Ultrasensitive Online SERS Detection of Structural Isomers Separated by Capillary Zone Electrophoresis

Pierre Negri, Ryan J. Flaherty, Oluwatosin O. Dada, and Zachary D. Schultz*

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556

*Corresponding Author email: Schultz.41@nd.edu

ABSTRACT

This supplement contains additional experimental details including the SERS substrate fabrication, the technical components used in our home-built Raman microscope, the LIF detector, and Figures S-1, S-2, S-3, S-4, and S-5. Figure S-1 depicts the CZE-SERS experimental setup as described in the text. Figure S-2 presents the electropherogram of the same three analyte mixture described in the main manuscript using LIF detection. Figure S-3 shows (A) the heatmap of the SERS intensity as a function of Raman shift and migration time and (B) the SERS electropherogram of the SERS intensity profile of the Raman band at 1357 cm^{-1} as a function of migration time for the CZE separation of the three rhodamine isomers following a 6 s (102 nL) injection of a sample containing 10^{-7} M R6G, 10^{-8} M RB, and 10^{-6} M 5-TAMRA. Figure S-4 shows shows 50 ms (left) and averaged (right) SERS spectra of the three analyte mixture extracted from Figure S-3A. Figure S-5 shows the chemical structures of the three rhodamine dyes, including their molecular weight.

Experimental Methods

Material and Reagents. Rhodamine 6G (R6G, ~99%), rhodamine B (RB, ~99%), 5-carboxytetramethylrhodamine (5-TAMRA, ~99%), and sodium tetraborate decahydrate (>99.5) were purchased from Sigma-Aldrich (St. Louis, MO). Ultrapure water (18.2 MΩ cm) was obtained from a Barnstead Nanopure filtration system. All other chemicals were of analytical grade and used without any further purification.

Substrate Preparation. SERS-active substrates were fabricated by a previously reported thermal evaporation procedure.[1] These substrates were incorporated into a custom-built flow cell by affixing individual substrates onto a standard microscope slide with two 3 mm diameter holes predrilled 35 mm apart along the center of the slide. Prior to its use, the SERS substrate on the glass slide was soaked overnight in 0.1 M NaOH (Sigma-Aldrich, 99.99%) to dissolve the anodized aluminum oxide (AAO) filter. The resulting SERS-active substrate was thoroughly rinsed with ethanol (Sigma-Aldrich, 99.5%) followed by a final rinse with ultrapure water.

Raman Measurements. Raman measurements were performed using a previously described home-built system.[1] The sample was illuminated through a 40X water-immersion objective (Olympus, NA= 0.8), resulting in a spot size of approximately 0.4 μm^2. The power of the 632.8 nm HeNe laser was ~1.2 mW, as measured at the sample. Raman back-scattering signal was collected into the same objective lens and directed to the spectrograph and EMCCD (Newton 970, Andor). The spectral resolution of the Raman measurement is about 3 cm^{-1} based on the grating (600 gr/mm), entrance slit (25 μm), monochromator pathlength (320 mm), and CCD pixel size.

CZE-SERS Setup. Figure S-1 shows a schematic diagram of the CZE-SERS experimental setup. The homebuilt flow cell consists of a FEP plastic base plate, a SERS substrate, and a 250 μm thick silicone gasket with a 2 mm slit to define the flow channel, and a stainless steel top plate. The end of a 50 cm bare fused silica capillary (Polymicro Technologies, Phoenix, AZ) with 72 μm i.d., 143 μm o.d. was tightly clamped in between the gasket and the substrate to deliver the sample into the detection region. The capillary dimensions were chosen so that the ratio of the o.d. to i.d. is as close to one as possible. Under these conditions, the distance between the molecules eluting from the capillary and the SERS-active substrate is minimized, which increases detection sensitivity.[2] The sample injection was pressure driven through the capillary at a flow rate of 1 μL/min using a custom-made injection block.[3] Hydrodynamic focusing of the sample stream inside the flow chamber was achieved by pumping the sheath liquid (15 mM sodium tetraborate buffer, pH 9.4) continuously at a flow rate of 10 μL/min through the flow chamber via the inlet port located on the base plate. The sheath liquid flow rate was controlled using a syringe pump (Model NE-500 OEM, New Era Pump Systems Inc., Farmingdale, NY) controlled by LabView (National Instruments, Austin, TX). The liquid was drained out of the flow chamber via the outlet channel connected to the waste reservoir. The flow channel was sealed with a standard cover glass, pressed by the top plate, and secured using 4 tensioning screws. The system was grounded directly from the SERS substrate during the CZE separations. 6000 spectra were recorded in kinetic series with 50 ms acquisition times.

LIF Measurements: A high dynamic range LIF detector was used in this experiment that has been described elsewhere.[4] Briefly, fluorescent molecules were excited in a sheath

flow cuvette using a 25 mW CW 532 nm diode-pumped laser (CrystalLaser, Model CL532-025) and fluorescence emission was collected through a 600 nm DF 40 bandpass filter at a 90° angle relative to the incident laser beam. Fluorescence was detected using cascaded single-photon counting avalanche photodiode modules (Perkin Elmer, Montreal, PC Canada). The dye concentrations used for the CZE-LIF experiments were identical to those used in the CZE-SERS experiments to provide a direct comparison.

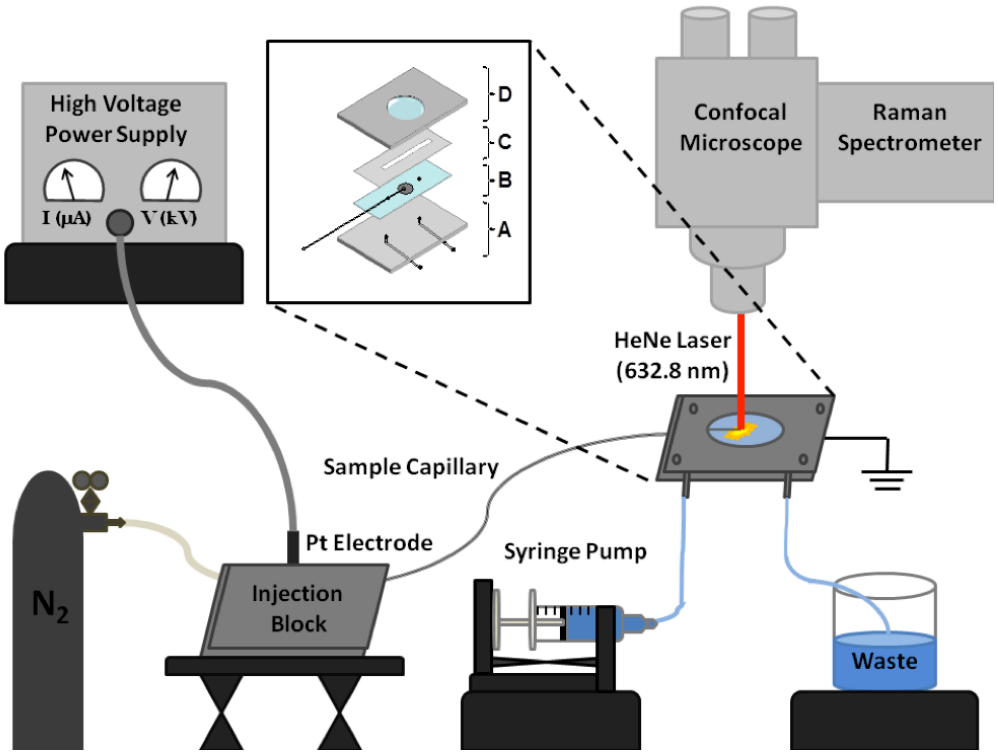

Figure S-1. The schematic diagrams the experimental CZE-SERS setup including the Raman microscope, the syringe pump, the waste reservoir, the nitrogen gas tank, the high voltage power supply, and the Pt electrode embedded in the custom-built injection block used to transport the sample through the fused silica capillary to the sheath-flow SERS detector. The inset shows the different components of the flow cell. The inlet and outlet ports located on the base of the flow cell are used to accommodate the sheath flow (A). Two holes are drilled in a microscope slide defining the sheath flow path and matching the dimensions of the ports. A sample capillary is pinned on a SERS-active substrate mounted in the center of the glass slide (B). A silicone gasket is cut defining the flow channel between the inlet and outlet ports (C). The top of the flow cell is sealed with a glass coverslip held in place by the top plate (D).

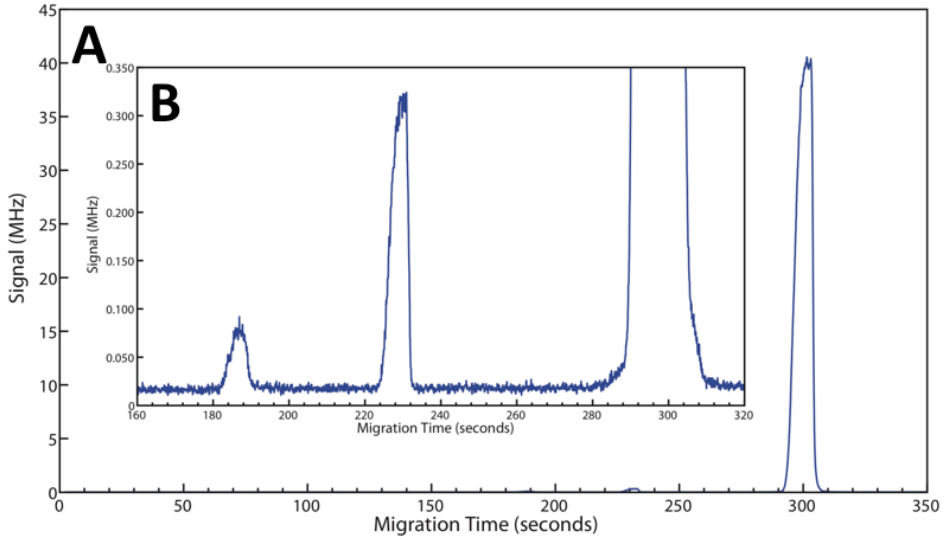

Figure S-2. Electropherogram of the three analyte mixture resulting from the CZE separation and detection by LIF. The attenuated signal presented here was extracted from the third avalanche photodiode (APD) on a high dynamic range system equipped with a total of five APDs in series.[4] The signal was treated to account for photon counter dead time (50 ns),[5] background corrected, and a three-point median filter was applied to remove spikes resulting from particulates passing through the detection volume. The experimental conditions (injection time, analyte concentrations, and CZE separation) were kept identical to those used for the CZE-SERS experiments to provide a direct comparison. The LIF electropherogram shows (A) the full scale of the 5-TAMRA peak and (B) all three peaks associated with the elution of R6G (t=187 ± 17 s), RB (t=229 ± 26 s), and 5-TAMRA (t=296 ± 23 s), respectively. The separation efficiencies were calculated from the first APD (no attenuation) using the peak width at baseline and were determined to be 2630 ± 400 theoretical plates for R6G, 6200 ± 600 theoretical plates for RB, and 900 ± 25 theoretical plates for 5-TAMRA. The poor column efficiencies are attributed to the large injection volume and the high concentration of analytes.

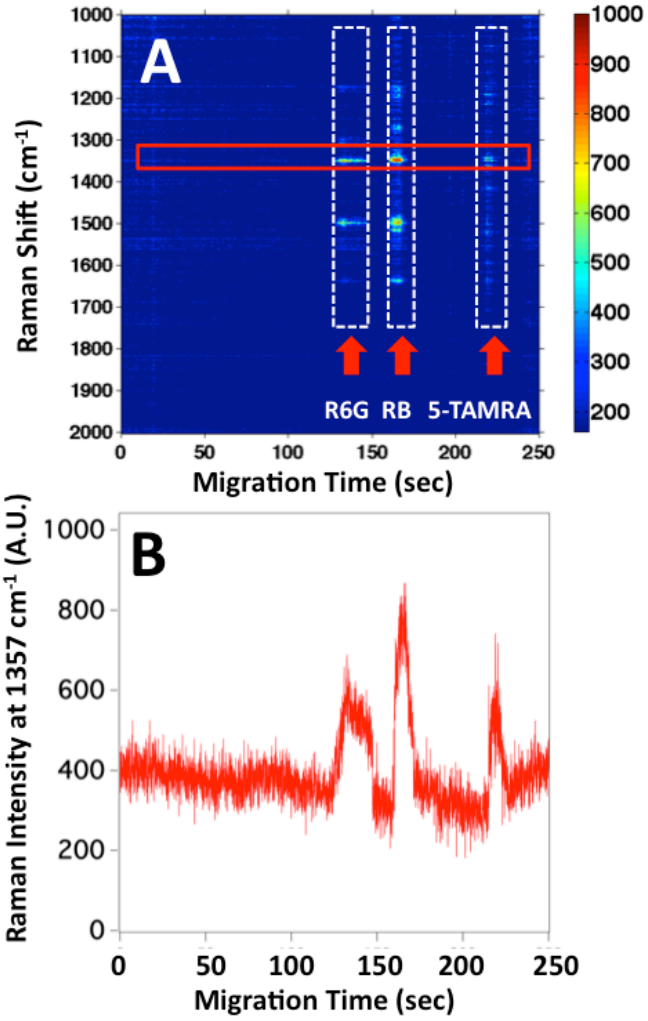

Figure S-3. (A) Heatmap of the observed SERS intensity of each Raman shift as a function of migration time for the electophoretic separation of a 102 nL (6 s injection) sample mixture containing 10^{-7} M R6G, 10^{-8} M RB, and 10^{-6} M 5-TAMRA. (B) SERS intensity profile of the Raman band at 1357 cm^{-1} as a function of migration time extracted from the red rectangle shown in (A). This band is attributed to the combined aromatic C-C and C=N stretching modes in rhodamine compounds. The dashed vertical rectangles in (A) highlight the detection of each analyte.

The analytes are assigned based on the observed Raman spectrum, indicating that R6G migrates at t=135 ± 13 s, RB at t=160 ± 15 s, and finally 5-TAMRA at t=220 ± 10 s. Clearly, the spectrally resolved SERS electropherogram of the three rhodamine dyes is characterized by a low and constant background. Figure S-3B shows the SERS electropherogram constructed from the SERS intensity at 1357 cm^{-1} as a function of migration time. The electropherogram peak for R6G shows a broad feature at t=135 s with a full width at half max (FWHM) of 12 s, which corresponds to a separation efficiency N = 700 ± 160 theoretical plates. The SERS electropherogram peak for RB at t=160 s shows a more symmetric peak with a FWHM of 6 s. This corresponds to N = 3,940 ± 1200 theoretical plates. The electropherogram peak for TAMRA at t= 220 s has a FWHM of 4 s and a separation efficiency N = 16,760 ± 3600 plates. Similarly poor numbers of theoretical plates was observed with CE-LIF and corresponds the long initial injection time and the high concentration of the sample into the capillary. The strong adsorption of R6G to the SERS substrate is clearly evident by the significant tailing noted in this measurement. This is in contrast with the sharper migration peaks and resulting higher column efficiencies observed for RB and 5-TAMRA, which suggests a faster desorption mechanism.

The SERS response from the substrate is highly reproducible; however, the signal is also a function of the where the laser is focused in relation to where the sheath flow confines the molecules in the detection area. We have found it is important to collect signal from this optimum point in the sheath flow.[2]

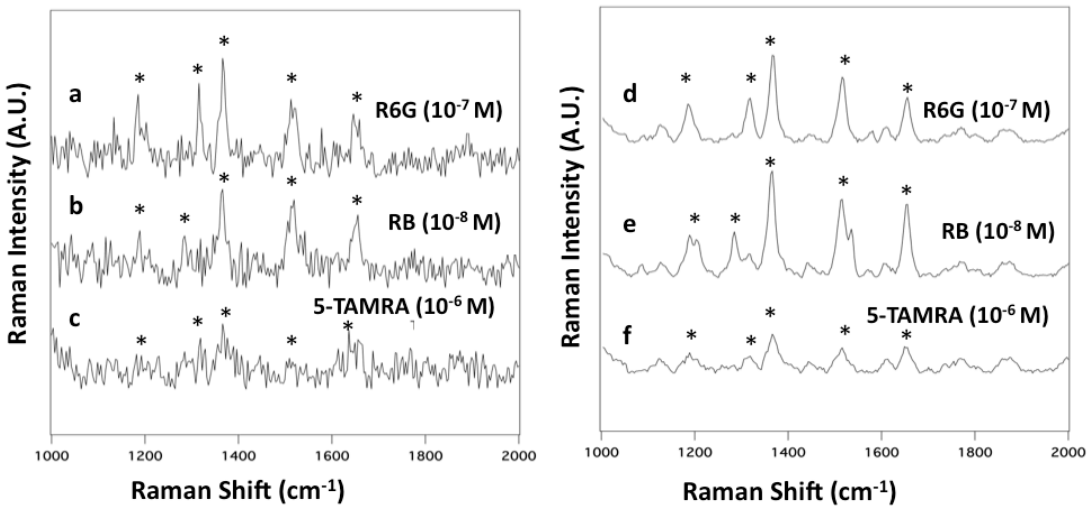

Figure S-4. Single 50 ms SERS spectrum of (a) R6G (10^{-7} M) extracted from Figure S-3A at t=135 s, (b) RB (10^{-8} M) extracted from Figure S-3A at t=165 s, and (c) 5-TAMRA (10^{-6} M) extracted from Figure S-3A at t=225 s. Averaged SERS spectrum of (d) R6G (10^{-7} M) extracted from Figure S-3A between t=133 and 145 s, (e) RB (10^{-8} M) extracted from Figure S-3A between t=160 and 168 s, and (f) 5-TAMRA (10^{-6} M) extracted from Figure S-3A between t=220 and 226 s. Asterisks indicate the bands intrinsic to each analyte.

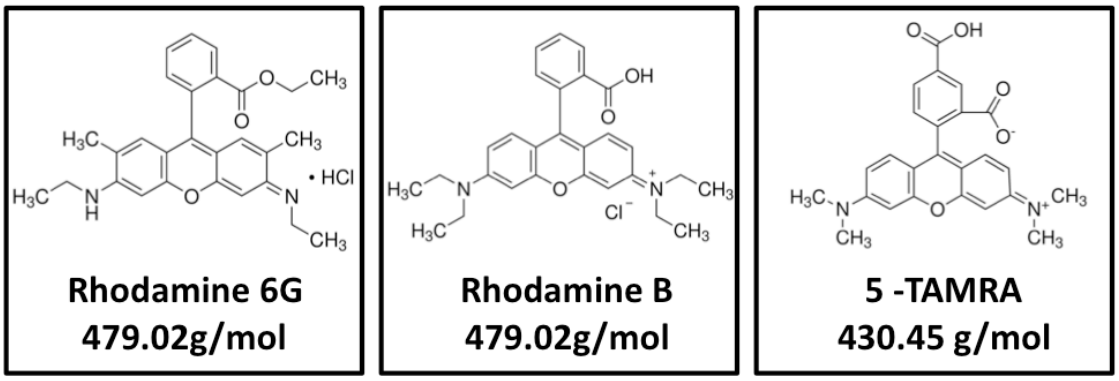

Figure S-5. Molecular structures and corresponding molecular weights of the three rhodamine dyes used in this study. Of note, rhodamine 6G and rhodamine B have identical molecular weights. Only rhodamine 6G has a secondary amine, to which we attribute its strong affinity for the silver SERS substrate.

References

1. S. M. Asiala and Z. D. Schultz, *Analyst*, 2011, **136**, 4472-4479.
2. P. Negri, K. T. Jacobs, O. O. Dada and Z. D. Schultz, *Anal Chem*, 2013, **85**, 10159-10166.
3. S. N. Krylov, D. A. Starke, E. A. Arriaga, Z. Zhang, N. W. Chan, M. M. Palcic and N. J. Dovichi, *Anal Chem*, 2000, **72**, 872-877.
4. O. O. Dada, D. C. Essaka, O. Hindsgaul, M. M. Palcic, J. Prendergast, R. L. Schnaar and N. J. Dovichi, *Anal. Chem.*, 2011, **83**, 2748-2753.
5. C. D. Whitmore, D. Essaka and N. J. Dovichi, *Talanta*, 2009, **80**, 744-748.