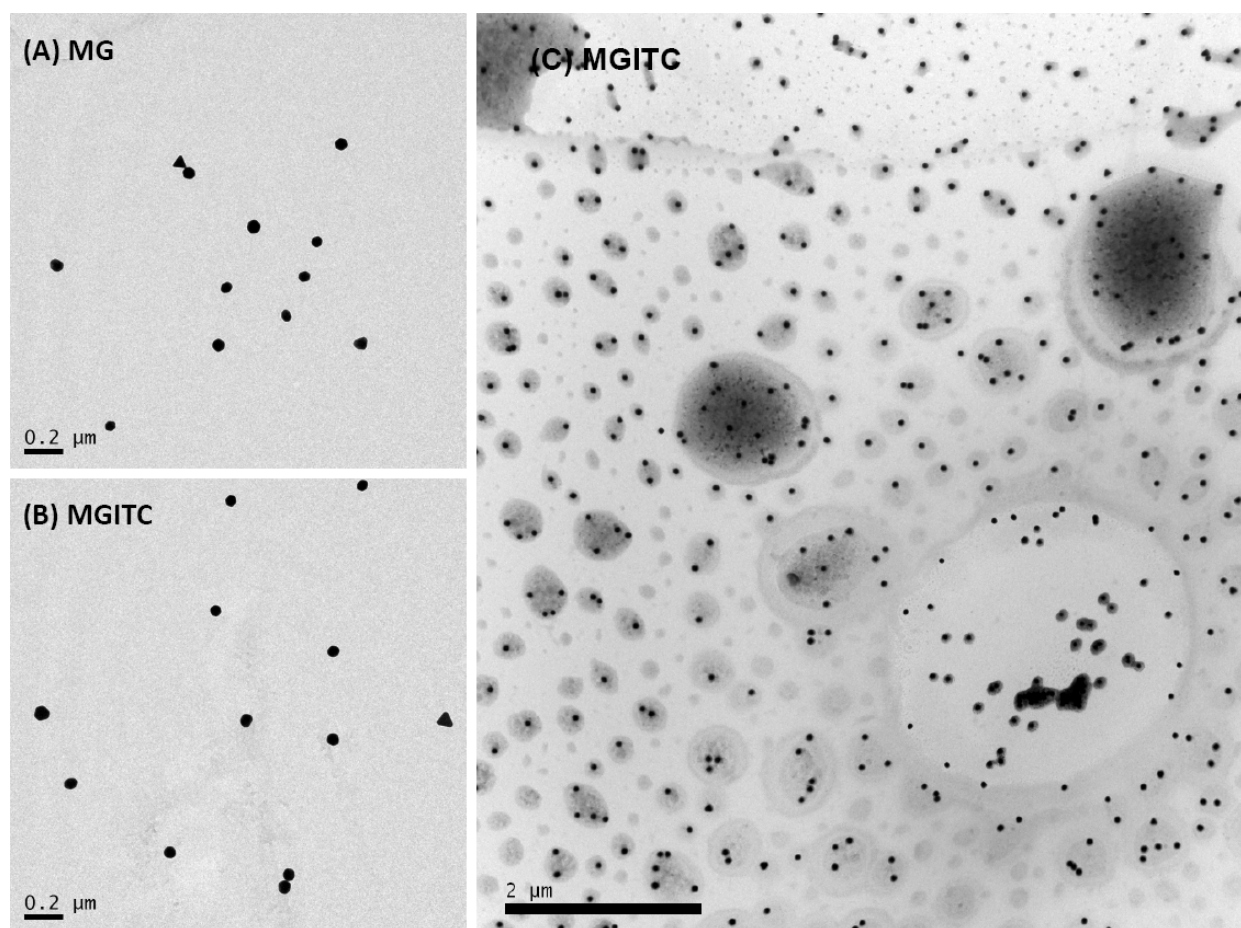
**Supporting Figure S4. UV-Vis absorption spectra of MGITC at different pHs.**



**Supporting Figure S5. SERRS spectra of MGITC (A) and MG (B) obtained with 633 nm laser excitation at acidic or basic pHs (pH = 6 or 13).** Note that the electronic structure of adsorbed MGITC is locked in its delocalized form and is no longer sensitive to pH, whereas the structure of adsorbed MG is still sensitive to pH and can be switched between its localized and delocalized forms. The laser power was 3 mW, and the integration time was 1 sec.



**Supporting Figure S6. UV-Vis absorption spectra of colloidal gold nanoparticles in the presence and absence of MG or MGITC reporter dyes (100 nM).** The data showed that the reporter dyes at low concentrations did not cause nanoparticle aggregation.



**Supporting Figure S7. Transmission electron micrographs of gold nanocrystals in the presence of MG or MGITC reporter dyes.** The same samples from Figure S5 were imaged without counterstaining. (A) and (B) are zoomed-in images to show uniform and well dispersed particles with little or no aggregation (scale bar: 200 nm). (C) shows a wider view micrograph of MGITC encoded gold nanoparticles with counterstaining (scale bar: 2 μm).