Single-molecule junction spontaneously restored by DNA zipper

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The electrical properties of DNA have been extensively investigated within the field of molecular electronics. Previous studies on this topic primarily focused on the transport phenomena in the static structure at thermodynamic equilibria. Consequently, the properties of higher-order structures of DNA and their structural changes associated with the design of single-molecule electronic devices have not been fully studied so far. This stems from the limitation that only extremely short DNA is available for electrical measurements, since the single-molecule conductance decreases sharply with the increase in the molecular length. Here, we report a DNA zipper configuration to form a single-molecule junction. The duplex is accommodated in a nanogap between metal electrodes in a configuration where the duplex is perpendicular to the nanogap axis. Electrical measurements reveal that the single-molecule junction of the 90-mer DNA zipper exhibits high conductance due to the delocalized \( \pi \) system. Moreover, we find an attractive self-restoring capability that the single-molecule junction can be repeatedly formed without full structural breakdown even after electrical failure. The DNA zipping strategy presented here provides a basis for novel designs of single-molecule junctions.
he structural, physical, and chemical properties of DNA at the nanoscale have attracted attention to the prospect of using DNA as a building block in nanoscience. In the field of molecular electronics, electron transport through a single DNA molecule has been extensively investigated. These studies exploit single-molecule junctions, in which a DNA molecule bridges a nanogap between the metal electrodes. For example, it was reported that A-form and B-form DNAs differ in the single-molecule conductance by an order of magnitude. Many studies have revealed that there is a change in the electron transport properties of DNA after ligand binding. These include an intercalator whose perturbation of base stacking leads to a dynamic manner.

DNA under the deliberate control of its structure paves the way for novel functionality and superior properties of nanoscale electronic devices. For example, we expect that electron transport through single DNA molecule conductance by an order of magnitude was reported that A-form and B-form DNAs differ in the single-molecule conductance that cannot be achieved using DNA junctions with the conventional configuration. The unzipping dynamics of the molecular junction were characterized by tunnelling currents in break-junction (BJ) experiments, based on scanning tunnelling microscopy (STM). The STM measurement and the molecular dynamics (MD) simulations of the unzipping dynamics revealed that the DNA junction with zipper configuration enables spontaneous restoration of the molecular junction after its electrical failure and thereby improves the reproducibility of the junction formation. Our study demonstrated that a DNA dynamic structural change could be applied to a single-molecule junction by using the zipper configuration. The findings pave way for novel functionality and superior properties of nanoscale electronic devices.

Results and discussion

Single-molecule conductance. First, STM-BJ experiments were performed to measure conductance of the single-molecule junction of DNA with a zipper configuration. Either 90-mer or 10-mer DNA was employed as a sample molecule. One strand of the sample was functionalized at the 3' end with a thiol linker, while the same linker was introduced at the 5' end of another strand. The DNA single-molecule junction differs from conventional ones in the DNA configuration; the present and conventional junctions contain a single DNA molecule, as exemplified above. However, these previous studies mainly focused on the transport phenomena in static DNA structure at thermodynamic equilibria.

Recently, rational control of DNA structures at the single-molecule level has been achieved by modern techniques and emerging technologies. Capturing desired conformation and separation of DNA are achieved by elaborate flow in nanofluidic systems. Sequencing analysis of DNA at the single-base resolution can be performed with nanopore devices during the constrained passage. Also, precise control of DNA structures by atomic force microscopy (AFM) has enabled accurate mapping of DNA unzipping dynamics and a free-energy landscape. This combination of electrical measurements and structural modulation of DNA could lead to the realization of the sophisticated functionality of electronic devices based on a single DNA molecule. For example, we expect that electron transport through DNA under the deliberate control of its structure paves the way for DNA electronic devices with functional controllability in a dynamic manner.

Here, we report the investigation of electron transport through the single-molecule junction of a zipper DNA that orthogonally clamps a metal nanogap (see Fig. 1a). The present DNA single-molecule junction differs from conventional ones in the DNA configuration; the present and conventional junctions contain a DNA molecule oriented in perpendicular and parallel directions to the axis of the nanogap, respectively. The DNA single-molecule junction with the present zipper configuration exhibited high single-molecule conductance that cannot be achieved using DNA junctions with the conventional configuration. The unzipping dynamics of the molecular junction were characterized by tunnelling currents in break-junction (BJ) experiments, based on scanning tunnelling microscopy (STM). The STM measurement and the molecular dynamics (MD) simulations of the unzipping dynamics revealed that the DNA junction with zipper configuration enables spontaneous restoration of the molecular junction after its electrical failure and thereby improves the reproducibility of the junction formation. Our study demonstrated that a DNA dynamic structural change could be applied to a single-molecule junction by using the zipper configuration. The findings pave way for novel functionality and superior properties of nanoscale electronic devices.

![Fig. 1 Single-molecule junction of DNA zipper.](image-url)

- **a** Schematic illustration of the scanning tunnelling microscopy-break-junction (STM-BJ) measurements.
- **b** Representative conductance traces for unmodified Au(111) substrate (gray dashed lines), and 90-mer and 10-mer DNAs (blue solid lines and purple dotted lines, respectively).
- **c** and **d** 2D histograms of the conductance-displacement (G–z) traces for 90-mer and 10-mer DNAs, respectively. The origin of the displacement was set at the point where the conductance decreased below 50 mG0. 2219 and 2635 traces were analyzed for histograms in **c** and **d**, respectively. Tip velocity, 31 nm/s; bias voltage, 20 mV.
shows conductance plateaus at 1.9 and 0.15 mG₀ for 90-mer and 10-mer DNA, respectively (Fig. 1c, d). The difference in the single-molecule conductance is discussed later. The single plateau and subsequent conductance decay in the traces indicate that these plateaus are attributed to the single-molecule junction that contains DNA. The tunnelling decay constants during and after the energy gap between the Fermi level of the electrode and that STM-BJ results). The decay constants are known to depend on Note 1 for the results of 10-mer DNA and detailed discussion of sequencing27.

Transition voltage spectroscopy. Next, current–voltage (I–V) curves of the single-molecule DNA junction with the zipper configuration were obtained to investigate the electron transport properties in detail. We previously developed a methodology to study electron transport induced by in situ hybridization of a single DNA duplex using an STM molecular tip34. This technique was also utilized in the present study: an Au STM tip was modified with a single strand of the 90-mer DNA zipper. An Au(111) substrate was separately modified with single-stranded DNA (ssDNA) complementary to the strand on the tip (Fig. 2a). After the molecular tip was brought near to, but never in contact with, the surface, the bias voltage was swept with the tip as the sample distance was held stationary to acquire the I–V curves (Fig. 2a). The I–V curves in Fig. 2a clearly exhibited two distributions, i.e., states with high and low conductance. The low-conductance state was common to those obtained in the control experiments, i.e., the measurements with unmodified tip and unmodified substrate (Fig. 1a). In contrast, the linker groups were conventionally located at the opposite ends of the duplex, and the junction accommodates the duplex aligned parallel to the gap axis. Electron transport through the latter conventional junction steeply attenuates with increased DNA length, since the electrons travel through the whole duplex33. No such attenuation happens in the present single-molecule junction. Indeed, the conductance of the 90-mer DNA zipper junction is larger than that of the 10-mer zipper junction. We measured the conductance of the zipper junction of a variety of DNA lengths ranging from 10 to 90 base pairs and confirmed that the conductance value increased as the DNA length increased (Supplementary Note 3). This length dependence, together with the self-restoring capability described later, are the advantages of the present configuration.

Fig. 2 Current–voltage (I–V) and transition voltage spectra. I–V 2D histograms and $V_{\text{trans}}$ histograms obtained with (a, e) molecular tip and modified substrate, (b, f) molecular tip and bare substrate, (c, g) unmodified tip and modified substrate, and (d, h) unmodified tip and bare substrate. Bias voltage ($V_{\text{bias}}$) was swept from −1.1 V to 1.1 V in 5 ms. Representative transition voltage spectra are shown in insets of e–h. Pink and blue spectra correspond to those with low and high $V_{\text{trans}}$, respectively.
high-conductance state was thus ascribed to the molecular junction of the DNA zipper. The conductance as estimated by the $I$–$V$ properties agrees with the conductance as determined by the BJ studies (Fig. 1c, d, see Supplementary Note 4), supporting the assignment of a high-conductance state to the DNA junction. It has been reported that ssDNA can adsorb to a metal surface via its bases. However, no state that could be attributed to ssDNA molecular junctions was found in the $I$–$V$ curves in Fig. 2b, c. STM-BJ study was also conducted with the unmodified tip and the ssDNA-modified substrate, and the conductance histograms without notable peaks were obtained (Supplementary Note 5). These results are most probably due to the significantly decreased conductance of ssDNA as compared to that of dsDNA because of base stacking is less ordered in ssDNA compared to that of dsDNA due to the delocalization of π stacking orbitals over its long base pairs, which agrees with previous research that used fragment molecular orbital and density-functional theory calculations. We attribute the small $V_{\text{trans}}$ that is, the decreased energy gap, to delocalization of the π-orbitals of DNA over its long base pairs. To prove this, molecular orbital calculations were performed based on density-functional theory (Supplementary Note 6). We indeed found that the energy gap between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) decreased with the increase in DNA length, in line with previous theoretical studies. The larger conductance of the 90-mer DNA zipper compared to the 10-mer counterpart (Fig. 1c) is consistent with the length-dependent decrease in the HOMO–LUMO gap. Thus, we conclude that the single-molecule junction of the DNA zipper attains high conductance due to the delocalized π system of the stacked DNA bases near the electrodes. The effect of this electron delocalization could explain the higher conductance of the 90-mer DNA zipper compared with that of the 10-mer counterpart (Fig. 1b), though this behavior merits further investigation.

For evaluation of the electronic structure of the molecular junction, the transition voltage ($V_{\text{trans}}$) was estimated using $I$–$V$ curves of the high-conductance state (Fig. 2e–h). It has been known that $V_{\text{trans}}$ is proportional to the energy gap between the Fermi level of the electrode (the tip or the substrate) and that of the conduction orbital of the molecule in the junction. In transition voltage spectra, $\log(I/V^2)$ is plotted against $V^{-1}$, and the voltage at which the plot reaches a minimum corresponds to $V_{\text{trans}}$. The mean value of $V_{\text{trans}}$ for the molecular junction of the 90-mer DNA zipper was found to be 0.4 V (Fig. 2e). In our previous work, a $V_{\text{trans}}$ value of 0.8 V was found for a single-molecule junction of 8-mer DNA in the conventional configuration. It was further observed that this value decreased to 0.5 V upon binding of an intercalator to the DNA. The $V_{\text{trans}}$ value for the molecular junction of the DNA zipper is smaller than in both these above-mentioned cases. This result suggests the decrease of the energy gap by delocalization of π stacking orbitals over its long base pairs, which agrees with previous research that used fragment molecular orbital and density-functional theory calculations. We attribute the small $V_{\text{trans}}$ that is, the decreased energy gap, to delocalization of the π-orbitals of DNA over its long base pairs. To prove this, molecular orbital calculations were performed based on density-functional theory (Supplementary Note 6). We indeed found that the energy gap between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) decreased with the increase in DNA length, in line with previous theoretical studies. The larger conductance of the 90-mer DNA junction compared to the 10-mer counterpart (Fig. 1c) is consistent with the length-dependent decrease in the HOMO–LUMO gap. Thus, we conclude that the single-molecule junction of the DNA zipper attains high conductance due to the delocalized π system of the stacked DNA bases near the electrodes. The effect of this electron delocalization could explain the higher conductance of the 90-mer DNA zipper compared with that of the 10-mer counterpart (Fig. 1b), though this behavior merits further investigation.

![Fig. 3 Consecutive conductance–displacement (G–z) measurements for molecular junctions of DNA zipper. a Schematic illustration of in-situ hybridization using STM molecular tip. Initial set-point current, 7.5 nA; pull-up distance, 30 nm; tip velocity, 31 nm/s.](image-url)
**Self-restoring capability.** Highly feasible and reproducible formation of a single-molecule junction, in addition to high conductivity, is a critical step toward the realization of electronic devices using a single DNA molecule. The zipping configuration of the present molecular junction enabled us to employ a longer DNA duplex, which is known to improve the thermodynamic stability of the duplex in solution. Thus, we expect that the present strategy could improve the stability and/or the reproducibility of single-molecule junctions. To test this hypothesis, we investigated repeated formation of the single-molecule junction of the DNA zipper (Fig. 3a). An ssDNA molecule and its complementary strand were tethered to the STM tip and the Au(111) surface, similar to that in the I–V measurement. The molecular tip was first carefully brought in close proximity to the sample surface under the STM feedback loop. After waiting for 0.3 s to facilitate hybridization and formation of the zipper structure, the STM tip was pulled up by 30 nm to record the conductance trace. The whole process was repeated to record consecutive G–z traces. For each trace, the dwell length was determined as the trace length between 1.4 and 2.4 mG0 and between 0.14 and 0.16 mG0 for the 90-mer and 10-mer zipper, respectively. These ranges were determined on the basis of the conductance values and their standard deviations from the STM-BJ measurements (see Fig. 1b). Figure 3b shows the time course of the dwell length obtained by successive 700 G–z traces for 10-mer and 90-mer DNA. The dwell length was then compared to the plateau length of the single-molecule junctions (dashed lines in Fig. 3b) of the 90-mer or 10-mer zipper DNA (Supplementary Note 1) to determine whether the molecular junction was successfully formed. It is clear that the 90-mer DNA zipper structure significantly enhances the repeatability of formation of the single-molecule junction compared with that of the 10-mer DNA zipper structure. The maximum number of repeated junction formations of the 90-mer DNA zipper reached 78; the single-molecule junction of the 90-mer DNA zipper was repeatedly reproduced for approximately 100 s despite the repeated perturbation of the junction by the tip displacements (Fig. 3c). The time course of the dwell length was quantitatively analyzed using joint probabilities (Supplementary Note 7). The analysis led to the same conclusion: the repeated and random formation of the molecular junction for 90-mer and 10-mer DNAs, respectively. The repeated formation of the DNA zipper junction was also confirmed by measuring mechanical forces exerted on the junction with AFM (Supplementary Note 8). A plausible model of DNA dynamics in this experiment was proposed in Fig. 3d. Repeated formation of the molecular junction for the 90-mer DNA could be due to partial preservation of the DNA duplex during pull-up procedures in the current measurements. This is not the case for the single-molecule junction of the 10-mer DNA zipper, since the pull-up distance of 30 nm is enough to break this junction considering the length of the duplex. The displacement dependence of the restoration behavior demonstrates the participation of the partially hybridized duplex and thus corroborates this model (Supplementary Note 9). The self-restoring behavior found for the present zipper junction opens up a way for reliable operations of single-molecule devices.

**MD simulations.** Course-grained MD simulations were performed to confirm partial preservation of the duplex for the single-molecule junction in the DNA zipper configuration. The...
CafeMol software\textsuperscript{42} with 3SPN.2 C model\textsuperscript{43,44} was used for these calculations. It has been shown that this model successfully reproduces melting phenomena, persistence lengths, and major and minor grooves of dsDNA\textsuperscript{13–15}. In the present work, one end of the 90-mer DNA duplex in the single-molecule junction was linked to two springs to represent the STM experiments (Fig. 4a, b). Then, the terminal base in one strand of the duplex (Chain A in Fig. 4b) was gradually pulled up by 30 nm in the simulation, as in the experiments (Fig. 3d). This base was then moved down to the initial position after the waiting time, as shown in the top panel of Fig. 4c. The distances between the selected complementary bases and the number of base pairs contained in the duplex region were plotted in the middle and bottom panels of Fig. 4c, respectively. Figure 4d shows selected snapshots of the single-molecule junction. Crucially, the DNA duplex is partially preserved after retraction (Fig. 4c, (II)), and the partial duplex, containing 44 base pairs, persisted after the waiting time (Fig. 4c, (III)). As the tethered end was pushed back to its original position after retraction, the unwound portion of DNA gradually reannealed to restore the complete duplex (Fig. 4c, (IV); see also Supplementary Movie 1). The distances between the complementary bases remained unchanged at the DNA end opposite to the tethered base (Fig. 4c, middle), demonstrating again the partial preservation of the DNA duplex. We carried out additional simulations, where the tethered end was released after the pull-up of 30 nm. The reannealing also occurred in this case, as found for the simulations above, and the complete duplex emerged (Supplementary Movie 2). These findings prove that spontaneous base-pairing accounts for duplex restoration, and not the external force exerted by the spring, which represents the STM tip in the experiments.

The lifetime of conventional single-molecule junctions, such as those of alkanedithiol, is typically short (in the order of milliseconds at room temperature), which poses fundamental problems for the realization of single-molecule electronics. These junctions break at the interface between the constituent molecule and the electrode, because the bonding between the functional group of the molecule and the electrode, e.g., Au–S bond, is weakest in the junction\textsuperscript{46}. It has been reported that the force required to break the Au–S or Au–Au bond is 1–2 nN\textsuperscript{47}, while the DNA unzipping takes place under the force of 10–50 pN\textsuperscript{22–24}. Thus, in the present junctions, the breakdown is attributed to be via DNA unzipping. The unzipping relaxes the tensile stress on the junction. Therefore, the duplex of long DNA zippers can be partially preserved, leading to prolonged preservation of the single-molecule connection with a DNA zipper. These results demonstrate that the DNA zipper offers a means to achieve both reproducible formation by the self-restoring capability and high conductivity.

In summary, we developed a DNA single-molecule junction where the duplex orthogonally clamped the metal nanogap. The electrical measurements based on STM revealed that the molecular junction of the DNA zipper attains high conductance, originating from the delocalized π system of the DNA near the electrodes. Moreover, we found an attractive self-restoring capability that the single-molecule junction can be repeatedly formed without breakdown of the whole structure even after an electrical failure. This advantage arises from the partial unzip of the DNA within the junction, which is supported by the coarse-grained MD simulations. It is also worth noting that conductance of the molecular junction exhibited no steep dependence on DNA length, which is distinctly different from the conventional single-molecule junction of DNA. Therefore, we expect that further functionalization could be possible through the use of a long DNA sequence as a scaffold to form conjugates with a wide range of functional (bio)molecules.
