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Wavelength-Scanned Surface-Enhanced Resonance Raman Excitation Spectroscopy

Jing Zhao,† Jon A. Dieringer, Xiaoyu Zhang,‡ George C. Schatz,* and Richard P. Van Duyne*
Northwestern University, Department of Chemistry, Evanston, Illinois 60208-3113
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We explore the correlation between localized surface plasmon resonance (LSPR) of triangular Ag nanoparticles, molecular resonance, and the surface-enhanced resonance Raman excitation profile of molecules adsorbed on these nanostructures. Ag nanoparticles with various LSPRs were fabricated with nanosphere lithography (NSL). A monolayer of tris(2,2′-bipyridine)-ruthenium(II) (Ru(bpy)32+) was introduced to the NSL Ag nanoparticles, and its effect on the LSPR was monitored by UV−vis spectroscopy. Wavelength-scanned surface-enhanced resonance Raman excitation spectroscopy (WS SERRES) profiles of Ru(bpy)32+ adsorbed on Ag nanoparticle arrays were measured for excitation wavelengths in the range of 400−500 nm. The WS SERRES profiles are correlated, both spatially and spectrally, with the corresponding LSPR spectra of the nanoparticle arrays and with the solution absorption spectrum of Ru(bpy)32+. The WS SERRES profile peak position depends on the relative spectral position of LSPR and the molecular resonance. Quasi-static electrodynamics modeling was applied to simulate the WS SERRES profiles, and the calculations are in agreement with the experimental results, demonstrating that the WS SERRES profiles involve multiplicative electromagnetic and resonance Raman enhancements.

Introduction

Localized surface plasmon resonance (LSPR) excitation is one of the characteristic optical properties of noble metal nanostructures. It arises when light induces a collective oscillation in the conduction electrons.1−3 The LSPR intensity, line width, and wavelength are determined by the composition, size, and shape of the nanoparticles as well as being extremely sensitive to the surrounding dielectric medium.4−12 A variety of chemical/biological sensors have been developed based on the refractive index sensitivity of LSPR.13−19 Recent investigations showed that when resonant molecules adsorb on nanoparticles, strong coupling between the molecular resonance and LSPR leads to a LSPR wavelength shift that is dependent on the spectral overlap between the plasmon resonance of nanoparticles and the molecular resonance of the adsorbed species.20−25 In particular, a very small LSPR shift is observed when the LSPR directly overlaps with the molecular resonance wavelength, and an amplified LSPR shift is observed when the LSPR is slightly to the red of the molecular resonance. By analyzing the wavelength-dependent LSPR wavelength shifts, one can study the electronic resonances of molecules that are adsorbed on a metallic surface under conditions where absorption by the molecules is too weak to be observed.25

When excited at the plasmon resonance wavelength of the noble metal nanostructures, significantly enhanced electromagnetic (EM) fields arise at the noble metal surface. The enhanced EM fields are responsible for the major enhancement (the electromagnetic enhancement mechanism) in surface-enhanced Raman spectroscopy (SERS).26−29 SERS and its mechanisms have been extensively studied by experiment and theory for the past 30 years.30−39 For nanoparticles of the appropriate shape and size, the EM enhancement can give rise to enhancement factors up to 108.40−42 On the other hand, when excited at the analyte’s electronic resonance frequency, the Raman scattering intensity is enhanced by 10−100 or more in comparison to off-resonance excitation, commonly referred to as resonance Raman enhancement.40−43−46 Under certain conditions, the contribution from the EM and resonance Raman mechanisms can be combined, leading to surface-enhanced resonant Raman scattering (SERRS) detection at a single-molecule level, where EM and resonant enhancement factors of 1010−11 and 104, respectively, are achieved.47−57

The LSPR spectrum and the frequency dependence of the SERS intensity are closely related to each other. To study the correlation between the LSPR of nanoparticles and the SERS excitation profile, McFarland et al. performed wavelength-scanned surface-enhanced Raman excitation spectroscopy (WS SERES) studies of a monolayer of benzenethiol (a nonresonant molecule) on Ag nanoparticles with varying LSPR.41 WS SERES profiles were taken at different wavelengths throughout the visible region of the electromagnetic spectrum. The experiments revealed that the maximum SERS enhancement factor occurs at excitation wavelengths that are shifted higher in energy than the spectral location of the LSPR extinction maximum by half the Raman Stokes shift of that band. This observation agrees with the SERS intensity wavelength dependence predicted by the electromagnetic mechanism.41

For resonant molecules, the SERS signal intensity is not only determined by EM enhancement from the nanoparticles but is also affected by the molecular absorption. In this work, we explore the relationship between the surface-enhanced resonance Raman excitation spectroscopy (SERRES) profile, the LSPR of the nanoparticles, and the molecular resonance. Tris(2,2′-bipyridine)-ruthenium(II) (Ru(bpy)32+) is chosen as the SERRS probe molecule. Ru(bpy)32+ and its derivatives have been widely applied to a variety of molecular devices due to its a key role in the development of photochemistry,58−60 electrochemistry,61,62
photoelectrochemistry, chemi- and electrochemiluminescence. The Ru(bpy)$_3^{2+}$ absorption spectrum has a maximum ground-state transition at 452 nm and a shoulder at 425 nm from a metal-to-ligand charge-transfer transition. It fluoresces at wavelengths greater than ~500 nm, which will not interfere strongly with its resonant Stokes Raman spectrum. SER(R)S has been intensively studied over the past 2 decades, and its vibrational modes are clearly assigned. When excited on and off resonance, Ru(bpy)$_3^{2+}$ has very different SERS and SERRS spectra that make it easy to distinguish the resonance effect in SERS.

The work presented in this paper addresses two goals: (1) to present a more complete data set for the coupling between resonant molecules and nanostructures with tunable surface plasmon resonances; (2) to understand the relationship between laser excitation, molecular resonance excitation, LSPR excitation, and SERRS intensity. Three necessary experiments for these studies include the following: (1) Measure the surface coverage of Ru(bpy)$_3^{2+}$ on Ag nanoparticles to ensure it is one monolayer or less so that the SERRS measurements are performed only on molecules that are directly adsorbed to the surface. (2) Study the LSPR wavelength shift of Ag nanoparticles induced by a monolayer of Ru(bpy)$_3^{2+}$ to determine how the two electronic transitions of Ru(bpy)$_3^{2+}$ couple to the LSPR. (3) Measure the WS SERRES profile of Ru(bpy)$_3^{2+}$ on the Ag nanoparticles while monitoring the correlated LSPR of the sample. This experiment reveals how LSPR and excitation wavelength affect SERRS intensity. In addition, theoretical studies using a quasi-static electrodynamics model are performed to understand the LSPR wavelength shift caused by a layer of Ru(bpy)$_3^{2+}$ and to predict how the EM enhancement together with the resonance enhancement lead to the experimental SERRES profile.

**Experimental Methods**

**Materials.** Silver shot was purchased from Alfa Aesar (no. 11357 1–3 mm diameter, Premion, 99.9999%). Tungsten vapor deposition boats were acquired from R. D. Mathis (Long Beach, CA). Polystyrene nanospheres with diameters of 280 ± 4 and 390 nm ± 19.5 nm were received as a suspension in water (Interfacial Dynamics Corporation, Portland or Duke Scientific, Palo Alto, CA) and were used without further treatment. Fisherbrand no. 2 glass coverslips with 18 mm diameters were obtained from Fisher Scientific (Pittsburgh, PA). For all steps of substrate preparation, deionized water was purified with the Milli-Q water. A Ag wire was used as the quasi-reference electrode.

**Nanoparticle Sample Preparation.** Glass substrates were cleaned in piranha solution (1:3 30% H$_2$O$_2$/H$_2$SO$_4$) for half an hour at 80 °C. The nanoparticles were sonicated for 3 min to remove the nanosphere mask. Following metal deposition, the samples were sonicated for 3–5 min in ethanol to remove the polystyrene nanosphere mask. The height of the nanoparticles was varied by depositing varying amounts of Ag onto the sample.

**Electrochemistry of Ru(bpy)$_3^{2+}$ Coverage on a Ag Elect rode.** Homemade silver electrodes were masked with Torr-Seal from Varian, Inc. to expose an area of ~0.2 cm$^2$. Prior to use, surfaces were polished with 0.3 and 0.05 µm alumina successively (Buehler Ltd., Lake Bluff, IL) and sonicated in Milli-Q water. The Ag electrode was used as the quasi-reference electrode, typically used in nonaqueous media. A BAS 100B/W electrochemical workstation was purchased from Bioanalytical Systems, Inc. (West Lafayette, IN). Solutions were deoxygenated with nitrogen for a minimum of 2 min prior to electrochemical experiments. A blanket of nitrogen, which was presaturated with solvent by passing it through a solvent saturator, was maintained in the electrochemical cell by continuous purging. The silver electrodes were incubated in ~0.1 mM Ru(bpy)$_3^{2+}$ in acetonitrile for 10–60 min. Double potential step chronocoulometry measurements (DPSCC) of the surface coverage of Ru(bpy)$_3^{2+}$ were made in 0.1 M TBAH in acetonitrile on those Ag electrodes by the application of a potential step waveform. The starting potential for the chronocoulometry measurement was −0.8 V. A potential step to −2 V past the 3e$^-$ reduction of Ru(bpy)$_3^{2+}$ for 250 ms duration was applied. The data were analyzed using Anson plots of the charge $Q_t$ versus $t^{1/2}$ for the forward step.

**LSPR Shift Induced by Ru(bpy)$_3^{2+}$ on Ag Nanoparticles.** Macroscale UV–vis extinction measurements were collected using an Ocean Optics (Dunedin, FL) SD2000 fiber optically coupled spectrometer with a CCD detector. All spectra in this study are performed in standard transmission geometry with unpolarized light. Nanoparticles with various LSPR wavelengths were fabricated with NSL by varying the size of the nanospheres and the deposited metal thickness. The nanoparticles were solvent-annealed with methanol for 10–20 min before being exposed to Ru(bpy)$_3^{2+}$. The nanoparticles were then incubated in 0.1 mM Ru(bpy)$_3^{2+}$ ethanol solution for 30 min, then rinsed by excessive neat ethanol and dried in N$_2$. After introducing Ru(bpy)$_3^{2+}$, the LSPR of the nanoparticle red shifts by different amount depending on the LSPR of the bare nanoparticles.

**SERRES Profile of Ru(bpy)$_3^{2+}$ on Ag Nanoparticles.** In order to perform correlated LSPR wavelength shift spectroscopy forming a monolayer in a close-packed hexagonal formation, which served as a deposition mask. The samples were then transferred to the evaporation chamber. The pressure in the vacuum chamber was maintained below 1 × 10$^{-5}$ Torr during the evaporation. A silver film was evaporated on the slides. The deposition rate Ag was 1.0 to ~1.5 A/s. A Leybold Inficon XTM/2 quartz crystal microbalance (East Syracuse, NY) was used to measure the thickness of the Ag film deposited over the nanosphere mask. Following metal deposition, the samples were sonicated for 3–5 min in ethanol to remove the polystyrene nanosphere mask. The height of the nanoparticles was varied by depositing varying amounts of Ag onto the sample.

**Figure 1.** Structure (left) and absorption spectrum of Ru(bpy)$_3^{2+}$ (right). The absorption spectrum is 0.01 mM Ru(bpy)$_3$(PF$_6$)$_2$ in 0.1 M TBAH acetonitrile solution.
and WS SERRES, it was necessary to perform LSPR microextinction on the inverted microscope such that the excitation profile was measured from the same spot on the sample due to sample heterogeneity. As such, the LSPR of the Ag nanoparticles before and after exposure to Ru(bpy)$_3^{2+}$ was measured with the microextinction setup without moving the sample. In situ measurement of the LSPR spectrum was achieved by illuminating the sample with the microscope lamp and analyzing the transmitted light with a fiber optically coupled miniature spectrometer (model SD2000, Ocean Optics, Dunedin, FL). SERR spectra of Ru(bpy)$_3^{2+}$ from the same spot were measured at different excitation wavelengths. Figure 2 shows a schematic of the instrumentation used for the WS SERRES experiments. All optical measurements were performed using a Nikon Eclipse TE300 inverted microscope (Fryer Co., Huntley, IL) equipped with a 20× objective (XLWD, NA 0.5, plan fluor). Substrates were mounted on a piezoelectric stage (model P-517.3CD, Polytech PI, Auburn, MA) to allow for sample positioning and raster-scanning during spectral acquisition. The light scattered by the samples was analyzed with a TriplePro three-stage spectrograph equipped with a liquid-nitrogen-cooled, deep-depletion Spec-10:400BR CCD detector (Princeton Instruments, Trenton, NJ). The deep-depletion CCD detector was not necessary for this experiment, but since this apparatus is also used for near-infrared SERS, it was chosen to prevent etaloning at those wavelengths. A color video camera was also attached to the front port of the microscope to facilitate laser alignment and positioning of the samples. Laser excitation was provided by a Spectra-Physics (Mountain View, CA) Milennia Xs ($\lambda_{\text{ex}}$ 532 nm) pumping a Spectra-Physics Tsunami with a GWU harmonic generator ($\lambda_{\text{ex}}$ 700–1000 nm, 350–500 nm). The laser light from the tunable laser system was filtered using Pellin–Broca prisms to ensure monochromatic illumination of the sample. Furthermore, the SERRS intensity was normalized to the normal Raman scattering of cyclohexane following the procedure of McFarland et al. to correct for the instrument throughput, the detector quantum efficiency, and the inherent $\nu^4$ dependence on the Raman scattering cross section. It is worthwhile to note that all illumination powers reported in this work were the laser powers incident on the microscope beam splitter, not the power incident on the sample. On the basis of experimental measurements, approximately 5–10% of the reported power is incident on the sample.

Figure 2. Schematic diagram of the WS SERRES apparatus.

Figure 3. (A) Cyclic voltammogram of 0.10 mM Ru(bpy)$_3^{2+}$ in acetonitrile at a silver electrode with 0.10 M TBAH as the supporting electrolyte. The scan begins at 0 mV and first moves in the negative direction at 100 mV/s. The three electron reductions of Ru(bpy)$_3^{2+}$ occur at −1347, −1539, and −1769 mV. (B) A representative double potential step chronocoullogram in acetonitrile at the silver electrode with 0.10 M TBAH as the supporting electrolyte. Prior to the measurement, the electrode was incubated in 0.10 mM Ru(bpy)$_3^{2+}$ solution for 5 min. Starting potential: −800 mV. Ending potential: −2000 mV. Step width: 250 ms. (C) Anson plots of $Q_f$ vs $t^{1/2}$ (forward) and $Q_r$ vs $\theta$ (reverse) for (B). (D) The plot of Ru(bpy)$_3^{2+}$ adsorbate coverage vs incubation time. As the incubation time is increased from 5 to 140 min, the surface concentration increases from 0.20 $\times$ 10$^{14}$ to 0.94 $\times$ 10$^{14}$ molecules/cm$^2$. 
The surface coverage of Ru(bpy)₃²⁺ was determined to be 0.20 mM Ru(bpy)₃₃(PF₆)₂ and 0.1 M TBAH acetonitrile solution. Using eqs 1 and 2 and experimental LSPR shift (the solid black line with filled dots). Ru(bpy)₃²⁺ surface concentration translates to 0.3 monolayer of adsorbed molecules/cm² as observed. Then the LSPR shift gradually decreases to 15 nm. As the incubation time increase from 5 to 140 min, the surface coverage of Ru(bpy)₃²⁺ adsorbate level off at ~0.94 × 10¹⁴ molecules/cm² (Figure 3D). The error bars represent the standard deviation of at least three replicated DPSCC measurements, which may be mainly caused by variation in electrode surface area and roughness. From this measurement, we determined that a incubation for 30 min yields a monolayer coverage of Ru(bpy)₃²⁺ on the Ag surface (~0.65 × 10¹⁴ molecules/cm²); therefore, this incubation time was used in the following experiments.

**Results and Discussion**

**Electrochemistry of Ru(bpy)₃²⁺ Coverage on a Ag Electrode.** Figure 3A shows a cyclic voltammogram which results when a silver electrode is placed in a solution containing 0.1 mM Ru(bpy)₃²⁺ and 0.1 M TBAH in acetonitrile. Ru(bpy)₃²⁺ has three continuous one-electron reduction peaks on Ag electrodes. Good reversible behavior was observed for all three reductions. The reduction of Ru(bpy)₃²⁺ to Ru(bpy)₃⁺ occurs at about −1.3 V versus a Ag quasi-reference electrode. The concentration of an electroactive species adsorbed on an electrode surface can be determined by DPSCC and Anson plots. An example of the DPSCC data and Anson plots for the adsorption of Ru(bpy)₃²⁺ on a smooth Ag electrode is shown in Figure 3, parts B and C. We determined that 0.20 × 10¹⁴ molecules/cm² were adsorbed on the smooth Ag electrode from 0.1 mM Ru(bpy)₃₃(PF₆)₂ and 0.1 M TBAH acetonitrile solution. Given the radius of Ru(bpy)₃²⁺ of 0.7 nm, the measured surface concentration translates to 0.3 monolayer of adsorbed Ru(bpy)₃²⁺. As the incubation time increase from 5 to 140 min, the surface coverage of Ru(bpy)₃²⁺ adsorbate level off at ~0.94 × 10¹⁴ molecules/cm² (Figure 3D). The error bars represent the standard deviation of at least three replicated DPSCC measurements, which may be mainly caused by variation in electrode surface area and roughness. From this measurement, we determined that a incubation for 30 min yields a monolayer coverage of Ru(bpy)₃²⁺ on the Ag surface (~0.65 × 10¹⁴ molecules/cm²); therefore, this incubation time was used in the following experiments.

**LSPR Shift Induced by Ru(bpy)₃²⁺ on Ag Nanoparticles.** Previous studies demonstrate that the shift in the LSPR of nanoparticles induced by resonant adsorbates is highly dependent upon the spectral overlap between the plasmon and molecular resonances. To explore the effect of a monolayer of Ru(bpy)₃²⁺ on the LSPR of Ag nanoparticles, nanoparticles with varying LSPR were fabricated with NSL and then incubated in 0.1 mM Ru(bpy)₃₃(PF₆)₂ solution for 30 min, followed by thorough rinsing. Figure 4A shows the plot of the LSPR shift induced by Ru(bpy)₃²⁺ versus the LSPR position of bare Ag nanoparticles. When 𝜆茕 max is blue of Ru(bpy)₃²⁺; the LSPR shift is ~20 nm. When 𝜆茕 max is at ~445 nm, close to the Ru(bpy)₃²⁺ long-axis (~452 nm), the LSPR shift is very small, roughly 5 nm. As 𝜆茕 max is tuned to be slightly red of the Ru(bpy)₃²⁺ resonance at 453 nm, an amplified LSPR response of 50 nm was observed. Then the LSPR shift gradually decreases to 15 nm as 𝜆茕 max is moved further to red of the Ru(bpy)₃²⁺ resonance.

The sharp transition of the LSPR shift from 5 to 50 nm within 10 nm of the molecular resonance is similar to that previously observed for several resonant molecules. In the present case, the two electronic transitions of Ru(bpy)₃²⁺ are polarized differently, and only the transition polarized perpendicular to the nanoparticle surface is strongly coupled with the LSPR. This phenomenon was previously observed for nanoparticles covered with a molecular layer of Fe(bpy)₃²⁺. In addition, theoretical predictions for an ellipsoidal particle coated with a layer of resonant molecules showed that the coupling between the plasmon and molecular resonances depends on the orientation of the molecules on the surface.

**Theoretical Modeling of the LSPR Shift.** In earlier studies, the LSPR wavelength shifts induced by a monolayer of resonant molecules were simulated by electrodynamics theory both numerically by the discrete dipole approximation (DDA) and analytically in the quasi-static limit. These methods calculated the extinction of a bare nanoparticle and a nanoparticle with a layer of resonant molecules to get the 𝜆.qml max shift. Results from both numerical and analytical methods agreed well (at least qualitatively) with the experimental data. Since the DDA method is computationally expensive, the quasi-static method is applied in this work. Briefly, in the quasi-static limit, the extinction cross section of a metallic spheroid is proportional to the imaginary part of its polarizability. For a bare spheroid embedded in a homogeneous medium, its extinction cross section is given by eq 1:

$$ C_{\text{ext}} \propto \frac{1}{\lambda} \Im \left\{ \frac{\varepsilon_1 - \varepsilon_0}{\varepsilon_1 + \chi \varepsilon_0} \right\} $$

where \( \varepsilon_1 \) is the dielectric constant of the spheroid (which is Ag for the current application), \( \varepsilon_0 \) is the dielectric constant of the surrounding medium (which is 1 for N₂), and \( \chi \) is a shape factor for the particle that has the value 2 for a sphere and increases with increasing aspect ratio. For a spheroid coated with a layer, the extinction cross section becomes:

$$ C_{\text{ext}} \propto \frac{1}{\lambda} \Im \left\{ \frac{\xi}{Q_1(\xi)} \frac{\varepsilon_1 - \varepsilon_0}{\varepsilon_1 + \chi \varepsilon_1} G - \frac{\xi}{Q_1(\xi)} (G - 1) \right\} $$

where \( \varepsilon_1 \) is the dielectric constant of the layer (which is Ru(bpy)₃²⁺ for the current application). The spheroidal coordinate \( \xi \) is defined by \( \xi = (r_1 + r_2)/2 \) with \( r_1 \) and \( r_2 \) being the distance from each focus of the spheroid to the specified position and \( f = (c^2 - a^2)^{1/2} \) with \( c \) and \( a \) being the major and minor
axis, respectively. \(\xi_0\) and \(\xi_1\) are the values of \(\xi\) at the inner and outer surfaces of the layered spheroid. \(Q(x)\) is the Legendre polynomial of the second kind. \(G\) is a correction factor that comes from solving the Laplace equations of the layered sphere. For expressions for \(G\), \(Q(x)\), and \(\chi\), please refer to the Supporting Information. To correct the static result for radiation damping and depolarization, we used the approach developed by Meier and Wokaun\(^{86}\) for spheres and generalized by Zeman and Schatz for spheroids.\(^{85}\) Details of this correction method are available in the Supporting Information.

In the experiments, the NSL-fabricated nanoparticles are on a glass substrate. By assuming the nanoparticles are truncated tetrahedral with in-plane width of 65 nm and out-of-plane height of 50 nm, it is estimated that \(\sim 25\%\) of the nanoparticle surface is exposed to substrate. This effect is treated by an effective medium theory\(^2\) where the dielectric constants of the layer are expressed as \(\epsilon_{\text{eff}} = \epsilon_{\text{mol}}x + \epsilon_{\text{mol}}(1 - x)\) where \(\epsilon_{\text{mol}}\) is the dielectric function of the molecule and \(x\) is the relative amount of the molecules in the layer, which is 0.75 in this case. The effect of the glass substrate on the LSPR is not considered because from effective medium theory and previous work,\(^7\) the substrate will result in a constant red shift in the nanoparticle LSPR. When comparing the difference in the LSPR of bare nanoparticles and nanoparticles with adsorbates, the constant red shift in the LSPR induced by substrate will be canceled.

For bare nanoparticles, \(\epsilon_o\) is set to 1 for the dielectric constant of \(N_2\). For the layered spheroid, the dielectric constant of the resonant layer of \(\text{Ru(bpy)}_3^{2+}\) was obtained from its absorption spectrum in solution using a Kramers–Kronig transformation\(^{3,88}\) where the real part of the refractive index is expressed as an integral of the absorption coefficients. This method has been applied in previous publications and is detailed in the Supporting Information. Note that only the electronic transition at 452 nm of \(\text{Ru(bpy)}_3^{2+}\) couples strongly to the LSPR. To get the dielectric constant corresponding to electronic transition at 452 nm of \(\text{Ru(bpy)}_3^{2+}\), the absorption spectrum was deconvoluted with two Gaussian curves at 452 and 425 nm. The Kramers–Kronig transformation was performed with the 452 nm Gaussian curve.

To simulate the LSPR wavelength shift, the semiminor axis of the spheroid is chosen as 40 nm and the semimajor axis is varied to alter the shape factor \(\chi\). This accounts for the varying LSPR of nanoparticles in the experiments. Figure 4B shows the predicted LSPR shift in comparison with the experimental results. The predicted LSPR shift curve captures the shape transition around the molecular resonance from a very small (<10 nm) to an amplified LSPR shift (~50 nm). However, the rapid change in \(\Delta\lambda_{\text{max}}\) occurred at ~450 nm in the experiments, whereas the predicted change occurred at ~430 nm. In addition, the magnitude of the predicted LSPR shift does not match the experimental data accurately. At a wavelength shorter than the molecular resonance, the predicted LSPR shift is negative. At an off-resonance wavelength (>480 nm), the predicted LSPR shift is much larger than the experimental data. This discrepancy has been seen in previous work\(^{25}\) using a similar method because nanoparticles with a larger aspect ratio show a larger LSPR shift even if the layer has a dielectric constant that is constant with wavelength. In the experiments, the LSPR of the nanoparticles is tuned by both size and aspect ratio. Therefore, using only the aspect ratio to vary the nanoparticle LSPR overestimates the nanoparticle aspect ratio and leads to a LSPR shift that is larger than the experimental data. Furthermore, as discussed in the last section and the Supporting Information, the dielectric constants of \(\text{Ru(bpy)}_3^{2+}\) obtained from Kramers–Kronig transformation have some uncertainty in absolute value.

**Figure 5.** Representative SERR spectrum of \(\text{Ru(bpy)}_3^{2+}\) on an NSL-fabricated Ag nanoparticle substrate. \(\lambda_{\text{ex}} = 457.9\, \text{nm, power} = 0.131\, \text{mW, acquisition time} = 60\, \text{s. An atomic force micrograph image of the sample is shown in the inset.}

**WS SERRS Profile of \(\text{Ru(bpy)}_3^{2+}\) on Ag Nanoparticles.** A representative SERR spectrum of \(\text{Ru(bpy)}_3^{2+}\) on Ag nanoparticles is shown in Figure 5. The peak at 1487 cm\(^{-1}\) is the most enhanced band under resonant conditions. Figure 6 shows three excitation profiles for the 1487 cm\(^{-1}\) peak of \(\text{Ru(bpy)}_3^{2+}\), each with an LSPR \(\lambda_{\text{max}}\) at different locations. The WS SERRS profile in Figure 6A consists of nine data points measured over the spectral range of 420 to ~497 nm. The LSPR \(\lambda_{\text{max}}\) of this substrate was measured to be 434.7 nm (23 004 cm\(^{-1}\)) with a monolayer of \(\text{Ru(bpy)}_3^{2+}\). Fitting a Gaussian line shape to the data reveals that the peak of the excitation profile is at \(\lambda_{\text{ex,max}} = 445.1\, \text{nm (22 467 cm}^{-1}\)) for the 1487 cm\(^{-1}\) mode and \(\lambda_{\text{ex,max}} = 428.1\, \text{nm (23 359 cm}^{-1}\)) for the 1602 cm\(^{-1}\) mode. The WS SERRS profile in Figure 6B consists of nine data points measured over the spectral range of 415–495 nm. The LSPR \(\lambda_{\text{max}}\) of this substrate was measured to be 465.2 (23 996 cm\(^{-1}\)) with a monolayer of \(\text{Ru(bpy)}_3^{2+}\). Fitting a Gaussian line shape to the data reveals that the peak of the excitation profile is at \(\lambda_{\text{ex,max}} = 466.4\, \text{nm (24 441 cm}^{-1}\)) for the 1487 cm\(^{-1}\) mode and 454.3 nm (22 012 cm\(^{-1}\)) for the 1602 cm\(^{-1}\) mode. The WS SERRS profile in Figure 6C consists of eight data points measured over the spectral range of 415–495 nm. The LSPR \(\lambda_{\text{max}}\) of this substrate was measured to be 480.1 nm (20 829 cm\(^{-1}\)) with a monolayer of \(\text{Ru(bpy)}_3^{2+}\). Fitting a Gaussian line shape to the data reveals that the peak of the excitation profile is at \(\lambda_{\text{ex,max}} = 466.4\, \text{nm (24 441 cm}^{-1}\)) for the 1487 cm\(^{-1}\) mode and \(\lambda_{\text{ex,max}} = 466.4\, \text{nm (24 441 cm}^{-1}\)) for the 1602 cm\(^{-1}\) mode. The intensities are normalized for laser power, acquisition time, and the ratio of SERRS intensity of \(\text{Ru(bpy)}_3^{2+}\) to the normal Raman intensity of cyclohexane. SERRS enhancement factor (EF) values were calculated by comparing the intensity of the peak (1487 or 1602 cm\(^{-1}\)) measured in the SERRS experiments to the peak (1444.4 cm\(^{-1}\)) measured from liquid cyclohexane and normalized to the resonance Raman cross section of \(\text{Ru(bpy)}_3^{2+}\). An example of the SERRS EF is described in the Supporting Information.

According to McFarland et al., for a nonresonant Raman scatterer adsorbed to a SERS active surface with well-defined LSPR, the maximum excitation, \(\lambda_{\text{ex,max}}\), occurs at higher energy than the LSPR extinction maximum, \(\lambda_{\text{max}}\), with the magnitude of the displacement being approximately half of the Raman Stokes shift.\(^{31}\) This is in line with the theoretical predictions for the electromagnetic enhancement mechanism. As such, different modes of a molecule will have maximum enhancement at different excitation frequencies. In this study, the molecule’s...
electronic resonance is both resonant with the laser excitation frequency and with the oscillation frequency of the LSPR. This leads to deviation from the nonresonant case due to the resonance Raman effect. A previous report for a Ru(bpy)$_3^{2+}$ analogue, Fe(bpy)$_3^{2+}$, shows that the 1491 cm$^{-1}$ mode (corresponding to the 1487 cm$^{-1}$ mode of Ru(bpy)$_3^{2+}$) undergoes a large resonance Raman enhancement biased toward the lower energy band of the electronic absorption spectrum (the high-energy sideband does not contribute to resonance Raman enhancement), whereas the 1607 cm$^{-1}$ mode (corresponding to the 1602 cm$^{-1}$ mode of Ru(bpy)$_3^{2+}$) has a broad, weak resonance enhancement. As a rough estimate, we note that the data for the 1487 cm$^{-1}$ appears to be weighted toward the spectral location of the Ru(bpy)$_3^{2+}$ electronic absorption, whereas the 1602 cm$^{-1}$ mode resembles nonresonant behavior.

Figure 6. LSPR and SERRES profiles of the 1487 cm$^{-1}$ peak (A, C, and E) and 1602 cm$^{-1}$ peak (B, D, and F) of Ru(bpy)$_3^{2+}$ with cyclohexane as the intensity standard. (A and B) LSPR $\lambda_{\text{max}} = 434.7$ nm, profile fit maximum at (A) $\lambda_{\text{ex,max}} = 445.1$ nm and (B) $\lambda_{\text{ex,max}} = 428.1$ nm. (C and D) LSPR $\lambda_{\text{max}} = 465.2$ nm, profile fit maximum at (C) $\lambda_{\text{ex,max}} = 466.4$ nm and (D) $\lambda_{\text{ex,max}} = 454.3$ nm. (E and F) LSPR $\lambda_{\text{max}} = 480.1$ nm, profile fit maximum at (E) $\lambda_{\text{ex,max}} = 464.1$ nm and (F) $\lambda_{\text{ex,max}} = 460.6$ nm.

Figure 7 shows two Ru(bpy)$_3^{2+}$ SERR spectra at different excitation frequencies. In the spectrum excited at lower energy close to the molecular resonance (top purple spectrum), the 1487 cm$^{-1}$ to 1602 cm$^{-1}$ mode intensity ratio is 2.5. This ratio changes to 1.2 as the excitation frequency is moved to higher energy and away from the molecular resonance (bottom blue spectrum). The same or an even more prominent phenomenon, where the 1602 cm$^{-1}$ mode becomes more intense than the 1487 cm$^{-1}$ mode, was reported previously, and also for an analogue.
Fe(bpy)$_3^{2+}$, because the 1487 cm$^{-1}$ mode is more resonantly enhanced, we propose a theoretical model for this mode to understand the relationship between the LSPR, molecular resonance, and its SERR excitation profile that is based on multiplicity of EM and resonance enhancement mechanisms.

**Theoretical Modeling of the SERRES Profile.** Previous work by Kerker and co-worker$^{90,91}$ showed that the SERRS enhancement is proportional to the surface electromagnetic field at incident frequency and the Stokes-shifted frequency and the resonant Raman scattering tensor. The enhancement of the electric field is the ratio of the squared field ($|E|^2$) to the square of the applied field ($|E_0|^2$). Therefore, the EM part of the enhancement is expressed as

$$\frac{|E(\omega)|^2 |E(\omega - \omega_{vib})|^2}{|E_0|^2}$$

where $|E(\omega)|^2$, $|E(\omega - \omega_{vib})|^2$ are the square of the electromagnetic fields at the incident and Stokes-shifted frequencies, respectively.

From the quasi-static theory model of a spheroid,$^{92}$ the average of the square of the electric field over the spheroid surface is expressed by the following equation (see the Supporting Information) derived by Zeman et al.$^{84}$

$$\langle |E|^2 \rangle = \langle |E_0|^2 \rangle \left[ 1 - \frac{2\text{Re}(1 - \gamma_0)\gamma_0^*}{Q_1(\xi_0)} + \frac{\gamma_0^2}{Q_1^2(\xi_0)(\xi_0^2 - 1)} \left( -\frac{\gamma_0^2 - 1 + \xi_0^2 \sin^{-1} \frac{1}{\xi_0}}{\sqrt{\xi_0^2 - 1 + \xi_0^2 \sin^{-1} \frac{1}{\xi_0}}} \right) \right]$$

where

$$\gamma = \frac{\varepsilon_1 - \varepsilon_0}{\varepsilon_1 + \varepsilon_0}$$

In the present application, we use $\chi$ as a parameter to tune the plasmon resonance wavelength of the spheroid to be at the plasmon resonances in the experiments (434, 465, and 480 nm) and calculate the EM enhancement for the three cases presented in Figure 7.

In addition to the EM enhancement factor, we also include for resonance Raman enhancement. Many studies have shown that the resonant Raman enhancement profile at different excitation wavelengths has the same line shape as the absorption spectrum of the molecule.$^{43,89}$ As discussed in the previous section, the report on Fe(bpy)$_3^{2+}$ shows that the 1491 cm$^{-1}$ mode undergoes a resonance Raman enhancement with the line shape similar to the lower energy band of the electronic absorption spectrum and a maximum resonance enhancement of 40.$^{40,89}$ Therefore, we assume that the resonance Raman enhancement profile of Ru(bpy)$_3^{2+}$ has the same shape as the 452 nm band of its absorption spectrum with a maximum resonance enhancement of 40. We then multiply the EM and resonant enhancement factors to get the WS SERRES profile for each experiment.

Figure 8 shows the three predicted SERRES profiles of the 1487 cm$^{-1}$ mode (where $\omega_{vib}$ is set to be 1487 cm$^{-1}$) for the three samples at different LSPR wavelengths. The experimental data and the Gaussian fitting are shown in the same plots for comparison. From Figure 8, the predictions agree well with the experimental WS SERRES profiles except for the width of the profiles, which are narrower in the theory. The difference in width probably arises because of inhomogeneous effects and because of differences between the shape of the nanoparticles in the theory and experiment. The results indicate that the WS SERRES profiles involve multiplicative electromagnetic and resonance Raman enhancements.

When there is a resonant molecular layer on the spheroid, the rigorous expression for the average of the square of the electric field is different from eq 4 due to coupling between the molecular and plasmonic resonances. This leads to the correction factor $G$ in eq 2, and the expression for $\gamma$ is also different. The effect of a resonant molecular layer on the electric field is calculated and discussed in the Supporting Information. It is found that the absorption by a layer of the resonant molecule Ru(bpy)$_3^{2+}$ is overestimated by this approach, leading to changes in the extinction spectrum that are not observed in the experiments (Figure S2 in the Supporting Information). Similar errors arise in the SERRES profile line shape (Supporting Information Figure S3). This indicates the absorbing layer is more weakly coupled to the plasmon resonance than is described by the layered spheroid model. This could be a problem with the use of continuum theory to describe the adsorbed layer, and it could also reflect uncertainties in our use of adsorbate dielectric constants that are obtained from a Kramers–Kronig analysis of solution spectra. In any event, the present analysis clearly shows that the best model of the observed SERRES profiles assumes that the molecular and plasmonic contributions to the enhancement are multiplicative.

**Conclusions**

In this work, we studied the contribution of electromagnetic and resonance enhancement to surface-enhanced resonant Ra-
man intensity. A monolayer of Ru(bpy)$_2^{2+}$ molecules was adsorbed on nanoparticles with different localized surface plasmon resonances, and the coupling between molecular resonance and LSPR was probed by LSPR shift spectroscopy. One of the electronic transitions of Ru(bpy)$_2^{2+}$ is strongly coupled to the LSPR and leads to wavelength-dependent LSPR shifts. SERR spectra of Ru(bpy)$_2^{2+}$ were collected on three samples with different LSPR wavelengths for 400–500 nm Raman excitation wavelengths. Unlike nonresonant adsorbates, Ru(bpy)$_2^{2+}$ WS SERRS profile peak positions depend on the relative spectral position of the LSPR and molecular resonance. Quasi-static electromagnetic simulations of the WS SERRS profile based on multiplication of the EM and resonant enhancement factors showed good agreement with the experimental results.

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Supporting Information Available: Detailed description of Kramers–Kronig transformation, the relationship between LSPR shift and refractive index, the spheroid model, and electric field and SERS EF calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes
