Supplementary Note 1 | Cleanliness during FM-TERS measurement

Initial cleanliness of the whole system (before TERS measurement) can be assured using the protocol in the Methods sections concerning tip and sample preparation. Then during our TERS measurements, contamination will come only from the air. These contaminants usually have a weaker affinity for the gold(111) substrate than 4bipy, and it takes some time for them to displace the probe.

Intermittent TERS measurements demonstrate that reliable TERS signals could be obtained even when the system has been exposed to air up to 50 min (Supplementary Fig. S3a). We attribute the decay of the signal to desorption rather than decomposition because it does not coincide with the appearance of carbon bands at 1350 and 1580 cm$^{-1}$. Carbon bands appear later, and we believe they arise from degradation of organic contaminants adsorbed from the air.

We found that continuous exposure of the gold tip-4bipy-gold(111) substrate system to a laser power of 5 mW will cause the TERS signal to decrease, and finally disappear after about 330 s, without the appearance of peaks at 1350 and 1580 cm$^{-1}$ (Supplementary Figs. S3b and S3c). When the tip was moved to a new position on the gold(111) substrate, the signal was recovered (Supplementary Fig. S3c). These results indicate that laser-induced desorption of 4bipy occurs locally in the laser spot.

In contrast, SERS substrates prepared by wet-chemical or lithographic methods are usually covered by a layer of reductants (for precipitation of the metal), surfactants (to prevent aggregation), or photoresist. Probe molecules must coexist with these contaminants on the surface of the substrate, and as a result, complex photochemical reactions of the probe, of the contaminants, and of the probe with the contaminants are all possible during SERS. These photochemical processes can yield a very complicated SERS signal, and thus hinder efforts to understand the system of interest.
Supplementary Figure S1 | Principle of FM-STM. a, A schematic diagram. The solid red curve is the working trace of the tip, and the dashed blue lines indicate undesirable tendency of movement due to thermal or mechanical instrument drift. b, The response time of the piezo under different feedback conditions. The percentage values given in the figure represent the set feedback relative to the normal feedback condition. The time values given in the figure indicate the response time. The initial bias was 100 mV, and response curves were recorded when the bias was switched to 900 mV. The current was 300 pA.
Supplementary Figure S2 | Comparison of the fishing-mode STM and STM break-junction methods for measuring conductance. Gold-1,8-octanedithiol-gold time-sequence conductance curves (a and c) are given with their respective histograms (b and d). **Left**, those obtained by our fishing-mode STM method in about half a second. **Right**, those obtained by the STM break-junction method for approximately 1000 data points, which normally take tens of minutes or hours to acquire.
Supplementary Figure S3: Time-dependence of the FM-TERS signal from 4bipy adsorbed on gold(111). **a**, The sample was illuminated with 5 mW of helium-neon laser light only during spectral acquisition, and each spectrum was measured with an acquisition time of 1 s. **b**, Decreasing intensity of 4bipy’s 1609 cm\(^{-1}\) band during continuous illumination. **c**, Some representative TERS spectra obtained at different times during continuous irradiation (data such as these were used to create the curve in **b**). The tip was later moved, and the spectrum obtained from a fresh point on the gold(111) surface is shown in blue. Complete loss of the 4bipy signal is emphasized with red in **a** and **c**.
Supplementary Figure S4 | Bias-dependent conductance, and TERS signals in the OFF state.  
**a,** Current vs. time curve obtained with normal feedback (the integral gain and proportional gain were both set to 3). The bias voltage was 200 mV and the tunneling current was 300 pA.  
**b,** TERS spectra obtained with different bias voltages.
Supplementary Figure S5 | The cluster-models used to simulate the OFF state and the ON states at different bias voltages.
Supplementary Figure S6 | Experimental setup of the FM-TERS instrument. Left: electronic circuitry. Right: Optical setup.
Supplementary Figure S7: An SEM image of a typical TERS gold tip prepared by electrochemical etching of a gold wire (diameter = 0.25 mm) in a mixture of fuming hydrochloric acid and anhydrous ethanol (1:1/v:v).
### Supplementary Table S1

Derived polarizabilities and Raman activities for our model system in the OFF and ON states.

|                | OFF / Br−Au₄bipy |          |          |          | Assignment |
|----------------|-----------------|----------|----------|----------|------------|
| Freq(cm⁻¹)     | $\mathcal{A}^2$ (Å⁴/amu) | $\gamma$ (Å⁴/amu) | $\alpha_{zz}^2$ (Å⁴/amu) | $S_i$(Å⁴/amu) |            |
| 1228           | 3.50×10⁰        | 1.04×10¹ | 1.55×10¹ | 2.31×10³ | $\nu_{9a}$ |
| 1287           | 9.12×10⁰        | 6.23×10¹ | 6.86×10¹ | 8.47×10³ | $\nu_{C-C}$ |
| 1513           | 9.76×10⁻¹       | 2.44×10⁰ | 3.96×10⁰ | 6.10×10¹ | $\nu_{19a}$ |
| 1614           | 2.03×10¹        | 2.43×10² | 2.22×10² | 2.61×10³ | $\nu_{8a}$ |

|                | ON / Br−Au₄bipyAu₄ |          |          |          | Assignment |
| Freq(cm⁻¹)     | $\mathcal{A}^2$ (Å⁴/amu) | $\gamma$ (Å⁴/amu) | $\alpha_{zz}^2$ (Å⁴/amu) | $S_i$(Å⁴/amu) |            |
| 1224           | 1.94×10³        | 1.69×10⁴ | 1.46×10⁵ | 2.06×10⁵ | $\nu_{9a}$ |
| 1288           | 8.41×10²        | 7.55×10³ | 6.46×10³ | 9.08×10⁴ | $\nu_{C-C}$ |
| 1515           | 1.28×10²        | 1.08×10³ | 9.42×10⁵ | 1.33×10⁴ | $\nu_{19a}$ |
| 1609           | 4.45×10³        | 4.11×10⁴ | 3.46×10⁴ | 4.87×10⁵ | $\nu_{8a}$ (S-ring) |
| 1621           | 4.37×10³        | 3.38×10⁴ | 2.86×10⁴ | 4.02×10⁵ | $\nu_{8a}$ (D-ring) |
Supplementary Table S2: Optimized bond lengths (\( B \), in Angstroms) and the dihedral angle formed between the two rings (in degrees) are given for our model system in the OFF and ON states. \( B(C, C) \) indicates the length of the parallel C-C bonds, \( B(C, C) \) indicates the length of the angled C-C bonds, and \( B(C, C') \) indicates the length of the C-C' bond between the rings.

| OFF State          | Low Bias Voltage         | High Bias Voltage         |
|--------------------|--------------------------|----------------------------|
| \( \text{Br}^- \text{Au}_4\text{4bipy} \) | \( \text{Br}^- \text{Au}_4\text{4bipy}\text{Au}_4\text{Cl}^- \) | \( \text{Br}^- \text{Au}_4\text{4bipy}\text{Au}_4\text{F}^- \) |
| Lower Ring         | Upper Ring               | S-ring                     | D-ring                     | S-ring | D-ring | S-ring | D-ring | S-ring | D-ring |
| B(N, Au)           | 2.3646                   | 2.3380                     | 2.3334                     | 2.3374 | 2.3322 | 2.3459 | 2.1527 |        |        |
| B(N, C)            | 1.3418                   | 1.3404                     | 1.3433                     | 1.3433 | 1.3433 | 1.3421 | 1.3492 |        |        |
| B(C, H)            | 1.0867                   | 1.0887                     | 1.0864                     | 1.0864 | 1.0864 | 1.0867 | 1.0842 |        |        |
| B(C, C')           | 1.3936                   | 1.3952                     | 1.3934                     | 1.3934 | 1.3935 | 1.3934 | 1.3888 |        |        |
| B(C, C')           | 1.4034                   | 1.4036                     | 1.4041                     | 1.4041 | 1.4041 | 1.4036 | 1.4048 |        |        |
| dihedral angle     |                          |                            |                            |        |        |        |        |        |        |

In the ON state, we call the 4bipy ring that binds to the negatively charged gold substrate the “source ring” (“S-ring”) and we call the one that binds to the positively charged tip the “drain ring” (“D-ring”). See also the labeling in main text Fig. 4c. In the OFF state, it may be more appropriate to call them the “lower ring” and the “upper ring” respectively.
**Supplementary Table S3** | Band assignments for some important vibrational modes of 4bipy in the OFF and ON states based on our own results as well as the literature. All Raman shift frequencies are in cm⁻¹.

| OFF State Calc. | OFF State Exp. | ON State / Low Voltage Calc. | ON State / High Voltage Calc. | ON State Exp. | Assignment |
|-----------------|----------------|-----------------------------|-------------------------------|---------------|------------|
| Br⁻Au₄-4bipy     | 1001(s)        | 1006(s)                     | 1005(s)                       | 1012(s)       | 1011       |
|                 |                | 1073(vw)                    | 1073(vw)                      | 1075(vw)      | 1068       |
|                 | 1228(s)        | 1226(s)                     | 1225(s)                       | 1224(vvs)     | 1215       |
|                 | 1287(vs)       | 1287(vvs)                   | 1285(vs)                      | 1291(vs)      | 1293       |
|                 | 1513(vw)       | 1512(vw)                    | 1513(vw)                      | 1515(s)       | 1535       |
|                 | 1614(vvs)      | 1614(vvs)                   | 1615(vss)                     | 1609(vvs), 1621(vvs) | 1609, 1631 |

Here vvs = very very strong, vs = very strong, m = medium, w = weak, vw = very weak.
**Supplementary Table S4** | NBO perturbation theory energy analysis ($E_2$ in kcal/mol) for three model structures in different molecule to electrode charge-transfer states

| Bond Type | OFF State (S-Ring) | ON State (S-Ring) | ON State (D-Ring) | Bond Type | Low Bias Voltage | High Bias Voltage | Low Bias Voltage | High Bias Voltage |
|-----------|-------------------|-------------------|-------------------|-----------|-----------------|-----------------|-----------------|-----------------|
| $lp(N_{\text{lower ring}}) \rightarrow 6p_{\downarrow}(\text{Au})$ | 24.5 | | | | | | | |
| $lp(N_{\text{S-ring}}) \rightarrow 6p_{\downarrow}(\text{Au})$ | 25.91 | 24.89 | | | | | | |
| $lp(N_{\text{D-ring}}) \rightarrow 6s+6p_{\downarrow}(\text{Au})$ | 47.24 | 70.20 | | | | | | |
| $lp(N_{\text{lower ring}}) \rightarrow \sigma^{\pi}(\text{Au-Au})$ | 7.93 | | | | | | | |
| $lp(N_{\text{S-ring}}) \rightarrow \sigma^{\pi}(\text{Au-Au})$ | 8.56 | 7.85 | | | | | | |
| $\sigma(N-C) \rightarrow 6p_{\downarrow}(\text{Au})$ | 6.25 | 6.60 | | | | | | |
| $\sigma(N-C) \rightarrow 6p_{\downarrow}(\text{Au})$ | 8.68 | 10.94 | | | | | | |
| $\sigma(C-C) \rightarrow 6p_{\downarrow}(\text{Au})$ | 3.41 | 3.47 | | | | | | |
| $\sigma(C-H)_{\text{down}} \rightarrow 6p_{\downarrow}(\text{Au})$ | 2.30 | 2.24 | | | | | | |
**Supplementary Table S5** NBO perturbation theory energy analysis (E$_2$ in kcal/mol) for three model structures in different electrode to molecule charge-transfer states

| Bond Type | OFF State | ON State (S-Ring) | ON State (D-Ring) | Bond Type | OFF State | ON State (S-Ring) | ON State (D-Ring) |
|-----------|-----------|-------------------|-------------------|-----------|-----------|-------------------|-------------------|
| Br$^-$Au$_4$ 4bipy | Bond Type | Low Bias Voltage | High Bias Voltage | Bond Type | Low Bias Voltage | High Bias Voltage | Bond Type |
| Br$^-$Au$_4$ 4bipy | Br$^-$Au$_4$ 4bipy Au$_4$Cl$^-$ | Br$^-$Au$_4$ 4bipy Au$_4$Cl$^-$ | Br$^-$Au$_4$ 4bipy Au$_4$Cl$^-$ |
| $\sigma$(Au-Au)$\rightarrow\sigma^*$(C-H)$_{near N}$ | 2.03 | $\sigma$(Au-Au)$\rightarrow\sigma^*$(C-H)$_{near N}$ | 1.89 | 2.17 | $d_{zz}$(Au) | $\rightarrow\sigma^*(N-C)_L$ | 0.78 | 1.49 |
| $\sigma$(Au-Au)$\rightarrow\sigma^*(N-C)_L$ | 0.72 | $\sigma$(Au-Au)$\rightarrow\sigma^*(N-C)_L$ | 0.69 | 0.75 | $6\sigma$(Au) | $\rightarrow\sigma^*(N-C)_L$ | 1.29 | 1.22 |
| $d_{zz}$(Au)$\rightarrow\sigma^*(N-C)_L$ | 0.68 | $6\sigma$(Au)$\rightarrow\sigma^*(N-C)$_L$ | 1.24 | 1.26 | $d_{zz}$(Au)$\rightarrow\sigma^*(N-C)$_L$ | 0.24 | 0.36 |
| $d_{zz}$(Au)$\rightarrow\pi^*(N-C)$_L$ | 1.58 | $d_{zz}+d_{zz}$(Au)$\rightarrow\pi^*(N-C)$_L$ | 1.67 | 1.76 | $d_{zz}$(Au)$\rightarrow\pi^*(N-C)$_L$ | 1.77 | 2.66 |
Supplementary Table S6: NBO perturbation theory energy analysis ($E_2$ in kcal/mol) for three model structures in different source-ring/drain-ring charge transfer states

| Bond Type | OFF State | ON State |
|-----------|-----------|----------|
| $\pi$(C-C)$_{D}$ → $\pi^*$($\pi$-$\pi$)$_S$ | Br^{-}Au$_4$4bipy | Low Bias Voltage | High Bias Voltage |
| | | Br^{-}Au$_4$4bipyAu$_4$Cl$^-$ | Br^{-}Au$_4$4bipyAu$_4$ |
| | 9.21 | 10.01 | 9.14 |
| $\pi$(C-C)$_{S}$ → $\pi^*$($\pi$-$\pi$)$_D$ | 9.83 | 10.00 | 11.63 |
Supplementary Methods

Calculation model

We adopted a cluster-model based DFT method to simulate structure and bonding of 4bipy with gold in the OFF and ON states (Supplementary Fig. S5). Our strategy was to create a cluster-model using the OFF-state TERS spectrum as a guide, then alter it to obtain the ON-state TERS spectrum. We focused on the differences between OFF- and ON-state spectra because they yield key information about the differences between OFF- and ON-state electronic structures, which determine conductance in the molecular junction and the related Raman response.

Instead of using a partially charged Au$_n$ cluster, which is conceptually appropriate but practically unfeasible, we used a halide ion ($X^-$) modified Au$_4$ cluster to represent the negatively charged gold(111) source electrode. By choosing the proper halide ion, we were able to tune the charge on the Au$_4$ cluster and the position of the Fermi level. We found that our experimentally measured OFF-state TERS spectrum (Fig. 4a curve II in the main text) could be reproduced well using either Br$^-$-Au$_4$-4bipy or Cl$^-$-Au$_4$-4bipy (see Supplementary Fig. S5 for the models). It was decided that the former (Br$^-$-Au$_4$) would be fixed as the source electrode.

We then modeled the gold tip drain electrode with Au4F$^-$ and Au4Cl$^-$ in two low-bias cases and with Au4 in a high-bias case. Thus, our model had a static electric field pointing from the tip (+) to the substrate (−) with several appropriate bias voltages.

Calculation details

All calculations were carried out using the Becke three-parameter exchange – Lee, Yang and Parr correlation functional (B3LYP)$^{37,38}$ in Gaussian 09.$^{39}$ The basis sets employed were 6-311+G(2d) for Br, and 6-31+G(d) for C, N and H. These basis sets contained polarization functions and diffuse functions on C, N and Br. Au was described by the relativistic LANL2DZ pseudopotentials with a small core approximation combined with the associated basis functions for valence electrons.$^{40-42}$

Geometry optimization was performed by relaxing all atoms in each model system. The convergence criterion for maximum force was $1.0 \times 10^{-5}$ Hartrees/Angstrom, and no imaginary
frequencies were present after optimization. A factor of 0.978 was used to scale the theoretical vibrational frequencies. A natural bond orbital (NBO) analysis was performed in Gaussian 09. Normal Raman (rather than pre-resonance Raman or resonance Raman) scattering factors were calculated analytically, and the differential Raman scattering cross-section was calculated as described in ref. 44:

$$
\left( \frac{d\sigma}{d\Omega} \right)_i = \frac{(2\pi)^4}{45} \frac{h}{8\pi^2 c v_i} \frac{(v_0 - v_i)^4}{1 - \exp(-hc v_i / k_B T)} S_i
$$

(S1)

where $S_i = 45 \left( \frac{d\alpha}{dQ_i} \right)^2 + 7 \left( \frac{d\gamma}{dQ_i} \right)^2$ is the Raman scattering factor (in Å$^4$/amu), and $d\alpha/dQ_i$ and $d\gamma/dQ_i$ are the isotropic and anisotropic polarizability derivatives of the $i^{th}$ normal mode respectively. In Equation S1, $v_0$, $v_i$, and $T$ are the frequency of the incident light (15802.6 cm$^{-1}$, i.e. $\lambda = 632.8$ nm from a helium-neon laser), the frequency of the Raman shifted light of the $i^{th}$ normal mode in cm$^{-1}$), and temperature (298.15 K), respectively.

### NBO perturbation theory energy analysis

Supplementary Tables S4 to S6 summarize the results of a second-order perturbation theory natural bond orbital (NBO) energy analysis. The total perturbation energy is more than double in the ON state what it is in the OFF state, and this is in line with the fact that the 4bipy molecule becomes more polarizable when it participates in a molecular junction (Supplementary Table S1).

The data in Supplementary Table S2 reveals that the N-Au bond is shorter in the ON state than it is in the OFF state. This suggests a stronger interaction between the 4bipy N atom and Au, which is consistent with the results of the NBO perturbation energy analysis. The shortening of the N-Au bond brings the nearest set of C-H bonds closer to the gold substrate and increases the chance that these bonds will become polarized by it. This effect leads to a stronger Raman signal from the $v_9a$ mode and a downshifting of the frequency from ~1228 to ~1224 cm$^{-1}$ (Supplementary Table S1). This calculated result is in good agreement with experimental findings (Supplementary Table S3, and Fig. 4a curves I and II in the main text).

The calculations also reveal that in the ON state, the N-Au D-ring to tip bond is shorter than the corresponding S-ring to substrate bond (Supplementary Table S2). This is in keeping
with a stronger back-bonding effect between the tip and the D-ring, and it indicates a stronger interaction of the Au with the D-ring than the S-ring. As a result, the two pyridine rings of 4bipy are no longer symmetrical. Indeed, we find that the two parallel C-C bonds are shorter in the D-ring than they are in the S-ring (Supplementary Table S2), and a 12 cm$^{-1}$ upshift occurs for the $\nu_{8a}$ band when a high bias voltage is applied (Supplementary Tables S1 and S3). This leads to the appearance of a $\nu_{8a}$ doublet (1609 cm$^{-1}$ for the S-ring and 1621 cm$^{-1}$ for the D-ring; Supplementary Tables S1 and S3, and main text Fig. 4a curves VI and VII) which agrees well with the experimentally observed one (1609 and 1631 cm$^{-1}$; Supplementary Table S3, and main text Fig. 4a curves I to V). Fig. 4a curves VII and II show that the splitting does not occur in the OFF state. We suggest that splitting of the $\nu_{8a}$ band can be used as a signature for the creation of a 4bipy molecular junction.

The Raman activities of different vibrational modes in the OFF and ON states

For selected vibrational modes of 4bipy in the OFF and ON states, Supplementary Table S1 gives the square of the isotropic polarizability derivatives ($\bar{\alpha}^2$), the anisotropic polarizabilities ($\gamma$), the square of the zz component of the polarizabilities ($\alpha_{zz}^2$) and the Raman activities ($S_i$). In general, the Raman activities of the ON state are two or three orders of magnitude greater than those of the OFF state (a similar result was obtained for pyrazine by Zhao et al.$^{33}$). As we have explained in the main text, this additional enhancement allows the acquisition of a distinct Raman signal from the single-molecule in the junction despite a background signal from the large number of surrounding molecules which do not make contact with the tip.
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