Supporting Information

Single-molecule conductance of double-stranded RNA oligonucleotide

Subrata Chandra, Keshani G. Gunasinghe Pattiya Arachchillage, Evgenii Kliuchnikov, Farkhad Maksudov, Steven Ayoub, Valeri Barsegov, and Juan M Artes Vivancos

Department of Chemistry, University of Massachusetts, Lowell, 01854 MA, United States

Materials and Methods

Oligonucleotide Preparation

DNA and RNA oligonucleotides were purchased from Biosynthesis Inc. (USA). Some sequences have thiol linkers and C6 spacers on both 5' and 3' termini in the same strand for electrode binding (RNA probes). Single-stranded oligonucleotide tubes were spun down and then resuspended in 100mM Phosphate buffer. dsRNA and DNA:RNA were prepared by hybridizing them with their respective complementary strands (RNA probe with DNA/RNA target) in 100mM phosphate buffer. Thermal hybridization was done by heating the mixture to 80^0 C degrees in a water bath. After that, the hybridized samples were left to cool down to room temperature within several hours.

Solution and Substrate Preparation

Piranha solution was used to clean all the glassware and Teflon STM cells. The solution was prepared by adding 98% sulfuric acid and 30% hydrogen peroxide in a 3:1 v/v ratio*. **Caution: Piranha solution is extremely corrosive and dangerous, direct contact will burn human skin, and both liquid and vapor phases are highly corrosive to skin and the respiratory tract.***

Other parts of the STM cell were cleaned by Acetone (HPLC plus), and Ethyl Alcohol (pure) were purchased from Sigma Aldrich. Before each experiment, Au single crystal was electropolished in 0.1 M sulfuric acid (Sigma Aldrich) using two thin gold wire loops as electrodes. Au single crystal was placed on top of one gold wire loop (working electrode), and it was connected to the DC power supply. The other gold wire loop (counter electrode) was placed closer to the single crystal (preferably less than 0.5 cm) and connected to the same DC supply. We used the DC power supply to achieve about 10V and 1 Amp current. The current was applied for about 30 seconds until we observed the Au single crystal had oxidized to form an orange layer on top. Then, the oxidized Au single crystal was rinsed in Milli-Q water multiple times. After that, the rinsed Au single crystal was placed into 1M HCl solution and rinsed again in Milli-Q water until we observed the pure gold color back, as previously described.[1] Finally, the cleaned Au single crystal was flame annealed.[2]

Na_2HPO_4 and NaH_2PO_4 were purchased from Sigma-Aldrich to prepare 100 mM pH 7.4 phosphate buffer with Milli-Q water. Then the buffer solution was filtered with 20 nm pore size filters (Whatman Anotop 25 plus). The filtered buffer solution was then ready to be used in experiments.

STM-Break junction measurements

All experiments were performed at room temperature. Experiments were done using a Molecular Imaging Pico-STM head connected to a Digital Instruments Nanoscope IIIa controller. In the STM-BJ method, the STM tip was repeatedly brought into contact with the substrate and then pulled away (Tapping method).[3] This process forms a tip-substrate gap that is narrow enough to be bridged by a molecule. In this method, a large number of individual molecules and their conformations can be probed by repeating the experiment many times. Thousands of current-distance traces were recorded during each experiment. Some of these traces show steps, which indicate the presence of oligonucleotide junctions between tip and substrate.

Before measuring oligonucleotide sequences, a control experiment in a phosphate buffer solution was performed. Then, small volumes of DNA:RNA hybrid or dsRNA were injected into the cell to achieve ~ nM final concentrations for the conductance measurements.

Tris (2-carboxyethyl) phosphine (TCEP) was used to reduce the disulfide bond. TCEP selectively and completely reduces even the most stable water-soluble alkyl disulfides over a wide pH range. This treatment produces free thiol groups from the oligonucleotide sequence, which will eventually bind the gold electrodes.

Labview Software

A customized LabView (National Instruments) program was used to control the movement of the STM tip during the measurements[4]. This program performs a logarithmic binning of each recorded current-distance trace and then selects those whose residual values meet the specified criteria. All the filtered curves were added together to obtain a semi-logarithmic conductance histogram, which revealed the most probable conductance of the specific molecular junction.

Computational Molecular Modeling

Modeling of RNA and DNA sequences

SimRNA software[5] was utilized to model RNA sequences in dsRNA. A DNA strand in DNA:RNA hybrid was constructed by replacing 2'-OH group by 2'-H group in ribose rings using VMD package[6]. The topology and coordinate files for dsRNA and DNA:RNA hybrid were prepared using tleap module in AMBER 20[7]. The system was neutralized using Na^+ and Cl^- ions and excess ions (0.1 M) were added to mimic the experimental conditions. The force fields $bsc0_{\chi OL3}$[8] used for RNA and bsc0[9] for DNA is a part of ff14SB[10]. Using TIP3P water molecules, the dsRNA and DNA:RNA systems were solvated up to 8Å from any of the solute atoms[11]. The number of water molecules in both systems were ~3,700.

MD simulation protocol

The water molecules and ions in the solvated systems were minimized in 10,000 steps of the steepest descent and in 50,000 steps of the conjugate gradient with 50 kcal/mol restraint on the solute atoms. The system was heated from 0K to 300K in 50 ps. Next, a 100 ps of restrained (0.5 kcal/mol on solute atoms) MD simulations were performed to equilibrate each system. The

production MD simulations were performed for 1 μs using CUDA version of pmemd[12] utilizing a GPU acceleratation[13,14] of AMBER 20[7]. The Particle Mesh Ewald (PME) method was utilized for calculating the contributions from the nonbonded interactions with a cutoff of 10Å. SHAKE algorithm was used to treat the bonds involving light (hydrogen) atoms. Next, the unrestrained MD simulations were performed in an NPT-ensemble with the time step of 2 fs. A constant external pressure of 1 atm was maintained using Bendersen weak-coupling barostat with the time constant of 0.5 ps[15]. The MD simulation temperature (300K) was maintained using a Bendersen thermostat with a time constant of 4 ps. The MD trajectories were saved every 20 ps.

Analysis of MD simulation output

The root mean square deviation (RMSD) was calculated for all atoms of the solute using the RMSD Trajectory Tool in VMD[2]. RMSDs were calculated for the backbone heavy atoms of DNA/RNA (P, O5′, C5′, C4′, C3′, and O3′). All the frames (structure snapshots) were superimposed with the structure corresponding to 500 ns (initial structure) after removing the water molecules from the MD output. The snapshots depicting the averaged structures (shown in the background in the Supplementary Movie1) were calculated using the Tcl script in Tk Console in the VMD. The end-to-end distance X was calculated as a distance between the first and last (11th) P-atoms in the passenger strand in dsRNA or DNA strand in DNA:RNA hybrid. The total number of base pairs and base stackings were calculated using Barnaba software[12]. Bases were classified as stacked if ($|z_{ij}|$ and $|z_{ji}| > 2$Å) and (ρ_{ij} or $\rho_{ji} < 2.5$Å) and ($|\theta_{ij}| < 40°$). Here, $\rho_{ij} = \sqrt{x_{ij}^2 + y_{ij}^2}$, where x- and y-axis are in the plane of the base and z-axis is normal to the xy-plane, z_{ij} is the distance between the centers of mass of the two bases, and θ_{ij} is the angle between the normal vectors of the two bases[12]. All the non-stacked bases are considered base-paired if $|\theta_{ij}| < 60°$ and there exists at least one hydrogen (H) bond. We assume that the H-bond D–H…A between the hydrogen donor atom (D) and acceptor atom (A) is formed if the donor–acceptor distance d_{DA} is less than the 3.3Å cutoff and the bond angle is larger than the 140°cutoff[12]. We also analyzed the torsion angles in RNA and DNA backbones, in particular, torsion angle δ formed by C5'-C4'-C3'-O3' atoms, and glycosidic angle χ formed by O4'-C1'-N9-C4 and O4'-C1'-N1-C2 atoms for purine and pyrimidine bases.

Calculation of thermodynamic state functions

Van der Waals interaction energies between two strands in the duplex (dsRNA or DNA:RNA hybrid) were computed utilizing MMPBSA pairwise energy decomposition analysis using a reported procedure[17,18] implemented in MMPBSA.py program[19] in AMBERTools[7]. For this calculation, structure snapshots were extracted from the last 500 ns part of MD trajectories taken at a time interval of 5 ns.
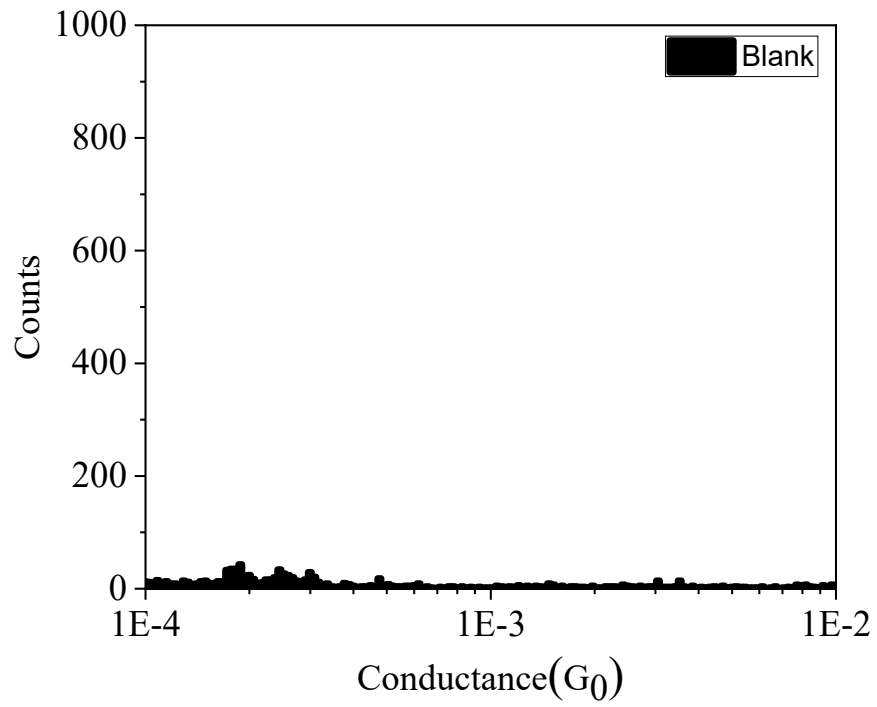
Figure S1. Logarithmic conductance histogram obtained from control experiments in phosphate buffer solutions.
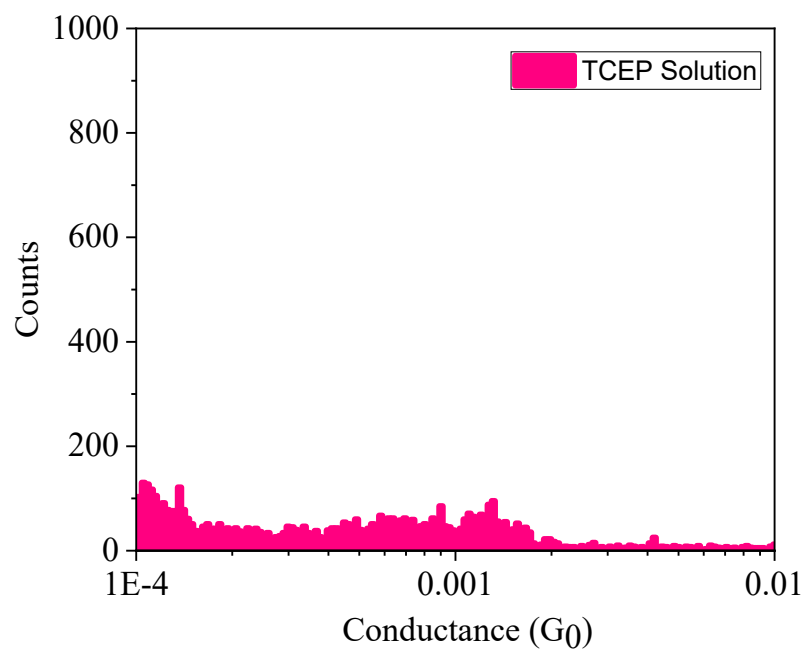
Figure S2. Logarithmic conductance histograms of control experiment on Tris (2-carboxyethyl) phosphine (TCEP) solution (used to reduce thiol linkers).
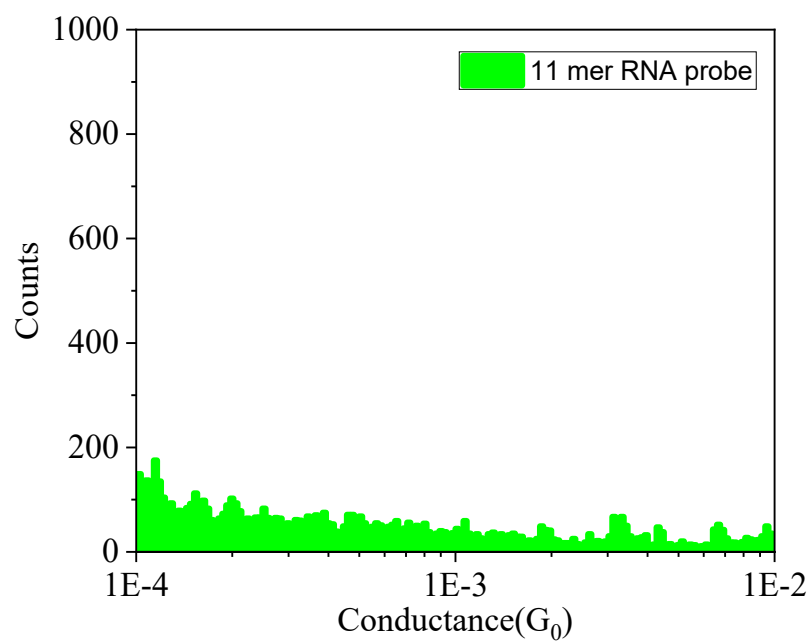

Figure S3. Logarithmic conductance histograms corresponding to control experiments on 11-bp dithiol-functionalized ssRNA.
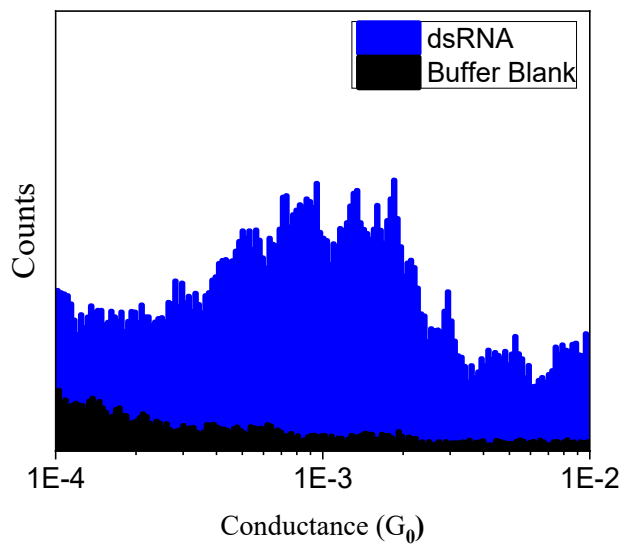
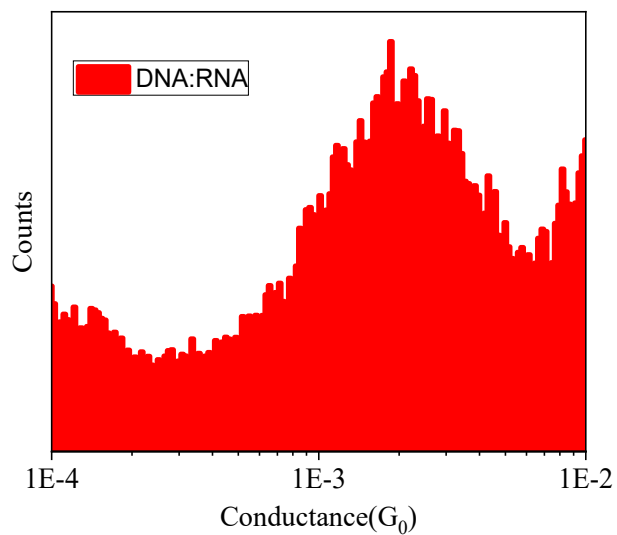*Figure S4. Conductance histogram of 11 -mer dsRNA (Blue) compared with phosphate buffer blank (black).*

Figure S5. Conductance histogram for the 11mer DNA:RNA oligonucleotide.

Supplementary Movie 1: MD simulations of dsRNA and DNA:RNA hybrid

The movie shows conformational fluctuations in dsRNA (left) and DNA:RNA hybrid (right) as observed in MD simulations. Transient structures are superposed with the average structure displayed in the background. The MD simulation runs were carried out in explicit water with 0.1 M NaCl, but the movie shows only nucleic acids for clarity. Each duplex displays the secondary structure and chemical bonds with "NewCartoon" and Bonds representations implemented in VMD (the blue color is for dsRNA and red color is for DNA:RNA). Shown are 500-ns long MD simulation runs. The length of the movie is 24 s. The movie is played 48,000,000 times slower than the computational experiment.

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