Probing single-molecule conformational heterogeneity at room temperature via hyperspectral tip-enhanced Raman imaging

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Abstract

Cryogenic tip-enhanced Raman spectroscopy (TERS) studies have revealed single-molecule dynamics; however, understanding the nature of single molecules in ambient conditions has remained challenging. Here, we demonstrate the hyperspectral TERS imaging of single brilliant cresyl blue (BCB) molecules, along with quantitative spectral
analyses, revealing their conformational heterogeneity in ambient conditions. Robust single-molecule imaging is enabled by encapsulating the molecules with a thin Al₂O₃ film, which suppresses spectral diffusions and inhibits chemical reactions and contaminations in air. For the single molecules resolved spatially in the TERS image, a clear Raman peak variation up to 7.5 cm⁻¹ is observed, which cannot be found in molecular ensembles. From density functional theory-based quantitative analyses of the varied TERS peaks of single molecules, we reveal the single-molecule conformational heterogeneity at room temperature. This work provides a facile way to investigate the single-molecule properties in interacting media, expanding the scope of single-molecule vibrational spectroscopy studies.

Introduction

The fundamental properties of molecules, e.g., conformations, spectral diffusions, and intra- and inter-molecular couplings, in different chemical environments can be understood via Raman scattering, based on their characteristic spectral "fingerprint." Through conventional Raman spectroscopy, however, spectroscopic responses are only obtained from a number of molecules or a molecular ensemble due to extremely low scattering cross-sections, which cannot provide detailed information corresponding to single molecules. Single-molecule detections were enabled by surface-enhanced Raman scattering (SERS) with a highly enhanced local field at the plasmonic gap, but its diffraction-limited spatial resolution does not allow the measurement of single molecules in heterogeneous chemical environments. By contrast, tip-enhanced Raman spectroscopy (TERS) gives access to extremely low vibrational responses of single molecules and even individual chemical bonds in a single-molecule using a strongly localized optical field at the plasmonic nano-tip, controlled by scanning probe microscopy approaches. In pioneering single-molecule TERS studies, a few groups technically detected single molecules at room temperature, however, only limited molecular properties were revealed due to the rapid structural dynamics of molecules in
ambient conditions. Recently, single-molecule TERS experiments were performed at cryogenic temperatures under ultrahigh vacuum (UHV) conditions; these experiments revealed the fundamental nature of single molecules, such as conformational heterogeneity, intramolecular coupling, vibrational dephasing, and molecular motion. However, single-molecule TERS experiments at room temperature and ambient conditions are still significant and should be further investigated because molecular properties can change depending on temperature and atmospheric conditions. Specifically, understanding the conformational heterogeneity of single molecules in the non-equilibrium state is highly desirable because it can address many fundamental questions regarding the structure and function of many biological systems, such as protein folding and RNA dynamics.

To perform single-molecule TERS measurements in ambient conditions, a well-designed TERS setup and appropriate sample preparation are required for the robust detection of Raman scattering responses of single molecules with the highest optical sensitivity. Among three types of common TERS geometries, i.e., the side-, bottom-, and top-illumination modes, the bottom-illumination TERS setup provides the largest excitation rate by using a high numerical-aperture (NA) objective lens in combination with a radially polarized excitation beam. However, the metal substrate for the bottom-illumination mode should not only be transparent, for high transmittance of excitation and scattering signals, but also have enough thickness to induce strong dipole-dipole interactions between the tip dipole and the image dipole emerging in the metal film; therefore, the thickness of metal film needs to be optimized to achieve TERS sensitivity at the single-molecule level.

Here, we perform TERS experiments on brilliant cresyl blue (BCB) molecules under ambient conditions, revealing the conformational heterogeneity of single molecules through the spatio-spectral TERS imaging. To obtain a highly sensitive TERS response, we use the bottom-illumination mode with an oil-immersion objective lens (NA = 1.3) and radially polarized excitation light. We then carefully design and prepare the single-molecule sample onto an optimized Au/Cr metal film (thickness $t \sim 11$ nm) deposited on a coverslip, with a
thin dielectric capping layer (0.5 nm thick Al₂O₃), for robust TERS measurements. Through this optimization process, we obtain a single-molecule TERS map at room temperature, enabling the probing of the spatial heterogeneity of the single BCB molecules adsorbed on the Au surface. Furthermore, through the quantitative analysis of the measured TERS frequency variation through density functional theory (DFT) calculations, we provide a comprehensive picture of the conformational heterogeneity of single molecules in interacting media.

Results and discussion

Experimental section

For highly sensitive single-molecule detection at room temperature, we used bottom-illumination mode TERS, as illustrated in Fig. 1a. As a sample system, BCB molecules were spin-coated on the thin metal film and covered by an Al₂O₃ capping layer to suppress rotational and spectral diffusions. We used an electrochemically etched Au tip attached to a tuning fork for normal-mode AFM operation (see Methods for details). Using an oil-immersion lens (NA = 1.30), we could obtain a focused excitation beam with a sub-wavelength scale, which can highly reduce the background noise of far-field signals in TERS measurements. Furthermore, in combination with the radially polarized excitation beam, we achieved strong field localization in the normal direction with respect to the sample surface, i.e., a strong out-of-plane excitation field in parallel with the tip axis. The excitation field, with a wavelength of 632.8 nm, is localized at the nanoscale tip apex, and the induced plasmon response gives rise to the resonance Raman scattering effect with the BCB molecules. Note that the thin dielectric capping layer (0.5 nm thick Al₂O₃) on the molecules also prevents possible contamination of the Au tip, e.g., adsorption of the probing molecules on to the tip surface, which can cause artifact signals (see Fig. S1).

Fig. 1b shows the far-field and TERS spectra of BCB molecules measured with linearly
Figure 1: (a) Schematic illustration of bottom-illumination mode TERS. The encapsulated BCB molecules on the Au surface are excited by a radially polarized beam ($E_{\text{Exc.}}$) and the back-scattered TERS responses are collected. (b) Far-field (black) and TERS spectra with different excitation polarization conditions. The TERS response with the radially polarized excitation laser (red) gives a larger enhancement compared to that obtained with the linearly polarized laser (blue). (c, d) AFM topography images of thin metal films fabricated with different deposition rates and cleaning methods of the substrate (coverslip). The coverslip for the non-optimal metal film (c) is cleaned using piranha solution, and the metal film is fabricated with a deposition rate of 0.01 nm/s. By contrast, the coverslip for the optimal metal film (d) is cleaned by ultrasonication in acetone and isopropyl alcohol along with O$_2$ plasma, and the metal film is fabricated with a deposition rate of 0.1 nm/s. (e) Topographic line profiles of two thin metal films, derived from (c) and (d).

and radially polarized excitation beams. With the exposure time of 0.5 s, we hardly observed the far-field Raman response of molecules (black), due to the extremely low Raman scattering cross-section. By contrast, we observed a few distinct Raman modes via the TERS measurements with a linearly polarized excitation (blue). Moreover, through the radially polarized excitation,[41,42] we observed most of the normal modes with a substantially larger TERS intensity (red) compared to the TERS spectra measured with the linearly polarized excitation. For example, the C-H$_2$ scissoring mode at $\sim$1360 cm$^{-1}$ is clearly identified in the TERS spectrum measured with the radially polarized light, whereas it is not present in the TERS spectrum measured with the linearly polarized light.
Optimization of the metal substrate for TERS

In bottom-illumination mode TERS, the deposition of thin metal films on the coverslip is required to preserve the transparency of the substrate. The surface of the thin metal films should be flat, to avoid SERS and fluorescence signals originating from the metal nano-structures. This is because these background noise signals interrupt the clear TERS signals of target molecules. To prepare the aforenoted thin metal film on the coverslip, we need to optimize the cleaning method, adhesion layer, and metal deposition condition. To demonstrate the influence of the surface condition of metal films, we performed a control experiment based on Au films fabricated with different conditions (see Table S1 for detailed control parameters). For the first substrate, we cleaned the coverslip using a piranha solution and initially deposited a Cr layer with a thickness of 2 nm to improve adhesion between the Au film and the glass surface. We then deposited the Au film with a target thickness of 5 nm at the rate of 0.01 nm/s (see Methods for details). Fig. 1c shows an AFM topography image of the deposited metal film. Instead of a flat surface, island-like metal nano-structures are formed due to the low deposition rate and the damaged surface of the coverslip. For comparison, we cleaned another coverslip through ultrasonication in a bath and fabricated the metal with a higher deposition rate. Specifically, we sonicated the coverslip with acetone and isopropanol, followed by oxygen plasma treatment to remove the remaining surface residues. We then deposited the Au film with a Cr layer on the glass at the rate of 0.1 nm/s with the same thickness (5 nm). The surface roughness of the fabricated metal film was characterized through AFM topography, as shown in Fig. 1d. In contrast to the first substrate, the island formation is not found, and the surface roughness is decreased significantly to \(~0.3\) nm. The line profiles in Fig. 1e clearly show the differences in height and shape for the two fabricated metal films (see Table S1 for more results with various fabrication conditions). In addition, from the optical characterization of these two metal films, we verify the significant influence of the surface roughness on the results of TERS experiments (see Fig. S2 for comparison results of transmittance and TERS response). Therefore, fabrication of flat metal thin films
Another important parameter for bottom-illumination mode TERS is the metal film thickness, because a sufficiently thick metal film is required to induce strong dipole-dipole interactions between the tip dipole and the mirror dipole of the metal film. However, the light transmission decreases with increasing metal thickness, which gives rise to a reduced excitation rate and collection efficiency in TERS. To experimentally determine the optimal thickness, we deposited Au films on O$_2$-plasma-cleaned coverslips with a Cr adhesion layer. We prepared six metal films with various thicknesses of 5, 7, 9, 11, 13, and 15 nm. Among these metal substrates, we could not perform TERS experiments with the 15 nm metal film.

with the optimized deposition rate and cleaning method is important to perform highly sensitive and reproducible TERS experiments.
because it was difficult to align the tip apex to the laser focus due to low light transmission. Regarding the 5 and 7 nm metal films, we could barely observe TERS signals from the BCB film because the TERS enhancement factor was too low. Therefore, we performed a control experiment with three different metal substrates, namely with metal thicknesses of \( t = 9, 11, \) and 13 nm. To compare the relative TERS intensities of BCB film for these three metal substrates, we obtained the TERS spectra for these substrates with three different Au tips, i.e., each sample was measured using three Au tips. Fig. 2a shows a comparison of the measured TERS intensities with respect to the thickness of the metal films. We consider the strongest TERS peak at \( \sim 580 \text{ cm}^{-1} \) and determine the relative TERS intensities for different metal films. When we used three different tips for this control experiment, the TERS enhancement factors in each case were different; nevertheless, the metal film with 11 nm thickness yielded the strongest TERS signal for all the tips. Therefore, we normalize the TERS intensity measured for the 11 nm metal film to \([0, 1]\) for all three tips and compare the relative TERS intensities measured for the 9 and 13 nm metal films for each tip, as displayed in Fig. 2a. The black circles indicate the average TERS intensities for the three tips, for each substrate. The TERS intensities of the \( \sim 580 \text{ cm}^{-1} \) peak, measured for the 9 nm and 13 nm thick metal films, are \( \sim 30 \% \) and \( \sim 60 \% \) lower than that measured for the 11 nm metal film.

We then verified the ideal metal film thickness through theoretical approaches. First, we calculated the localized optical field intensity between the Au tip apex and the Au surface with respect to the metal film thickness using finite-difference time-domain (FDTD) simulations (see Methods for details). Fig. 2b and c show the simulated \(|E_z|^2\) distributions for the metal film thicknesses of 9 nm and 11 nm, respectively. When we set the distance \( d \) between the Au tip and Au surface to \( d = 3 \text{ nm} \) (i.e., the expected gap in tuning fork-based AFM), we achieve the maximum excitation rate for TERS, \(|E_z|^2 \approx 1000\), with the metal thickness of 11 nm. Fig. 2d shows the expected optical signal \((|E_z|^2 \times T^2\), where \( T \) is the calculated transmittance\) as a function of the metal film thickness in the bottom-illumination
geometry. $T$ is calculated with the following formula:\cite{46}

$$T = \left| \frac{E(t)}{E_0} \right|^2 = e^{-4\pi\kappa t/\lambda},$$

(1)

where $E_0$ and $E(t)$ are incident and transmitted optical field amplitudes, $\kappa$ is the extinction coefficient of Au at the given wavelength $\lambda$ (632.8 nm), and $t$ is the thickness of the metal film (see also Fig. S3 for the experimentally measured transmittance).\cite{47} $|E_z|^2$ at each film thickness is obtained from FDTD simulations and multiplied by $T^2$, as the light passes through the metal film twice. $|E_z|^2 \times T^2$ is gradually enhanced with an increase in thickness up to $t = 11$ nm, but interestingly, it starts to decrease from 12 nm. To understand this behavior, we performed the same thickness-dependence simulations for different gaps between the tip and the metal surface (see Fig. S4 for simulated results). Through these simulations, we found that the optimal metal film thickness varies slightly depending on the gap; nevertheless, the optimal metal film thickness is $\sim 11 - 12$ nm irrespective of the tip-surface gap (see SI for details).

**Single-molecule TERS imaging at room temperature**

We then performed the hyperspectral TERS imaging of single isolated BCB molecules adsorbed on the optimal metal film ($t = 11$ nm). As we described in detail in the experimental section, the thin dielectric capping layer (0.5 nm thick Al$_2$O$_3$) allowed us to stably detect single-molecule responses under ambient conditions. Fig. 3a and b show the TERS integrated intensity images of the vibrational modes at $\sim 580$ cm$^{-1}$ (in-plane stretching mode of C and O atoms in the middle of the molecule) and $\sim 1160$ cm$^{-1}$ (in-plane asymmetric stretching mode of O atom), which are only two recognizable TERS peaks of a single or a few BCB molecules, due to the short acquisition time (0.5 s) in our TERS mapping.\cite{22,23} In the TERS images of both the $\sim 580$ cm$^{-1}$ and the $\sim 1160$ cm$^{-1}$ modes, the TERS intensity of the detected regions shows a spatial variation even though the responses are detected in
Figure 3: TERS mapping images of single BCB molecules measured with the excitation laser power of 220 µW and acquisition time of 0.5 s at each pixel under ambient conditions. TERS peak intensity images for in-plane symmetric stretching mode of O-C\textsubscript{2} and N-C\textsubscript{2} observed at ∼580 cm\(^{-1}\) (a) and in-plane asymmetric stretching mode of O-C\textsubscript{2} observed at ∼1160 cm\(^{-1}\) (b). (c) TERS peak-to-peak intensity ratio image of ∼580 cm\(^{-1}\) and ∼1160 cm\(^{-1}\) peaks, arithmetically calculated from TERS images (a) and (b) after filtering a background fluorescence signal. Yellow dashed circles in (a-c) indicate the same positions in the TERS images.

similar nanoscale areas. This spatially heterogeneous intensity distribution originates from the difference in the number of probing molecules and/or the molecular orientation on the Au surface. Since the apex size of the electrochemically etched Au tip is larger than ∼15 nm, a few molecules under the tip can be detected together, which gives rise to a strong TERS response. Alternatively, although some of the observed TERS responses are from single molecules, the Raman scattering cross-section can differ from molecule to molecule due to their orientations and the corresponding TERS selection rule.\[^{11}\] Specifically, because the excitation field in our TERS setup has a strong out-of-plane polarization component, the peak-to-peak Raman scattering intensity changes depending on the conformation of a molecule. Hence, the conformational heterogeneity of probed molecules can be best exemplified with the TERS peak-to-peak intensity ratio, as shown in Fig. 3c.

From the TERS response corresponding to the nanoscale regions in the TERS image, we can infer the possibility of single-molecule detection; nevertheless, more substantial evidence is needed to verify this possibility. In addition to the aforementioned molecular orientation and the selection rule, the vibrational energy of the normal modes of an adsorbed molecule
Figure 4: (a) TERS peak intensity image of the vibrational mode at $\sim 580 \text{ cm}^{-1}$. (b) TERS spectra measured at spots 1, 2, and 3 indicated with red circles in (a). TERS spectra measured at spots 1-3 (c) and spots 4-9 (d) fitted with the Voigt line shape function in the range from 565 cm$^{-1}$ to 595 cm$^{-1}$. From the observed TERS peak shift in Fig. 4d, the TERS spectra at spots 1-3 (red circles in Fig. 4a) and spots 4-9 (dark blue circles in Fig. 4a) are possibly measured from a few BCB molecules and single BCB molecules, respectively. In Fig. 4c and d, the dots and lines are experimental data and fitted curves, and the TERS spectra at each spot are also shown as background 2D contour images.

can change due to coupling with the atoms of the metal surface, leading to peak shift and intensity change signatures.$^{15,50}$ Therefore, we analyze the spectral properties of the observed spots in the TERS image to obtain the evidence of single-molecule detection. First, we classify the observed TERS spots in Fig. 3 into two groups, as shown in Fig. 4a. We surmise that the TERS response in the first group (red circled spots 1-3) was measured from a few BCB molecules because the TERS signal of both the $\sim 580 \text{ cm}^{-1}$ and the $\sim 1160 \text{ cm}^{-1}$ modes is pronounced, as shown in Fig. 4a (see also Fig. 3a and b). Furthermore, a significant TERS peak shift is not observed in these three spots (Fig. 4c) since the observed TERS response is from the ensemble average. By contrast, we observe a much weaker TERS response in the second group (blue circled spots 4-9 in Fig. 4a) with a significant peak variation corresponding to $\sim 580 \text{ cm}^{-1}$, as large as $\sim 7.5 \text{ cm}^{-1}$, as shown in Fig. 4d. Based on these spatio-spectral analyses (Fig. 3 and 4), we believe the observed TERS response
possibly originated from single isolated BCB molecules.

**DFT calculation of vibrational modes in different chemical environments**

To reveal the possible origins of the observed TERS peak variations, we calculated the normal vibrational modes of a BCB molecule through DFT simulations. Since the BCB molecules are encapsulated using a thin dielectric layer, we presume the spectral diffusion is suppressed. Indeed, we did not observe spectral fluctuations in our time-series TERS measurements of BCB molecules, which indicates the suppressed spectral diffusion (see Fig. S5 for details). Based on this assumption, we design two kinds of fixed conformations of a BCB molecule, i.e., horizontal and vertical geometries with respect to the Au (111) surface. Regarding horizontally laying molecules, we additionally consider the position of the BCB molecules...
(especially C atoms vibrating with a large amplitude for the $\sim 580$ cm$^{-1}$ mode) with respect to the Au atoms since the substrate-molecule coupling effect can be slightly changed (see Methods for calculation details). Fig. 5a shows the calculated normal modes (colored vertical lines) of a BCB molecule with the measured TERS spectra at spots 4-9 (from Fig. 4a and d) for different chemical environments described in Fig. 5b-d. In the frequency range of 570 - 590 cm$^{-1}$, two theoretical vibrational modes ($\nu_1$ and $\nu_2$) are observed even though only a single peak was experimentally observed due to the limited spectral resolution and inhomogeneous broadening at room temperature. As individual atoms in a BCB molecule involve additional coupling to Au atoms on the surface, the Raman frequencies of two vibrational modes are varied depending on the conformation and the position of the molecule. When the C atoms of the molecule are vertically aligned with the Au atoms (as indicated with red circles in Fig. 5b), $\nu_1$ is calculated as 575.29 cm$^{-1}$ with the out-of-plane bending vibration mode of the C atoms, and $\nu_2$ is calculated as 576.94 cm$^{-1}$ with the in-plane stretching mode of the C atoms. By contrast, when these C atoms are mis-located with respect to the Au atoms (red circles in Fig. 5c), the Raman frequency of the out-of-plane bending mode of the C atoms is increased to 579.23 cm$^{-1}$ ($\nu_1$ in Fig. 5c). It is likely that the two strongly oscillating C atoms located near the hollow sites of Au atoms (Fig. 5c) experience stronger restoring forces than the C atoms located near the top sites of Au atoms (Fig. 5b). On the other hand, both $\nu_1$ and $\nu_2$ are significantly increased for a vertically standing molecule (Fig. 5d) due to the lessened molecular coupling with the Au atoms (see Fig. S6 for the normal mode of a BCB molecule in the gas phase). From these simulation results, we can deduce that the experimentally observed single molecules in S4, S5, and S9 (in Fig. 3 and 4) have a different chemical environment or molecular orientation compared to the single molecules in S6, S9, and S7. In other words, we experimentally probed the conformational heterogeneity of single molecules under ambient conditions through highly sensitive TERS imaging and spectral analyses with DFT simulations.
Conclusions

In summary, we demonstrated the hyperspectral TERS imaging of single molecules at room temperature by optimizing experimental conditions, such as the surface roughness and thickness of a thin metal film and the excitation polarization in the bottom-illumination geometry gap-mode TERS setup. These carefully selected and optimized conditions led to a substantial enhancement of the excitation field and high collection efficiency of the near-field Raman signals. In addition, the thin dielectric Al₂O₃ layer encapsulating the single molecules adsorbed onto the Au (111) surface played a significant role in enabling room temperature single-molecule TERS imaging. This is because the thin dielectric layer can suppress the rotational and spectral diffusions of molecules and inhibit the chemical reactions and contaminations in air, including potential physisorption of molecules onto the Au tip. Through this room-temperature single-molecule TERS imaging approach, we examined the conformational heterogeneity of BCB molecules with supporting theoretical DFT calculations. We envision that the presented optimal experimental setup for single-molecule TERS measurements will be broadly exploited to investigate unrevealed single-molecule characteristics in ambient conditions.

Methods

Sample preparation

Coverslips (thickness: 170 µm) for non-optimal metal films (Fig. 1c) were cleaned with piranha solution (3:1 mixture of H₂SO₄ and H₂O₂) for > 60 mins and ultrasonicated in deionized water. The other coverslips for optimal metal films (Fig. 1d) were ultrasonicated in acetone and isopropanol for 10 mins each, followed by O₂ plasma treatment for 10 mins. The coverslips were then deposited with a Cr adhesion layer with a thickness of 2 nm (a rate
of 0.01 nm/s) and subsequently deposited with Au films with varying thicknesses (0.01 nm/s to 1.0 nm/s) at the base pressure of $\sim 10^{-6}$ torr using a conventional thermal evaporator. The deposition rate of metals was precisely controlled using a quartz crystal microbalance (QCM) detector. The transmittance spectra of the substrates were measured by a UV-Vis spectrometer (UV-1800, Shimadzu). Next, BCB molecules in ethanol solution (100 nM for the single-molecule experiment and 1.0 mM for the thickness optimization process) were spin-coated on the metal thin films at 3000 rpm. Finally, an Al$_2$O$_3$ capping layer with a thickness of 0.5 nm was deposited on the sample surface using an atomic layer deposition system (Lucida D100, NCD Co.).

TERS imaging setup

For TERS experiments, we used a commercial optical spectroscopy system combined with an AFM (NTEGRA Spectra II, NT-MDT). The excitation beam from a single-mode-fiber-coupled He-Ne laser ($\lambda = 632.8$ nm, optical power $P$ of $\geq 1$ mW) passed through a half-wave plate ($\lambda/2$) and was collimated using the two motorized lenses. The beam passed through a radial polarizer and was focused onto the sample surface with an oil immersion lens (NA = 1.3, RMS100X-PFOD, Olympus) in the inverted optical microscope geometry. The electrochemically etched Au tip (apex radius of $\sim 10$ nm) attached on a tuning fork was controlled using a PZT scanner to alter the position of the Au tip with respect to the focused laser beam. The backscattered signals were collected through the same optics and transmitted to a spectrometer and charge-coupled device (Newton, Andor) after passing through an edge filter (633 nm cut off). The spectrometer was calibrated using a mercury lamp and the Si Raman peak at 520 cm$^{-1}$, and its spectral resolution was $\sim 4.3$ cm$^{-1}$ for a 600 g/mm grating.
FDTD simulation of optical field distribution

We used finite-domain time-difference (FDTD) simulations (Lumerical Solutions, Inc.) to quantify the optical field enhancement at the apex of the Au tip with respect to the metal film thickness. The distance between the Au tip and the Au film was set to 2 - 4 nm based on our experimental condition. As a fundamental excitation source, monochromatic 632.8 nm light was used with linear polarization parallel to the tip axis. The theoretical transmittance of thin gold films was calculated using the material properties obtained from Olmon et al.\textsuperscript{47}

DFT calculation of BCB vibrational modes

DFT calculations were performed using the Vienna Ab initio Simulation Package (VASP) to identify the normal vibrational modes of a single BCB molecule. In our simulation model, the chemical environment and molecular orientation were critically considered; i.e., the vibrational modes for a BCB molecule placed in a free space and adsorbed onto the Au (111) surface in different orientations and positions were calculated. Specifically, molecules oriented normal and parallel to the Au surface were modeled. For a molecule placed parallel to the Au surface, the lateral position was varied, as shown in Fig. 5, to consider the effect of an interaction between the atoms of the BCB molecule and those of the Au surface. To describe the adsorption of a molecule on the Au (111) surface, Tkatchenko and Scheffler (TS) dispersion correction\textsuperscript{52} with the Perdew-Burke-Ernzerhof (PBE) exchange functional\textsuperscript{53} was employed. Regarding the Au (111) slab supercell, four layers of Au atoms were considered, with the top two layers optimized in the gamma point. Furthermore, 1.5 nm vacuum space was considered to avoid an interaction between periodic slabs, and the plane-wave energy cutoff was set to 550 eV. The vibrational modes of the BCB molecule were shown using visualization for electronic and structural analysis (VESTA).
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Supporting Information Available

Supporting Information is available free of charge.

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