

# Surface-Enhanced Hyper-Raman Scattering from Single Molecules

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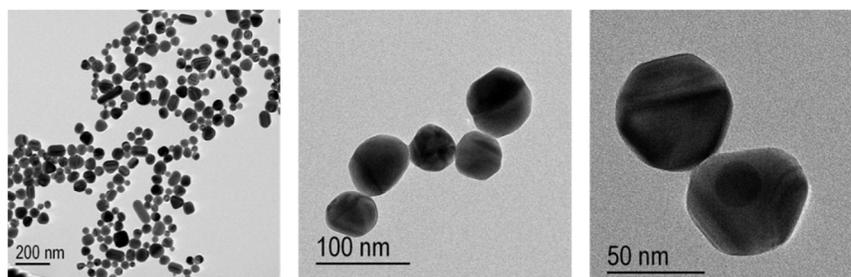
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## Supplementary Materials:

### Ag colloid synthesis and preparation of SEHRS samples

Silver colloids were synthesized using the Lee and Meisel method.<sup>1</sup> Specifically, 100 mg of silver nitrate was dissolved in 200 mL of water, and then brought to a boil. 50 mg of sodium citrate were then added, and the solution was allowed to reflux for 30 minutes. High resolution transmission electron microscope (HRTEM) images of SM-SEHRS colloids were obtained using a Carl Zeiss Libra<sup>®</sup> 200MC transmission electron microscope equipped with a monochromator. A 3 $\mu$ L aliquot of the silver nanoparticle colloids was deposited onto a copper TEM grid (SPI supplies #3520C-CF) and allowed to dry in air. The nanoparticle shape distribution was heterogeneous with an average size of 50 nm. The colloids were then diluted by a factor of 80 to give a final concentration which was approximately  $1 \times 10^{-10}$  M, which was estimated from the average shape and size determined via TEM. A mixture of CV-d<sub>0</sub> (Sigma-Aldrich) and CV-d<sub>30</sub> was added to the colloids, to give the concentrations reported in Fig. 2. Sodium bromide salt was added to the solution, to give a final concentration of 0.01 M. This solution was then flowed through a cell, utilizing a peristaltic pump. 1260 nm light was focused into the cell through the use of an inverted microscope (Nikon, Ti-U; 20X, NA=0.5). The back-scattered light was analyzed and detected via a spectrometer and back-illuminated CCD (Princeton Instruments).



*Figure S1: TEM images of the Ag colloids used for the SM-SEHRS studies.*

### Experimental Set-up

The experimental set-up, similar to ones used in previous experiments.<sup>2</sup> In detail, the oscillator is an optical parametric oscillator (Picoemerald, APE), which frequency doubles the  $\sim 5$  ps 1064 nm pulse generated by an internal laser and then splits the resulting frequency doubled radiation

into a signal, whose wavelength can be selected between 690 and 980 nm, and an idler, which is generated at a wavelength proportional to the energy a photon of the doubled light minus the energy of a photon from the signal. Only the idler is utilized in this experimentation as an excitation beam, so a long pass 1064 nm optic (Semrock) is utilized to block the “signal” radiation generated by the laser. The idler intensity is modulated, using a combination half-wave plate (Thor Labs) and Glan polarizer (Thor Labs). The horizontal polarization used to irradiate the sample. The excitation beam is directed into the back port of an inverted microscope (Ti-U, Nikon), through a 20x, NA = 0.5, objective (Nikon) which is focused on the sample. A 1350 nm short pass filter (Edmund Industrial Optics) is used to reduce the intensity of the laser line before it is reflected to the spectrometer (Acton, Princeton Instruments). The detector is a back-illuminated deep depletion CCD camera (Spec-10, Princeton Instruments) which is operated using either the commercially available Winspec (Princeton Instruments) or a homebuilt LabView program.

### Data Analysis

The spectra were analyzed by a home-built Labview program. The program first identified a SEHRS “event” by detecting the number of peaks in each spectrum. Peaks were identified through the standard peak detector subroutine in Labview, which uses a y threshold and x-axis window to identify the presence of a peak. Only spectra with fewer than a threshold number of peaks were considered to be a SEHRS “event”. Once identified, the event spectrum is compared to the SEHRS spectra obtained from high concentration samples of CV-d<sub>0</sub>, CV-d<sub>30</sub>, and CV-d<sub>0</sub>+CV-d<sub>30</sub> in a 1:1 ratio. The window of comparison spanned the 1100 to 1400 wavenumber region, to include the areas where the CV-d<sub>0</sub> and CV-d<sub>30</sub> spectra show the greatest difference. The comparison is performed by the linear correlation  $\rho$  which runs a Spearman correlation between the two sequences and outputs a coefficient which ranges from 1 (absolute correlation) to -1 (absolute negative correlation). Spectra which obtained a correlation higher than an empirically determined threshold were assigned as either “Combined” or “CV-d<sub>30</sub>”.

The program thresholds were optimized by creation of a training data set, which consisted of an assortment of CV-d<sub>0</sub>, CV-d<sub>30</sub>, and [CV-d<sub>0</sub>+CV-d<sub>30</sub>] spectra which contained varying levels of random noise. These spectra were analyzed by the program using multiple thresholds, until an optimized set was found, which would identify the most number of spectra correctly without incorrectly categorizing spectra. Using the optimized threshold and correlation parameters, the program was able to successfully sort and identify all spectra up to a Signal-to-Noise level of 0.5. Even when the noise was increased such that the Signal-to-noise ratio was 0.33, the program was still 60% successful at identifying spectra. We further emphasize that the Signal-to-Noise level of most of our single molecule SEHRS spectra acquired is estimated to be ~1.

### Synthesis of Crystal Violet-d<sub>30</sub>

Crystal violet-d<sub>30</sub> was synthesized using a procedure that was adapted from Van Duyne’s modification<sup>3</sup> of Lohmann’s synthetic method<sup>4</sup> for CV. A 25 mL round bottom flask was flamed dried, capped with a septum, and flushed with nitrogen, to which anhydrous aluminum trichloride (22 mg, 0.17 mmol) was quickly added. The flask was recapped with a septum, and

the air was removed under vacuum, after which an argon balloon was added to the flask. Carbon tetrachloride (250  $\mu$ L, 2.6 mmol) was next added via syringe, and the solution was allowed to stir and was heated in an oil bath to 70°C. Once the temperature reached 70°C, *N,N*-dimethylaniline- $d_{11}$  (1.0 mL, 8.0 mmol) was added dropwise via syringe to the stirring solution, after which heating and stirring were continued for 15 min. Afterwards, the solution was uncapped and quenched by adding the crude to 5g of ice in a 50 mL Erlenmeyer flask. Next, 10 mL of diethyl ether was added to the Erlenmeyer flask, and the solution was extracted with water (3 x 5 mL). The aqueous layers were combined, 10 mL of diethyl ether were added, and the aqueous layer was transferred to a 50 mL Erlenmeyer flask. To remove aluminum, sodium bicarbonate (120 mg, 1.43 mmol) was added to the Erlenmeyer flask, which was shaken to precipitate aluminum hydroxide. This precipitate was then vacuum filtered using a fritted filter into a 250 mL round bottom flask, and the Erlenmeyer was rinsed several times with 10 mL aliquots of water. The filter was then washed with 100% ethanol (2 x 10 mL) to ensure all the CV- $d_{30}$  was removed from the filter. The resulting filtered solution was then concentrated to yield crude CV- $d_{30}$ , which was redissolved in 10 mL of ethanol and filtered while washing with ethanol. The filtered CV- $d_{30}$  was concentrated under vacuum, and was then purified by column chromatography on silica gel using gradient elution of 27% methanol/dichloromethane. This provided CV- $d_{30}$  as a purple film (13.9 mg, 1.3%). HRMS (JEOL AccuTOF<sup>TM</sup> DART) calculated: 402.43228 m/z found: 402.43177 m/z. <sup>13</sup>C NMR (500 MHz, CD<sub>3</sub>OD):  $\delta$  179.72, 157.31, 140.36 (t, J = 95 Hz), 127.64, 113.03 (t, J = 95 Hz), 39.64 (m, J = 85 Hz) ppm.

- 1 Lee, P. C. & Meisel, D. Adsorption and Surface-Enhanced Raman of Dyes on Silver and Gold Sols. *J. Phys. Chem.* **86**, 3391-3395, (1982).
- 2 Milojevich, C. B., Silverstein, D. W., Jensen, L. & Camden, J. P. Surface-Enhanced Hyper-Raman Scattering Elucidates the Two-Photon Absorption Spectrum of Rhodamine 6g. *J. Chem. Phys. C* **117**, 3046-3054, (2013).
- 3 Kleinman, S. L. *et al.* Single-Molecule Surface-Enhanced Raman Spectroscopy of Crystal Violet Isotopologues: Theory and Experiment. *J. Am. Chem. Soc.* **133**, 4115-4122, (2011).
- 4 Lohmann, G. Y. J. U. S. Patent 3,689,495. (1969).