

## Introduction

### Background:

- Surface-enhanced Raman spectroscopy (SERS) is an embodiment of Raman spectroscopy that provides enormous signal enhancement while retaining all molecular specificity of regular Raman scattering<sup>1</sup>.
- The SERS effect occurs when the incident electromagnetic field of the laser induces localized surface plasmon resonance (LSPR) in silver nanoparticles (AgNPs)<sup>1,2</sup>.
- The size, shape and aggregation geometry of the AgNPs, along with excitation wavelength, are among the most important parameters responsible for the large signal enhancement observed in SERS<sup>3</sup>.

### Main Scientific Goal:

To determine the optimum combination of AgNP size and excitation wavelength for single-molecule detection.

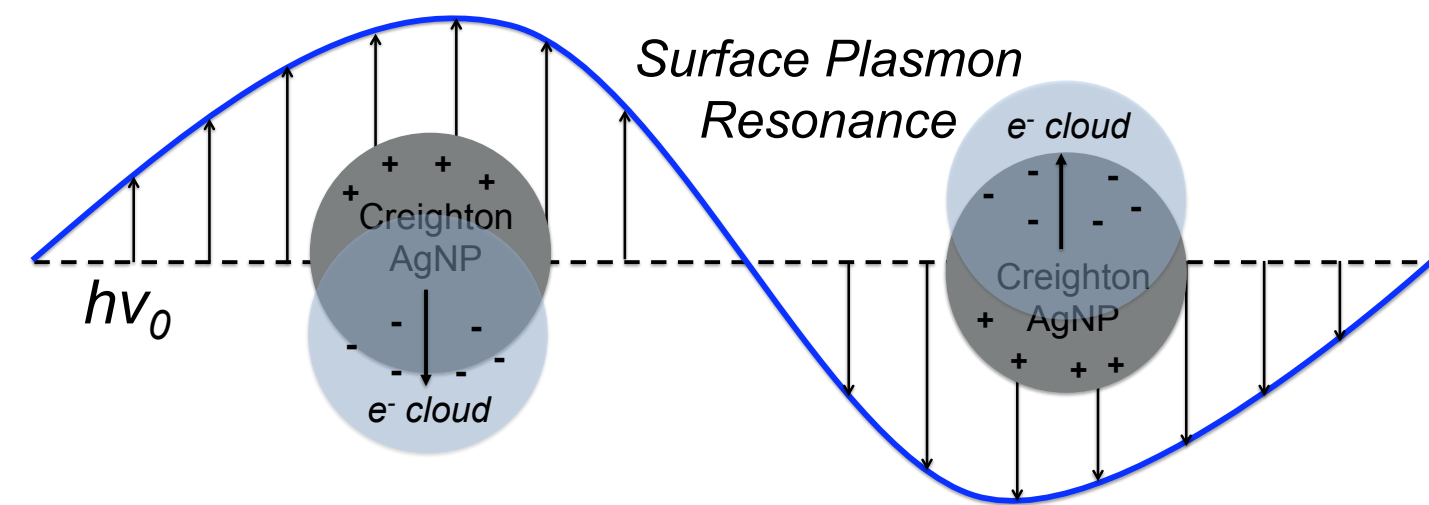


Figure 1. Schematic representation of the LSPR effect in Creighton AgNPs<sup>3,4</sup>.

### Scientific Aims:

- Specific Aim #1:** Synthesize and characterize a large volume (4.0 L) of Creighton colloidal AgNPs of moderate size distribution.
- Specific Aim #2:** Size-select, concentrate, and purify the original Creighton AgNPs via "green" tangential flow filtration (TFF), quantify the nanosilver concentrations by inductively coupled plasma optical emission spectroscopy (ICP-OES), and confirm fractionation using transmission electron microscopy (TEM).
- Specific Aim #3:** Characterize the SERS and SERRS enhancement as a function of AgNP geometry and excitation wavelength (632.8 nm and 532.1 nm) using a Raman reporter, rhodamine 6G (R6G).

### Synthesis and Characterization of Creighton Colloidal AgNPs:

- A large volume (4.0 L) of polydisperse, colloidal AgNPs was synthesized using the Creighton method<sup>5</sup> through the aqueous reduction of silver nitrate (1 mM of  $\text{AgNO}_3$ ) with sodium borohydride (2 mM of  $\text{NaBH}_4$ ).
- The resulting colloidal suspension was characterized via ultraviolet-visible absorption spectrophotometry (UV-VIS), Raman spectroscopy, X-ray diffraction

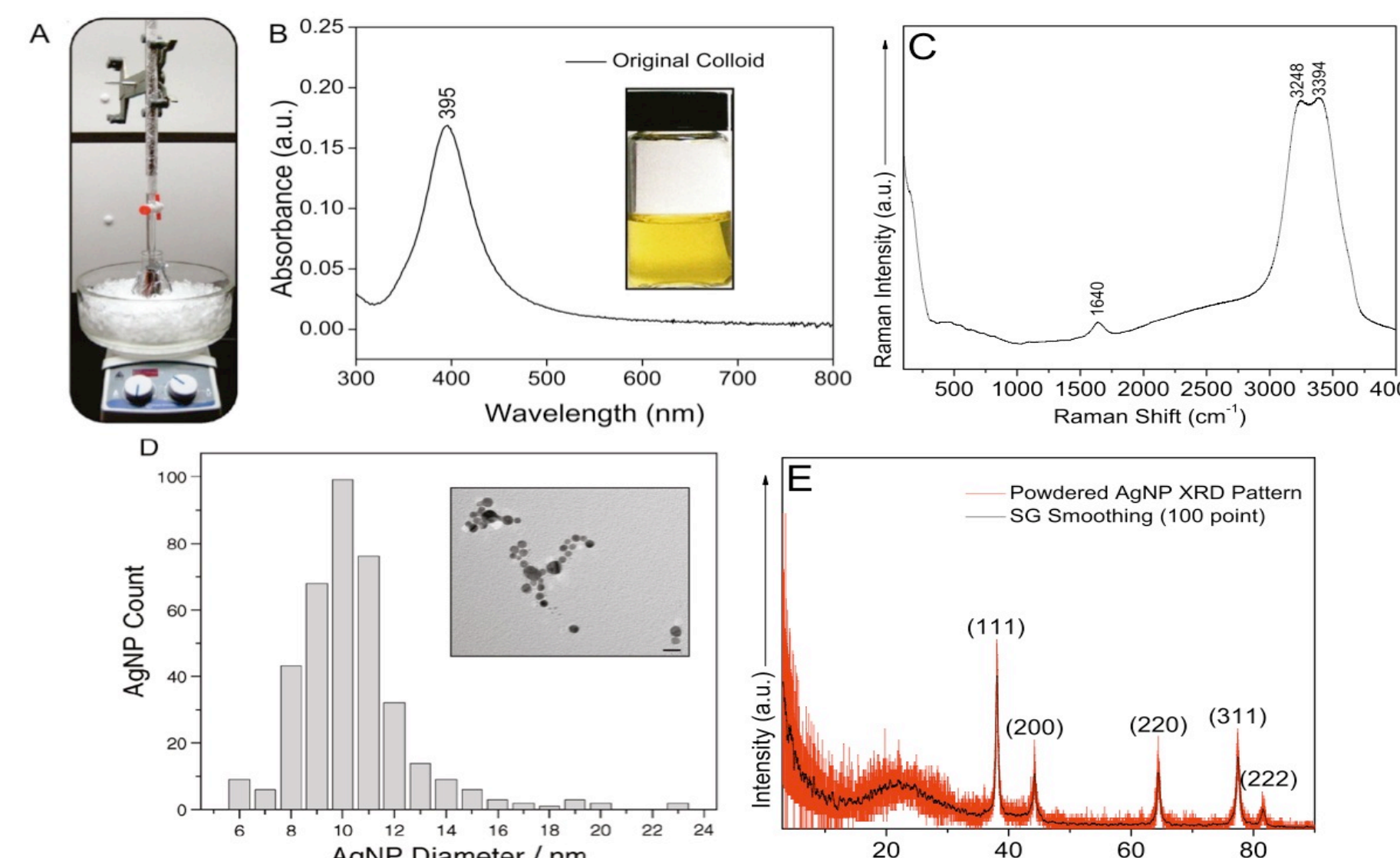


Figure 2. (A) Experimental setup for the synthesis of Creighton AgNPs<sup>5</sup>. (B) UV-VIS absorption spectrum, (C) Raman spectrum, (D) TEM size histogram (Inset: TEM micrograph)<sup>6</sup>, (E) XRD powdered diffractogram of AgNPs.

## Experimental Methods

### Three-step TFF based Filtration of Creighton AgNPs:

- The original AgNP suspension (Ori) was subjected to a 3-step TFF fractionation and purification procedure, according to the scheme in Figure 6.
- The resulting TFF solutions were then characterized by ICP-OES, Raman spectroscopy, TEM, and UV-VIS to confirm the size-selection and concentration capabilities of the TFF procedure.

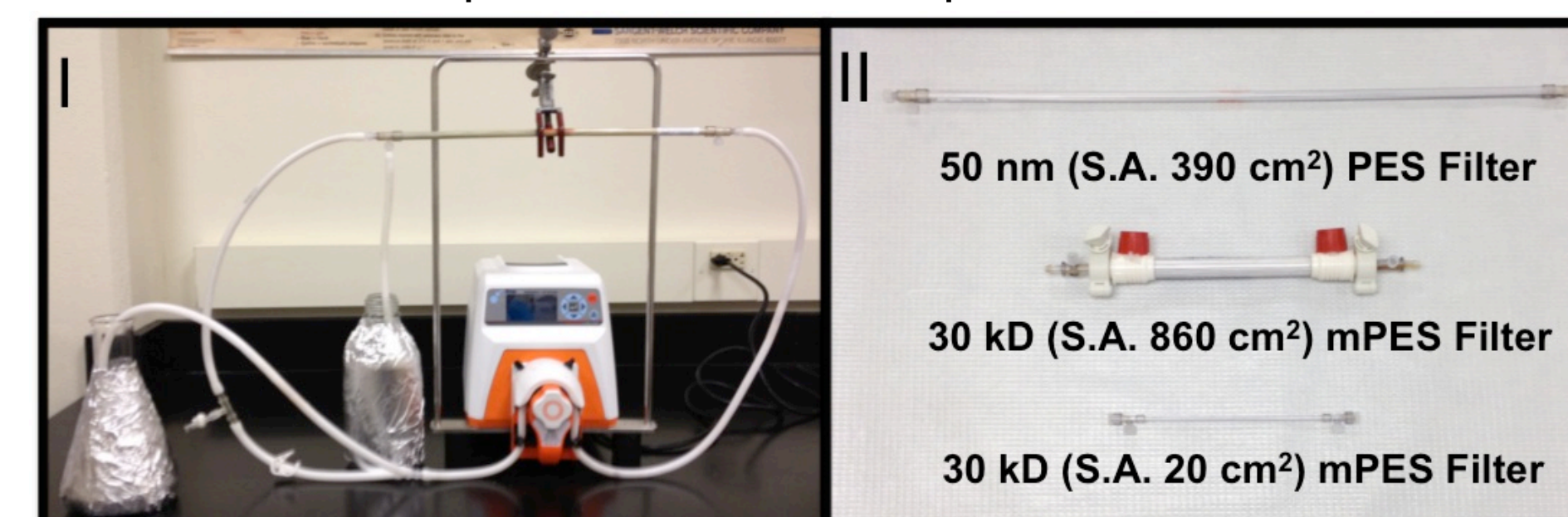


Figure 3. (I) KrosFlo Research II/ TFF system. (II) The three hollow fiber filter modules utilized in this study.

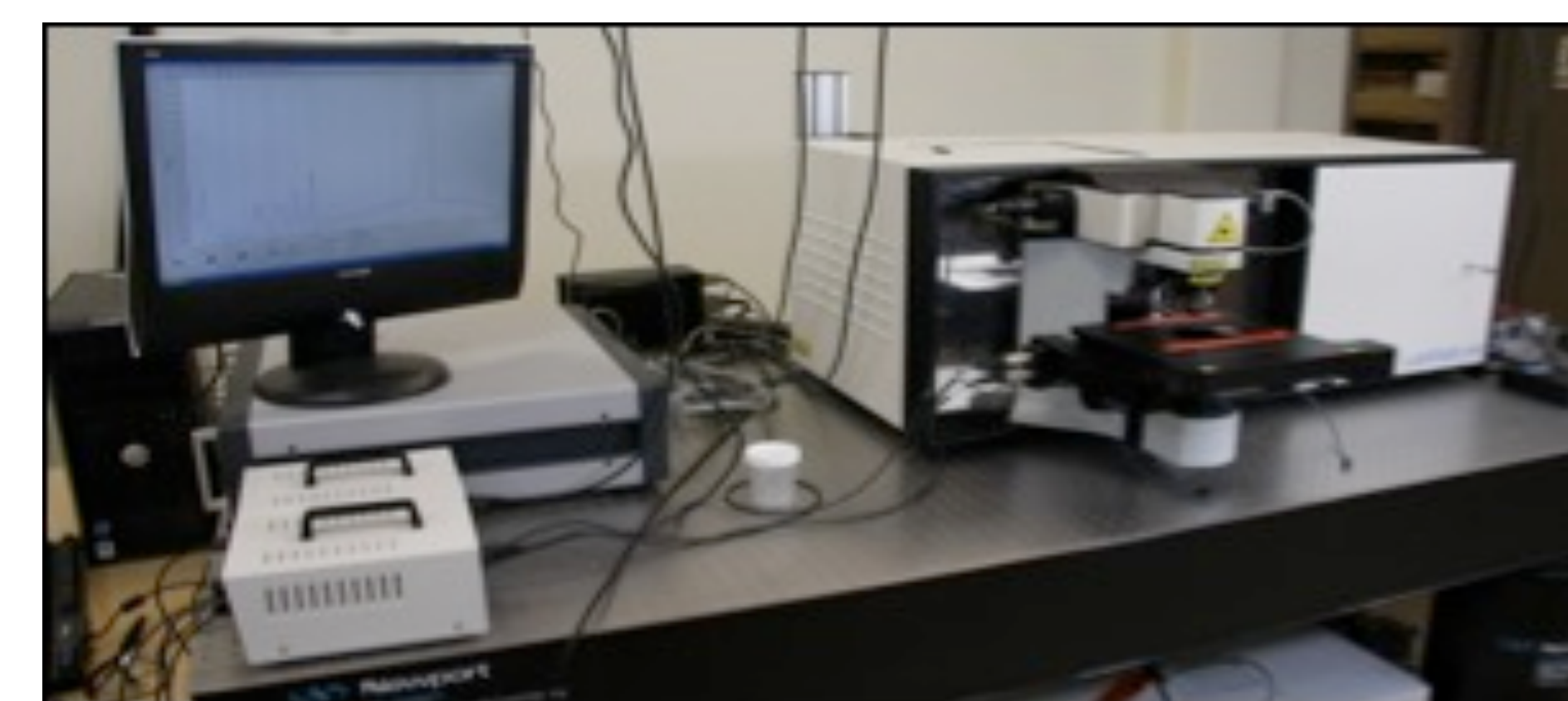


Figure 5. LabRamHR800 system (Horiba, Inc.). Acquisition parameters: 632.8 nm He-Ne (SERS) and 532.1 nm diode-pumped Nd:YAG (SERRS) excitation, 15 mW laser power at sample, 300 μm confocal hole, 3 s integration time.

### Fluorescence Emission Spectroscopy:

- The absorption of R6G onto AgNPs in the Ori,  $\text{Ag}_{50\text{R}}$ , and  $\text{Ag}_{30\text{R}2}$  suspensions was quantified by measuring its fluorescent emission (530 nm excitation) in the presence and absence of AgNPs.



Figure 4. (A) Cary Eclipse fluorescence spectrophotometer (Agilent Technologies). (B) 710-ES ICP-OES spectrometer (Varian, Inc.). (C) Miniflex II X-ray diffractometer (Rigaku Corp.).

### SERS and SERRS Measurements:

- Solutions of known concentrations of R6G ( $10^{-6}$  to  $10^{-15}$  M) were incubated with Ori,  $\text{Ag}_{50\text{R}}$ ,  $\text{Ag}_{30\text{R}2}$ , and potassium bromide (1.0 M of KBr, a "hot-spot" promoter)<sup>7</sup>. SERS/SERRS scattered photons were collected in a 180-degree backscattering geometry after 24 hours.

### Spectral Data Analysis:

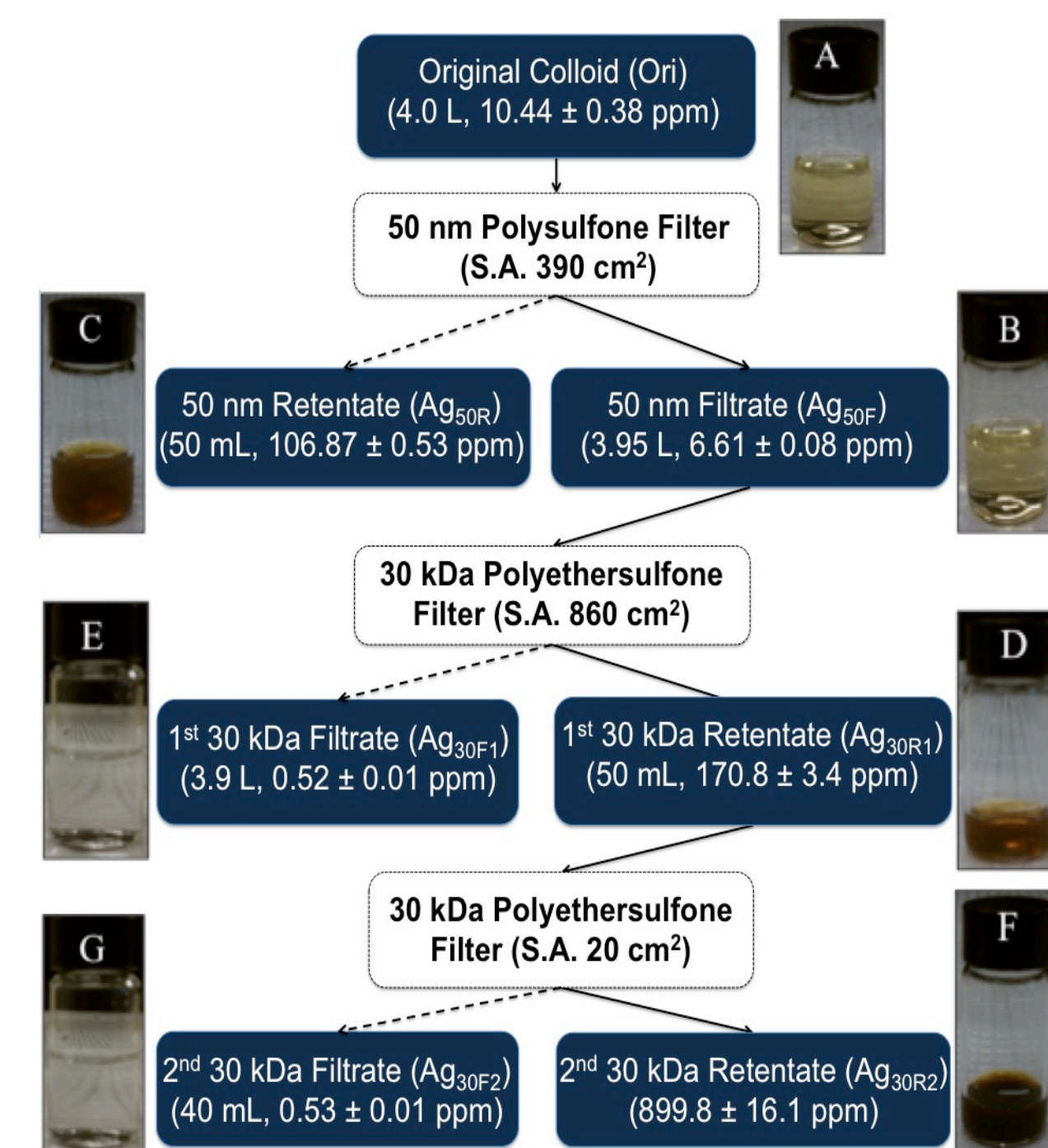
- Spectra were baseline-corrected using a BSpline interpolation mode in Origin Lab 8.5 software.
- The magnitude of the SERS/SERRS signal was quantified via integration of the 611  $\text{cm}^{-1}$  marker band of R6G (i.e., C-C-C backbone stretching).
- The analytical and surface enhancement factors<sup>8</sup> (AEF and SEF, respectively) were calculated for each size-selected AgNP solution, with respect to excitation wavelength.

## Results & Discussion

### Concentration, Size-Selection, and Purification of AgNPs:

- The TFF procedure yielded two highly concentrated, unfunctionalized AgNP suspensions:
- A 50 nm retentate ( $\text{Ag}_{50\text{R}}$ )
- A 30 kD retentate ( $\text{Ag}_{30\text{R}2}$ ) (Figure 6C & F).
- The TFF process retained 61% of the total AgNPs in the Ori. solution.

Figure 6. TFF scheme employed to fractionate and concentrate the Creighton colloid.



### Quantification of AgNP Size and Concentration in TFF Suspensions:

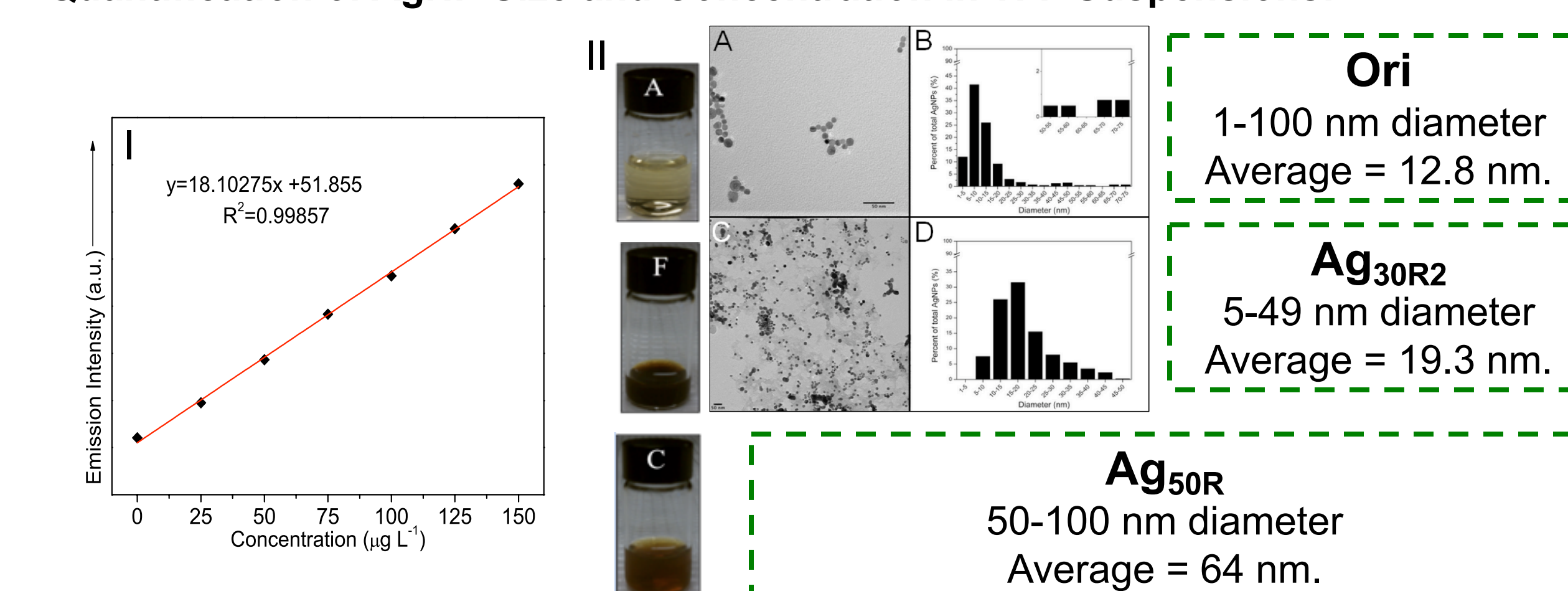


Figure 7. (I) ICP-OES standard calibration curve constructed using seven silver standards. (II) TEM micrographs<sup>4</sup> and the corresponding size histograms for two of the TFF-fractionated suspensions of AgNPs utilized in the SERS/SERRS measurements.

### Estimating the Percentage of R6G Molecules Adsorbed on AgNPs:

- AgNPs effectively "quenched" the fluorescence of surface-complexed R6G. This resulted in a reduced emission intensity when compared to the emission of the R6G bulk solution.

$$C_{R6G, AgNP\ surface} = C_{R6G, Bulk} \left[ 100\% - \left( \frac{I_{R6G, SERS\ sample}}{I_{R6G, Bulk}} \times 100\% \right) \right]$$

Table 1. Calculated Adsorption Ratios of R6G on to Creighton AgNPs

SERS/SERRS Substrate	Percentage of R6G Adsorbed onto AgNPs
Original Colloid (Ori.)	79.9% ( $10^9$ M of R6G)
50 nm Retentate ( $\text{Ag}_{50\text{R}}$ )	92.5% ( $10^9$ M of R6G)
30 kD Retentate ( $\text{Ag}_{30\text{R}2}$ )	91.6% ( $10^9$ M of R6G)

### Estimating the SERS and SERRS Signal Enhancement Factors:

- The C-C-C backbone stretching of the R6G xanthine ring at 611  $\text{cm}^{-1}$  was utilized as a marker band to quantify the SERS/SERRS signal enhancement for each AgNP size range.
- The lowest SERS detection limit was  $10^{-9}$  M of R6G ( $\text{Ag}_{50\text{R}}$  substrate).
- The lowest SERRS detection limit was  $10^{-15}$  M of R6G (all substrates)
- The analytical and surface enhancement factors<sup>8</sup> (AEF and SEF, respectively) were estimated to determine the overall SERS/SERRS signal enhancement.

$$AEF = \frac{d\sigma_{R6G, SERS}}{d\Omega} \times \frac{t_{Raman}}{d\Omega} \times \frac{1}{t_{SERS}}$$

$$SEF = \frac{1}{C_{R6G\ on\ AgNP\ Surface}} \times 8 \times \frac{AEF}{C_{R6G, Solution}}$$

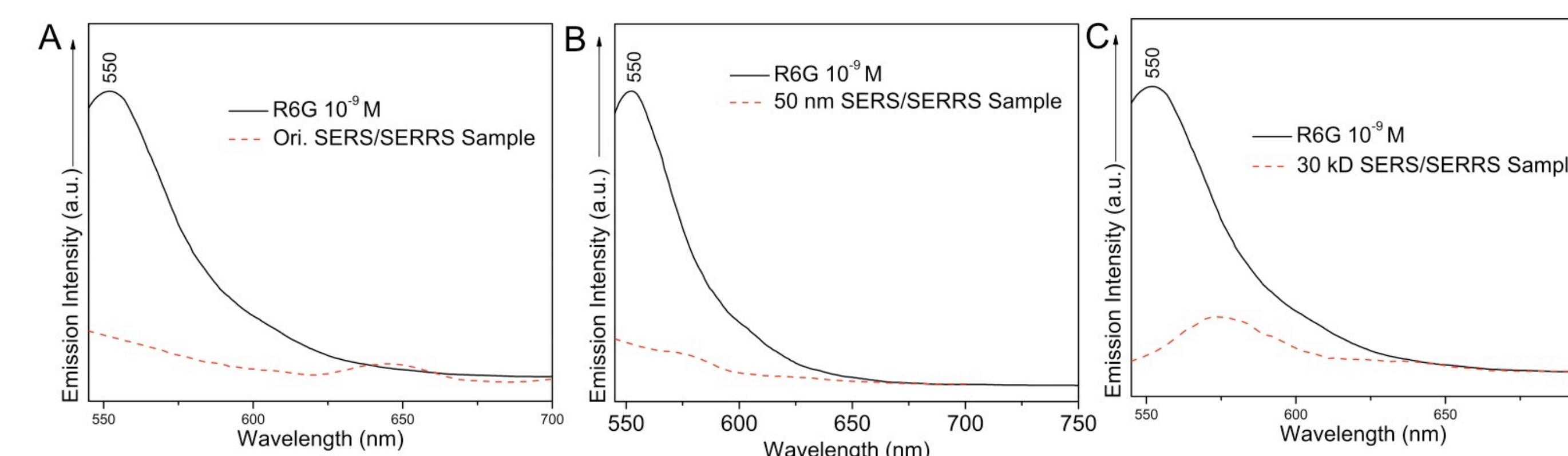


Figure 8. Fluorescence emission spectra of R6G in bulk solution and adsorbed onto the AgNPs in the (A) Ori., (B)  $\text{Ag}_{50\text{R}}$ , and (C)  $\text{Ag}_{30\text{R}2}$  suspensions.

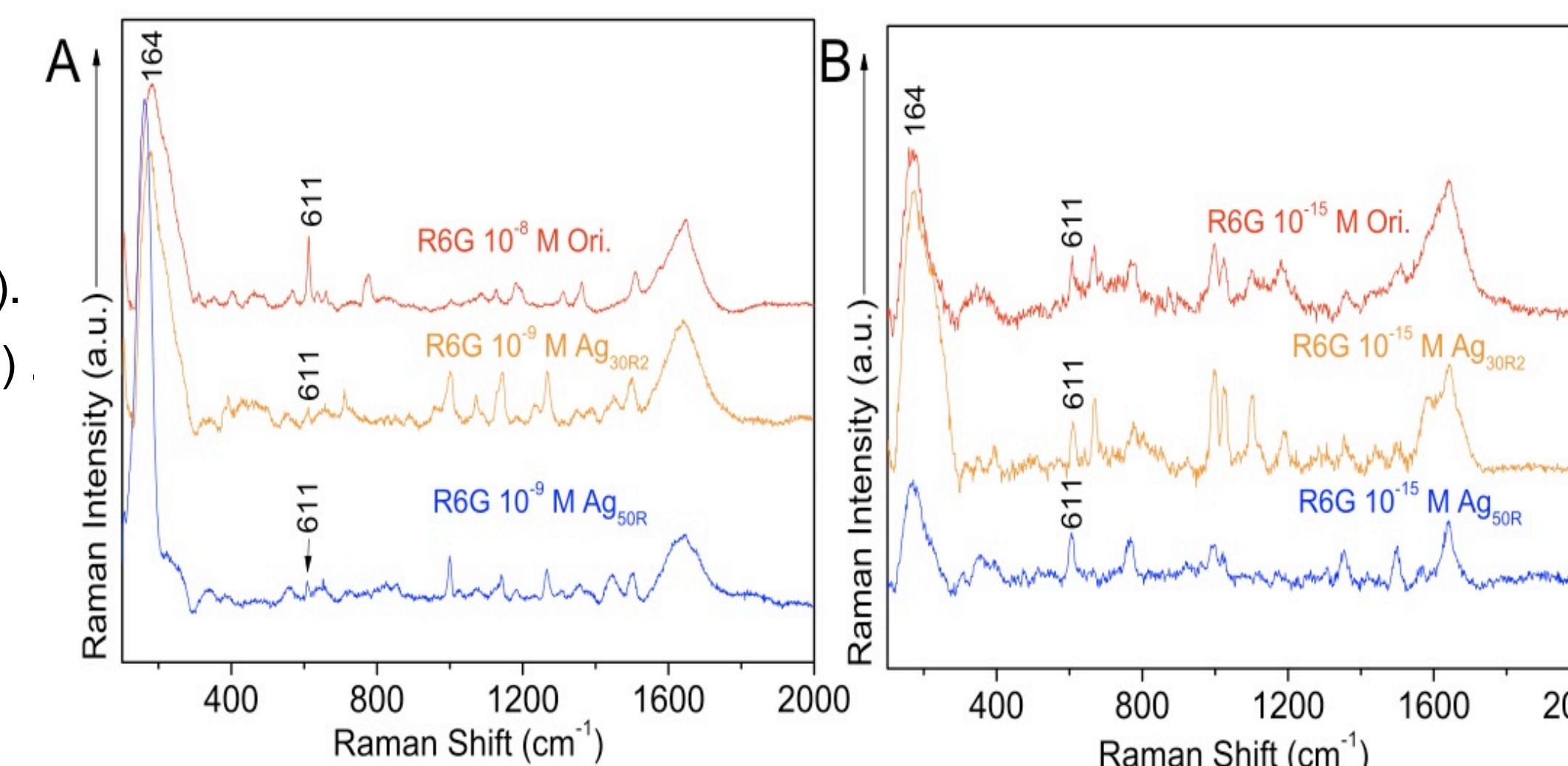


Figure 9. (A) SERS spectra of R6G depicting the lowest detection limit achieved for each AgNP size range. (B) SERRS spectra of R6G exhibiting a 6-fold lower detection limit. Spectra are intensity shifted for clarity.

## Conclusions

- The largest AEF and SEF values were observed for the  $\text{Ag}_{50\text{R}}$  substrate (i.e., AgNPs in the 50-100 nm size range with an 64 nm average diameter).

Table 2. AEF and SEF for AgNP Suspensions in this Study

SERS/SERRS Substrate	AEF	SEF	AEF	SEF
Original Colloid (Ori.)	$2.6 \times 10^4$	$2.1 \times 10^4$	$1.57 \times 10^9$	$1.25 \times 10^9$
50 nm Retentate ( $\text{Ag}_{50\text{R}}$ )	$2.2 \times 10^6$	$2.1 \times 10^6$	$2.85 \times 10^9$	$2.64 \times 10^9$
30 kD Retentate ( $\text{Ag}_{30\text{R}2}$ )	$1.1 \times 10^6$	$1.0 \times 10^6$	$2.2 \times 10^9$	$2.0 \times 10^9$

- The SERRS SEF values obtained for all substrates correspond to "single-molecule" detection events<sup>8</sup> (i.e., under resonant conditions, 532.1 nm excitation).
- Currently, finite element modeling is being implemented to quantitatively explain the observed AgNP size and excitation wavelength dependence of the EM field enhancement at the nanogap.

## Acknowledgments

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