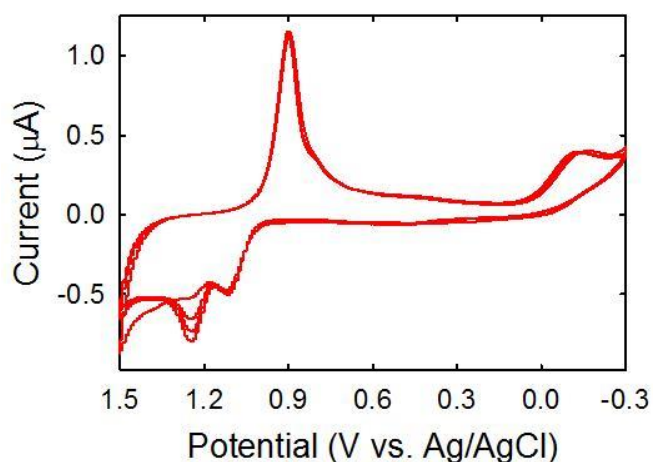


## Supporting Information

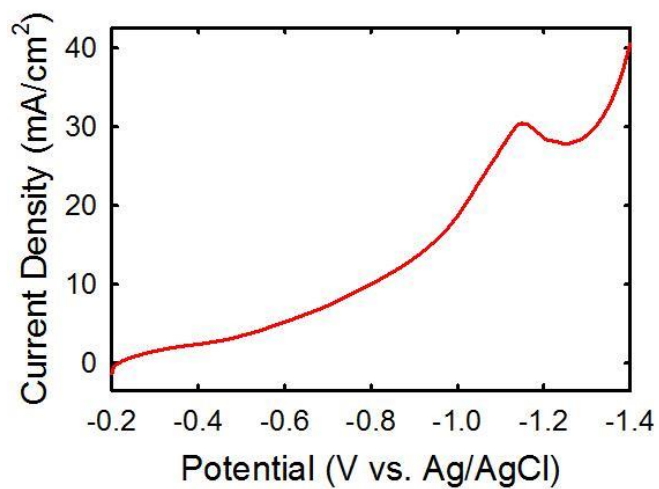
# Modification of the Electrochemical Properties of Nile Blue through Covalent Attachment to Gold as Revealed by Electrochemistry and SERS

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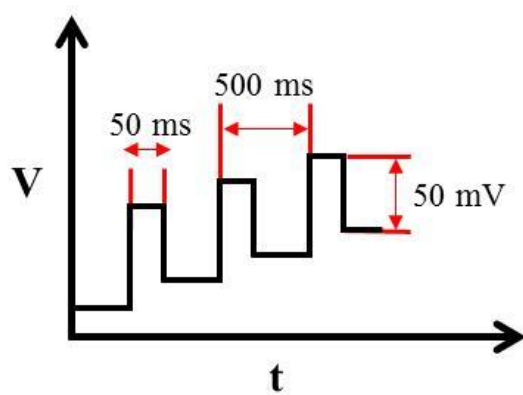
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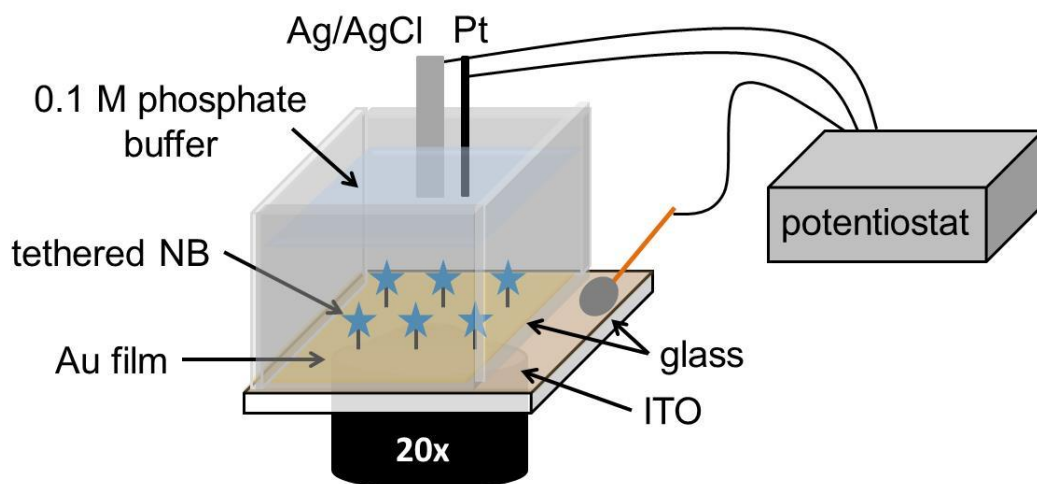
**Figure S1.** Cyclic voltammogram of a 100 nm Au film on an ITO substrate in 0.3 M H<sub>2</sub>SO<sub>4</sub>. The scan rate is 100 mV/s.



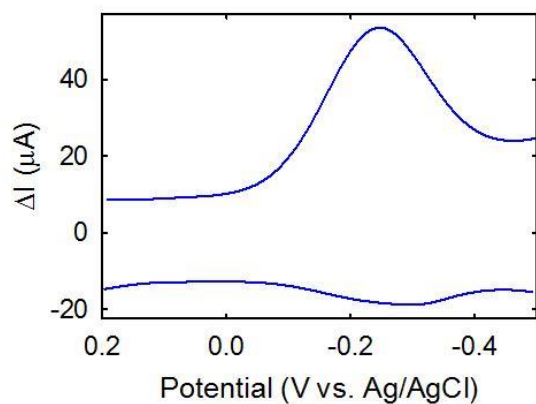
**Figure S2.** Cathodic linear sweep voltammogram showing reductive desorption of 8-mercaptooctanoic acid at -1.15 V from a 100 nm Au film in 0.1 M NaOH. Scan rate is 100 mV/s.



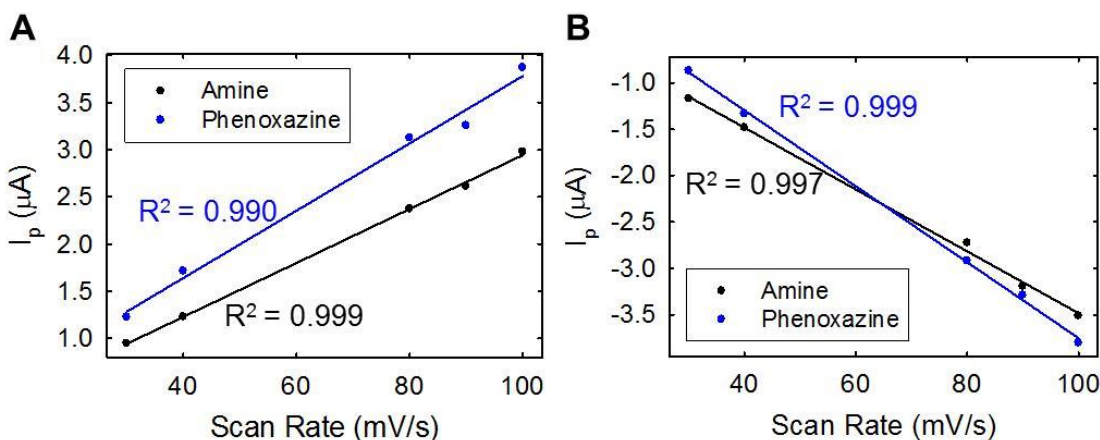
**Figure S3.** Differential pulse voltammetry waveform used for all experiments in the manuscript.



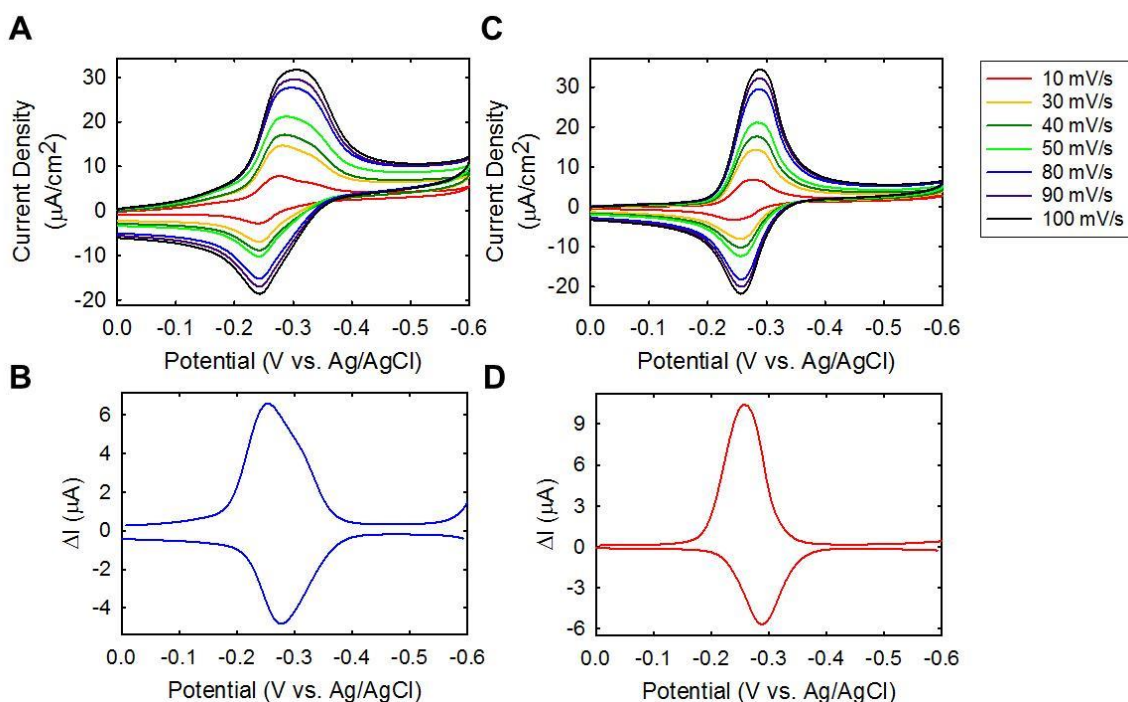
**Figure S4.** Spectroelectrochemical cell.



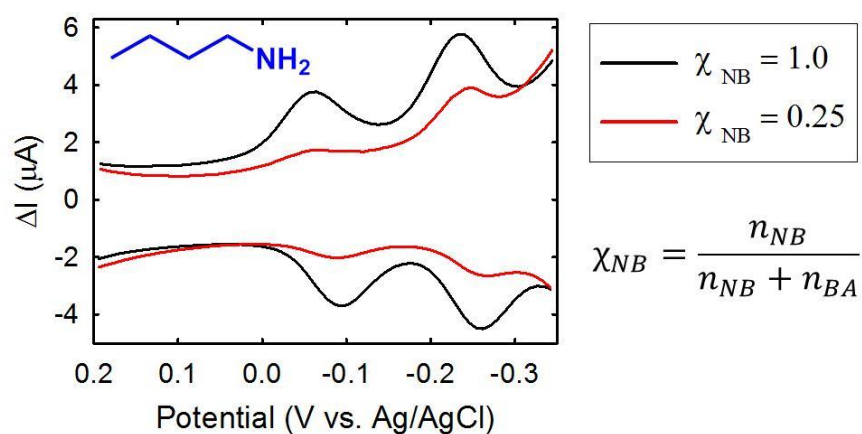
**Figure S5.** Differential pulse voltammogram of physisorbed Nile Blue on a 100 nm gold electrode supported by ITO. Adsorption was achieved by incubating the gold electrode in a 10  $\mu$ M solution of Nile Blue at pH 5 for 4 hours. Asymmetry in the peak current values indicates desorption of NB in the reduced state.



**Figure S6.** Peak cathodic (A) and anodic (B) current for both redox reactions of Nile Blue immobilized via EDC/NHS coupling to a gold electrode as a function of scan rate. Linear dependences highlight successful coupling of the redox probe to the electrode surface.

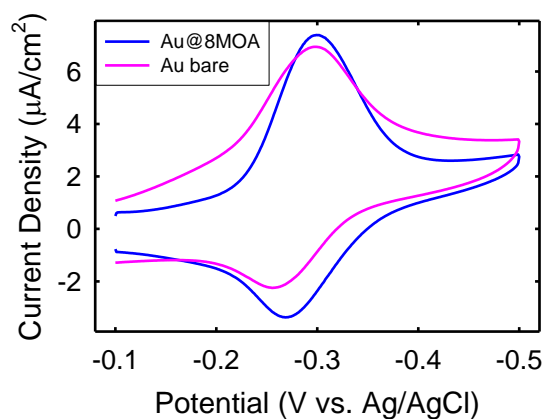


**Figure S7.** Cyclic voltammograms of 0.5 mM Nile Blue in 0.1 M phosphate buffer at pH 5 for scan rates between 10 and 100  $\text{mV/s}$  using a (A) bare 2 mm gold disc electrode and a (C) 2 mm gold disc electrode coated with a self-assembled monolayer of 8-mercaptopoctanoic acid. Differential pulse voltammograms (B and D) of Nile Blue using the same conditions as A and C, respectively.

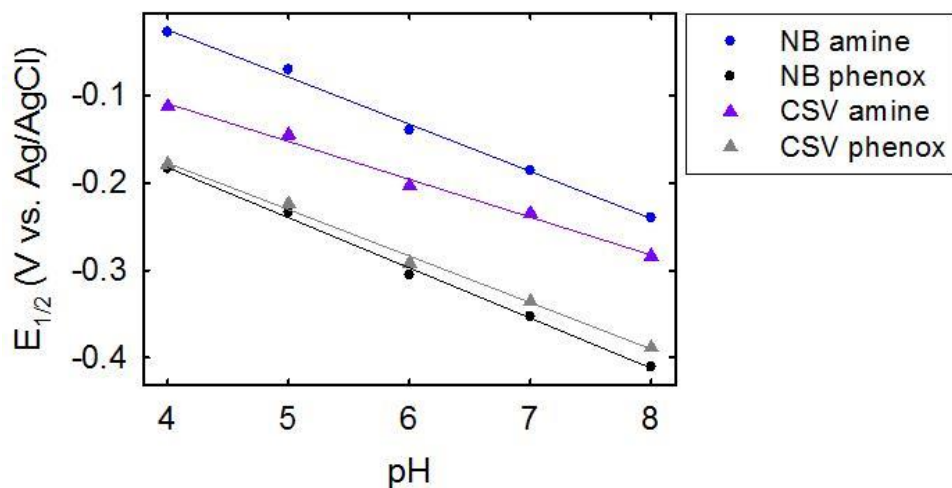


**Figure S8.** Differential pulse voltammograms of Nile Blue tethered to a 100 nm gold thin film electrode via 8-mercaptopoctanoic acid and EDC/NHS at relatively high (black) and low (red) coverages. Inset (blue) is the structure of butylamine used to compete in EDC/NHS coupling to lower Nile Blue coverage.

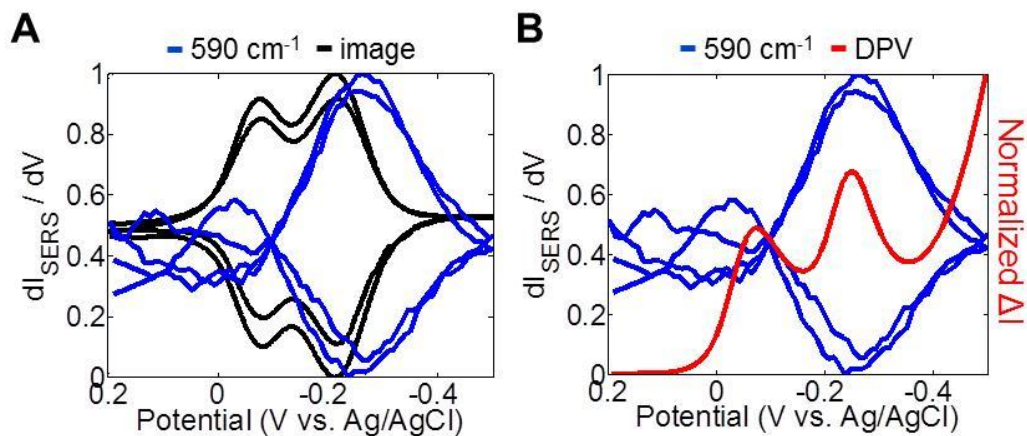
Differential pulse voltammograms of Nile Blue covalently immobilized on gold electrodes via EDC/NHS coupling show cathodic and anodic peaks that do not occur at the same potential for either of the two redox reactions. To eliminate the possibility of intermolecular interactions causing these deviations from ideal behavior, we reduced the Nile Blue coverage by co-incubation with a competing primary amine which would also couple to the carboxylic acid of the self-assembled monolayer (SAM) formed over the gold electrode. First, a SAM was formed by incubating a 100 nm gold film supported by an indium tin oxide substrate in a 10 mM solution of 8-mercaptopoctanoic acid dissolved in ethanol for 48 hr. Next, the terminal carboxylic acid was activated by incubating the electrode in 0.02 M EDC and 0.04 M NHS for 1 hr. Finally the electrode was immersed in a 0.1 M phosphate buffer solution (pH 5) containing two primary amines: Nile Blue and butylamine. The total concentration of primary amines in solution was kept constant at 10  $\mu$ M. The mole fraction of Nile Blue ( $\chi_{NB}$ ) was varied by mixing butylamine in solution as a competitor in the EDC/NHS coupling reaction.



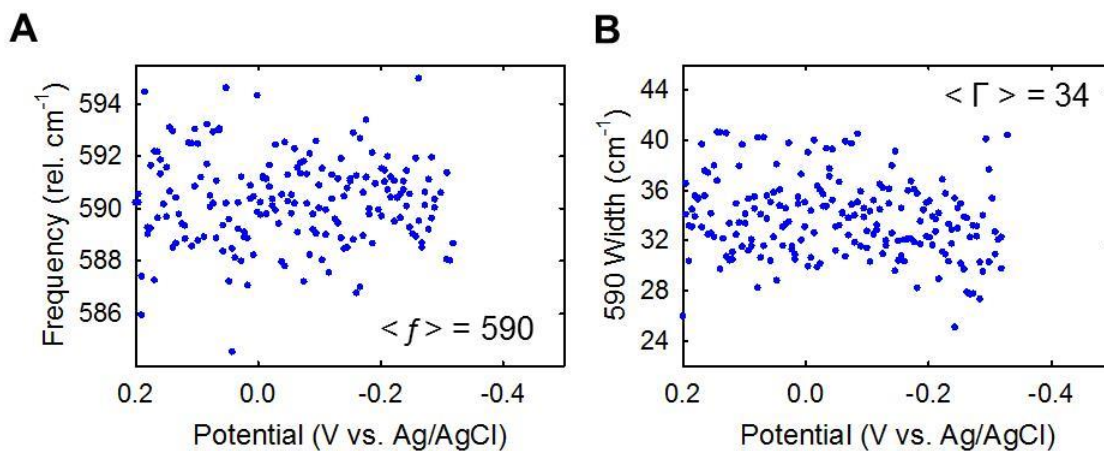
**Figure S9.** Cyclic voltammograms of 1 mM Cresyl Violet in 0.1 M phosphate buffer at pH 5 using a 2 mm diameter bare gold disc working electrode (pink) and coated with 8-mercaptopoctanoic acid (blue). In solution a single cathodic and anodic peak is observed.



**Figure S10.** Half-wave potentials of the amine and phenoxazine redox reactions for Nile Blue (circles) and Cresyl Violet (CSV, triangles) tethered to a gold electrode via 11-mercaptoundecanoic acid and EDC/NHS coupling.

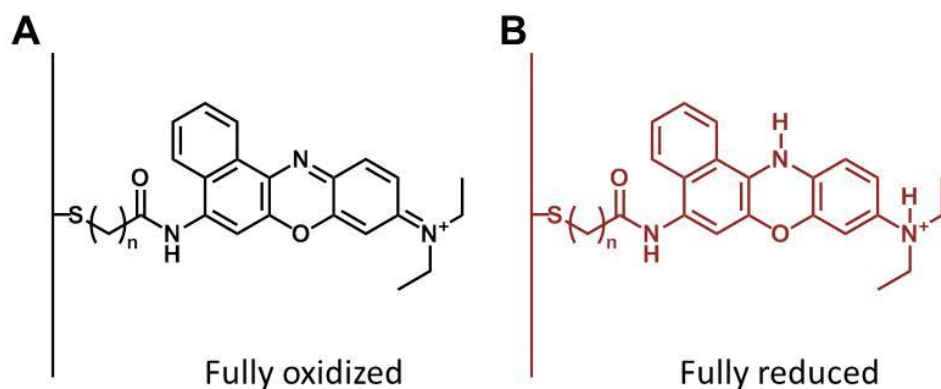


**Figure S11.** Derivatives of surface-enhanced Raman scattering (SERS) cyclic voltammograms from EDC/NHS tethered NB on gold island film electrodes showing the overlap of the (A) 590  $\text{cm}^{-1}$  Nile Blue spectral mode with Nile Blue SERS images and the (B) 590  $\text{cm}^{-1}$  mode with the corresponding cathodic differential pulse voltammogram.

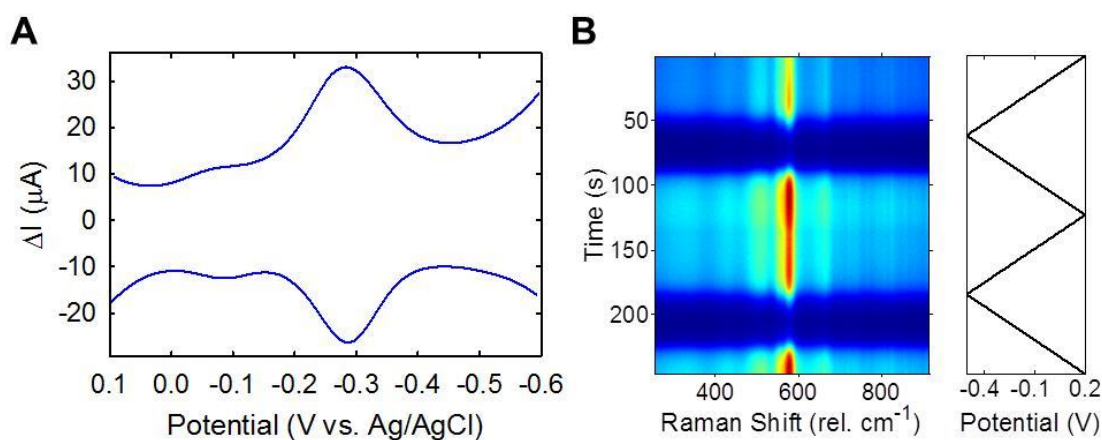


**Figure S12.** Spectral fits for the (A) frequency and (B) width of the prominent NB SERS mode when tethered to a gold island film with 8-mercaptopoctanoic acid and EDC/NHS.



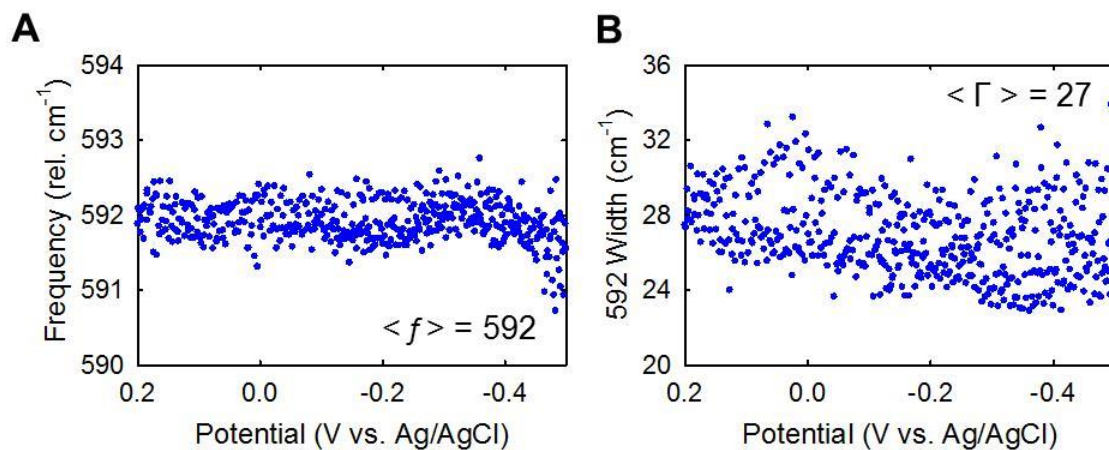


**Figure S13.** Structure of Nile Blue tethered to a gold electrode with a carboxylic acid-terminated alkanethiol and EDC/NHS in its fully (A) oxidized and (B) reduced forms.



**Figure S14.** (A) Differential pulse voltammogram of Nile Blue immobilized on a gold electrode using click coupling. (B) Waterfall spectra of click coupled Nile Blue modulating with applied potential. A 0.1 M phosphate buffer at pH 5 was used in both experiments.





**Figure S15.** Spectral fits for the (A) frequency and (B) width of the prominent NB SERS mode when tethered to a gold island film with click coupling.