Electrochemical TERS Elucidates Potential-Induced Molecular Reorientation of Adenine/Au(111)

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Abstract: Electrochemical surface activity arises from the interaction and geometric arrangement of molecules at electrified interfaces. We present a novel electrochemical tip-enhanced Raman spectroscope that can access the vibrational fingerprint of less than 100 small, non-resonant molecules adsorbed at atomically flat Au electrodes to study their adsorption geometry and chemical reactivity as a function of the applied potential. Combining experimental and simulation data for adenine/Au(111), we conclude that protonated physisorbed adenine adopts a tilted orientation at low potentials, whereas it is vertically adsorbed around the potential of zero charge. Further potential increase induces adenine deprotonation and reorientation to a planar configuration. The extension of EC-TERS to the study of adsorbate reorientation significantly broadens the applicability of this advanced spectroelectrochemical tool for the nanoscale characterization of a full range of electrochemical interfaces.

The adsorption geometry of (re)active molecules is a crucial parameter that determines surface reactivity or device functionality in a large variety of applications, such as heterogeneous catalysis, electrochemical (EC) energy conversion, biotechnology, and molecular electronics. For example, it has been suggested that the spatial tilt of π-bonds with respect to the substrate strongly influences the catalytic activity or electron conductance of adsorbates.[1–3] Accessing adsorbate orientation in relation to specific surface sites in situ or in operando is a first crucial step toward controlling interfacial geometries for improved device architecture. However, suitable in situ techniques to study molecular orientation at well-defined adsorption sites are still scarce. Surface-specific in situ Raman- (EC-SERS) or IR-based (EC-SEIRAS) linear and nonlinear (EC sum frequency generation, SFG) vibrational spectroscopies provide the required sensitivity and chemical specificity for investigating molecular geometries at (potential-controlled) solid/liquid interfaces at the ensemble level, that is, the signals represent an average response from a large number of molecules and surface sites present in the focus spot.[4–7] EC scanning probe microscopy (EC-SPM) enables the visualization of individual adsorbate structures with nanometer spatial resolution, albeit at the expense of chemical specificity.[8,9]

Tip-enhanced Raman spectroscopy (TERS) offers an elegant solution to achieve both the required surface (sub)-monolayer chemical sensitivity and nanometer spatial resolution. TERS is based on the combination of an SPM with a Raman optical platform in which a metallic SPM probe located in close vicinity above a surface acts as a nanoantenna to create a plasmon-enhanced field and, consequently, strong Raman scattering from adsorbates located in the tip–sample gap at a spatial resolution of typically below 10 nm.[10–12] While TERS in UHV and ambient conditions is increasingly used in surface science to interrogate adsorbate orientation,[13–16] the study of solid/liquid interfaces has long remained a huge technical challenge.[17,18] Extending the technique to spectro-electrochemical experiments requires additional electrical contacting and control of the working electrode under investigation, thus rendering the setup even more complex and technically challenging. Recently, the groups of Bin Ren and Richard Van Duyne reported EC-TERS setups that allow the in situ investigation of surface redox or chemical conversion processes steered by the electrode potential.[19–21] While these works demonstrate the impressive potential that EC-TERS holds for studying interfacial molecular processes in situ, the versatility of the instruments was limited by either the required tilt of the sample[19] or the use of optically transparent substrates,[20] the former complicates TERS mapping, while the latter is needed because of the chosen transmission illumination geometry.

Herein, we report an alternative EC-TERS approach that enables the study of the potential-dependent behavior of small, non-resonant molecules adsorbed onto an opaque substrate. As a proof of concept, we monitor the EC-TERS response of a monolayer of the DNA base adenine adsorbed onto a well-defined Au(111) single crystal. Given the importance of understanding the interactions between DNA bases and noble metals for biosensing applications and for the development of biocompatible materials,[22,23] it is surprising that the DNA base orientation with respect to the Au surface (for example, upright, tilted, or flat) and the chemical state of the DNA base (for example, (de)protonated or anionic) has remained a point of controversy, despite extensive efforts to characterize the system with traditional cyclo-voltammetric (CV) approaches in combination with SPM or (ensemble) spectro-electrochemistry.[24–32] EC-TERS offers the unique advantage of placing the near-field probe at a location of interest chosen according to the SPM topographic information gathered simultaneously, in
contrast to EC-SERS and EC-SEIRAS studies in which substrate roughness leads to an inherent variation of the geometry of the surface adsorption sites and thus to a broad distribution of probed molecular adsorption geometries. Our approach thus enables selective monitoring of potential-dependent TER spectral changes from less than 100 adenine molecules adsorbed onto a Au(111) terrace with uniform adsorption geometry in which an averaging effect from different substrate/adsorbate geometries is limited. We complement the experimental EC-TERS results with density functional theory (DFT) gas-phase approximations for data analysis and interpretation.

Figure 1A depicts our scanning tunneling microscope (STM)-based EC-TERS experimental configuration. The potentials of the sample (working electrode 1, WE₁) and tip (WE₂) can be controlled independently with a biopotentiostat versus a Pt/H₂ reference electrode (RE) (all potentials are reported vs. Ag/AgCl). The TER signal is generated by side-illuminating the tip-sample gap in tunneling mode with a 632.8 nm excitation HeNe laser through a 50 x air-immersion objective (N.A. = 0.5) and is collected through the same objective in backscattering mode as described previously. The sample consists of an adenine self-assembled monolayer on a Au(111) single crystal in adenine-free 0.01M H₂SO₄. For details about the EC-TERS setup and sample preparation, see Section 1 of the Supporting Information. All spectra were recorded with the tip located in the center of a flat Au(111) terrace (Figure 1B, white dot) as confirmed by EC-STM imaging prior to and after spectral acquisition.

Figure 1C shows EC-TERS spectra recorded at different sample potentials, E_{sample}, in chronological order of acquisition from bottom to top. At low potentials, one prominent peak is present at circa 736 cm⁻¹ (ring breathing), and weaker bands appear at around 1320 cm⁻¹ (CN and CC stretch) and 1464 cm⁻¹ (N7–C8 stretch, C8–H bend, and NH₂ scissor), in agreement with previous reports for adenine/Au(111). At all potentials, a broad band is observed below 300 cm⁻¹ that can be decomposed, with help of Lorentzian band fitting, into three components (Figure 1D): The two peaks located at 172 and 220 cm⁻¹ that are also observed in all far-field spectra acquired upon tip retraction and do not show any potential-dependent changes; as their molecular origin is unclear, they will not be discussed further. Notably, the fitting reveals a near-field band at circa 260 cm⁻¹ at potentials greater than 0.6 V that has been assigned to Au–N interactions.⁶¹

To quantify the spectral changes as a function of E_{sample}, we plot amplitude and peak frequency of the 260, 736, and 1464 cm⁻¹ modes obtained from Lorentzian fits (Figure 2, details in the Supporting Information, Section 1). The corresponding CV recorded at 50 mVs⁻¹ inside the EC-TERS cell is shown as an inset in Figure 2B. The CV exhibits the two well-known peaks at 0.36 and 0.62 V (0.34 and 0.59 V) in the anodic (cathodic) scan direction.²⁷,²⁸ The first peak can be attributed to an increase in capacitance as a result of the molecular reorientation from a physisorbed tilted arrangement to a vertical geometry around the potential of zero charge,²⁷ such that more Au surface is exposed to the adenine-free electrolyte, in agreement with previous studies.²⁵,²⁷,²⁸ The second peak at around 0.6 V results from deprotonation and partial charge transfer from adenine to the Au electrode (around 0.68 electrons per molecule as determined by Aldaz and coworkers)⁹⁰,⁹¹,²⁵,²⁷,²⁸ Note that oxidation of adenine to its oxo forms occurs at potentials higher than 1.2 V outside our potential window.²⁶ Importantly, the CV was identical before and after the EC-TERS experiment, that is, after ramping up to 1.2 V several times, indicating that (partial) Au oxidation does not alter the behavior of adenine at the electrode (Supporting Information, Section 2).

The Au–N mode at circa 260 cm⁻¹ only appears after the second anodic peak and blue-shifts from 245 cm⁻¹ at 0.6 V to 274 cm⁻¹ at 1.2 V (Figure 2A, blue) while maintaining a constant intensity (variations within the noise level, Figure 2B, blue). In contrast, the intensity of the 736 cm⁻¹ mode shows a maximum at 0.3 V (Figure 2B, purple). In the region between the two anodic peaks, the band significantly falls steeply in intensity and further decreases after the second

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**Figure 1.** A) EC-TERS cell and electrode configuration; STM = scanning tunneling microscope; WE/CE/RE = working/counter/reference electrodes. B) Left: 228 x 228 nm² STM image (E_{sample} = 0.5 V, E_{bias} = 0.4 V, I = 1.35 nA); dot indicates EC-TERS position. Right: Au-step line profile as indicated in STM image. C) EC-TERS spectral evolution upon WE₁ potential ramp (1.77 mW laser power, 5 s acquisition time). Inset: neutral adenine. D) Lorentzian band fitting examples for the low wavenumber region.
peak at a less pronounced rate before disappearing at potentials above 1 V. Interestingly, the band shows a small, but measurable blue shift from 734.6 cm\(^{-1}\) at 0.3 V to 736.4 cm\(^{-1}\) at 1 V (Figure 2A, purple). The 1464 cm\(^{-1}\) mode shows a constant intensity between 0.2 and 0.6 V, it vanishes at 0.8 V, and reappears at 1 V (Figure 2, green). This mode blue-shifts by circa 10 cm\(^{-1}\) at high potentials.

Different phenomena, like changes in surface coverage, chemical conversion, molecular reorientation, and charge effects, could account for the observed potential-dependent spectral changes. Similar intensity variations of the ring breathing mode have been attributed to changes in the coverage in an adenine-containing electrolyte in an ensemble study.\(^{[13,14]}\) As we work in an adenine-free electrolyte, an increase in coverage would be uniquely due to molecular diffusion. By integrating over 5 s per spectrum and averaging over 5 spectra per data point, however, we average over random fluctuations produced by diffusion into and out of the hot spot. Furthermore, the recovery of the 736 cm\(^{-1}\) upon sweep reversal (Supporting Information, Section 3) and the constant intensity of the other Raman peaks as well as of the CV features (Supporting Information, Section 2) corroborate that the surface coverage in the near-field region remains constant throughout the hour-long experiment set. According to the literature, one adenine molecule occupies a surface area between 0.41 nm\(^2\) (upright) and 0.55 nm\(^2\) (flat).\(^{[39]}\) The TERS spatial resolution for small, non-resonant molecules in a Au-tip/Au-sample gap is on the order of a few nanometers, in which the near-field is sufficiently strong to provide detectable TER scattering.\(^{[42–45]}\) With an effective TERS scattering diameter of 6 nm, we can estimate the number of probed adenine molecules to be between 50 and 70, demonstrating the extreme sensitivity our EC-TERS setup holds particularly for non-resonant species.

In acidic media, (de)protonation from N1-protonated to neutral adenine has been suggested to occur upon potential sweep at around 0.6 V, facilitating chemisorption with a partial charge transfer from N lone-pairs of adenine to the d\(^{4}\)-band of Au(111) at higher potentials.\(^{[26–28,31]}\) As a transition between protonated, neutral, and partially oxidized adenine may induce band shifts, we have performed gas-phase DFT calculations (details in the Supporting Information, section 1) to compare the Raman shifts predicted for cationic, neutral, and N1-protonated molecules. We assign the experimental mode at 736 cm\(^{-1}\) to the simulated ring-breathing mode around 723 cm\(^{-1}\) and the experimental 1464 cm\(^{-1}\) band to the calculated mode at circa 1513 cm\(^{-1}\) based on the mode displacements returned by the calculations (Supporting Information, Section 4) in agreement with previous reports.\(^{[33,34]}\) DFT predicts a blue-shift from 721.69 (1511.89) cm\(^{-1}\) for the protonated molecule to 725.71 (1514.03) cm\(^{-1}\) for the neutral case to 731.72 (1569.53) cm\(^{-1}\) for the cation. Note that adenine is not fully oxidized within our potential window as discussed earlier; the cation calculation merely serves to confirm the trend of the Raman shift due to deprotonation and partial charge transfer upon chemisorption. This trend is in qualitative agreement with the blue-shifts of 2 and 10 cm\(^{-1}\) observed experimentally for the 736 and 1464 cm\(^{-1}\) modes, respectively. In addition, previous pH-dependent SERS experiments showed that at low pH, the intensity ratio of the 1320 and 1350 cm\(^{-1}\) bands is higher for the protonated species than for neutral adenine.\(^{[36]}\) We observe the same trend upon potential increase (Supporting Information, Section 5), which further supports the conclusion that at potentials below 0.6 V, protonated adenine molecules are present before deprotonation occurs at more positive potentials. While potential-induced deprotonation explains the observed band shifts and small variations in relative band intensities, it cannot account for the drastic intensity changes of the prominent ring-breathing mode.

Since the Raman scattering intensity is proportional to the forth power of the polarizability, potential-dependent molecular reorientation is expected to manifest itself in strong intensity changes as observed in the EC-TER spectra, provided the tensor elements exhibit anisotropy that is largely maintained upon adsorption. Note that in TERS, the excitation (near-)field is oriented parallel to the surface normal.\(^{[14]}\) As such, with knowledge of the respective Raman polarizability tensors of the vibrational modes of interest, the molecular orientation of the few molecules in the nanometer-confined near-field spot on the flat Au(111) terrace can be deduced from the observed intensity changes.\(^{[13,14]}\) As a first approximation, we have calculated the transition polarizability tensors of the 723 and 1513 cm\(^{-1}\) modes of an isolated

![Figure 2. A) Raman shift and B) integrated intensity of the the 260 cm\(^{-1}\) (blue), 736 cm\(^{-1}\) (purple), and 1464 cm\(^{-1}\) (green) bands as a function of sample potential. Error bars result from fittings. CV of a monolayer of adenine on Au(111) in 0.01 M H\(_2\)SO\(_4\) \(\nu = 50\) mV s\(^{-1}\) (B, inset).](image-url)
molecule in protonated and neutral forms by using DFT. The complete tensors are shown in the Supporting Information, Section 6. In our calculations, the z-axis is perpendicular to the rings’ plane (containing the x-y-axes, with y (short axis) parallel to the C4–C5 bond; Figure 3A, inset). The diagonal elements of the Raman tensors for the 723 cm\(^{-1}\) mode show a pronounced anisotropy between the x-y-plane of the adenine rings and the z-component for both protonated and neutral species with a ratio of $\alpha_{xx}:\alpha_{yy}:\alpha_{zz} = 3:3:1$, while the Raman tensor of the 1513 cm\(^{-1}\) mode shows a pronounced anisotropy in the x,y-plane with a ratio of 14:1:1 for the neutral form of adenine and 20:2:1 for the protonated case.

Figure 3A displays the simulated intensity variation of the 723 and 1513 cm\(^{-1}\) modes for different orientations of the adenine molecule with respect to the electrode surface (Supporting Information, Section 7). The quantitative deviation between the absolute intensity values of experiments and simulations is in line with weakly physisorbed adenine in which the adsorbate–substrate interaction is expected. Interestingly, the Au–N mode only appears at potentials above 0.6 V after deprotonation; the Au–N bond is expected to be more affected by the exclusion of the electrode in the simulations than the delocalized ring-breathing mode. Simulations of adenine at solid/liquid interfaces beyond gas-phase calculations could give polarizability tensors that better describe the experimental results.

Adenine reorientation from vertical to horizontal upon a potential increase above 0.3 V is further supported by the analysis of the low-wavenumber mode at circa 260 cm\(^{-1}\). This band has previously not been investigated experimentally in detail, despite the wealth of information it contains about the adsorbate–substrate interaction. Interestingly, the Au–N mode only appears at potentials above 0.6 V after deprotonation. The significant blue-shift from 245 to 274 cm\(^{-1}\) can be attributed to a strengthening of the Au–N bond with increasing potential, that is, an increasing interaction between the N lone pairs and the metal d*-band that results in a planar orientation of adenine. Coordination of neutral adenine through the amino group N10 and N1 at potentials above the anodic peaks in the CV is consistent with all observed trends. The lack of the Au–N mode at potentials below 0.6 V is in line with weakly physisorbed adenine in which the monolayer is stabilized by π-d* adsorbate–substrate or π–π intermolecular interactions at potentials below or around 0.3 V, respectively.[25,30,32] Figure 4 summarizes the adsorption/reaction model of the potential-dependent, reversible adenine (de)protonation and reorientation as deduced from our EC-TERS results.

To conclude, we have developed a novel EC-TERS setup to study the electrochemical behaviour of small non-resonant molecules at electrified interfaces with a spatial chemical resolution of a few nanometers and sensitivity below 100 mol-
ecules. For the adenine/Au(111) system, EC-TERS in combination with DFT results allow us to deduce a reversible adsorption/reaction model in which protonated adenine is adsorbed onto atomically flat Au(111) in a tilted geometry at low potentials and in an upright configuration with its short molecular axis perpendicular to the surface around the potential of zero charge. Upon potential increase, the molecule adopts a flat adsorption geometry with strong Au–N interactions between deprotonated adenine molecules and the gold substrate. With this important extension of EC-TERS capabilities to evaluate adsorbate orientation and chemical conversion as a function of potential, in situ local chemical information at the nanoscale becomes accessible for a full range of electrochemical systems.

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Conflict of interest

The authors declare no conflict of interest.

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Figure 4. Proposed potential-dependent reversible adsorption/reaction model for adenine/Au(111).