Single-Molecule Chemistry with Surface- and Tip-Enhanced Raman Spectroscopy

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ABSTRACT: Single-molecule (SM) surface-enhanced Raman spectroscopy (SERS) and tip-enhanced Raman spectroscopy (TERS) have emerged as analytical techniques for characterizing molecular systems in nanoscale environments. SERS and TERS use plasmonically enhanced Raman scattering to characterize the chemical information on single molecules. Additionally, TERS can image single molecules with subnanometer spatial resolution. In this review, we cover the development and history of SERS and TERS, including the concept of SERS hot spots and the plasmonic nanostructures necessary for SM detection, the past and current methodologies for verifying SMSERS, and investigations into understanding the signal heterogeneities observed with SMSERS. Moving on to TERS, we cover tip fabrication and the physical origins of the subnanometer spatial resolution. Then, we highlight recent advances of SMSERS and TERS in fields such as electrochemistry, catalysis, and SM electronics, which all benefit from the vibrational characterization of single molecules. SMSERS and TERS provide new insights on molecular behavior that would otherwise be obscured in an ensemble-averaged measurement.

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1. INTRODUCTION

Molecular systems and nanoscale environments are neither static nor simple. Ensemble averaging obscures the vast complexity and heterogeneity of all accessible molecular microstates. Important information with regard to site-specific behavior, distributions in molecular dynamics and interactions, and chemistry cannot be obtained on the ensemble level. Over the past several decades, several analytical techniques have been used to overcome the effects of ensemble averaging, including single-molecule fluorescence spectroscopies, scanning probe microscopies (SPM), and force spectroscopies, such as magnetic and optical tweezers. While many of these single-molecule (SM) techniques can provide information about the molecular electronic states, surface topography, or even the behavior of a single molecule under stretching or torsional mechanical force, most are limited by their lack of chemical specificity. Unlike the aforementioned techniques, Raman spectroscopy can access the chemical content of a molecular system through the observation of molecular vibrations. As such, Raman scattering provides great chemical specificity and yields what is often referred to as a chemical or vibrational “fingerprint”. In 1997, Nie and Emory and Kneipp et al. both claimed SM detection of resonant dyes by surface-enhanced Raman spectroscopy (SERS). These first observations of single-molecule SERS (SMSERS) opened new possibilities toward obtaining the vibrational spectrum of a single molecule. SMSERS is ideal for improving the fundamental understanding of SERS mechanisms and probing single-molecule chemistries.

Despite the chemical sensitivity of SERS, it cannot, by itself, provide subnanometer spatial resolution. Overcoming the diffraction limit of light and achieving simultaneous subnanometer spatial resolution with SERS is a major challenge for investigating complex molecular systems and their environments at the SM level. SPM is commonly used to study surface-bound single molecules with atomic resolution, but SPM alone cannot capture detailed chemical information such as adsorbate–adsorbate interactions and adsorbate–surface interactions except for few-atom molecules using inelastic electron tunneling spectroscopy at few to milli-kelvin temperatures. In contrast, tip-enhanced Raman spectroscopy (TERS) combines the imaging ability of SPM, achieving subnanometer spatial resolution, along with the single-molecule chemical sensitivity provided by SERS. Both SMSERS and TERS are ideal approaches for obtaining deeper insight into the mechanisms of SERS, the behaviors of single molecules on surfaces and in plasmonic cavities, and chemistries obscured by ensemble averaging.

1.1. Background

Raman spectroscopy is a method for studying the inelastic scattering of light. An excitation photon induces a dipole in the molecule, yielding inelastic light scattering. Stokes scattering occurs when the scattered photon is lower in energy than the incident photon (positive Raman shift relative to excitation), leaving the molecule in the first vibrational state ($v = 1$ to $v = 0$). Anti-Stokes scattering occurs when the scattered photon is higher in energy than the incident photon (negative Raman shift relative to excitation), leaving the molecule in the ground state ($v = 0$ to $v = 1$). Under equilibrium conditions, the intensity of the anti-Stokes scattering is related to the populations of excited vibrational states, which follow a Boltzmann distribution.

Raman spectroscopy provides detailed chemical and structural information on molecular systems. The efficiency of the inelastic scattering process, however, is very low. To enhance the inelastic scattering probability, resonance Raman (RR) scattering occurs when the incident laser is near an electronic transition of the molecule of interest, increasing the signal by an additional factor of $10^5$–$10^6$. To achieve single-molecule detection with Raman spectroscopy requires the large signal enhancements of SERS. The two main mechanisms of signal enhancement are the electromagnetic (EM) mechanism and the chemical enhancement (CE) mechanism. Enhancement factors (EF) are used to quantify the signal enhancement and are generally defined as the ratio of the normalized SERS signal over the normal Raman signal of the same molecule, as follows

$$EF_{\text{SERS}} = \frac{I_{\text{SERS}}}{I_{\text{NRS}}} \cdot \frac{N_{\text{SERS}}}{N_{\text{NRS}}}$$

Figure 1. (A) Energy diagram showing the spectroscopic transitions involved in Rayleigh, Raman, and resonance Raman scattering. Two pathways can be found in Raman: either the scattered photon has a lower energy than the adsorbed photon (Stokes scattering) or, inversely, the scattered photon has a higher energy than the scattered photon (anti-Stokes scattering). (B) Schematic drawing (not to scale) of a plasmonic nanosphere when the electric field component of incident light induces an oscillation of the electron cloud at a frequency defined by the nanosphere size and shape, known as the localized surface plasmon resonance.
where $I_{\text{SERS}}$ and $I_{\text{NRS}}$ are the intensities of the SERS and normal Raman spectroscopy (NRS) signals, respectively, and $N_{\text{SERS}}$ and $N_{\text{NRS}}$ are the number of molecules contributing to the SERS and NRS signals, respectively.

The EM mechanism of SERS relies on a phenomenon known as the localized surface plasmon resonance (LSPR). The LSPR is the collective oscillation of surface conduction electrons of noble-metal plasmonic nanoparticles that are smaller in size compared to the wavelength of incoming light. A physically intuitive diagram of the LSPR is shown in Figure 1B. The LSPR creates regions of intense EM field about a nanoparticle surface or tip-sample junction, known as hot spots, which can produce EFs ranging from $10^5$ to $10^{10}$.\(^{26}\) The EM mechanism combined with RR is the most common strategy used to achieve single-molecule sensitivity with SERS/TERS or, more accurately, surface/tip-enhanced resonance Raman spectroscopy (SERRS/TERRS). For simplicity, however, we will use the terms SERS/TERS throughout the remainder of this review.

The CE mechanism is typically the smallest contributor to the signal enhancement, with EFs on the order of $10^1$ to $10^2$.\(^{32-37}\) The CE is postulated to arise from two main processes: (1) enhancements from charge transfer resonances between the molecule and the SERS substrate\(^{33}\) and (2) nonresonant changes in the molecular polarizability\(^{35,36}\) upon surface binding. Combining the chemical contribution with EM enhancement is a strategy for boosting the signal of nonresonant molecules for SMSERS.

Additional background relevant to SMSERS and TERS will be provided as necessary throughout this review.

### 1.2. Scope of Review

In this review, we describe progress in SMSERS and TERS toward the ultimate goal of understanding the fundamental behavior of single molecules on surfaces with high chemical sensitivity and spatial resolution. The first half of this review will focus on SMSERS, with an emphasis on the development and understanding of the phenomena underlying SMSERS, which includes hot spots and plasmonic substrate design, signal fluctuations and heterogeneity, and verifying SM detection. Then, we will highlight the application of SMSERS toward studying chemistry at the single-molecule limit. The second half of this review will focus on the current imaging capability of TERS and its applications to various SM studies, which include SM electronics and isomerization processes. To orient the reader and provide an accurate account of the field, selected TERS experiments that were performed on low-dimensional materials and biological samples are described, as they show comparable lateral resolution to SM systems. For a thorough coverage of the field of SMSERS and TERS, we direct the reader to other relevant reviews on SERS and TERS EFs,\(^{38,39}\) characterizing hot spots,\(^{40-42}\) and TERS setups and applications,\(^{43}\) including catalysis.\(^{43}\)

## 2. SINGLE-MOLECULE SERS

### 2.1. Hot Spots and Plasmonic Nanostructures

After the first demonstrations of SMSERS, early studies set out to understand the specific nanoparticle structures giving rise to SM detection, including the connection with SERS hot spots. These investigations focused on the most commonly used SMSERS.
high as $10^8$, and the sharp tips of Au nanostars$^{46}$ as high as $10^9$.

A subsequent study correlated SMSERS-active nanoparticles with AFM and found that all of the SERS-active nanoparticles were aggregates,$^{46}$ supporting the importance of aggregation to obtain SMSERS detection. AFM correlation, however, provided only limited structural information for elucidating the nature of the SMSERS hot spots.

Camden et al. completed the first comprehensive characterization of hot spots using a correlated experimental and theoretical approach. After locating SMSERS-active nanoparticle aggregates, they conducted high-resolution transmission electron microscopy (HRTEM) to image the aggregate structures, followed by theoretical calculations to model the EM field enhancements of the aforementioned structures.$^{26}$ Figure 2 shows correlated (A) HRTEM images, (B) SMSERS spectrum, and (C and D) discrete dipole approximation (DDA) calculations of the EM field enhancement calculated as $\frac{E_{\text{loc}}^2}{|E_0|^2}$. The DDA calculations indicate that the dimer structure in panel A can provide enhancements of $10^7$ at 532 nm excitation in the crevice sites of the nanoparticle dimer junction (i.e., hot spots). These results have been corroborated in multiple studies.$^{26,27,31}$

It is important to remember that all of these studies were performed on electronic resonance with the molecule of interest. Rhodamine 6G (R6G), for example, has a RR cross section$^{25}$ integrated over all observable modes ($514 \text{ nm}$ excitation) of $2.3 \times 10^{-22} \text{ cm}^2\text{ molecule}^{-1}$ compared with a nonresonant molecule at $\sim 10^{-28} - 10^{-30} \text{ cm}^2\text{ molecule}^{-1}$. Assuming the total enhancement is a multiplication of the RR contributions (up to $10^9$ for R6G) and the EM enhancement ($10^7$), these values are in good agreement with the total EFs of $10^{14}$, claimed in earlier studies.$^{12,13}$

Hot spots in plasmonic structures are not exclusive to nanoparticle junctions. The EM enhancement of individual nanoparticles can be increased with the introduction of sharp features. For example, EFs at the corners of nanobars$^{31}$ have been calculated to reach as high as $10^5$, the tips of nanopyramids$^{45}$ as high as $10^6$, and the sharp tips of Au nanostars$^{34}$ as high as $\sim 10^8 - 10^9$. We can therefore define hot spots as local areas of intense ($10^5 - 10^9$) EM field occurring at sharp edges, nanoparticle gaps and crevices, or other geometries with a sharp nanoroughness (typically <10 nm).

A demonstration of SM sensitivity from individual nanoparticles was performed with SERS substrates fabricated by nanosphere lithography (NSL).$^{46-50}$ The NSL substrates consisted of an array of nanopyramids with an average nanoparticle distance of $83 \pm 20$ nm and sharp tips (Figure 6B).$^{51}$ The large distances observed between the nanoparticles indicate that the SM sensitivity is the result of individual nanopyramids and not nanoparticle junctions or nanogaps (i.e., gaps <10 nm). The highest enhancements of the nanopyramids have been shown with DDA calculations to occur at the tip and can be as high as $10^8$. While the understanding of the plasmonic structures that are SMSERS-active has improved, challenges in the rational design and reproducibility of SERS substrates still remain and will be addressed in section 2.1.3.

2.1.1. Enhancement Factor Distributions. Enhancement factors are not uniform across a SERS substrate or even within an individual hot spot. This implies that molecular positioning is an important consideration for fundamental and analytical SERS studies and quantifying EFs. To investigate this further, Fang and co-workers$^3$ used photochemical hole burning on a Ag film over nanosphere (AgFON) SERS substrate to compare the percentage of molecules contributing to the SERS signal with respect to the contributions from the EM enhancement. Essentially, laser pulses were used to photochemically damage molecules adsorbed to the AgFON. Molecules in the highest enhancing regions (i.e., experiencing the highest EFs) are photodamaged first, resulting in a loss of signal. As the electric fields created by the laser pulses are increased, molecules in increasingly lower enhancing regions will be subsequently photodamaged, leading to further decreases in the SERS signal. By monitoring the signal loss with respect to the fields created by the laser pulses, they found that ~25% of the SERS signal is generated by less than 0.01% of the molecules on the substrate surface, which were located in the highest enhancing regions of up to $10^9$. This study highlights the importance of molecular location in SMSERS experiments, but it was focused on EF distributions across the entire SERS substrate with ensemble coverage. For SMSERS, a molecule is only detectable when located in a hot spot; thus, it is important to consider the EF distribution within an individual hot spot.

Interrogation of individual hot spots was performed by Le Ru and co-workers for the hot spot created by a nanoparticle gap, theoretically$^{58}$ and experimentally.$^{55}$ In both studies, they found the probability distribution of the nanoparticle gaps followed a long-tail distribution with an average EM enhancement of $\sim 10^7$ and a maximum as high as $\sim 10^{10}-10^{11}$. EFs as low as $10^{-8}$ were found to be sufficient to observe the SER scattering of a single resonant molecule, but nonresonant molecules require higher EFs ($\sim 10^{8}-10^{11}$), as visualized in Figure 3. These EF values are assuming typical experimental conditions with no additional contributions from chemical enhancements.

An important take-away of these studies is that the calculated EF is critically affected by molecular location with respect to the hot spots of a SERS-active substrate. A difference of only 2 nm in the location of the molecule can result in an order of magnitude
change in the EF, which can ultimately dictate whether a molecule is detected or not. It is also difficult to distinguish between multiple molecules experiencing a weaker EF and a single molecule experiencing a higher EF. The large intensity distributions observed for SM events spanning as high as 3 orders of magnitude for R6G and just over 2 orders of magnitude for crystal violet (CV) at 532 nm excitation are a consequence of the EF distribution across a SERS substrate. Thus, molecular binding affinity is an important consideration when using two analytes to verify SM detection, as we will discuss in section 2.2. All of these findings imply that molecular binding affinity and positioning of molecules within a hot spot are important considerations for fundamental and analytical SERS studies and quantifying EFs.

A second consideration is the influence of enhancement gradients in the hot spot on the observed molecular behaviors. For example, molecular diffusion across EM fields on the nanoparticle surface has been proposed as a mechanism for the origin of blinking (i.e., the on–off cycling of SM SERS signal). A wavelength-dependent near-field enhancement has also been demonstrated to strongly contribute to the anti-Stokes-to-Stokes intensity ratios of SMs. Accordingly, understanding the role of molecular coupling with variable optical near fields is essential for accurately describing molecular behaviors and should be pursued in future theoretical models.

2.1.2. Super-Localization of SMSERS Hot Spots. Spatially resolving the location of a single molecule on a nanoparticle would aid in understanding the coupling between a single molecule and the optical fields of a nanoparticle and how the location and density of nanoparticle hot spots affect the overall optical response of the molecule. Obtaining nanometer scale spatial resolution, however, from optical spectroscopies like SERS is restricted by the diffraction limit, or approximately half of the emission wavelength (~250 nm with 532 nm excitation). One means of achieving subdiffraction-limited resolution is to fit the diffraction-limited emission from a single emitter, such as a noble-metal nanoparticle aggregate labeled with a SERS-active probe, using super-resolution imaging techniques. Super-resolution fluorescence techniques, including photoactivated localization microscopy (PALM), stochastic optical reconstruction microscopy (STORM), and stimulated emission depletion (STED) microscopy, have demonstrated sub-20-nm resolution of fluorescent molecules in biologically relevant systems.

Willets et al. first combined principles of super-resolution fluorescence microscopy with SERS, coining the term super-localization SERS, where they imaged the spatial profile of R6G SMSERS signal on single Ag colloidal nanoparticle aggregates with sub-15-nm spatial precision. The position of the R6G SERS emission was determined by fitting the signal to a two-dimensional Gaussian point spread function (PSF), with the peak of the fit defined as the centroid position. The emission of the R6G molecule can be readily separated from any background emission, potentially originating from the citrate molecules capping the Ag colloidal nanoparticles. The authors found that the spread of centroid positions of the SMSERS-active regions on a single nanoparticle aggregate (20–100 nm) was much greater than that of the expected nanoparticle aggregate hot spot junction size (1–10 nm). They concluded that the larger SMSERS active area was caused by the R6G molecule being located between multiple hot spots, greatly extending the SMSERS-active area on the nanoparticle aggregate.

In order to precisely elucidate and understand the spatial origin of the SERS centroids suggests that the molecule is confined to the surface of a single nanoparticle within an aggregate system. Using the isotopologue approach for SMSERS can further illuminate the behavior of distinct molecules on a nanoparticle surface: Weber, Willets, and their co-workers used this approach to prove that observed shifts in the centroid position from previous work were due to movement of R6G molecules on the surface and not molecular reorientation or random nanoparticle emission. Using super-localization SERS to watch the centroid positions of distinct molecules on a single nanoparticle aggregate with sub-5-nm spatial precision lends itself to exploring the nanoscale reactivity of nanoparticles, which has been applied to studying the site specificity of electro-chemical reactions and will be discussed in section 2.5.1.

Super-localization SERS is an extremely promising means of imaging single molecules on nanoparticle surfaces with sub-5-nm spatial precision. However, there are challenges with regard to accurately modeling the position of a SERS-active emitter that must be addressed in order to interpret the results. As described above, the position of an emitter is most commonly modeled with a two-dimensional (2D) Gaussian PSF, where the emitter position is approximated to be the peak position of the fit. Despite its utility in determining centroid positions, the 2D Gaussian PSF simply models the symmetric shape of a diffraction-limited spot and is not an accurate physical representation of the emitter, especially in the case of a SERS-active molecule adsorbed on a nanoparticle surface. Additionally, experimental conditions such as the microscope objective used can induce aberrations in the emission and therefore skew the calculated centroid positions. Accurately modeling molecule—
plasmon coupling is not trivial; one must take into account that higher-order aggregates (e.g., dimers, trimers, tetramers) or elongated nanoparticles such as nanorods have multiple plasmon modes. Alternative PSFs have been explored and compared to the standard 2D Gaussian PSF in an attempt to model the complexity of plasmon–molecule coupling and the resulting SERS emission spatial profile. For example, PSFs derived from a 2D centroid/single dipole and an intensity-weighted sum of three orthogonal dipoles were both used to model the centroid positions of SERS emission on Ag colloidal nanoparticles in the many- and single-molecule regimes.74 As shown in Figure 5, the fits of the 2D Gaussian and three-dipole-derived PSF have stark differences in the single-molecule regime, whereas the high molecule coverage is very similar. Overall, the three-dipole PSF was the most successful in providing a precise fit, albeit more computationally demanding than the other fits. This study highlights that the choice of PSF model will alter the spatial profile of the emission centroid and that performing correlated structural measurements is crucial to gain a complete understanding of how molecule–plasmon interactions effect the observed emission centroid profile. Improving the ability to accurately model SERS-active molecule interactions with plasmonic nanoparticle systems could help extend super-localization SERS to studying chemical reactions at the few- to single-molecule level with sub-5-nm spatial precision.

2.1.3. Rational Design of SMSERS Substrates. The design of new substrates for SMSERS has focused primarily on improving the reproducibility of nanoparticle size, shape, and degree of aggregation, increasing hot spot density, and controlling the positioning of molecules into a hot spot. Lithographically prepared substrates such as nanoantennas (Figure 6A) and nanopyramid arrays (Figure 6B)31 are one avenue to improve substrate reproducibility. Nanoantennas have been reported to have EFs of up to $\sim 10^{13}$ due to the combination of an optimized local EM field enhancement ($10^{11}$) and antenna directionality ($10^2$).75,76 with excellent control over hot spot positioning. However, nanoantenna fabrication is complicated and costly. The multistep, top-down fabrication requires electron beam lithography and alignment with nanoscale precision. On the other hand, NSL used to fabricate arrays of nanopyramids is a facile, cost-effective, and easily tunable approach to prepare nanoparticles. However, due to the simplicity of NSL, nanoparticle arrays are subject to packing defects due to small variations in nanosphere size.27−49,52,53 In NSL, nanospheres are dried into a close-packed monolayer on glass coverslips, onto which a layer of metal is deposited. Removal of the nanospheres leaves behind an array of nanopyramids created in the interstitial sites of the close-packed monolayer (Figure 6B). Outside of NSL and nanoantenna fabrication, other efforts have focused on improving the reproducibility of chemically synthesized Ag nanoparticles. One common strategy is to use DNA self-assembly and other linking molecules77 to form controlled nanoparticle dimers or larger aggregates.78,79

Another strategy to improve reproducibility is gap-mode SERS (GM-SERS), a simple nanoparticle-based analog to TERS (section 3). In GM-SERS, the analyte of interest is adsorbed onto a smooth metallic thin film, upon which nanoparticles are deposited. The molecules of interest are consequently located in the film–nanoparticle gap, as shown in Figure 6D.81 The benefit of GM-SERS is that it does not require the random aggregation of nanoparticles, which improves substrate reproducibility, assuming the nanoparticles are monodisperse in size. An additional advantage of GM-SERS is the possibility to observe site-specific chemistry on thin film surfaces that are not inherently plasmonic.84−86 GM-SERS has recently been used to study single-molecule host–guest interactions, which we discuss in detail in section 2.5.3.

Increasing the hot spot density of SERS substrates is also an important parameter in substrate design. For aggregated Ag colloids, typically only 1% of the nanoparticles are SMSERS-active,26 making the collection of statistics in SMSERS studies challenging. Attempts to improve hot spot density include fabricating close-packed monolayers of Ag28,80 (Figure 6F) and Au87 nanoparticles, templated Ag nanocube arrays,88 and synthesizing porous Au−Ag nanospheres80 (Figure 6C). Porous Au−Ag nanoparticles, for example, create a high density of hot spots across the nanoparticle surface through the introduction of nanoscale features. The single particles were reported to have EFs on the order of $\sim 10^4$ compared with “smooth” single, spherical nanoparticles at $\sim 10^3$.28,80,89−91

Another major challenge is the controllable positioning of molecules within the plasmonic hot spot. One strategy addressing this challenge was the fabrication of cetyltrimethylammonium bromide (CTAB)-coated bipyramids (Figure 6E). The CTAB bilayer prevents the adsorption of positively charged dyes (crystal violet in this study) to the nanoparticle surface except on the tip, where CTAB was not present. In principle, this design would only allow the binding of molecules to the regions of highest enhancement.82 They observed that every crystal violet molecule experienced an EF close to the maximum value expected for the bipyramid tips, suggesting the success of this strategy. This bilayer approach, however, is not necessarily adaptable to gap-containing nanoparticles and is highly dependent on the analyte’s adsorption affinity. More recently, a plasmonic substrate was designed to reversibly trap single molecules in hot spots.92 The substrate was fabricated through the electrostatic self-assembly of Au nanoparticles onto a Au- and silica-coated silicon platform. The hot spots were isolated by using a thermoresponsive polymer, which acts as a gate for

Figure 5. AFM images of Ag colloidal nanoparticle aggregates and SERS spatial intensity maps that indicate the average intensity of all fitted centroids. (A and D) AFM images with the three-dipole fit estimate (green dashed line) with the same scale bar. (B and E) SERS spatial intensity maps using the 2D Gaussian PSF model. (C and F) Spatial intensity maps using the three-dipole model. A white x on panels C and F indicates the average position of the 2D Gaussian PSF. Panels A−C shows an example of single-molecule coverage, and panels D−F shows many-molecule coverage. The scale bars in panels B, C, E, and F represent 10 nm. Adapted with permission from ref 74. Copyright 2013 American Chemical Society.
trapping the molecules through the heating and cooling of the substrate.

Over the past several years, great strides have been made toward improving SERS substrates. The majority of SMSERS experiments, however, still rely on the random aggregation of Ag nanoparticles. As the field moves forward, utilizing more reproducible nanoparticle substrates will allow for the systematic improvement of our fundamental understanding of SERS mechanisms and provide more reliable means of interpreting chemistry at the single-molecule level. In addition, the ability to increase hot spot density while simultaneously controlling the positioning of molecules within those hot spots will greatly improve the collection of statistics for SMSERS.

2.2. Verification of SMSERS

2.2.1. Early Strategies. The first SMSERS studies adapted proof methodologies from SM fluorescence. SM detection by fluorescence spectroscopy is unequivocally proven through the observation of photon antibunching, which is based on the phenomenon that single molecules cannot emit two fluorescence photons simultaneously.59 However, this strategy is not possible for SMSERS because the lifetime of Raman scattering is short (10−14 s) relative to that of fluorescence (10−9 s).94 Another strategy to confirm SM detection, also common in SM fluorescence, is to sufficiently dilute the target molecule concentration to achieve, on average, one or fewer molecules within the probe volume. While this is an effective strategy for fluorescence spectroscopy, the addition of metallic nanoparticles complicates the process. Unequivocally proving SMSERS detection using only ultralow concentrations would require knowledge of the contributions from molecular binding affinity and diffusion, molecule and nanoparticle concentrations, the position of the molecule relative to plasmonic hot spots, and the number of nanoparticle hot spots within the probe volume. As a consequence, ultralow concentrations as a justification for SMSERS are inadequate. This is further highlighted by Darby and Le Ru, who found that the dilution procedure used to prepare samples could artificially create the appearance of SMSERS due to the competition between molecule diffusion and adsorption to the nanoparticles.75 Instead, most strategies have employed ultralow concentrations with additional protocols to prove SMSERS ranging from the observation of fluctuations to a statistical analysis.

As an alternative to using ultralow concentrations to prove SMSERS, dramatic fluctuations in the SERS signal were viewed as validation of SM detection in many early studies. Fluctuations include spectral wandering,56,96 large relative intensity fluctuations of particular vibration modes,97 and blinking.52,59,99–102 Nie and Emory, for example, noted that the Raman signals would suddenly disappear or change during a period of continuous illumination.12 This blinking behavior, or the on–off fluctuation of the SERS signal, was initially used as a verification of SM detection.59,103 Andersen et al., however, demonstrated that Ag colloids exhibit blinking that is independent of the molecule being probed and that this phenomenon was observed in both the presence and absence of a probe molecule.104 A second study observed large spectral fluctuations for an amorphous carbon layer deposited on SERS substrates.105 Furthermore, blinking has been observed at surface coverage above the SM level.30,98 While fluctuations are a characteristic behavior of single molecules, they do not exclude the possibility of multiple molecules or other phenomena104,105 causing the fluctuations. Therefore, the use of a fluctuation argument is inadequate evidence for SM detection. Another indirect strategy to verify SM detection is the observation of a polarization-dependent SER signal.12 This, however, requires the ability to deconvolve the polarization dependence of the plasmonic structures from that of the SER scattering of the analyte.
Others have provided evidence for SM detection using a combination of ultralow analyte concentration and a statistical analysis of the SERS intensities. At ultralow concentrations the number of molecules that bind to the nanoparticle surface should follow a Poisson distribution. The Poisson distribution assumed that the difference in the SERS intensity between events could be attributed to zero, one, two, or three molecules being probed. However, it was demonstrated that a valid SMSERS Poisson distribution requires the variation in intensity between SERS events to be less than a factor of 2, and a minimum of 10,000 sample events needs to be recorded, which is a highly impractical feat for SMSERS. Furthermore, the variation in intensity between SM events has been shown to span as many as 3 orders of magnitude, considerably greater than a factor of 2. As a consequence, the existence of a Poisson distribution of SERS intensities alone is not a valid proof of SM detection.

2.2.2. Current Methodologies.

In 2006, Le Ru et al. introduced a bianalyte approach for proving SM detection. This approach involves dosing the SERS-active substrate with two analytes at equimolar concentrations and determining whether the SERS events contain a single analyte or a combination of both analytes. While the bianalyte approach is a useful means of proving SMSERS detection, there can be experimental problems, such as different Raman scattering cross sections or molecular affinity for the SERS-active substrate. An alternative of the bianalyte approach is the isotopologue approach. The isotopologue approach uses a molecule and its isotopically edited analog as a bianalyte pair (i.e., isotopologues). This approach is advantageous because isotopologues inherently have identical surface binding affinities, extinction coefficients for molecular electronic resonances, and Raman scattering cross sections, while bianalyte partners will not have identical values of these properties.

The ratio of counts of individual analyte events to events with both analytes is used to verify SM detection. This ratio can be described using a Poisson binomial distribution. At sufficiently low coverage, the probability that a molecule is bound to the nanoparticle aggregate follows a Poisson distribution. Once bound, the probability that the molecule detected is either analyte 1 or analyte 2 follows a binomial distribution. Therefore, the overall probability that analyte 1 and/or analyte 2 will be detected in a given spectrum is the product of both distributions

\[
P(n_1, n_2 | \alpha) = \frac{e^{-\alpha \beta} \alpha^{n_1} (1 - \beta)^{n_2}}{n_1! n_2!}
\]

(2)

where \(\alpha\) is the average number of molecules per spectrum, and \(n_1\) and \(n_2\) are the number of analyte 1 and analyte 2 molecules detected in the SERS spectrum, respectively. \(P\) is the probability that \(n_1\) analyte 1 and \(n_2\) analyte 2 molecules at \(\alpha\)-coverage will be detected for a given spectrum. The probability that a given molecule detected in the spectrum corresponds to analyte 1 or analyte 2 is designated with an empirical variable \(\beta\). The value of \(\beta\) is a function of the binding affinities, concentrations, and Raman cross sections of analytes 1 and 2.

In order to test the influence of \(\beta\) on the overall proof of SM detection with bianalyte pairs, a multianalyte experiment was conducted using R6G and CV isotopologues (R6G-d_0/R6G-d_4 and CV-d_0/CV-d_12). This allows for the direct comparison of statistics for the bianalyte pair with the corresponding
isotopologue statistics as an internal standard. Figure 7 shows two representative bianalyte data sets A and B and their analogous multianalyte data sets C and D, respectively. All of the data sets were collected with equimolar mixtures of R6G and CV, where data sets B and D were at $1 \times 10^{-8}$ M and data sets A and C were at $1 \times 10^{-7}$ M overall concentration. In both A and B, the majority of spectra showed individual analyte character, but R6G was observed an order of magnitude more frequently than CV, demonstrating the different detection probabilities of the analytes. As shown in analogous multianalyte data sets C and D, despite both A and B having primarily individual analyte character, only data set B was at the SM level. Spectra with both isotopologues were observed more frequently than those of the individual isotopologues in data set C (analogous to data set A), but not in data set D (analogous to data set B). These results demonstrate that bianalyte partners require careful statistical analysis in order to rigorously prove SM detection.

Recently, a SMSERS study using porphycene isotopologues ($\text{Pc-d}_0$ and $\text{Pc-d}_{12}$) to verify SM detection observed the preferential observation of $\text{Pc-d}_0$ over $\text{Pc-d}_{12}$. They observed a similar discrepancy in counts between the isotopologues as discussed above for bianalyte partners, showing the importance of careful analysis for every SM proof. Zrimsek et al. further discussed experimental considerations for SM proofs, including correcting for differences in analyte behavior through altering the analyte concentration ratios and defining more rigorous thresholds for verifying SM detection. Appropriate implementation of SM proofs will greatly advance the field of SMSERS.

**2.2.3. Reliable Sample Preparation.** Along with a rigorous statistical analysis, proper sample preparation is essential to ensure consistent coverage of molecules on the SERS substrates. A typical SMSERS experiment involves incubating resonant dye molecules at ultralow concentrations (typically nanomolar or picomolar) with Ag colloidal nanoparticles. Salt solutions such as NaCl or KCl are used to aggregate the colloids, creating hot spots for EFs on the order of $10^7$–$10^8$. Darby and Le Ru investigated the effect of sample preparation on SMSERS experiments for dye/colloidal mixtures. Figure 8 demonstrates two tested dilution procedures: (1) large dilution factors (LDF) and (2) half-half dilution (HHD). Due to the competition between molecular diffusion and adsorption, LDFs lead to uneven nanoparticle coverage and the appearance of SM detection when it is not actually achieved. Premixing the dyes and using HHDs is recommended to obtain uniform coverage. Figure 8A shows experimental results from samples prepared using HHD. Figure 8B shows samples prepared with premixing of the dyes and a 100× dilution factor. Figure 8C shows samples in which the dyes were added to the colloid mixture sequentially with a 100× dilution factor. The overall SERS signal intensities in A are weaker than in B and C, suggesting more uniform coverage, as indicated in the schematic representations. Most strikingly, the majority of events in Figure 8C show SERS spectra with strong
signals of only one isotopologue, when the majority of spectra should have had both analytes. This work illustrates that the proper dilution procedure is crucial to obtain the appropriate molecular coverage and accurate verification of SM detection.

Since a simple dilution procedure can significantly influence molecular coverage and EF distributions exist across a SERS substrate or hot spot (discussed in section 2.1.1), all studies should clearly define their experimental procedures to ensure accurate interpretation of results and reproducibility. Other practical considerations when preparing SMSERS samples are the choice of bionalyte pair for proving SM detection,\textsuperscript{32} and spin-coating.\textsuperscript{51} or Langmuir Blodgett film deposition\textsuperscript{109,110}, and laser power to limit photodegradation.\textsuperscript{57}

Therefore, in order to expand the scope of molecules that can be studied with SMSERS, it is necessary to gain a bianalyte partner to verify SM detection.\textsuperscript{56} For BPE, EFs of \(10^{2}\) were necessary to achieve SM detection. In this study, the authors also attempted SM detection of adenine, but it was only possible in the rare cases when EFs reached as high as \(10^{9}\). This study demonstrates that SMSERS detection of nonresonant molecules is possible; however, the rarity of observing a SM event due to the high EF requirements makes these experiments very challenging. Therefore, in order to expand the scope of molecules that can be studied with SMSERS, it is necessary to gain additional enhancement from avenues besides molecular resonance.

One strategy is to use highly enhancing SERS substrates with EFs of \(10^{7}\) or higher, such as the nanoantennas discussed in section 2.1.3. Wang and co-workers claimed that the nanoantennas\textsuperscript{55,76} had EFs as high as \(10^{15}\), combining an optimized local EM field enhancement (\(10^{15}\)) and antenna directionality (\(10^{3}\)), which would allow the detection of nonresonant single molecules. Another strategy is to take advantage of the chemical enhancement (CE) mechanism in addition to the EM mechanism. The CE mechanism in SERS is generally accepted to contribute enhancements of \(10^{−1}–10^{10}\) and is based on the chemical properties of the molecule around the hot spot, and/or photobleaching.

Kitahama et al. postulate that blinking statistics in SERS will be approximated using a one-dimensional random walk model.\textsuperscript{98} Theoretical calculations have also found that by tailoring the molecule with different functional groups, thereby altering the energy difference between the highest occupied molecular orbital (HOMO) of the metal and the lowest unoccupied molecular orbital (LUMO) of the analyte, the chemical enhancement can surpass \(10^{3}\).\textsuperscript{36} Utilization of CE in combination with EM enhancement, may help probe a wider spectrum of molecular systems with SMSERS.

2.4. Signal Fluctuations and Heterogeneity in SMSERS

One advantage of studies at the SM limit is that they provide a viable route for investigating principle mechanisms of the SERS enhancement, the fundamental properties of spectroscopic phenomena, and single molecular properties otherwise obscured by ensemble averaging. We will discuss several recent studies focused on improving our fundamental understanding of signal fluctuations, such as blinking and spectral wandering and the heterogeneity of SMSERS events.

2.4.1. Blinking. SMSERS signal fluctuations were originally used to validate SM detection. Despite the fact that this idea was later disproven,\textsuperscript{60,96,104,105} the origin of signal fluctuations in SMSERS spectra is still a highly debated topic in the SERS community. Blinking is described as the on–off cycling of the SMSERS signal and can last from milliseconds to minutes.\textsuperscript{59,98–102} The roles of illumination power,\textsuperscript{102} electrolyte concentration,\textsuperscript{102} temperature,\textsuperscript{61,117} EM mechanism,\textsuperscript{114,115} and excitation wavelength\textsuperscript{60,99} have all been explored in relation to blinking behavior, some of which we will discuss further in this section.

Itoh and co-workers conducted a quantitative analysis of blinking in SERS and surface-enhanced fluorescence (SEF) using Ag nanoparticle dimers functionalized with R6G.\textsuperscript{114} They observed that the SERS and SEF signals varied from dimer to dimer but could be divided into three main categories: (1) stable, (2) fluctuating, and (3) intermittent. Stable refers to a SERS signal with minimal changes in intensity throughout the entire temporal trajectory. Fluctuating refers to a SERS signal that blinks continuously, and intermittent refers to a SERS signal that switches between periods of being stable and blinking. Correlated SER, SEF, plasmon resonance spectra, and temporal trajectories of the SERS and SEF were collected. The authors used intense NIR laser pulses to induce fluctuations in an otherwise stable signal. These laser pulses induced a 50 nm blue shift in the LSPR, which coincided with the onset of the unstable SERS signal. On the basis of these results and their reproduction of the SERS and SEF signals using the EM mechanism (see ref 114 for more details on their quantitative analysis), they proposed two insights into blinking behavior. First, an unstable adsorption of R6G to the Ag nanoparticles causes instability in the SERS and SEF signals. Unstable adsorption can alter the distance between the molecule and the nanoparticle surface overtime, leading to changes in the EF that the molecule experiences and, therefore, the signal. Second, changes to the plasmon resonance of the Ag nanoparticles can induce blinking because it also changes the EF. Due to these insights, they suggest that the underlying cause of blinking can be attributed to photoinduced effects, such as thermal heating of nanoparticle hot spots leading to plasmon resonance shifts, diffusion of the molecule around the hot spot, and/or photobleaching.

Kitahama and co-workers analyzed the bright (on) and dark (off) states of SERS blinking for thiacyanine using a power law analysis.\textsuperscript{98–100} Power law statistics have been used to analyze long-range ordered nonexponential behavior in quantum dots (QD). The power law for blinking of a single QD fluorescence can be explained by a distribution in the passage time required for a random walker to return to its starting point, which is approximated using a one-dimensional random walk model. Kitahama et al. postulate that blinking statistics in SERS will be similar due to the random molecular walk of an adsorbed molecule on the nanoparticle surface. Indeed, they observed that the power law reproduced the probability distribution of the occurrence of the bright and dark states over time. Next, the authors monitored the power law exponent as they swept the excitation wavelength across the nanoparticle aggregate LSPR. When excited at the LSPR wavelength of the nanoparticle aggregate or at high laser power, the power law exponent of the bright and dark blinking events approach \(-1.5\), similar to what is expected for QD blinking. When excited off the LSPR wavelength and at lower excitation laser powers, the power law
deviated from $\sim$1.5. They propose that the high EM fields created when excited at the LSPR wavelength restrain the molecule by a surface-plasmon-enhanced optical trapping potential well around the nanoparticle junction. Subsequent studies by Kitahama and co-workers observed that a truncated power law reproduces the probability distribution of dark SERS events versus their duration time.\textsuperscript{95,99} They proposed that the truncation of the power law indicates that a high-energy barrier must be overcome for the molecule to undergo a transition from the nonemissive state to the emissive state and that fast random molecular walking (i.e., surface diffusion into the nanoparticle junction) helps to overcome this energy barrier. The energy barrier is suggested to originate from the coupling of multipolar surface plasmon resonances around the nanoparticle junction. This research highlights the importance of considering both the bright and dark SERS states to fully explain SERS blinking behavior.

In addition to on–off cycling, blinking behavior includes sudden, sharp changes in the Raman peak intensities and their ratios.\textsuperscript{116} For example, in CV the low-frequency region of the Raman peak intensities and their modes was maintained.\textsuperscript{102} These would blink in tandem, while the intensity of the remaining events versus their duration time.\textsuperscript{98,99} They proposed that the power law reproduces the probability distribution of dark SERS studies by Kitahama and co-workers observed that a truncated potential well around the nanoparticle junction. Subsequent molecule by a surface-plasmon-enhanced optical trapping created when excited at the LSPR wavelength restrain the observed that the on

Additional proposed mechanisms for blinking include (1) thermal diffusion in-and-out of the hot spot,\textsuperscript{59–61} (2) thermally stimulated molecular reorientation and chemical processes,\textsuperscript{85} (3) photoionization due to charge transfer states,\textsuperscript{102} and (4) metastable and nonemissive states of the molecule.\textsuperscript{117} On the basis of the current studies, the overall consensus for the origin of blinking is thermally stimulated movement of the molecules on the nanoparticle surface, likely accompanied by photoinduced effects such as electron transfer or other chemical processes.

\subsection{2.4.2. Spectral Wandering.} Spectral wandering is the shift in frequency or spectral position for a particular vibrational mode. The isotopically sensitive phenyl band at 600 and 610 cm\textsuperscript{−1} for SM events of undeuterated R6G (R6G-$d_0$) and its deuterated isotopologue (R6G-$d_4$), respectively, have been observed in multiple studies,\textsuperscript{51,58,96} showing that the peak center can shift within a range of $\pm$5 cm\textsuperscript{−1} from event to event. Similarly, spectral wandering was explored by Etchegoin and Le Ru for Nile Blue (NB) and Rhodamine 800 at 77 K.\textsuperscript{118} Using a high-resolution grating, the authors were able to observe inhomogeneous broadening of the 590 and 2226 cm\textsuperscript{−1} modes of NB and Rhodamine 800, respectively. Figure 10 shows the individual SM events and average spectra for (A) NB and (B) Rhodamine $800$. Multiple SM spectra of the $\sim$590 cm\textsuperscript{−1} peak of NB and the $\sim$2226 cm\textsuperscript{−1} mode of Rhodamine 800. The spectra were collected at 77 K with 633 nm excitation and a high-resolution 2400 lines/mm grating. Individual single-molecule events are shown to fall within the average signal of over 7500 spectra (black trace) for both NB and Rhodamine 800. Adapted with permission from ref 118. Copyright 2010 American Chemical Society.

![Figure 9](image1.png) Figure 9. Representative time-dependent SERS intensity fluctuations of a single R6G molecule illuminated with a power of 10 W/cm\textsuperscript{2}. Each row is a color-coded spectrum based on the intensity. The on–off cycling of the 615 and 774 cm\textsuperscript{−1} modes can be seen to blink in tandem. Adapted with permission from ref 116. Copyright 2001 American Chemical Society.

![Figure 10](image2.png) Figure 10. Inhomogeneous broadening observed for (A) the $\sim$590 cm\textsuperscript{−1} peak of NB and (B) the $\sim$2226 cm\textsuperscript{−1} mode of Rhodamine 800. The spectra were collected at 77 K with 633 nm excitation and a high-resolution 2400 lines/mm grating. Individual single-molecule events are shown to fall within the average signal of over 7500 spectra (black trace) for both NB and Rhodamine 800. Adapted with permission from ref 118. Copyright 2010 American Chemical Society.
14N to 15N.119 These studies show that small frequency shifts observed in SMSERS spectra are generally attributed to different local environments and isotopic effects.119 Additional studies have observed homogeneous broadening and overtone and combination bands121 with SMSERS.

### 2.4.3. Distribution in the Anti-Stokes-to-Stokes Scattering Ratio

Broad distributions in the anti-Stokes-to-Stokes scattering ratio (\(aS/S\)) have been observed in SMSERS studies.62,122–124 Anti-Stokes scattering depends on the population of the excited vibrational states. Increases in the anti-Stokes scattering intensity (i.e., increases in \(aS/S\)) can be attributed to a few factors, including vibrational pumping, heating, and resonance effects, that variably enhance the Stokes and anti-Stokes scattering. Several studies have focused on understanding how these effects can lead to fluctuations in the anti-Stokes scattering intensity that cannot be accounted for by thermal population of the excited vibrational states alone.62,122–125

Vibrational pumping is the increase of the population of excited states through Stokes scattering.125 The first conclusive demonstration of vibrational pumping in SERS was done by Maher et al., who used temperature scans to separate out the contributions of resonance effects and heating from those of vibrational pumping.123 Building on this study, Galloway and co-workers, used low temperatures (77 K) and high power densities (4.48 \(\times\) \(10^8\) \(\text{W}\text{m}^{-2}\)) to verify the effect of vibrational pumping on the \(aS/S\) ratio.124 They showed that even though both NB and CV Stokes signals were present for many events, the anti-Stokes scattering could often be observed for only NB or only CV in the same, simultaneously collected spectrum. This result suggests that molecules can experience different contributions from vibrational pumping. While vibrational pumping is an important consideration at cryogenic temperatures, it is not a main contributor to the \(aS/S\) ratio at room temperature, which is dominated by thermal population or resonance effects.

Two subsequent studies investigated the role of local EM fields and plasmonic heating on the \(aS/S\) ratio of SMs. dos Santos and co-workers studied the \(aS/S\) ratio for brilliant green (BG) and CV shown in Figure 11.122 They used a normalized \(\kappa\) value, defined as the \(aS/S\) ratio of a SM divided by the ensemble-averaged \(aS/S\) ratio. The mean \(\kappa\) values were 1.00 and 1.04 for BG and CV, respectively. The equal \(aS/S\) ratios for the SM and ensemble regime show that the average SERS signal is the combination of contributions from all the individual hot spots illuminated during the SERS experiment. They also found that the distribution in \(\kappa\) values was the same for both analytes. As a consequence, the variability of the \(aS/S\) ratio for different SM events is expected to result from nonequivalent enhancement of the Stokes or anti-Stokes scattering.

This conclusion was later supported by an investigation by Pozzi et al., where the distribution of \(aS/S\) ratios for R6G was found to span more than 1.5 orders of magnitude.62 Using finite-difference time-domain (FDTD) simulations of a nanoparticle aggregate, the theoretical EF as a function of the scattered photon wavelength was calculated for both crevice sites of a nanoparticle junction. In each crevice, two locations at a distance of 1 nm were calculated (i.e., four locations total). A molecule diffusing 1 nm in the first crevice site would see a decrease in the \(aS/S\) ratio from 1.9 to 1.6 and in the second crevice site a decrease from 0.17 to 0.09. Considering all four locations in the single nanoparticle junction, the ratio of the anti-Stokes-to-Stokes scattering could vary by a factor as high as 21, providing further evidence for the role of local EM fields on the \(aS/S\) ratio. Pozzi and co-workers also investigated the possibility that local heating contributed to \(aS/S\) ratio based on two assumptions.62 (1) molecules residing in higher EM fields will give higher SERS signals and (2) molecules in more intense local EM fields experience a greater extent of heating. Essentially, this means that higher SERS intensity would be correlated with greater heating and a higher \(aS/S\) ratio. A positive correlation was found, suggesting that heating is observed, but since it was only partially correlated, the heterogeneous enhancement of the anti-Stokes and Stokes scattering is believed to be the main contributor.

Another recent study by dos Santos et al. approaches this phenomenon from a different perspective and uses the \(aS/S\) ratio of single CV molecules to probe the aggregation state of the Ag colloids.123 The experimental results were interpreted within the framework of generalized Mie theory (GMT) simulations. Figure 12 shows the electric field enhancement profiles \(E/E_0\) as a function of incident wavelength) for the hot spots of Ag nanospheres 25 nm in diameter. With increasing aggregation state, the resonances are red-shifted and the maximum \(E/E_0\) decreases. From these simulations, it is expected that for Ag dimer at 633 nm excitation the anti-Stokes scattering would be enhanced relative to the Stokes scattering. For larger aggregates, the Stokes scattering would be preferentially enhanced. They tested these predictions by using different KBr concentrations to influence the aggregation state of the Ag nanoparticles. Small aggregates (i.e., dimers and trimers) dominated with [KBr] \(\leq 7.5\) mM, and larger aggregates dominated at [KBr] > 7.5 mM. At low KBr concentrations (2.5 mM), the anti-Stokes SERS signal was
observed at 633 nm excitation but not at 785 nm, as seen in Figure 12C. As the KBr concentration was increased, the nanoparticle resonances red-shifted due to the formation of larger nanoparticle aggregates. As a result, the SERS intensities with 785 nm excitation increased, while the SERS intensities with 633 nm excitation decreased. Furthermore, the anti-Stokes signal at 633 nm excitation only extended out to \(-600 \text{ cm}^{-1}\), while at 785 nm the Raman shifts were visible out to \(-800 \text{ cm}^{-1}\). Their experimental results are in good agreement with the model presented in Figure 12, where, for larger aggregates, the plasmon resonance broadens. These works suggest that the local structure of nanoparticle aggregates can be probed with SMSERS because, as previously discussed, the SMSERS signal is strongly dependent on the local field properties of the nanostructure.

2.4.4. Pressure-Induced Blue Shift of Vibrational Modes. The ability to study single molecules under high pressure would deepen our understanding of the broadening and blue shifting of vibrational modes in Raman spectroscopy. Pressure-induced blue shifts of molecular vibrations can arise from anharmonic coupling between intramolecular vibrations and the environment. When molecules are present in disordered media, such as Ag colloids under pressure, individual molecules will experience different anharmonic coupling to the environment, resulting in varied pressure-induced blue shifts for the vibrational modes. Fu and Dlott investigated the effect of pressure on vibrational modes in SERS and SMSERS from 1 to 4 GPa.126 For these studies, the SERS substrate consisted of aggregated Ag nanoparticles embedded in a poly(vinyl alcohol) matrix, shown in Figure 13A. The substrate was then placed in a diamond-anvil cell (DAC) to accommodate the high pressures, which resulted in \(\sim 25\%\) compression of the sample at the highest pressure of 4 GPa. SM detection was verified using the R6G isotopologues. As shown in Figure 13A, the brightness of each pixel in the scan correlates to the integrated intensity of the \(~1650 \text{ cm}^{-1}\) R6G mode (in-plane stretching of the xanthene moiety), which shifts \(\sim 5 \text{ cm}^{-1}\cdot\text{GPa}^{-1}\). An initial, rapid drop in SERS intensity at 1.5 GPa is believed to result from a pressure-induced destruction of numerous hot spots by compression of the sample. With the surviving hot spots, the authors were able to observe the effect of pressure on the SERS spectra of R6G. They observed a broadening in line width and a blue shift in peak frequency for the ensemble-averaged \(1650 \text{ cm}^{-1}\) mode shown in Figure 13B. Figure 13C shows the histogram of the \(~1650 \text{ cm}^{-1}\) peak location for the SMSERS spectra at different pressures. The magnitude of the blue shift varied between SM events, as expected, and became more pronounced as the environment was further compressed under pressure. The line width increase in the ensemble spectra of R6G results from the variability in blue shift from molecule to molecule. Each molecule is located in a different hot spot structure (i.e., environment), leading to differences in anharmonic coupling at each molecular site and, therefore, different blue shifts. Using insights from SMSERS, the pressure-dependent ensemble SERS measurements were explained by the anharmonic shifts of various molecule–environment interactions.

2.4.5. Excitation-Wavelength Dependence of SMSERS. The excitation-wavelength dependence of SMSERS was characterized using a tunable optical parametric oscillator to
finely tune the excitation wavelength across the molecular resonance of a single R6G molecule (i.e., ∼500–575 nm). The Raman excitation profiles (REPs) for ensemble R6G (black line) and a SM of R6G (black circles) are shown in Figure 14. The SM REP was obtained from the sum of all the observed Raman modes and was fit to a Lorentzian function (red), producing a fwhm of ∼400 cm⁻¹. As expected, the SM REP is narrower than the ensemble and was dominated by homogeneous broadening. This study was the first example of a high-density REP (i.e., finely tuned wavelength scan) across the molecular resonance of a SM. Collecting a distribution of REPs with correlated nanoparticle structures could help improve our understanding of how local environments influence the energy, line width, and population distribution of SMs that cannot be observed with ensemble averaging.

2.4.6. Summary. While numerous studies have focused on explaining the fluctuations observed in SMSERS spectra, there is still much to be learned. Their origin appears to be a convolution of photochemical, plasmonic near field, and chemical (e.g., charge transfer) effects and is highly molecule/system dependent, complicating our fundamental understanding. For example, there are several proposed mechanisms for the origin of blinking, ranging from thermally stimulated molecular diffusion to the formation of charge-transfer states, but robust theoretical models describing these mechanisms are still insufficient. Determining the role of each of these factors in signal fluctuations requires isolating the individual contributions. This may include tailoring molecular systems to isolate molecular diffusion, charger transfer effects, etc. In addition, correlated nanoparticle structure, theory, and SMSERS signal can help elucidate the various contributions from plasmonic near fields to SMSERS signal fluctuations.

2.5. Chemistry at the Single-Molecule Limit

Now that SMSERS has become a relatively well-established technique, researchers have extended the technique and have begun answering questions about molecular reactivity at its most fundamental limit. To this end, SMSERS has been applied to monitoring catalytic and electrochemical reactions, observing single-molecule tautomerization, and more, as we will discuss in this section.

2.5.1. Electrochemical SMSERS. As the study of electrochemical reactions shrinks to the nanoscale regime, there has been a recent spike of interest in monitoring the electrochemistry of single molecules or single nanoparticles with optical spectroscopy. This is achieved by monitoring the signal of an optically active redox probe as a function of applied potential. In particular, the advantages of utilizing SMSERS to study nanoscale electrochemistry are that single-molecule detection is readily attainable compared to traditional current detection techniques, the SMSERS signal does not require additional amplification, SMSERS provides rich chemical information, and, during the electrochemical reaction, it allows one to monitor structural changes of a molecule in close proximity to a nanostructured metallic surface. SMSERS was first combined with electrochemistry in 2010 (EC-SMSERS), where a bialyte SMSERS proof was done by adsorbing R6G and Nile Blue (NB) on a Ag mirror—Ag nanoparticle substrate working electrode. As the potential was swept positive from 0.1 V, the 2H⁺, 2e⁻ oxidation of NB was indicated by the appearance of the NB signal, and upon the negative sweep, the reduction of NB was indicated by the loss of the NB signal. The
authors observed slight differences in the bulk surface cyclic voltammogram versus the single-molecule electrochemistry. In later work, the authors extended the study to high-resolution SMSERS spectra of NB by observing changes in the NB vibrational frequencies as a function of applied potential.\textsuperscript{71} Observed NB vibrational mode shifts versus reduction potential were attributed to reorientation of the NB molecule in the electrochemical double layer and the orientation of NB on the Ag nanoparticle surface. EC-SMSERS was also implemented in a study by Wang et al. to understand photoelectrochemical charge transfer dynamics in hemin, an iron protoporphyrin, where it was found that local thermal fluctuations govern electron transfer dynamics in the protoporphyrin system.\textsuperscript{153}

More recently, EC-SMSERS was implemented to study single, heterogeneous electron transfer events of R6G in nonaqueous conditions.\textsuperscript{134} Using a sample consisting of R6G-physiosorbed Ag nanoparticle aggregates covalently tethered to an ITO coverslip, SMSERS detection was first validated by the isotopologue proof.\textsuperscript{36} Then, the electrochemical conversion of a single R6G molecule to its neutral radical form was observed by stepping the potential of the working electrode from 0 to −1.2 V in −0.1 V steps and monitoring the SMSERS spectra as a function of applied potential. The potential at which the R6G SMSERS signal was no longer observed was counted as a SMSERS loss event and indicated the reduction of R6G to its neutral radical form. However, the majority of SMSERS signal loss events did not have a subsequent oxidative signal return, indicative of electrochemically induced desorptive losses. This issue can be addressed using a molecule covalently tethered to the Ag nanoparticle surface, as demonstrated in recent work from the Willets group.\textsuperscript{57} Despite the occurrence of electrochemical desorptive losses, the total SMSERS loss events followed the profile of a surface voltammogram of R6G on Ag. Interestingly, there were a small number of SMSERS signal loss events that occurred in the underpotential region of the surface voltammogram, which are attributed to site-specific reduction potentials on the Ag nanoparticle surface. Observing single-electron transfer with EC-SMSERS strongly demonstrates the electrochemical heterogeneity of nanoparticles and elucidates the importance of site-specific electrochemical activity on surfaces.

The Willets group has exploited the 5−10 nm spatial precision of super-localization SERS, as discussed in section 2.1.2, to understand the spatial dependence of electrochemical events on Ag colloidal nanoparticles.\textsuperscript{70,135} They implemented NB physisorbed onto Ag colloidal nanoparticle aggregates to observe the 2H\textsuperscript{+}, 2e\textsuperscript{−} reduction reaction of NB, where the SERS intensity is lost and returns with NB reduction and oxidation, respectively. Three different concentration regimes were studied: single-molecule coverage, few-molecule coverage, and high molecule coverage. At single-molecule coverage, the fitted SMSERS emission centroid displays two unique reduction potentials that are observed upon subsequent potential sweeps, which correspond to two distinct positions of the molecule on the Ag nanoparticle aggregate. This result suggests that the reduction potential of NB is dependent on its location on the Ag nanoparticle surface. However, performing multiple potential sweep cycles at single-molecule coverage was challenging, due to factors such as the small number of potential sweep cycles where the molecule is emissive. The authors then confirmed the site-specific behavior of NB electrochemistry by performing the same experiment at few-molecule coverage, where the intensity-weighted SMSERS emission position shifts as a function of potential. This finding suggests that single NB molecules are being oxidized or reduced at unique potentials based on their location on the electrode. Later work from Weber et al. confirmed the site-specific redox activity of NB on Ag nanoparticles when performing super-localization EC-SERS with a spatially correlated SEM.\textsuperscript{70} At highly reducing potentials, the SERS emission is centered at junctions between nanoparticles and localized close to the center of the nanoparticle aggregates at more oxidizing potentials. The change in centroid position as a function of applied potential implies that higher energies are required to reduce NB molecules in junctions between Ag nanoparticles, corroborating the concept of site-specific redox potentials on nanoparticles first postulated by Wilson and Willets. Overall, the use of EC-SMSERS and super-localization EC-SERS demonstrates the power of SERS to study single-molecule electrochemical reactions at the few- to single-molecule level.

2.5.2. Observing Catalytic Reactions. As demonstrated by EC-SMSERS studies, SMSERS is a powerful tool for monitoring chemical reactions. In particular, SERS has been recently applied to study heterogeneous catalytic reactions because SERS-active nanoparticles can serve both as the catalyst material and Raman-enhancing substrate. Many recent studies have focused on examining the model dimerization reaction of p-nitrothiophenol (p-NTP) or p-aminothiophenol (p-ATP) to 4,4-dimercaptoazobenzene (DMAB).\textsuperscript{33,136−147} Recently, efforts have pushed toward studying the DMAB formation reaction at the single-molecule limit. The Deckert group explored the effect of p-NTp coverage on Au nanoparticles and how this affects the DMAB formation yield. Further, they explore the fate of a single p-NTp molecule if it cannot react with another molecule and dimerize to form DMAB.\textsuperscript{137} At incubation concentrations greater than 5 × 10\textsuperscript{−5} M of p-NTp, the coverage of p-NTp on the Au nanoparticle surface is sufficiently dense such that dimerization to DMAB still occurs upon illumination. Below 5 × 10\textsuperscript{−8} M, the coverage of p-NTp is too sparse and there is no apparent DMAB formation on the Au nanoparticle surface; the authors therefore assume that there are one or two molecules in the Au nanoparticle hot spot junction. Upon sample illumination, there is the appearance of new vibrational modes, indicating the cleavage of the nitro group of p-NTp to form thiophenol (TP) (Figure 15). They rationalize the formation of TP via plasmon-induced hot-electron generation, providing sufficient energy to cleave the nitro group from the p-NTp molecule. This work demonstrates the power of monitoring single-molecule reactions on a plasmonic nanoparticle surface using SERS. In a separate study, Choi et al. demonstrated the heterogeneity of reactivity of single pairs of p-NTp molecules dimerizing to DMAB, which they attributed to the relative location of the p-NTp molecules within the hot spot junction.\textsuperscript{157} They demonstrated “hot” and “mild” photo-switching regimes, as defined by two unique types of temporal fluctuations in the DMAB signal. In future work, SMSERS studies of catalytic reactions could be more rigorously performed using isotopically substituted molecules like p-ATP or p-NTp,\textsuperscript{136} where the dimerization of an isotope pair occurs to form a single molecule.

2.5.3. Additional Applications of SMSERS. In addition to potentially monitoring local temperature and observing single catalytic reactions, SMSERS can also be used to observe transient intermediates, such as the rare cis tautomeric form of porphycene, as studied by Gawinkowski and co-workers.\textsuperscript{108} SMSERS detection of porphycene was proven using the
isotopologue method. Statistically valid SMSERS detection occurred when the total isotopologue concentration of 1:1 porphycene-d$_6$:porphycene-d$_{12}$ was $10^{-9}$ or $10^{-10}$ M. It is interesting to note that in each SMSERS histogram collected, more porphycene-d$_6$ counts were measured than porphycene-d$_{12}$, indicating that the $d_6$ had a higher surface diffusion coefficient on the nanoparticle surface. This result highlights the importance of taking into account the subtle differences in molecular properties (e.g., Raman scattering cross section, diffusion coefficient) when conducting a statistically valid SMSERS proof.

The authors also found that the porphycene molecules exhibit 100 times higher photoactivity when adsorbed onto silver and gold nanoparticles as compared to solution-phase electron microscopy. They attribute the higher photoactivity of the adsorbed porphycene, as compared to the free molecule, to the decreased lifetime of the lowest excited singlet state, which decreases the probability of intersystem crossing to the molecule’s triplet states.

After proving SM detection, the authors sought to observe the cis tautomeric form of porphycene, which had previously only been observed on Cu(110) single crystals using STM. They rely on DFT calculations to predict that the major changes between the $d_6$ and $d_{12}$ spectra are a shift in the Raman band at 180 cm$^{-1}$ (trans) to the blue by 20 cm$^{-1}$ (cis) and the appearance of a Raman peak at 1420 cm$^{-1}$ upon formation of the cis form of the $d_{12}$ isotopologue. Time trajectories of a single nanoparticle aggregate were taken of both isotopologues, and instances of trans→cis→trans switching were observed, where the theoretically predicted peak shifts and new modes were observed. They find that the tautomerization is independent of temperature, as they observe the cis form at both low temperature and at room temperature. Instead, they attribute the tautomerization to a hot-spot-dominated effect. This work demonstrates the potential for studying similar molecules such as substituted porphycenes and porphyrins with SMSERS and how nanoparticle hot spot structure can affect the chemical behavior of molecules.

SMSERS can also monitor host–guest interactions, as demonstrated by Sigle and co-workers. They implement the host molecule cucurbit[7]uril (CB[7]) as a means of detecting single guest molecules within a Au mirror–Au nanoparticle junction. Cucurbit[n]urils (CB[n]) are macrocyclic molecules with a barrel-like structure consisting of a glycouril monomer unit where [n] is the total number of monomers in the CB[n] molecule. CB[n]s are ideal for sensing applications because a single molecule binds in the CB[n] cavity volume and the CB[n] subsequently undergoes structural changes when a guest molecule enters the CB[n] cavity, which is readily detectable using Raman spectroscopy. In the CB[7] SMSERS experiment, a Au mirror is incubated with CB[7] at submonolayer coverage and Au nanoparticles are then drop-cast onto the sample in a gap-mode SERS configuration (Figure 6D). The authors claim bianalyte SMSERS spectra of either the empty CB[7] or filled CB[7] with eight different molecules [methyl viologen (MV), adamantane, or ferrocene derivatives] were acquired. Binding events were indicated by the appearance of the guest molecule SERS spectra, as well as a 5 and 10 cm$^{-1}$ shift in the 440 cm$^{-1}$ mode of CB[7] (Figure 16). Despite the clear distinction between empty and filled CB[7] used to claim bianalyte SMSERS detection, nearly half of complex binding events detected were both filled and unfilled CB[7] for all guest molecules studied, indicating the presence of at least one CB[7] molecule in the Au mirror–nanoparticle junction. While the molecular coverage of the SERS-active substrate needs to be optimized in order to rigorously demonstrate SMSERS, this work is a promising step forward toward using SMSERS to probe biologically relevant molecules including cellular metabolites (e.g., pyruvate and lactate), proteins, and DNA.

3. SINGLE-MOLECULE AND HIGH-RESOLUTION TERS

SMSERS has been routinely achieved under favorable conditions, allowing the exploration of the fundamental mechanisms of SERS and spectroscopic phenomena and the investigation of molecular reactivity. Combining SMSERS with super-resolution microscopy has successfully overcome the optical diffraction limit; however, the super-resolution signals originate from the heterogeneity of the near field rather than from the physical environment in which the molecules are located. The direct imaging of surface-bound molecules with the capability of controlling hot spot location can be achieved using the plasmonic properties of a SPM tip made or coated with a plasmonic metal, known as TERS.

3.1. Background and Principles

In 1985, Wessel first proposed the utilization of a plasmonic nanoparticle as an STM probe. The nanoparticle amplifies and confines the electromagnetic field of incoming photon excitation to a small volume, defined by the tip–sample junction of the STM. This idea, now commonly known as TERS, integrated the high chemical sensitivity of SERS with the high precision spatial control of STM. However, the experimental realization of TERS was not demonstrated until decades later when several groups independently reported Raman spectra of surface-bound molecules with plasmonically enhanced fields from metal or metal-coated tips (Figure 17). Early TERS experi-
ments were performed on scanning probe microscopes (SPMs) mounted on inverted optical microscopes in which the laser was focused through the microscope objective below the sample and collected through the same optical path. While this approach utilizes the high numerical aperture of the optical microscope, it is limited to transparent samples. Later on, refractive side-illumination optics and parabolic mirrors were incorporated, enabling TERS on opaque samples.

Of the independently published original demonstrations of TERS at the turn of the century, several were performed on an...
AFM platform.\textsuperscript{16,17,20,152} Since its inception, AFM–TERS has enjoyed widespread use in spectroscopic characterization of large molecules, especially biomolecules \textsuperscript{46,157} and low-dimensional materials \textsuperscript{158–160} with nanoscale resolution. Due to the popularity for studying such systems, commercial AFM–TERS systems (e.g., the Nano-Raman system from Horiba) have recently been developed. While TER imaging of small molecules is generally performed with STM systems, AFM–TERS has been applied to some small molecular systems, including those relevant to molecular electronics,\textsuperscript{161} art conservation,\textsuperscript{162} and electrochemistry.\textsuperscript{163}

Similar to SERS, the physics responsible for the enhancements of the Raman scattering in TERS is mainly the EM and CE mechanisms. The EM mechanism derived from classical electrodynamics is the dominant factor and thus will be discussed in this section; however, it does not adequately explain the recently reported subnanometer resolution of TERS. The ongoing efforts on integrating quantum mechanics into TERS theories will be discussed in section 3.3.3.

In TERS, a noble-metal tip mostly made of, or coated with, Ag or Au serves as an analog to the enhancing nanostructures in SERS. The sharp geometry of the tip apex acts as the source of a LSPR, which generates an intense and highly localized electromagnetic field. As the plasmonic tip approaches the noble-metal surface and is brought within close proximity (\textit{A} \textasciitilde \textit{d} \textasciitilde \textit{nm}), the gap-mode effect between the tip and surface further increases the electromagnetic field intensity and confinement. Roughly speaking, the field enhancement in this narrow active region of the coupled tip–substrate follows a $d^{-10}$ dependence, where \textit{d} is the distance between tip and substrate.\textsuperscript{164,165}

EFs of resonant dye molecules exceeding 10$^7$ have been demonstrated in TERS,\textsuperscript{166–168} which provides sufficient sensitivity for SM detection if the molecule of interest has a strong electronic transition aligning with the incoming photon excitation. Quantification of EFs in TERS is important in order to understand the fundamental effects of the local electromagnetic field on the system. This is typically done with the same equation as used in SERS (eq 1) with slight modification

$$EF = \frac{I_{nf}}{N_{nf}} \frac{I_{ff}}{N_{ff}}$$

where $I_{nf}$ and $I_{ff}$ are the intensities of the near-field and far-field Raman signals, respectively, and $N_{nf}$ and $N_{ff}$ the number of molecules contributing to the near-field and far-field signals, respectively. Since the far-field Raman signal originating from all molecules within the laser spot always contributes to the total TERS signal, $I_{ff}$ cannot be directly measured. However, $I_{nf}$ can be calculated by the difference of the signal from when the tip is engaged to when the tip is retracted from the substrate, as $I_{nf} = I_{TERS} - I_{ff}$. In the cases of submonolayer to single-molecule coverage, the far-field signal is not strong enough to be detected. In this respect, the noise level can be utilized as an upper bound for the far-field intensity, and consequently, the EF will be underestimated. $N_{ff}$ can be obtained by using the SPM image to estimate the surface coverage of the analyte while factoring in the area of the laser spot. Due to the fact that there is no identical SPM probe, the enhancing region of the tip is not well-defined. $N_{nf}$ can only be estimated, except in the case of a SM, in which $N_{nf} = 1$. Pettinger et al. proposed to approximate $N_{nf}$ on the basis of half the radius of the curvature of the tip;\textsuperscript{169} however, this is not accurate because recent experimental results have demonstrated subnanometer lateral resolution.\textsuperscript{16,21} Currently, the most accurate method of determining the near-field area is through TER imaging, which will be discussed in section 3.3.

3.2. AFM and STM Tip Fabrication

Arguably the most challenging experimental aspect of TERS is the reliable fabrication of highly enhancing tips. Particularly in STM, the fabrication of a tip that yields high TERS enhancement and high-resolution STM imaging can be difficult and irreproducible. A comprehensive survey of all methods for tip fabrication, conditioning, and preservation is beyond the scope of this review. Instead, we will briefly survey the most common methods and those with which the highest TERS spatial resolution was obtained. We refer the interested reader to a recent review by Huang and co-workers exclusively devoted to the subject of TERS tips.\textsuperscript{170}

In STM-TERS, the most frequently used method for tip fabrication is electrochemical etching of a Ag or Au wire. For their submolecular resolution UHV-TERS work, the Dong group etched a 0.3 mm diameter Ag wire centered inside a Pt ring counter electrode immersed in 1:4 perchloric acid:methanol. A 1 V potential was applied to the Ag wire, which was etched for \textit{A} \textasciitilde 8 min.\textsuperscript{16,171} The Van Duyne group used a similar etching method for their 4 nm resolution TERS imaging of a dynamic phase boundary,\textsuperscript{41} etching a 0.25 mm Ag wire in 1:4 perchloric acid:ethanol with a 1.6 V applied potential versus an ethanolic Ag/AgCl reference, as shown in Figure 18. A comparable procedure is generally used for etching Au. For example, the Pettinger group etches 0.25 mm diameter Au wire in 1:1 HCl:ethanol at a potential of 2.4 V versus SCE.\textsuperscript{172} There are countless variations of the electrochemical etching fabrication strategy for TERS tips and all have been used with varying degrees of success. Implementation of potentiostatic control with current cutoffs and optical feedback for monitoring the etching process can help prevent overetching.\textsuperscript{173–175} Additionally, pulsed dc etching, ac etching, and etching within a thin film of solution are options for controlling tip shape and morphology.\textsuperscript{174,176,177} Automation techniques have also been employed in an attempt to increase etching throughput and reproducibility.\textsuperscript{174}

In AFM–TERS, the most common fabrication method for producing tips is thermal evaporation of Ag or Au onto a commercial Si or SiN AFM probe.\textsuperscript{40,163,178,179} The deposition thickness for Ag varies widely in the literature, generally in the range of 20–70 nm.\textsuperscript{30,177} However, Au AFM tips are used far less frequently in the AFM–TERS literature, with the optimal
thickness reportedly in the range of 50–80 nm. Alum

In ultraviolet (UV) TERS studies. Some common alternative methods for AFM–TERS tip fabrication include functionalizing AFM tips with single Au nanoparticles and electrodeposition of Ag and Au. The use of commercially available Au-coated AFM tips for TERS has also been reported. Tuning-fork-based AFM (shear force microscopy, SFM) has also been extensively used as a TERS platform. In general, tip fabrication for SFM experiments is identical to those discussed above for STM experiments, with the resulting tip then attached to a tuning fork. Batch fabrication methods have also been developed to increase tip reproducibility. A collaborative effort between the Oh group and the Novotny group has led to a promising template-stripping method for SFM probes. Large-scale fabrication methods such as this are promising techniques for expanding the utility of TERS toward routine and commercial use (Figure 19).

Figure 19. (A) SEM image showing massively parallel Au tip formation on a silicon wafer, where ~1.5 million nominally identical tips can be fabricated over a single wafer. (B) Close-up image of a single 200 nm thick gold tip resting in the inverted silicon mold after lift-off. The tips can be stored over an extended period of time without degradation. (C) SEM image after template stripping with epoxy and a thin tungsten wire. (D) The radius of the tip is 10 nm, suitable for high-resolution near-field imaging. Adapted with permission from ref 188. Copyright 2012 American Chemical Society.

3.3. Spatial Resolution

3.3.1. Early Development of Imaging/Environmental Control for Single-Molecule TERS. An early example of TERS imaging on single-walled carbon nanotubes (SWNTs) was reported by the Novotny group, wherein the authors demonstrated simultaneous AFM topographic and TERS imaging constructed by integrating the 1596 cm⁻¹ G’ band of SWNT at each pixel. They reported ~25 nm lateral resolution in the TERS image. Since then, significant improvements on the lateral resolution of TERS have been demonstrated, making TERS an ideal tool for single-molecule chemical imaging.

For molecular systems, however, TERS under ambient conditions often results in spurious signals in Raman spectra or rough features in SPM topographic images. SPM samples in ambient environments are easily exposed to airborne contaminants, which may interfere with the interpretation of data. Additionally, a several nanometer thick water meniscus on surfaces has been measured by AFM at moderate humidity. Other than introducing contaminants through diffusion, the presence of unwanted water may alter the molecule–surface interactions that otherwise would not be present under a controlled environment.

In 2007, the Pettinger group reported the design and operation of the first home-built TERS instrument under ultrahigh vacuum (UHV) conditions. All necessary optical elements were mounted on a rigid frame containing the STM inside the UHV chamber. In 2008, the same group demonstrated the first correlated UHV-TERS and STM mapping of a single Brilliant Cresyl Blue (BCB) molecule, reporting a lateral resolution of 15 nm (Figure 20A). When sample preparations are performed under UHV, atomically pristine surfaces are achieved with only the molecule(s) of interest present. Utilizing the benefits of working in a UHV environment, the Van Duyne group demonstrated detection of multiple vibrational modes in UHV-TERS of copper phthalocyanine (CuPc) on Ag(111) concurrent with molecular-resolution STM imaging (Figure 20B,C). Furthermore, Klingsporn et al. demonstrated UHV-TERS of immobilized R6G molecules on Ag(111) with liquid He cooling. The cryogenic temperature not only minimized the surface diffusion of the adsorbed R6G molecules, but it also increased the vibrational dephasing time, which resulted in narrower line widths of the observed Raman modes. Additionally, the spectral shifts observed in the low-temperature UHV-TERS were further analyzed by potential energy distributions of the corresponding vibrational modes, which allowed accurate determinations of the absorption geometry of R6G on Ag(111) at 19 K. Since then, UHV has been the desired environment for high-resolution TERS studies on molecular systems.

3.3.2. Single-Molecule TER Imaging with Sub-5 nm Resolution. After the first demonstration of single-molecule TERS imaging in 2008, the lateral resolution of TERS on molecular systems has been pushed to below 5 nm under UHV conditions. In UHV, correlated STM and TERS imaging have been applied to three distinct surface-bound molecular systems: (1) an isolated molecule, (2) two distinct, adjacent analytes, and (3) a dynamic molecular phase boundary. Dong and co-workers first demonstrated subnanometer lateral resolution on an isolated porphyrin molecule at 80 K. The obtained TERS images allow for direct visualization of the inner structure of a single molecule through optical spectroscopy (Figure 21A). In 2015, the same group, with the same instrument, unambiguously distinguished two distinct adjacent porphyrin molecules on a Ag(111) substrate by the difference in vibrational fingerprints in the TERS line scan (Figure 21B). Also performed under UHV conditions, a dynamic molecular phase boundary has been imaged by TERS at room temperature. In this study, the condensed phase and the 2D gas phase of a perylene diimide derivative coexist on a Ag(100) substrate. Collected TERS images exhibit ~4 nm lateral resolution over the dynamic phase boundary (Figure 21C). Here, the measured resolution is a convolution of the intrinsic TERS resolution and molecular diffusion on the phase boundary.

TERS has set the stage for future molecular-resolution chemical imaging studies. Clearly, the original description of lateral resolution derived purely from classical electrodynamics, where Pettinger and co-workers estimated that the lateral resolution is about half of the radius of the curvature of the tip, is insufficient to explain sub-5-nm resolution. A detailed
discussion of the recent attempts to explain this high-resolution with an advanced theoretical approach is presented in the following section.

3.3.3. Theoretical Explanations for High Spatial Resolution. As discussed in the previous section, Zhang et al.\textsuperscript{21} and Jiang et al.\textsuperscript{18} have demonstrated subnanometer lateral resolution in UHV-TERS. In an effort to explain this extraordinary lateral resolution, the theoretical community has vigorously pursued several concepts. As the papers discussed in the previous section have shown,\textsuperscript{18,21,195} extracting the most molecular information requires a combined theoretical and experimental approach. An interesting idea behind high-resolution TERS experiments originates from a reconsideration of plasmonic enhancement beyond the well-established EM mechanism.\textsuperscript{28} In this section, we discuss the ongoing theoretical investigations into the high-lateral resolution of TERS. To do this, we will divide the redevelopment of plasmonic enhancement in UHV-TERS as either invoking optomechanical cavities or more deeply exploring plasmonic enhancement effects, such as the image field effect,\textsuperscript{196} electric field gradients,\textsuperscript{197} and nonresonant chemical enhancements.\textsuperscript{198}

Theoretical approaches previously applied to SERS have been implemented to gain an understanding about TERS resolution, including the image field effect,\textsuperscript{196} electric field gradient effects,\textsuperscript{197} and nonresonant chemical effects.\textsuperscript{198} The image field effect of SERS\textsuperscript{199} was applied as a self-interaction of the
molecular dipole with an image dipole in the substrate. The higher lateral resolution coming from self-interaction relies on multiple elastic scattering events within the Ag tip–substrate gap and thus has a higher spatial dependence. While this theory does provide a higher lateral resolution than that predicted by conventional EM mechanisms, it does not account for the subnanometer resolution observed. Meanwhile, the effect of electric field gradients on lateral resolution in TERS was explored by Meng et al., who considered a system of meso-tetra(3,5-di-tert-butylphenyl)porphyrin (H2TBPP) molecules interacting with a plasmonic tip. The authors show that electric field gradients can lead to higher TERS lateral resolution than predicted by traditional EM mechanisms. However, both the image dipole and the electric field gradient effects rely on effects in a spatial regime where quantum plasmonic effects dominate.

The optomechanical cavity mechanism for UHV-TERS was explored by Roelli et al., and they observed nonlinear plasmonic enhancement within certain physical limits, such as high power densities. Applying the theory of optomechanical cavities to SERS and TERS relies on a coherent coupling between the molecular vibrations (mechanical oscillator) and the localized surface plasmon (electromagnetic cavity). The molecular vibration alters the plasmon such that the instantaneous plasmon occupancy changes and then acts back on the molecular vibration. If the incident laser intensity approaches a parametric instability threshold, at which the rate of backaction amplification exceeds that of intrinsic damping, a strong nonlinear Raman response is observed. This nonlinear Raman response is a possible explanation of the high lateral resolution demonstrated in TERS. However, the ~2 × 10^8 W/cm^2 power density giving rise to this effect exceeds power densities used in <5 nm TERS reports, which are in the range 10^6–10^7 W/cm^2.2

Stimulated Raman scattering (SRS) has also been suggested as a concept to account for the exceptional TERS lateral resolution. These reports develop a theory of SRS where a Raman pump field induces an optical plasmonic response and the electric field of this spatially confined plasmon is then taken as the Raman probe field. Using the higher-order confinement from SRS, Duan et al. calculated higher lateral resolution in TERS by the nonlinear Raman effects compared to normal SERS effects. However, UHV-TERS experiments were done using laser excitation on resonance with the molecule being studied, which leads to dispersive line shapes in resonant femtosecond stimulated Raman scattering (FSRS) experiments due to the interference of multiple Feynman pathways for the light–matter interaction. Thus, the explanation of high-resolution TERS by SRS suggests discrepancies with the experiments performed.

It is clear that explaining the physical origins of the high lateral resolution of TERS imaging in the subnanometer limit is not yet complete, and there exists ample opportunity for new developments. Existing theoretical studies, while convincing on some levels, all achieve their high lateral resolution at the expense of physicality. Specifically, optomechanical cavities suggest experimental power regimes that are experimentally inaccessible, SERS mechanisms lead to unobserved experimental signatures, and previous plasmonic enhancement mechanisms rely on spatial regimes that have stronger quantum plasmonic effects. Thus, further work on high-resolution TERS theories are necessary to fully understand the mechanism(s) responsible for the experimentally observed resolution. The studies analyzed in this section provide a solid foundation for future theoretical work that must overcome the nonphysical consequences discussed above.

3.4. Single-Molecule TERS Studies

Aside from TERS mapping of individual molecules, several other methods have been used to obtain TERS spectra of single molecules and study molecular properties without the effects of ensemble averaging. Using an isotopologue method analogous to SMSERS studies, Sonntag et al. presented a frequency-domain proof of single-molecule TERS (SMTERS). TERS spectra of protonated R6G (R6G-d0) and deuterated R6G (R6G-d1) adsorbed on a polycrystalline silver film were acquired using a side illumination ambient STM TERS system with an electrochemically etched silver tip. In the majority of spectra, one of the two isotopologues was observed. Few of the acquired spectra exhibited contributions from both molecules, verifying the SM nature of the measurement. Furthermore, the spectral wandering of the 612 cm\(^{-1}\) phenyl ring breathing mode of R6G-d0 (602 cm\(^{-1}\) for R6G-d1) was found to be ±4 cm\(^{-1}\), indicating that there is no spectral overlap between the two isotopologues in the distinguishing region. In the follow-up work, Sonntag et al. investigated the nature of relative intensity fluctuations in SMTERS spectra. In some spectra, the low wavenumber modes (e.g., 608 and 771 cm\(^{-1}\)) are the most intense, while in others, the higher wavenumber modes (e.g., 1362 and 1651 cm\(^{-1}\)) are strongest (Figure 22). Molecular orientation effects on the relative intensity were neglected due to the rotational invariance of the Raman tensor for resonant excitation. Additionally, covariance and cross-correlation analysis revealed that all modes fluctuated completely independently, further ruling out orientation or adsorption geometry as the cause of relative intensity fluctuation. Time-dependent density functional theory (TDDFT) calculations of the resonance Raman spectra of R6G revealed that fluctuations in the excited state geometry, energy, and lifetime could reproduce the experimental results remarkably well. Thus, the combination of SMTERS measure-
ments and TDDFT is a powerful tool that can reveal fundamental properties of single molecules that are obscured by averaging in ensemble measurements.

The fundamental interactions of single molecules with plasmons in molecular junctions can also be interrogated using TERS. Using cross-correlational analysis, El-Khoury et al. examined the relationship between the intensities of the vibrational bands of 4,4′-dimercapto stilbene (DMS) and the intensity of scattering from the charge transfer (CT) plasmon of the molecular junction in the conducting limit.208 Similar to what was observed by Sonntag et al.,97 each of the totally symmetric $a_g$ modes was found to fluctuate completely independent of all other modes and of the scattering from the CT plasmon. In contrast, the nontotally symmetric $b_u$ modes strongly correlate with each other and with the CT plasmon.208 The correlation of the nontotally symmetric modes with the CT plasmon was attributed to perturbation of the vibronic coupling terms of the Raman tensor by the tunneling plasmon. Since the intensities of the totally symmetric mode can be described solely by the Franck–Condon term of the induced dipole, modulation of the (higher order) Herzberg–Teller term by the CT plasmon affects only the nontotally symmetric $b_u$ modes.208 Therefore, SMTERS studies of molecules in plasmonic junctions can reveal detailed fundamental information about the interaction between the CT plasmon and the vibrational modes of the molecule bridging the junction.

In order to extract additional fundamental information about the structure, environment, and properties of single molecules, TERS at cryogenic temperatures can be employed to slow down molecular motion to measurable time scales. Park et al. recently used variable-temperature TERS and micro-Raman to observe rapid changes in molecular orientation and elucidate detailed information about intramolecular vibrational coupling in malachite green (MG).209 TERS line widths were found to be significantly narrower than those measured with micro-Raman at all temperatures (e.g., 3.5 cm$^{-1}$ for TERS versus 12 cm$^{-1}$ for micro-Raman at 90 K) due to the absence of inhomogeneous broadening in SM and small-ensemble measurements. The temperature dependence of the TERS and micro-Raman line widths and frequencies were fit to Arrhenius functions, and information about intramolecular coupling was extracted. The authors determined that the Arrhenius behavior of the line widths emerges from vibrational dephasing resulting from coupling of the observed Raman modes with low-frequency torsional modes of MG. Further, fundamental parameters governing this intramolecular coupling were extracted from the Arrhenius model, including activation energy, lifetime of the exchange mode, and exchange coupling strength. The activation barrier was indeed found to match the frequencies of the torsional modes of MG, rather than substrate phonons.209 Additionally, using symmetry arguments similar to those above, Park et al. and co-workers used cross-relation analysis of TERS modes at low temperature to observe pinwheel-like rotational motion of MG on the surface that is only observable when the molecular motions are slowed down to a measurable time scale.97,208,209 Therefore, variable-temperature TERS is a powerful tool for obtaining molecular information on time scales inaccessible by room-temperature continuous wave measurements.

SMTERS therefore has the flexibility to study single-molecule processes in a range of environments (e.g., ambient, UHV) and temperatures (e.g., room temperature, cryogenic temperatures). Further extension of TERS to study chemical reactions (e.g., in electrochemical systems) and dynamics at ultrafast time scales will offer an unprecedented view of fundamental heterogeneities at the single-molecule level. However, the methods commonly used for extracting information about single-molecule structure, environment, and dynamics from SMTERS results are still incomplete. For example, a recent work by Jiang and co-workers revealed through TDDFT that the orientation of the electronic transition dipole of a molecule in the tip–sample can significantly impact the appearance of the Raman spectrum.193 Thus, cross-correlation analysis and current TDDFT methods may not provide a complete picture of the nanoscale processes that contribute to SMTERS spectra. Further development of theoretical methods is needed to improve our understanding of the origins of characteristic SMTERS behaviors.

3.5. Applications of Single-Molecule TERS and TERS Nanoscopy

The single-molecule sensitivity and nanoscale resolution of TERS discussed in the previous sections have been exploited in a variety of practical systems. In this section, we will discuss recent applications of TERS for studying electrochemical systems, biomolecules, low-dimensional materials, heterogeneous catalysis, and single-molecule electronics. Since TERS is a relatively new research field, several of the experiments discussed in this section are still proof-of-concept. Nevertheless, these results serve as cornerstones and open up new frontiers of SM research with the ability to interrogate physiochemical phenomena with unprecedented spatial resolution.

3.5.1. Electrochemical TERS. A nanoscale understanding of electrochemical processes, including heterogeneities in electrochemical behavior across an electrode surface, is critical to understanding electrocatalysis, biological electron transfer, and energy production and storage. TERS is an ideal tool for elucidating structure–activity relationships in electrochemical systems at the nanoscale. Electrochemical TERS (EC-TERS) was first demonstrated on an AFM platform by Kurouski et al. and on an STM platform by Zeng et al.163,210 Kurouski et al. published the first TERS study of a redox reaction.163 Using an electrochemical AFM (EC-AFM) platform, the authors investigated the redox behavior of Nile Blue (NB) spontaneously adsorbed onto an indium–tin oxide (ITO) film. TERS spectra were acquired with a Au tip (70 nm Au on Si) using 632.8 nm excitation. The spectrum of NB was monitored during cyclic voltammetry in Tris buffer (50 mM Tris +50 mM NaCl at pH 7), scanning from 0 to −0.6 V versus Ag/AgCl and back with a scan rate of 10 mV/s. As the potential was swept from 0 to −0.6 V, the resonant oxidized form (NB$_{ox}$) was converted to the nonresonant reduced form (NB$_{red}$) and the intensity of the 591 cm$^{-1}$ mode decreased. Upon scanning the potential back to 0 V versus Ag/AgCl, NB$_{red}$ was oxidized back to NB$_{ox}$ and the signal increased back to its original intensity.163 In Figure 23, TERS spectra acquired at various potentials during the voltammogram are shown. By integrating the intensity of the 591 cm$^{-1}$ mode, TERS voltammograms were constructed. Remarkably, some TERS voltammograms, such as the one shown in Figure 23c, exhibit steplike features indicative of few- or single-molecule behavior. However, in other locations on the sample, the TERS voltammogram did not contain steps, demonstrating a nonuniform surface coverage of NB across the ITO surface.163 This work demonstrated the potential of TERS for studying redox reactions at the nanoscale, probing few- or single-molecule behavior across a nonuniform surface coverage inaccessible by SERS.
Simultaneously, Zeng et al. demonstrated TERS of (4′-(pyridine-4-yl)biphenyl-4-yl)methanethiol (4-PBT) self-assembled onto a Au(111) electrode in 0.1 M NaClO₄ (pH 3 and 10). The electrochemically etched TERS tip was insulated with polyethylene glue to prevent Faradaic current at the tip from interfering with the tunneling current. Interestingly, the authors observed no Faradaic processes attributed to 4-PBT, but they did observe changes in the TERS spectrum of 4-PBT as a function of potential at pH 10. With the sample held at −700 mV versus Pt, the 1592 cm⁻¹ mode is slightly visible as a shoulder of the 1601 cm⁻¹ mode. In contrast, the 1592 cm⁻¹ mode shifts to slightly lower frequency and is clearly resolved at 300 mV versus Pt. No changes in the 4-PBT spectra were observed using TERS at pH 3 or SERS on Au nanoparticles at pH 10. The authors proposed that the shift in frequency observed for the 1592 cm⁻¹ mode was due to protonation of the pyridine moiety terminating the 4-PBT adsorbed onto an ITO working electrode at the voltammogram in panel b. (b) Voltammogram of NB spontaneously adsorbed onto an ITO working electrode at ∼0.02 monolayer coverage. (c) TERS voltammogram produced by plotting the integrated intensity of the 591 cm⁻¹ mode as a function of potential. Steplike functions are observed in the TERS voltammogram, indicative of few-molecule behavior. The cathodic trace (blue dots) and anodic trace (red dots) are offset for clarity. Adapted with permission from ref 163. Copyright 2015 American Chemical Society.

Figure 23. (a) Selected TERS spectra of NB at different potentials along the voltammogram in panel b. (b) Voltammogram of NB spontaneously adsorbed onto an ITO working electrode at ∼0.02 monolayer coverage. (c) TERS voltammogram produced by plotting the integrated intensity of the 591 cm⁻¹ mode as a function of potential. Steplike functions are observed in the TERS voltammogram, indicative of few-molecule behavior. The cathodic trace (blue dots) and anodic trace (red dots) are offset for clarity. Adapted with permission from ref 163. Copyright 2015 American Chemical Society.

Characterized redox molecule (e.g., NB) are needed to fully integrate nanoscale TERS mapping into electrochemical measurements. Further, a microscopic model must be developed in order to explain the steplike behavior in TERS voltammo-grams.

3.5.2. Biological Samples: Amyloid Fibrils and DNA Sequencing. TERS is an inherently label-free, nondestructive chemical examination tool for complex biological systems with SM resolution. Specifically, TERS has been applied to various biological samples, such as viruses, cells, DNA, RNA, and amyloid fibrils. Identification of individual nucleobases on RNA and DNA strands has been demonstrated with exceptional spatial resolution, providing new insights into biological processes. For example, the termination group of DNA fragments has recently been interrogated by Lipiec et al. with TERS. Here, the authors irradiated a thin film of DNA in aqueous solution with a UV source before AFM and TERS measurement. They were able to unambiguously identify double-strand breaks of DNA from the observed TERS spectrum and hence proposed that the C–O double band cleavage was caused by the UV radiations. Moreover, TERS has unraveled the surface structure and composition of insulin fibrils. Instead of the expected dominance of β-sheets formation, only about one-third of the insulin fibrils’ surfaces was composed by β-sheets. The remainder was identified as a mixture of disordered proteins and α-helix. Thanks to its high spatial resolution, TERS can provide new insights into biological processes.

3.5.3. Low-Dimensional Materials. The robustness and high Raman cross section of low-dimensional materials, such as graphene, MoS₂, and carbon nanotubes, makes them ideal targets for imaging by TERS. After the first TERS imaging on SWNTs with ∼25 nm resolution reported by the Novotny group, carbon nanotubes remain one of the most common samples studied due to its high Raman cross section and robustness under high power laser illumination. Kawata and co-workers have demonstrated sub-2-nm resolution TERS images of SWNT under ambient conditions (Figure 24A). Peica et al. have identified SWNT within a carbon nanotube bundle containing seven nanotubes. Furthermore, Yano et al. utilized TERS to monitor the mechanical properties of carbon nanotubes by using the AFM tip to increase the pressure applied to the nanotube, which caused a ∼10 cm⁻¹ shift in the G band. In the most

Figure 24. (A) The 1.7 nm spatial resolution TERS of single carbon nanotubes. Adapted with permission from ref 203. Copyright 2014 Nature Publishing Group. (B) Molecular-resolution topographic STM imaging and UHV-TERS of graphene nanoribbons (GNRs) fabricated on Au(111) by the on-surface polymerization technique under UHV conditions. Adapted with permission from ref 222. Copyrights 2014 American Chemical Society.
recent TERS study of SWNTs, Dong and co-workers reported spectral changes due to strain. Two-dimensional materials are another popular system of study for TERS. It has been shown that TERS is capable of detecting small defects and contaminations in graphene sheets, which may alter the electronic properties of the sheets. The intensity of the graphene D band at 1350 cm\(^{-1}\) is significantly increased in the presence of defects and at the edges of graphene sheets. At the same time, in-plane vibrations (G band) were found to be weakly enhanced because their orientations are not aligned with the enhanced plasmonic field of the tip–sample junction. In 2014, the Wolf group reported a TERS study of graphene nanoribbons prepared on a Au(111) surface under UHV conditions (Figure 24B). Molecular-resolution imaging provided topographical characterization during the polymerization process and corroborates the signal detected in UHV-TERS. The submolecular resolution of TERS provides new capabilities in studying the relationship between the mechanical and electronic properties of carbon-based low-dimensional materials.

### 3.5.4. TERS Investigations of Single-Molecule Electronics

The possibility of combining TERS with STM break junction measurements makes TERS a natural tool for studying SM electronics. In recent years, two groups have demonstrated the potential of TERS for studying the structure of molecular break junctions, in which the tip–sample junction is bridged by a conducting molecule. Liu et al. acquired TERS spectra concurrently with conductance measurements in the “fishing-mode” STM configuration. Fishing-mode STM monitors current jumps associated with the formation of SM break junctions as in conventional STM break junction techniques but uses extremely slow feedback to maintain a stable tip–sample distance within a few angstroms before breaking the junction. This allows conductance measurements to be made for long periods of time while a tip–sample distance suitable for TERS is maintained. By acquiring TERS spectra of 4,4′-bipyridine (4bipy) simultaneously with conductance measurements, Liu et al. could elucidate differences in the structure of 4bipy when the junction was formed (ON state) versus broken (OFF state), as shown in Figure 25. In the ON state, several of the Raman modes of 4bipy, particularly the 1227 cm\(^{-1}\) mode, are broadened and shifted due to loss of symmetry. Further structural changes were observed for the on state as a function of bias voltage. Notably, 1609 cm\(^{-1}\) ring breathing mode reversibly splits into a doublet as the bias is changed from 0.01 to 0.5 V. However, in its current implementation, fishing-mode TERS (FM-TERS) cannot be used to directly correlate conductance measurements with molecular structure for a discrete SM junction. Instead, the 4bipy acquisition time averages over several hundred ON–OFF events, providing the average structure of a SM junction. The discrepancy between typical TERS acquisition times and the frequency of junction formation/breaking is a significant challenge that must be met before FM-TERS can be used to understand the relationship between structure and conductance in molecular junctions.

Recent work by El-Khoury et al. has taken steps toward overcoming this limitation at the expense of structural information. Using an AFM–TERS platform, with 0.1 s acquisition times, the authors observed a broad background peak in spectra, corresponding to the on state of the junction comprised of a gold tip and silver sample bridged by a bipyridilthiol (BPDT) molecule. The background peak was attributed to scattering from conducting plasmons across the molecular junction. However, the molecular structure in the ON state was not easily distinguishable above the broadened background when adding many spectra together. TERS trajectories (intensity–time transients) collected for BPDT resemble current–time transients typically collected in STM break junction measurements. However, even with a 0.1 s acquisition time, several tens of formation/breaking events are averaged over in each TERS spectrum. Nonetheless, the observation of direct scattering from the junction plasmon upon shorting through single BPDT molecules reveals the potential of TERS in probing the structural and electronic properties of molecular break junctions. Further improving acquisition time and collection efficiency will allow for the direct correlation of the structure and conductance of a single molecular junction.

### 4. CONCLUSION AND OUTLOOK

Over the past 2 decades, SMSERS and TERS have become established techniques for characterizing molecular systems in...
nanoscale environments. They combine the inherent chemical selectivity of Raman spectroscopy with plasmonically enhanced signals to achieve the ultimate limit of detection—a single molecule. Moreover, TERS is capable of both chemical specificity and imaging of single molecules with high lateral resolution. Throughout this review, we have discussed the development and understanding of the phenomena underlying SMERS and high-resolution TERS. For SMSERS, we covered hot spots and EF distributions, the rational design of plasmonic substrates, the rigorous verification of SM detection, and our current understanding of signal fluctuations and heterogeneity. For TERS, we covered AFM and STM tip fabrication, the physical origins of the high spatial resolution, including recent theoretical explanations, and fundamental SM studies with TERS.

To continue advancing our fundamental understanding of the SERS and TERS phenomena, future SM studies should improve the reproducibility of nanoparticle and tip fabrication for enhancing the Raman signal, develop methods for controlling molecular location, and increase hot spot density. Using more reproducible plasmonic structures would allow us to systematically investigate the SERS mechanisms and adsorbate—surface interactions. Moreover, coupling controlled molecular location with higher hot spot densities would increase the statistical power of SM studies, thereby strengthening experimental reproducibility and the interpretation of single-molecule chemistry. Similarly, applying rigorous SM proofs in future investigations of single-molecule chemistry will increase the robustness of SMSERS and TERS as analytical techniques. Furthermore, future studies should develop and improve the theoretical models used to describe observed SM behaviors.

Another challenge for SMSERS and TERS is broadening the scope of studyable molecules. Expanding the molecular “toolbox” will generalize these techniques for new and exciting applications. For instance, we highlight in this review recent efforts toward studying biologically relevant systems and nonresonant molecules with SMSERS and TERS. It also appears natural to use the added high resolution of TERS to follow reactions at the nanoscale. For example, dimerization reactions have been studied by TERS. While several steps have now been taken toward the monitoring of chemical reactions with TERS, the full potential of TERS to monitor single-molecule chemical reactions has yet to be realized. Recent developments in high-resolution UHV-TERS also suggest that it should be possible to achieve <1 nm resolution in monitoring chemical events for individual molecules in future studies.

Due to great strides in the fundamentals of SERS and TERS, SM detection is now widely accepted and is being used to study relevant chemistries at the SM level. Electrochemistry, site-specific catalysis, and SM electronics are a few fields covered in this review where the vibrational characterization of single molecules has provided new insight that cannot be gained from an ensemble-averaged experiment. In addition, single to few molecule level studies have been used to observe rare transient intermediates and monitor host–guest interactions. Future endeavors with SMSERS and TERS have the potential to answer questions in photochemistry, plasmon-driven chemistry, site-specific heterogeneous catalysis, and more. These are only a few of the exciting new prospects for application of SM detection that can benefit from the combination of site-specific chemical and structural information.
her doctoral thesis on the rational design and intrinsic properties of nanocrystal superlattices. She has extensive experience working with plasmonic nanomaterials, from their synthesis, assembly, and structure—function relationships to implementing strategies for harvesting their potential as optical sensors for defense and healthcare applications. Lately, her research has focused on the development of plasmonic sensors for bioanalyte detection using surface-enhanced Raman spectroscopy (SERS). She is also actively involved in managing research programs involving SERS biosensing and tip-enhanced Raman-spectroscopy-enabled nanoscale imaging. She holds a B.S. (2002) and a M.S. (2004) from University of Paris-Sud (Orsay, France) and a Ph.D. (2008) in physical chemistry (summa cum laude) from Pierre and Marie Curie University (Paris, France).

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