Distinguishing Individual DNA Bases in a Network by Non-Resonant Tip-Enhanced Raman Scattering

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Abstract: The importance of identifying DNA bases at the single-molecule level is well recognized for many biological applications. Although such identification can be achieved by electrical measurements using special setups, it is still not possible to identify single bases in real space by optical means owing to the diffraction limit. Herein, we demonstrate the outstanding ability of scanning tunneling microscopy (STM)-controlled non-resonant tip-enhanced Raman scattering (TERS) to unambiguously distinguish two individual complementary DNA bases (adenine and thymine) with a spatial resolution down to 0.9 nm. The distinct Raman fingerprints identified for the two molecules allow to differentiate in real space individual DNA bases in coupled base pairs. The demonstrated ability of non-resonant Raman scattering with super-high spatial resolution will significantly extend the applicability of TERS, opening up new routes for single-molecule DNA sequencing.

Nucleobases are the basic building block of life, and the sequencing of DNA is important for many biological and medical applications. Although many electrical and fluorescence methods have been used for DNA sequencing over the years,[1,2] it is always desirable to develop new techniques with better accuracy and precision.[3] Tip-enhanced Raman scattering (TERS) offers an attractive approach to this end, as it can reach nanometer resolution with chemical sensitivity to distinguish different molecules.[4–7] In recent years, the TERS technique has been applied to resolve DNAbases in many systems, such as self-assembled monolayers,[8,9] nanoclusters,[10–15] and even more complicated compounds including nucleobase polymers[16,17] and DNA-strands and bundles.[18–20] However, the spatial resolution reported in those TERS studies is still within several nanometers,[10,12,17,18] which is not adequate to realize the unambiguous identification of single DNA bases in real space. Recently, sub-nanometer spatial resolution has been demonstrated for the chemical identification of porphyrin molecules by resonant TERS in the visible region with a green laser.[21–23] When the same experimental setup is employed for DNA bases featuring optical absorption in the ultraviolet region (ca. 260 nm), the whole Raman process thus becomes non-resonant with reduced Raman intensities. Whether the non-resonant TERS could achieve the same spatial resolution as the resonant one is an open question that is yet to be answered. In other words, it is still highly challenging to resolve DNA bases at the single-molecule level by Raman spectroscopy.

In this work we address this issue by carrying out a proof-of-principle experiment for two complementary DNA bases, adenine (A) and thymine (T), using our STM-controlled TERS technique under ultrahigh-vacuum (UHV) and low-temperature (LT) conditions. To obtain the maximum plasmonic enhancement for DNA bases under the excitation of a green laser (532 nm), silver (Ag) was used as both tip and substrate materials, owing to its lowest optical losses in the visible region in comparison with other plasmonic materials.[25,26]

Our TERS experiments were performed with a custom LT-UHV STM under a base pressure of circa 1 × 10–10 torr at circa 80 K, equipped with a side-illumination confocal-type optical system, as detailed in the Supporting Information. Molecular samples were prepared by thermally depositing the A and T molecules onto freshly cleaned Ag(111) surfaces at room temperature separately. Figure 1a,b shows the high-resolution STM topographs of A and T molecules with atomically flat-lying adsorption geometry on the surface. These network patterns agree well with the structure of the molecular skeleton, suggesting a flat-lying adsorption geometry on the surface. These network patterns agree well with the reported assembly structures of DNA bases adsorbed onto Au(111), in which the molecules are physically adsorbed onto the surface and tightly bound through hydrogen-bonding (H-bonding) with three neighboring molecules in the case of the A-network[27,28] and by H-bonding as well as weak van der Waals (vdW) interactions in the case of the T-network.[29] The weak surface adsorption could also be the reason why we were unable to obtain isolated single A or T molecules at room temperature or even 80 K in the present experiments.[27]

Figure 1c shows typical TERS spectra acquired from the molecular networks shown in Figure 1a,b, which display...
distinctly different spectral profiles from each other. Specifically, the spectrum acquired from the A-network (blue curve) gives a pronounced peak at approximately 680 cm\(^{-1}\), whereas the TERS spectrum from the T-network (red curve) has a sharp and strong peak at approximately 881 cm\(^{-1}\). Such spectral differences provide reliable fingerprints to directly distinguish these two different DNA bases on the surface by TERS.

Due to the TERS selection rule\(^{21,22}\) and molecular interactions with the Ag substrate or tip\(^{11,13}\), the overall spectral features of the TERS spectra for both A and T bases on Ag(111) are quite different from their respective powder Raman spectra (see Figure S1 in the Supporting Information for details). The in-plane vibrational modes of the molecules, such as the characteristic in-plane ring-breathing modes of both bases (ca. 720 cm\(^{-1}\) for A and ca. 740 cm\(^{-1}\) for T), are considerably suppressed, which suggests a flat-lying configuration for both A and T molecules in the molecular networks. Such spectral changes are consistent with previous STM-TERS studies for DNA-base monolayers on the gold surface.\(^{23}\) The exact reason for the appearance of the predominant peaks such as those at 680 cm\(^{-1}\) for A and 881 cm\(^{-1}\) for T in the TERS spectra is still not completely clear but could possibly correlate with a chemical enhancement caused by the interaction with silver substrate or tip.\(^{11,13}\)

It is very encouraging that non-resonant TERS can indeed produce well-defined spectra with high signal-to-noise ratios for DNA bases. However, it is not clear how many DNA bases have contributed to the spectra. To verify this, the spatial resolution of TERS on the DNA-base molecular networks has to be determined, which could be done by sequentially acquiring a series of TERS spectra across the edges of the A- and T-network separately. Figure 2a,c shows the waterfall plots of 20 TERS spectra that were acquired along the line traces shown in the insets. A sharp drop in the TERS signals appears on the spectral map when the tip moves across the edge. Such an abrupt change on the optical contrast can be further observed from the analysis of the corresponding intensity profile of the featured peak for A (integrated over 623–742 cm\(^{-1}\)) and the 900 cm\(^{-1}\) mode of T (integrated over 834–939 cm\(^{-1}\)) taken from the TERS spectra in (a) and (c), respectively. The corresponding height profile of the line trace is also shown in the bottom blue curve for comparison.
The achieved spatial resolution of circa 0.9 nm is much smaller than previous results measured on DNA bases and related structures using AFM setups.[10,12,17,18] The involvement of highly confined plasmonic field inside the STM junction defined by the silver tip and silver substrate could be responsible for such a high resolution.[30] Note that this resolution value obtained under the non-resonant condition for DNA bases is only slightly larger than that achieved previously for porphyrin molecules and carbon nanotubes under the resonant condition with the same setup.[21,22,30] This is really a pleasant surprise, which implies that STM-TERS could in practice be applied to a very large number of systems for sub-nanometer resolved Raman studies.

With such a high resolution, it would be interesting to examine whether the non-resonant TERS can be employed to distinguish adjacent individual DNA bases in the base-pairing network. We have thus carried out TERS measurements on a mixed network structure with co-adsorbed A and T molecules. The co-deposited sample was fabricated by depositing a small quantity of T molecules onto a surface that already contained a submonolayer of A molecules on Ag(111), as shown in Figure 3a. Because both A and T bases show up as bright dots (Figure 3b, c), it is difficult to distinguish with certainty the A bases from the T bases at the boundary (black dash line) by STM imaging alone. Nevertheless, by comparing the mixed network STM image with the STM image of the pure A-networks (see Figure S2 in the Supporting Information), we can speculate that the co-deposited T molecules are likely located outside the boundary of the A-network (lower-right ordered area). Interestingly, some of the T molecules tend to form repeated trimer-like structures at the boundary (marked by an arrow and white square in Figure 3a).

We then carried out single-point TERS measurements around the boundary area that contains the trimer structures, as indicated in the STM inset of Figure 3b, with the tip positioned above selected individual DNA bases. As shown in Figure 3b, the TERS spectra acquired above the six different positions vary significantly and can be classified into two main types. The spectra measured at positions 1, 5, and 6 constitute one type featuring a strong TERS peak at circa 680 cm\(^{-1}\). These molecules can thus be identified as A bases by referring to the TERS spectral database provided in Figure 1c. Similarly, the TERS spectra measured at positions 2, 3, and 4 (that is, on the trimer) form another type featuring a strong peak at circa 880 cm\(^{-1}\) and thus these molecules can be identified as T bases. A model for the molecular arrangement can thus be proposed, as shown in the schematic below the STM inset in Figure 3b.

Figure 3. a) STM topograph showing T molecules adsorbed at the edge of an A-molecular network (−1 V, 5 pA). The black dashed line indicates the boundary of the A-network. b) Single-point TERS spectra (0.1 mW, −0.1 V, 0.3 nA, 30 s) acquired from the different positions indicated in the STM inset, the area of which is marked by the white square in (a). The schematic below the STM inset shows a proposed molecular arrangement for the numbered molecules. c) High-resolution STM imaging (−1 V, 5 pA) around the co-adsorption boundary. d) Corresponding spectral evolution for 20 sequential TERS measurements along the line trace marked by crosses in (c) (0.3 mW, −0.1 V, 1 nA, 30 s) with a step size of 0.27 nm. e) A proposed molecular arrangement along the line trace in (c).
indicates that the tip is likely located above the open space between the A and T molecules (Figure 3e).

In summary, we have demonstrated for the first time that the non-resonant TERS technique is also capable of achieving sub-nanometer spatial resolution with the ability to distinguish different molecules. With this technique, we have distinguished individual DNA bases in a hydrogen bonded DNA-base molecular network in real space. Our results open up new routes to realize single-molecule DNA sequencing on surfaces by Raman spectroscopy.

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Conflict of interest

The authors declare no conflict of interest.

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