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Tip-Enhanced Raman Spectra of Picomole Quantities of DNA Nucleobases at Au(111)

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The label-free detection of smallest sample amounts by enhanced Raman spectroscopy is gaining considerable attention in biochemical and biophysical sciences.[1−3] Recently, it was shown by Rasmussen and Deckert that the extremely high sensitivity of surface-enhanced Raman spectroscopy (SERS) renders possible the identification of the DNA base moieties even in their corresponding deoxynucleotides,[4] and Bell and Sirimuthu presented an improved SERS experiment for the analysis of mononucleotides in nanomolecular quantities,[5] important steps toward the investigation of DNA strands. The disadvantage of SERS, however, lies in the fact that rough sample surfaces or colloids of the coinage metals (Cu, Ag, Au) have to be employed for sufficient Raman enhancement.[6]

Tip-enhanced Raman spectroscopy (TERS) allows a highly sensitive vibrational analysis of molecules adsorbed in (sub)-monolayer quantities at atomically smooth surfaces, not limited to coinage metal substrates.[7] In addition to the chemical fingerprint of the investigated species, scanning tunneling microscope (STM) images can be obtained simultaneously from the same nanometer-sized sample. Our group recently demonstrated that even ≤ 5 dye molecules with vibrational excitations in resonance with the excitation laser line at 632.8 nm (resonant molecules) can be investigated with TERS.[8] In this Communication, we present the TER spectra of (sub)monolayers of the four DNA bases adenine, guanine, thymine and cytosine, respectively, adsorbed homogeneously at Au(111) in picomole quantities. The purine and pyrimidine bases of the nucleic acids absorb at wavelengths shorter than 280 nm.[9] With this study, we prove the applicability of TERS for biochemically highly relevant, optically nonresonant species and highlight the sensitivity of this technique.

The nucleobases were adsorbed at a freshly annealed Au(111) single crystal from 1 mM ethanolic solutions. After 1 h adsorption time, the crystal was rinsed with 10 mL of ethanol to remove multilayers. A sharp Au STM-tip (20 nm apex radius) was moved toward the sample into tunneling contact and illuminated by red laser light (632.8 nm) of approximately 2 mW incident power (laser focus: 1 μm diameter). A detailed description of the TERS experiment is given elsewhere.[8] The creation of a giant electromagnetic field (EF) underneath the tip apex ($r_{EF} \approx r_{tip} \approx 20$ nm)[8] leads to intense Raman scattering by the molecules adsorbed in the EF region (1260 nm^2), that is, directly beneath the tip apex.

According to the molecular dimensions and STM results presented in the literature,[10−13] the largest molecular density of adsorbed nucleobases can be estimated to around 2−2.5 molecules/nm^2 for closest packing in a monolayer. In our TERS experiment, this corresponds to approximately 3000 molecules present in the enhanced field region under investigation, that is, only 420 pmol/cm^2 of target species. Note that this value is a maximum value to find the most conservative TERS detection limit. As DNA bases adsorb only weakly at Au,[14,15] the real surface coverage is likely to be even smaller, because perfect packing order may not be achieved within 1 h adsorption time and rinsing the sample may wash off some molecules. Figure 1 shows the respective Raman vibrational fingerprints of monolayers of the four DNA bases after spectral background correction. The fact that TER spectra of slightly stronger adsorbed adenine give a much better signal-to-noise ratio than the other nucleobases' spectra supports the theory of enhancement to some extent through chemical adsorbate/substrate interaction as proposed by Otto.[16]

The near-field TER spectra can be compared to unenhanced (far-field) Raman spectra of bulk samples and to SER spectra (Ag substrate),[17−20] and unambiguously assigned to the respective nucleobase. The label-free sensoring of picomole quantities of small, nonresonant biomolecules is reproducible and straightforward with TERS. The main difference between the enhanced and unenhanced spectra are the Raman bands that appear at low wavenumbers around 220−250 cm^{-1}, which are assigned to the molecule−metal bond and thus cannot be found for powder or crystalline samples. In contrast to the ones obtained from nanocrystalline samples by Rasmussen and Deckert,[4] our TER spectra of homogeneously distributed nucleobases at Au(111) exhibit clear similarities with SER and not with unenhanced Raman spectra.

To illustrate the versatility of combining topographic and chemical information in TERS, an example STM image of a thymine monolayer at Au(111) is presented as an inset in Figure 1. The row structure observed after 12 h adsorption is in excellent agreement with results published by Boland and Ratner who determined the surface lattice structures of purine and pyrimidine DNA bases on Au(111).[21] Note that the STM image was obtained with a similar Au probe as employed in the TERS experiments.

To determine the TERS detection limit for optically nonresonant species, we lowered the concentration of the adenine adsorption solution to 7.6×10^{-8} M, thus considerably decreasing the surface coverage. The recorded Raman fingerprint allows the identification of adenine by the prominent band at 731 cm^{-1} assigned to its ring-breathing mode. Figure 2 compares (a) the TER spectrum obtained from full coverage, that is, maximal around 3000 molecules present in the EF region, and (b) the one from submonolayer coverage.

The integrated ring-breathing mode band intensities are 16500 cts/s in spectrum a and 730 cts/s in spectrum b, approximately 20 times smaller for the submonolayer coverage in comparison to the monolayer coverage. Taking into account a linear relation between the number of nonresonant (nonabsorbing) scatterers and the band intensity, that is, 5.5 cts/s per molecule, we are able to detect around 130 adenine molecules with TERS at low coverage, or approximately 18 pmol/cm^2.

Employing the same TER setup for the nonresonant adenine as for the resonant dye,[8] thus creating identical experimental conditions, enables us to compare resonant and nonresonant scatterers

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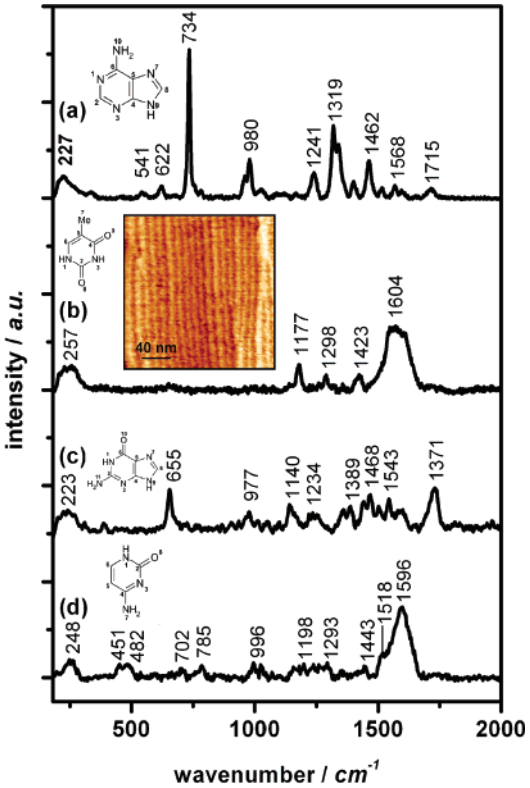

Figure 1. TER spectra (background-corrected) of DNA bases adsorbed at Au(111): (a) adenine; (b) thymine; (c) guanine; (d) cytosine; normalized to 1 s integration time at 2 mW incident power. The inset shows an example STM image of a thymine self-assembled monolayer on Au(111) recorded after 12 h adsorption with a Au probe; $I_t = 1$ nA, $E_{bias} = 150$ mV, scan area as indicated.

and to experimentally estimate the contribution of optical resonance to the overall Raman enhancement, as widely discussed in the literature.[22] Let us compare the scattering intensity per molecule obtained from the largest band, respectively, of adenine (5.5 cts/s per molecule) and of the dye (783 cts/s).[8] We find that the TER scattering cross section of the nonresonant molecule is reduced by approximately a factor of 140 in comparison to the one of the resonant species. This is direct experimental evidence for the pure resonance contribution to the Raman enhancement. The difference in the detection limit for resonant (single molecule)[8] and nonresonant (130) molecules agrees very well with this number. We conclude that the resonance contribution to TER scattering indeed plays a role in the overall Raman enhancement, apparently (still) a necessary one for the detection of single molecules. It is, however, rather small in comparison to the electromagnetic enhancement which could still be increased with sharper tips. In addition, better collection devices in the setup, as currently being constructed in our laboratory,[23] should improve the performance of TERS to allow single-molecule detection of arbitrary nonresonant species.

Our results elucidate the extreme sensitivity already reached with TERS, opening an interesting route toward label-free sensoring at atomically smooth substrates with well-defined adsorption sites. Working at atomically smooth substrates, orientational studies on adsorbates at surfaces, on folding mechanisms or on molecular

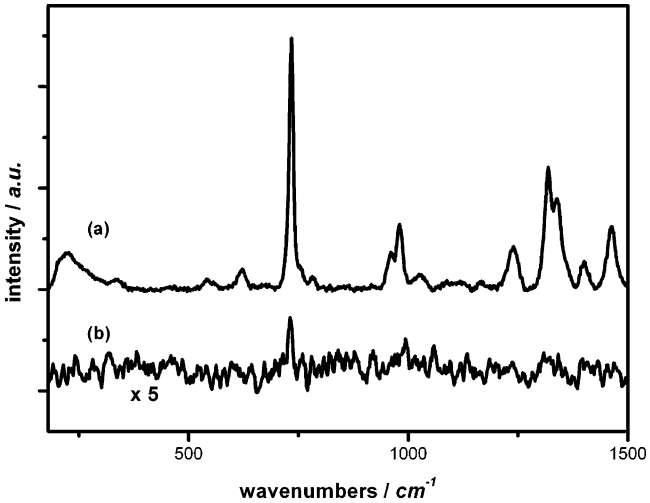

Figure 2. TER spectra of adenine adsorbed at Au(111) normalized to 1 s integration time at 2 mW incident power: (a) monolayer coverage of around 3000 molecules present in the enhanced-field region; (b) submonolayer coverage of around 130 molecules present in the enhanced-field region; intensity multiplied by a factor of 5 for easier band comparison.

switches, which are of great interest in (bio)chemical sciences, may be realized with this technique. Experiments on the adsorption geometry of the DNA bases at Au(111) and on the interaction of base pairs are in progress in our laboratory.

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