1. **Figure S1: SEM images of mechanically-configurable nanoelectrodes**

![Figure S1](image)

**Fig. S1.** Scanning electron microscopy images of the mechanically-configurable nanoelectrodes. **a,** A free-standing nano-scale gold junction fabricated on a polyimide-coated flexible metal substrate. **b,c,** Magnified views of the narrowest constriction of the nanojunction. The inter-electrode gap between the nanoelectrodes can be adjusted to arbitrary size at a sub-picometer resolution through finely manipulating the substrate bending\textsuperscript{S1-S3}.
2. Figure S2: Calibration measurements of inter-electrode gap distance

**Fig. S2.** Calibration measurements for inter-electrode gap distance adjustments.  

**a,** A schematic illustration describing the principle of operation of the inter-electrode gap distance control. The gap size $d_{\text{gap}}$ can be widened (narrowed) by pushing (retracting) the substrate through manipulating the actuation voltage $V_p$ exerted on the piezo-element.  

**b,** Calibration measurements of $d_{\text{gap}} - V_p$ conversion ratios.  

The tunneling current flowing through an electrode nanogap $I$ is measured in a vacuum during gap closure by ramping $V_p$. $I$ is exponentially dependent on the tunneling distance $d_{\text{gap}}$ as $I \sim \exp(\beta d_{\text{gap}})$ with the decay constant $\beta = -2\sqrt{2m\Phi}/\hbar$, where $m$ and $\Phi$ denote the electron mass and the work function of gold electrodes, respectively.  

The linear scaling of log($I$) with respect to $V_p$ in **b** thus indicates a linear $d_{\text{gap}}$–$V_p$ relationship $\Delta d_{\text{gap}} = \alpha \Delta V_p$ with the conversion coefficient $\alpha$. $\alpha\beta$ is obtained from a linear fit to the log($I$) – $V_p$ plots (red broken line).  

From this, we acquire $\alpha$ assuming bulk properties for $m$ (9.11 × 10$^{-31}$ kg) and $\Phi$ (5.3 eV). $d_{\text{gap}}$ adjustments is implemented through $V_p$ control via $d_{\text{gap}} = \alpha V_p^{S3}$. The calibration
measurements were conducted for each nano-MCBJ sample in prior to use it for single nucleotide detections.

3. **Figure S3: \( I - t \) curves obtained under various electrode gap distance conditions**

![Graph](image)

**Fig. S3.** \( I - t \) curves obtained at different inter-electrode gap conditions \( d_{\text{gap}} \). 

**A,** An example of \( I - t \) curve measured in a Milli-Q solution of GMP (50 \( \mu \)M) with \( d_{\text{gap}} \) adjusted to \( \sim \) 2.0 nm. The curve is featureless and includes no \( I \)-pulses. 

**B,** Only
when $d_{\text{gap}}$ is set close to an average nucleotide molecule length (~ 1.0 nm) did we observe the characteristic pulse signals that signify single-nucleotid e trapping between nanoelectrodes ($I-t$ was obtained in a 5 μM GMP solution). Pulse signals tend to appear as a cluster of several events with a relatively long (50 to 1000 seconds) time interval. This indicates that nucleotide molecule adsorbs on a gold electrode surface at their base unit, whose affinity to gold is well studied $^{S4,S5}$. When adsorbed nearby the electrode gap region, electrons tunnel through a nucleotid e molecule and yield $I$-pulses until gold-base unit binding thermally dissociates. After desorption, no signals appear until next molecules come across in the vicinity of the nanoelectrode gap space. c, Closer look to one of the cluster events shown in b. The $I$-pulses with various heights emerge in a random fashion with time.

4. Figure S4: Anomalous $I-t$ characteristics of AMPs

Fig. S4. Anomalous $I$-$t$ characteristics of AMPs at a relatively wide inter-electrode gap size of $d_{\text{gap}} \sim 2.5$ nm. Note that the vertical scale is nA. The $I$-$t$ curve is
characterized by a large offset of ~10 nA and irreversible ups and downs of current.

Further extending $d_{\text{gap}}$ to > 5 nm resulted in smooth decrease in $I$ with $d_{\text{gap}}$.

5. Figure S5: $I$-$t$ characteristics of single organic molecules other than nucleotides

![Figure S5](https://example.com/image)

**Fig. S5.** $I$-$t$ curves measured for organic $\pi$-conjugated molecules other than nucleotides. **a,** Three test molecules employed here: 1,4-benzenedithiolates
(BDTs), 1,4-benzenediamines (BDAs), and benzeneamines (BAs). Typical \( I-t \) curves obtained for BDTs (b), BDAs (c), and BAs (d) dissolved in 1,2,4-trichlorobenzene solvent (5 \( \mu \)M). Right panels are magnified views of characteristic current signals marked by arrows in left panels. The three molecules all demonstrated current ups and downs suggestive of single molecule trapping and detrapping between gold nanoelectrodes. However, they showed clear difference in the magnitude of the current rise \( I_r \) and the trapping duration \( t_d \). BDTs and BDAs exhibited relatively large \( I_r \) of \(~ 100 \) nA whereas BAs showed much smaller values of \(~ 0.1 \) nA. This reflects the fact that BDTs and BDAs have two anchoring groups whereas BAs possess only one, giving rise to weaker molecule-electrode coupling for BAs and hence lower electron transmissivity. Moreover, average \( t_d \) varies substantially among the molecules: \( t_d > 1 \) s, \(~ 0.1 \) s, and \(~ 0.01 \) s for BDTs, BDAs, and BAs, respectively. The longest \( t_d \) acquired for BDTs reflects the strong gold-thiol bonds (bonding energy \(~ 1.6 \) eV\(^{6}\)) formed at molecule-electrode contacts, while an order of magnitude lower \( t_d \) arises from the weak gold-amine links (bonding energy \(~ 0.7 \) eV\(^{7,8}\)). It is noticeable that \( t_d \sim 0.1 \) s of BDAs agrees plausibly with the recent natural lifetime measurements of gold-BDA-gold single molecule junctions\(^{8}\). Even lower \( t_d \) obtained for BAs presumably stems from mobile nature of one-side connected molecular contacts analogous to Au-nucleotide-Au junctions studied here. Overall, the difference in \( I_r \) and \( t_d \) among the three molecules can be consistently explained by the distinct
electronic coupling and chemical bonding strength at the molecule-electrode links unique to each type of molecules.

6. **Figure S6: Control current histograms for benzenedithiolates and benzenediamines**

![Current histograms for BDTs and BDAs](image)

**Fig. S6.** Current histograms constructed from \( I-t \) data for BDTs and BDAs. **a,** The histogram of BDTs shows a single peak at \( \sim 70 \) nA. The relatively broad \( I \) distribution of BDTs is attributable to wide variations of gold-thiol contact configurations involved that yield orders of magnitude diversity in single BDT molecule electrical conductance\(^{S9}\). **b,** On the other hand, the \( I \) histogram of BDAs reveals two peaks located at \( \sim 120 \times n \) nA \((n = 1, 2)\). The regularly positioned peaks signify bridging of \( n \) number of molecules over gold electrode nanogaps in parallel. From this, we deduce BDA single molecule conductance of 62 m\( G_0 \), which accords with the previously reported values\(^ {S7,S10} \).
7. Figure S7: $I-V_b$ characteristics of dried nucleotide thin films.

**Fig. S7.** $I-V_b$ characteristics of dried nucleotide thin films measured at room temperatures in a vacuum. We have prepared nucleotide thin film samples as follows. First, we formed a 1nm electrode gap in a Milli-Q solution of nucleotides (10mM) using the method described in the manuscript. After that, we wait for about 3 hours and subsequently evacuated a chamber to remove solvent and form a thin film of nucleotides. Evacuating for another 3 hours, we measured $I-V_b$ characteristics of the thin films in a bias range of $-0.75 \leq V_b \leq 0.75$ V at room temperatures. As shown above, we obtain linear $I-V_b$ curves for the four nucleotides with conductance order of AMP < CMP ~ TMP < GMP, the trend of which agrees with the order of HOMO-LUMO gaps of the four nucleotides mentioned in the text. However, this coincidence may be a fortuitous one as the number of current carrying molecules is not controlled here. Nonetheless, the fact that dried nucleotide thin films still carry relatively large current, $10^{-7}$ to $10^{-9}$ A, even
after removing solvent and eliminating ion pathways indicates little contribution of ionic current to the $I$-pulse signals we observed in a nucleotide solution.

8. Supplementary References


