

Self-Aligning Nanojunctions for Integrated Single-Molecule Circuits

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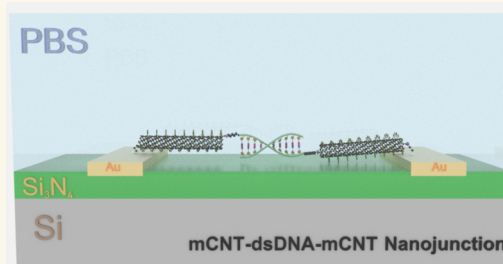
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ABSTRACT: Robust, high-yield integration of nanoscale components such as graphene nanoribbons, nanoparticles, or single-molecules with conventional electronic circuits has proven to be challenging. This difficulty arises because the contacts to these nanoscale devices must be precisely fabricated with angstrom-level resolution to make reliable connections, and at manufacturing scales this cannot be achieved with even the highest-resolution lithographic tools. Here we introduce an approach that circumvents this issue by precisely creating nanometer-scale gaps between metallic carbon electrodes by using a self-aligning, solution-phase process, which allows facile integration with conventional electronic systems with yields approaching 50%. The electrode separation is controlled by covalently binding metallic single-walled carbon nanotube (mCNT) electrodes to individual DNA duplexes to create mCNT-DNA-mCNT nanojunctions, where the gap is precisely matched to the DNA length. These junctions are then integrated with top-down lithographic techniques to create single-molecule circuits that have electronic properties dominated by the DNA in the junction, have reproducible conductance values with low dispersion, and are stable and robust enough to be utilized as active, high-specificity electronic biosensors for dynamic single-molecule detection of specific oligonucleotides, such as those related to the SARS-CoV-2 genome. This scalable approach for high-yield integration of nanometer-scale devices will enable opportunities for manufacturing of hybrid electronic systems for a wide range of applications.

KEYWORDS: single-molecule electronics, molecular devices, biosensors, carbon nanotubes, nanoelectronics, nanojunction, self-alignment



1. INTRODUCTION

The development of self-aligning transistors, where the gate electrode is used as a mask layer to pattern the source and drain, was a key breakthrough that greatly improved the manufacturing yield of semiconductor devices and enabled the continued scaling of electronic systems over many device generations.^{1–7} Now, in recent decades a variety of low-dimensional materials have been developed with intriguing electronic properties, including CNTs,^{8,9} semiconductor nanowires,¹⁰ graphene and other 2D materials,^{11,12} and molecular electronic components.¹³ However, despite the promise of these systems, the ability to manufacture these devices at-scale for useful applications and to integrate them into larger-scale lithography processes has remained challenging.^{14–18} This issue becomes increasingly prominent as the size-scale decreases to nanometer and molecular levels.^{4,15} For example, while an incredible variety of impressive, molecularly-enabled electronic functions have been demonstrated including quantum interference,^{16,19} neuromorphic and memristive activity,^{20–22} and optoelectronic control,²³ the utility of these devices has been restricted to understanding physical and chemical processes at the nanoscale due to the difficulties with

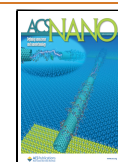
larger-scale integration.^{2,24,25} To realize the full utility of these systems, they must be incorporated into stable, robust, and reliable electronic systems. Thus, inspired by the techniques used for creating self-aligned transistors, we present a method for combining controlled, bottom-up self-assembly processes, with top-down lithographic techniques to create self-aligning single-molecule devices, where the molecule itself is used to define the gap between metallic electrodes, with an overall yield of 44.6% (Figure 1). We demonstrate the utility of this approach by creating a dynamic, high signal-to-noise ratio (SNR), single-molecule electronic biosensor capable of identifying specific oligonucleotide sequences with high fidelity. This approach provides a basis for developing reliable methods for integrating single-molecule circuits with conven-

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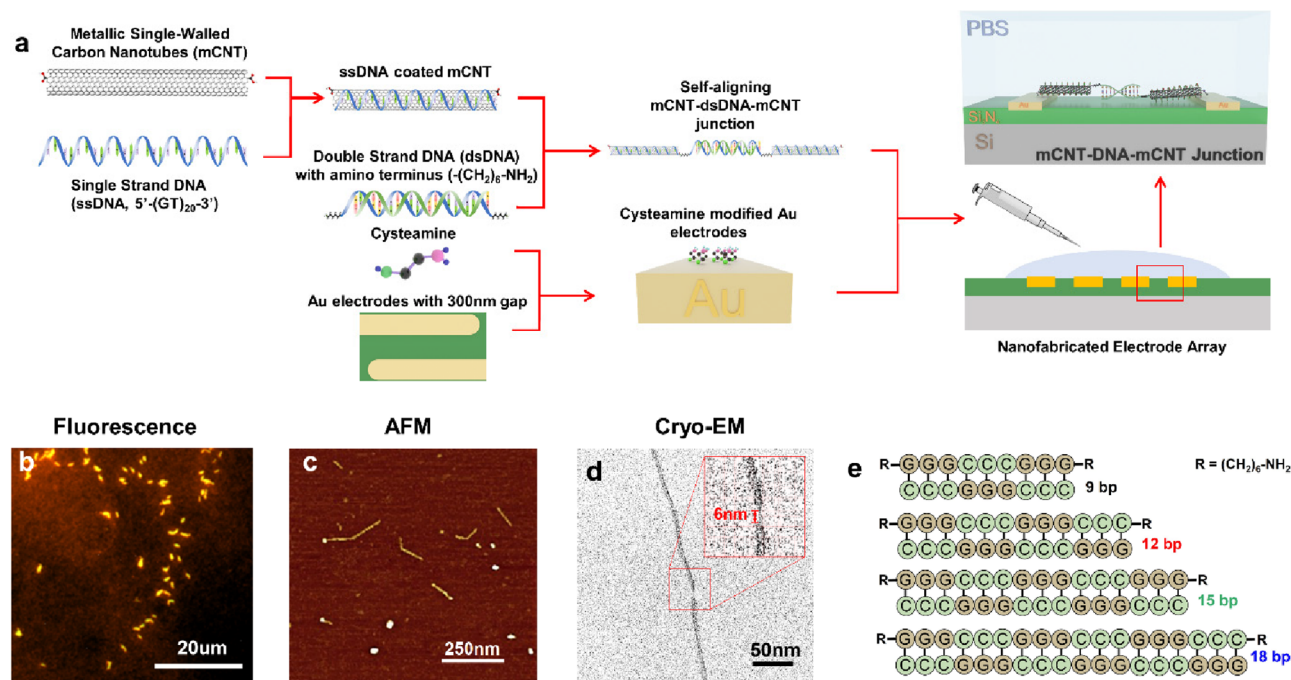


Figure 1. Development of mCNT-DNA-mCNT junctions. (a) Reaction scheme for assembling the self-aligning mCNT-DNA-mCNT junctions and integrating them into the electronic circuit. Carboxyl-terminated mCNT are first wrapped with (GT)₂₀ and then coupled to amino-functionalized DNA oligonucleotides in PBS buffer to form a self-aligning hybrid structure. Thereafter nanojunctions are drop-cast onto nanofabricated devices where cysteamine modified gold electrodes can anchor the nanojunction with an amide bond to create a robust single molecule junction. (b) Fluorescence micrograph of mCNT-DNA-mCNT structures. (c) Atomic force microscope image of mCNT-DNA-mCNT structures on a mica surface. (d) Cryo-EM micrograph of a mCNT-DNA-mCNT junction with a ~6 nm gap structure. (e) Series of alternating G:C triplet DNA sequences studied.

tional electronic systems to allow the distinctive functionality of molecular systems to be harnessed at scale.

To create robust single-molecule junctions, we use metallic carbon nanotubes (mCNTs) as nanoelectrodes, which are covalently linked to a molecular bridge in solution phase to create hybrid mCNT–molecule–mCNT junctions. This solution-phase chemical approach ensures that the gap between the mCNTs is atomically matched with the length of the molecule; this “self-aligning” chemical assembly process circumvents the difficulty of fabricating subnanometer precision single-molecule gaps using standard lithography processes.^{1,3,5} Instead, these mCNT–molecule–mCNTs can be integrated with conventionally nanofabricated Au electrodes with 300 nm gaps. This combination of bottom-up and top-down processing allows facile integration of single-molecule devices with conventional lithographic processes. Thus, by harnessing the inherent strengths of chemical control of molecular systems, this solution-phase approach allows straightforward junction self-alignment. To create these hybrid junctions, we utilize only metallic CNTs to improve the reproducibility and decrease the dispersion of the conductance properties. DNA is selected as an initial molecular target due to its extensively studied electronic properties and its potential utility as a biosensing element.^{26,27}

2. RESULTS AND DISCUSSION

To assemble the mCNT-DNA-mCNT structures, we begin with commercially available mCNTs that are soluble in aqueous solutions.²⁸ To protect the mCNT sidewalls during the impending chemical processes, they are wrapped with single-stranded (GT)₂₀ DNA (Figure 1a,b), which binds

strongly to the mCNTs through noncovalent interactions and ensures the continued solubility of the mCNTs in aqueous phases.^{29–32} After this step (see [Methods](#)), the DNA wrapped-mCNTs are added to a solution of the target DNA duplexes that are terminated on both ends with an amine group $-(\text{CH}_2)_6\text{-NH}_2$. Then, an amidation reaction between the terminal amines and the exposed carboxylic acid groups on the ends of the mCNTs is initiated using hexafluorophosphate azabenzotriazole tetramethyluronium (HATU) to create the final, self-aligned nanojunction in solution that can be subsequently placed on Au electrodes (Figure 1a).^{33,34}

To examine the morphological properties of these potential mCNT-DNA-mCNT structures, we first utilize fluorescence microscopy. By incubating the samples with ethidium bromide (EtBr) and comparing the resulting fluorescence images (Figure 1b) with control experiments (Figure S1), we find that the combination of single-stranded DNA wrapping and an amidation reaction with double-stranded (ds) DNA results in structures that have either single or multiple bends in the backbone, indicative of a linked structure with differing persistence lengths.^{35,36} These results are further corroborated by atomic force microscopy (AFM) images, where V-shaped features commonly appear (Figure 1c). The AFM images reveal that the resulting structures are typically on the order of 250–350 nm and that there are often locations within the structures that possess a smaller cross-section than the rest of the structures (Figure S2). However, it is difficult to determine whether these features are due to the presence of single-molecules between two mCNTs or due to defects in the single-stranded DNA wrapping process. Thus, to further investigate this possibility, we utilize cryogenic transmission electron microscopy (cryo-EM) to examine the structures. The cryo-

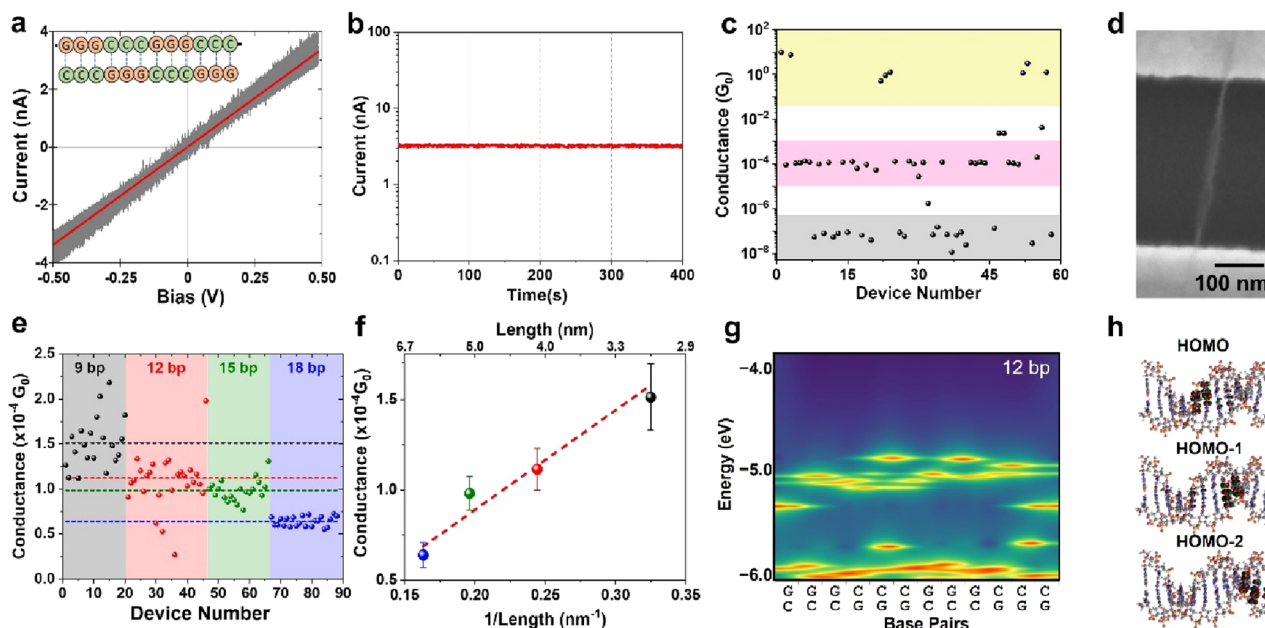


Figure 2. Charge transport properties of mCNT-DNA-mCNT structures. (a) 100 repeated I – V characteristics (gray background) for the 12 bp G:C sequence shown in the inset and a linear fitting to the data ($R^2 = 0.989$, red line) indicating ohmic transport. (b) Current through the device shown (a) over a 400 s time period. (c) Aggregated data from 58 (300 nm) gap devices with the 12 bp sequence. There are 3 distinctive regimes: high conductance ($>10^{-1} G_0$, yellow), medium conductance ($1 \times 10^{-4 \pm 1} G_0$, pink), and low conductance ($<10^{-6} G_0$, gray). 45% of the devices had a conductance value in the pink region. (d) SEM image of one of the devices in the pink region of (c) showing a single bridge between the electrodes. (e) Plot of all devices with a conductance in the range of $1 \times 10^{-4 \pm 1} G_0$ for all 4 G:C triplet sequences studied from 9 to 18 bp, and their average values (dashed lines). (f) Conductance of the G:C triplet sequences vs $1/L$. Error bars are standard deviations calculated from all measurements in plot (e). (g) 2D density of states plot for the 12 bp sequence showing a series of delocalized states in a narrow energy range. (h) Isoplots for the 3 highest occupied molecular orbitals (HOMO) demonstrating orbitals are delocalized over several bases.

EM images indicate that gaps appear in the mCNT structures (Figure 1e), which is again indicative of the formation of mCNT-DNA-mCNTs. Taken together, these characterization approaches consistently suggest molecularly aligned mCNT-DNA-mCNT structures are formed using the above procedure.

Next, we turn to electrical characterization techniques to demonstrate the single-molecule nature of these devices more conclusively. We start by analyzing the electrical properties of a 12 bp sequence with alternating G:C triplets (5'-GGG CCC GGG CCC-3' + complement) with amine linkers on each end (Figure 1f). To create robust mechanical contacts to the mCNTs, we incubated the nanofabricated substrate with 300 nm gap Au electrodes in a cysteamine solution overnight prior to adding the mCNT-DNA-mCNT solution (see Methods section for details). Figure 2a plots 100 consecutive I – V characteristics obtained from a single, bridged 300 nm gap measured in a 100 mM sodium phosphate buffer solution (PBS) at room temperature. For bias voltages swept over a ± 0.5 V range, the I – V curves of this 12 bp device are nearly linear, indicating ohmic transport behavior. And as can be seen by the reproducibility of the I – V characteristics and the time series measurements on these devices (Figure 2b), the structures and conductance values are very stable over time. To examine the reproducibility of the measured conductance values, we examined 58 pairs of 300 nm gap devices across three different chips, and the data from each of these gaps are plotted in Figure 2c. There are primarily three different conductance regimes visible in this plot. The gray region shows gaps that are open circuits, indicating no bridging occurred. The yellow region includes high-conductance values. These

electrode pairs are either short-circuited during fabrication or bridged by mCNTs (Figure S3) or bundled structures. The third, pink region, which spans a conductance range of $1 \times 10^{-4 \pm 1} G_0$ (where G_0 is the conductance quantum, $G_0 = 77.48 \mu\text{S}$), displays a series of devices with a narrow conductance distribution at $1.02 \times 10^{-4} \pm 0.17 \times 10^{-4} G_0$, which is in close agreement with published values for this sequence obtained using the single-molecule break junction approach.³⁷ Scanning electron microscopy (SEM) images of the devices from this conductance range verify that they are bridged by a single entity (Figure 2d). Notably, of the 58 electrode pairs measured, 26 devices (45%) have a conductance value within this window.

Upon verifying that the measured conductance values stem from individual bridges between the electrodes, the next step is to demonstrate that these conductance values are determined by single molecules in the mCNT-DNA-mCNT junctions. To do so, we take advantage of the self-alignment capabilities of this approach and systematically measure the conductance of these alternating G:C triplet sequences with lengths of 9, 12, 15, and 18 base pairs. For each molecule, mCNT-DNA-mCNT nanojunctions are first created in solution-phase using the procedure above to ensure that the gap between the mCNTs is perfectly aligned to each molecule's length. After this, the nanojunctions are drop-cast onto their respective chips for electrical characterization. As seen in Figure 2e the conductance of each of these molecules falls in a narrow region around $1 \times 10^{-4} G_0$, which is consistent with previous measurements on GC-rich DNA using Au electrodes.^{38,39} Moreover, it is also apparent that there is a systematic decrease

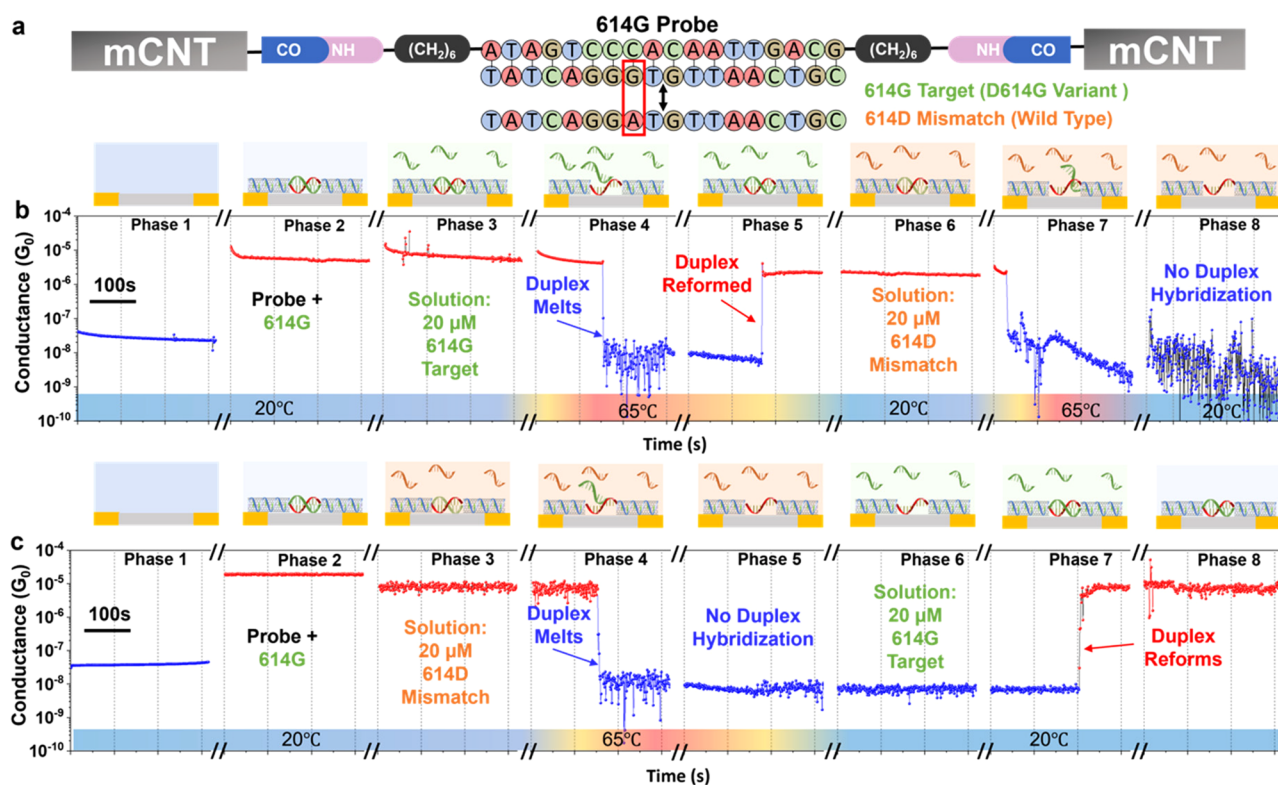


Figure 3. Electronic oligonucleotide detection using a single-molecule biosensor. (a) Schematic representation of the device with the 614G probe and target sequences (614G and 614D). (b) Conductance as a function of time for a single mCNT-614G probe-mCNT junction, with schematics above each phase indicating the process. Phase 1: open-circuit, blank device, prior to junction deposition. Phase 2: conductance of the 614G probe/target (PBS, 20 °C). Phase 3: Replace solution with 20 μ M 614G targets in PBS. Phase 4: Dehybridize the duplex by heating the system at a rate of 0.5 °C/s. A conductance drop of 3 orders of magnitude occurs when the solution temperature reaches 65 °C indicating DNA dehybridization, with only the single-stranded 614G probe remaining between the mCNTs resulting in a current below our detectable range. Phase 5: During cooling, the junction is re-formed when a 614G target from solution binds to the probe sequence to reform a DNA duplex and conductive pathway. Phase 6: Replace solution with the mismatched 614D sequence (WT). Phase 7: Repeat the dehybridization process by heating the sample, which again dehybridizes near 65 °C. Phase 8: Upon cooling, no device formation was observed. (c) Similar procedure as in (b) on a second device but with sample injection order reversed (first mismatched 614D followed by perfectly matched 614G). Mismatched DNA does not rehybridize in the junction in either case, but the matched 614G sequence does.

in the conductance with increasing length (L), and we find that this change in conductance is directly proportional to the $1/L$ (Figure 2f). This linear dependence of the conductance with length, coupled with the linearity of the I - V traces, is strongly indicative of a hopping-dominated transport mechanism, which has been widely attributed to GC-rich sequences.^{40–44} We note that if the DNA was not playing a role in the conductance of these systems, we would not expect any change in the conductance with molecular length because the nominal electrode gap is 300 nm in all cases. Thus, these measurements provide a clear indication that the circuits are derived from single-molecule junctions. Altogether, from the four molecules, we measured 197 electrode pairs and obtained an overall yield of 44.6% (88 bridged gaps).

To further examine hopping as the dominant transport mechanism, we performed molecular dynamics (MD) simulations, density functional theory (DFT) electronic structure calculations, and extracted the density of states for the system.^{26,45} As can be seen in Figure 2g,h, for the 12 bp case, the top several occupied molecular orbitals are delocalized over several guanines in the G-triplet regions (see Supporting Information Figure S4 for other molecules). We also find a relatively weak coupling between the triplets (average of 7.5 meV), indicating that a charge would likely

traverse these structures by systematically hopping between delocalized energy levels.

Finally, to unambiguously verify the single-molecule nature of these circuits, demonstrate their stability, and examine their utility to serve as active electronic components, we utilize the system as a single-molecule electronic biosensor (Figure 3). For these experiments, we test whether we can detect the presence of a specific oligonucleotide related to the D614G mutation (aspartic acid swapped for glycine at amino acid residue 614 on the spike protein) in the SARS-CoV-2 virus. The D614G modification was an early mutation that greatly increased the virulence of SARS-CoV-2,^{46,47} and by focusing on this coding sequence with a known point mutation difference between the wild type (WT) (GAT codon) and the variant (GGT codon), we can examine the specificity of this detection approach and its resilience when challenged with potentially interfering targets.

For the detection process, we selected an 18 bp DNA sequence identical to the SARS-CoV-2 genome that included several bases on either side of point mutation D614G, which we refer to as the 614G target sequence (Figure 3a), and then use a complementary DNA strand with amine linkers on either end as a probe molecule (614G probe). We then hybridize the probe and target and subsequently link it to mCNTs as above. This structure is then incubated on a microelectrode chip for

30 min before identifying a bridged device. For the device examined, the conductance was stable at $\sim 6 \times 10^{-6} G_0$ prior to sensing experiments (Figure 3b, phase 2). To decrease the possibility of additional mCNT-DNA-mCNT junctions bridging the electrodes during the sensing process, the sample solution is replaced with 20 μM 614G targets in PBS. The conductance remains close to the original value during this solution exchange process (Figure 3b, phase 3). Then, to complete the sensor preparation, we monitor the conductance of the junction while heating the system at a rate of 0.5 $^{\circ}\text{C}/\text{s}$. The conductance suddenly drops to the resolution of the current amplifier when the temperature reaches $\sim 65^{\circ}\text{C}$, which corresponds to the calculated melting temperature for this sequence ($T_m = 64.4^{\circ}\text{C}$). The change in the conductance at this temperature is likely due to the dehybridization of the DNA duplex, and a change in the circuit from transporting through double-stranded DNA is due to only the single-stranded 614G probe DNA, which is not conductive due to the lack of π -stacking (Figure 3b, phase 4).^{41,48} Now, with the conductance at effectively zero, we aim to detect the target sequence in the solution. To do so, we decrease the temperature of the cell back toward room temperature, and within several minutes of cooling, there is a sharp increase in the conductance of 3 magnitudes (Figure 3b, phase 5), which returned to $2.3 \times 10^{-6} G_0$ when the DNA probe rehybridizes with a target from the solution. This binary change in the conductance with the formation and breakdown of the device is a clear indicator of a single-molecule binding process^{49,50} and is commonly used in break-junction based experiments to identify single-molecule conductance values.^{51,52} We further note that this change in the conductance is not likely to be due to the breakdown and re-formation of the whole device or the contacts, as mCNT junctions are very stable over this temperature range (see Figure S3).

Next, to gauge the specificity of the system and verify that the binary conductance change occurs due to the molecule instead of changes to the overall bridged structure upon temperature cycling, we repeated this experimental procedure after replacing the 614G solution in the cell with a 20 μM concentration of the wild type, mismatched 614D sequence in PBS (Figure 3b, phase 6). Upon heating, the device again dehybridized at $\sim 65^{\circ}\text{C}$, but this time, upon cooling, no current increase was observed (Figure 3b, phases 6–8). We note that the conductance of the mismatched duplex is $\sim 1 \times 10^{-6} G_0$ (see Figure S6), so if the mismatched sequence binds *in situ* it will be measurable. Thus, this result suggests that the mismatched sequence is not binding to the probe in this configuration. We hypothesize that the lower melting temperature of the mismatched sequence coupled with the potential hindrances introduced by the rigid mCNT gap and the proximity to the surface may inhibit binding and duplex formation in this constrained configuration on-chip. Nevertheless, this obvious difference in device response between the 614G and 614D targets indicates that the sensing platform can be extremely sensitive to single nucleotide polymorphisms, though additional experiments will be required to determine if this is a general result for mismatched sequences in these devices. However, to confirm this result for sequence, a complementary experiment is performed on a second device but with the order of target injection reversed (Figure 3c). In this case, the junction conductance still decreases to zero upon heating, and again, no conductance increase occurred when cooling with an excess of 614D present in the solution (Figure

3c, phases 1–5). However, when the 614D solution is replaced with the perfectly matched 614G sequence (Figure 3c, phases 6–8), we again observe junction formation with a conductance of $4.8 \times 10^{-6} G_0$. We also note that the entire measurement process takes ~ 2 h, demonstrating the long-time stability of these single-molecule junctions. Two additional examples of the 614G probe/target binding process are shown in Figure S7.

3. CONCLUSION

Solution-phase self-alignment of single-molecule electronic devices followed by deposition onto nanofabricated electrodes provides the ability to create dynamic yet stable single-molecule circuits that are easily integrated with conventional lithographic processes. We obtain yields on the order of 50% using only standard, low-resolution photolithography without the addition of a low-throughput electron beam or focused ion beam lithography, which enables manufacturing nanoscale electronic circuits based on single-molecule or other nanoscale devices. We show that the resulting DNA-based sensors can reliably detect targeted sequences with very high SNRs and limited possibilities for false positives from interfering or mismatched sequences. This approach offers several potential advantages over conventional viral diagnostic tests. Compared to antigen tests, this approach could quickly pivot to different targets by simply changing the DNA probe sequences as new threats emerge, and it can also obtain strain-level information (i.e., distinguish between α and wild-type variants as done here). Typically, to obtain strain-level information, it is necessary to do RT-qPCR (reverse transcription quantitative polymerase chain reaction); these assays are expensive and time-consuming and require highly trained personnel. This platform also has the potential to be compact and field-deployable. Moreover, this target-agnostic diagnostic platform will allow DNA and RNA sequence detection to be implemented for a wide range of applications in biology, healthcare, and security for examining the presence of and identifying pathogens, performing liquid biopsies, and tracking disease progression. Moreover, the straightforward chemistry for creating these self-aligning structures provides additional opportunities for integrating other molecular systems with electronic platforms including proteins, catalysts, molecules that undergo field-controllable reactions, and more conventional molecular electronic devices such as switches, molecular memory devices, and diodes.

4. METHODS

Preparation of mCNT-DNA-mCNT Nanostructures. Metallic single wall carbon nanotubes (mCNTs) were purchased from Nano Integrus, Inc. Phosphate buffer solution (PBS) and hexafluorophosphate azabenzotriazole tetramethyl uronium (HATU) were purchased from Thermo Fisher Scientific. All other analytical-grade chemicals were purchased from Sigma-Aldrich. All DNAs were obtained from Alpha DNA, and sequences are listed in the Supporting Information.

The mCNT wrapping process is driven primarily by favorable interactions between the ssDNA bases and the mCNT due to van der Waals and hydrophobic interactions. 0.1 mL of the mCNT (1 mg/mL, with sodium dodecyl sulfate (SDS) and sodium lauryl ether sulfate (SLES)) surfactants solution is first diluted with 2.5 mL of PBS, then 2.5 mL of 5 μM (GT)₂₀ is added. After overnight incubation at room temperature, the mixture is placed in a clean tube surrounded by ice for at least 2 h under sonication (at the power level of 3 W) which is then centrifuged at 5000 rpm (Eppendorf 5415C) for 2 h to remove insoluble material. The supernatant is collected and

diluted with DI water (Milli-Q), and the volume of (GT)₂₀-dispersed mCNT solutions was kept to 2 mL.

To covalently attach DNA duplexes to (GT)₂₀ wrapped mCNTs, we begin with amino-terminated DNA prepared at a concentration of 5 μ M in 2 mL of PBS buffer and mix it with 2 mL of (GT)₂₀ wrapped mCNTs and $\sim$ 0.13 mM hexafluorophosphate azabenzotriazole tetramethyluronium (HATU, coupling agent). The mixture was left to react at room temperature for 3 h under weak sonication. Subsequently, any insoluble material is removed via 15 min centrifugation at 12 000 rpm. The mCNT-DNA-mCNT hybrids are then extracted from the supernatant and readied for transfer onto nanofabricated Au electrodes with 300 nm gaps.

Single-Molecule Device Fabrication. The microelectrode platform used for electrical tests of the nanostructures was fabricated by using standard optical lithography techniques. In brief, a Si/Si₃N₄ wafer is first spin-coated with hexamethyldisilazane (HMDS) as an adhesive layer, followed by PID controlled baking at 110 $^{\circ}$ C for 1 min. Then microelectrodes are patterned on the wafer using photolithography with a KL5302 hi-res photoresist (Kem Lab). After $\sim$ 15 s of development in CD-26 (TMAH), the wafer is gently rinsed with DI water and blow-dried with nitrogen to stop the development process. Then, a PETS-RIE plasma etcher is employed to etch $\sim$ 65 nm trenches into silicon nitride. A Cr(adhesive)/Au multilayer of thickness 10 nm/55 nm is deposited by an e-beam evaporator. The sample is then immersed in MICROPOSIT Remover 1165 for 90 min and ultrasonicated to lift off residual resist and metal. After that, a 100 nm thick Si₃N₄ insulating layer is deposited using plasma-enhanced chemical vapor deposition (PECVD), to cover the gold electrodes. Micrometer-sized windows for the probing and sensor areas are patterned using the same photolithography process used previously for the microelectrodes. Finally, a $\sim$ 100 nm etch into the PECVD Si₃N₄-cover layer is performed using the Plasma-Therma RIE to open the windows and expose the gold electrodes in the desired areas.

To couple the mCNT-DNA-mCNT structures to the Au electrode surfaces, we began by immersing the freshly made substrates in a 10 mM solution of cysteamine overnight after 5 min of UV-ozone treatment. The substrates were then removed from the solution, gently rinsed with DI water, and dried with a stream of nitrogen gas. After device characterization (blank test), a silicone ring was mounted and sealed to the sensor area as a sample reservoir. Thereafter, the modified Au electrodes were reacted with the mCNT-DNA-mCNT nanostructures through the same amidation reaction in a 100 mM PBS solution to covalently bridge the gaps and create a mechanically robust device.

Characterization. All the electrical device characterization is performed using a Cascade micromanipulator probe station equipped with a semiconducting parameter analyzer (Keithley 4200-SCS) at room temperature with a consistent moisture content (30%). For thermal cycling measurements, a PID-controlled heater stage ($\pm$ 0.01 $^{\circ}$ C) was employed to adjust the temperature (20–65 $^{\circ}$ C) at a constant rate. Sample surface temperatures were monitored by using an infrared thermometer (Fluke-561). Current sampling measurements were conducted with 0.5 V bias and at least 3 min of monitoring for each electrode. Voltammetry scans (–0.5 V to 0.5 V) were conducted subsequently. To ensure that any unattached or nonspecifically bound molecules are completely removed during the sample exchange, we pipet out the spent solution and then rinse the sample reservoir with PBS buffer before introducing a new sample.

Fluorescence. The fluorescence studies of the mCNT-DNA-mCNTs structures with an ethidium bromide (EtBr) intercalating agent were performed with a ZEISS Axio Imager Widefield Fluorescence Microscope. The stock solution of EtBr–nanohybrids is prepared in PBS buffer (containing 1 μ M mCNT-DNA-mCNTs and at pH 7.2) and incubated for 30 min at 25 $^{\circ}$ C before being dropped on a microscope slide for imaging.

AFM. The sample solution is deposited onto a piece of mica. Then mica is baked at 60 $^{\circ}$ C under vacuum for 30 min and rinsed with water to desalt the mica surface. Tapping mode AFM is used to

acquire the images under ambient conditions using a Digital Instruments multimode AFM.

Cryo-EM. Cryogenic electron microscopy (cryo-EM) measurements are carried out using a ThermoFisher Titan Krios electron microscope (Hillsboro, OR). The samples for cryo-TEM are prepared by plunge freezing. A 3 μ L sample solution is applied to a graphene oxide coated lacey carbon grid (EMS, Hatfield, PA, USA) and then glow-discharged for 15 s with 15 mA current. A thin vicinal film of the CNT sample is formed by blotting with Whatman no. 1 filter paper for approximately 6 s, and the grid is immediately plunged in liquid ethane held at a liquid nitrogen temperature. The grid is then transferred to a microscope. Images are recorded at an accelerating voltage of 300 keV and magnifications ranging from 11 500 $\times$ to 50 000 $\times$ using K2 summit DED camera in low-dose imaging mode, with the electron dose not exceeding 60 electrons/ $\text{\AA}^2$. The magnifications result in final pixel sizes ranging from 1 to 3 $\text{\AA}$, and the typical value of the sample under focus ranged from 1 to 3 μ m.

Molecular Dynamics Simulations. 9, 12, 15, 18 base pair long DNA molecules are generated using AMBER Nucleic Acid Builder. All structures are neutralized with Na⁺ counterions and added into an octahedral water box, which had a 15 $\text{\AA}$ cutoff from the DNA molecules.

First, water molecules and counterions are subjected to 500 steps of energy minimization, while DNA molecules are restrained with a 50 kcal/mol force. Then, 5000 steps of energy minimization are applied to the whole system without any restraint on the molecules. Then, the system is heated to 300 K in the NVT ensemble within 100 ps while a 50 kcal/mol restraint force is applied to the DNA molecules. Next, the system equilibrated for 100 ps while a 0.5 kcal/mol restraint force is applied only to the DNA. Finally, the entire system is simulated in an NPT ensemble for 100 ns without any restraining force applied via the AMBER 16⁵³ pmemd CUDA module. For all simulations, bsc1⁵⁴ and TIP3P⁵⁵ force fields are used to describe DNA and water molecules as well as the counterions, respectively. The particle mesh Ewald⁵⁶ algorithm is used for long-range electrostatic interactions and a cutoff value of 10 $\text{\AA}$ is used for the van der Waals interactions. The simulations are performed and recorded every 2 fs, resulting in 50,000 conformations in each trajectory. All bonds with the hydrogen atoms are constrained using the SHAKE algorithm.⁵⁷

To evaluate the electronic properties of each structure, we employ a RMSD based clustering algorithm using VMD software,⁵⁸ which categorizes the DNA conformations observed throughout the simulation time. A cutoff value of 1.75 $\text{\AA}$ RMSD was chosen to cluster the DNA structures. We select the centroid structures from the most populated cluster (top cluster) which have a minimum RMSD difference from the rest of the conformations within the top cluster. Subsequently, energy minimization is performed on the selected structures to relax residual strains resulting from thermal fluctuations during molecular dynamics before quantum mechanical calculations.

DFT Calculations. To perform DFT calculations, water molecules and counterions are removed from the previously energy-minimized representative structures. The total charge of each system is set to –16, –22, –28, and –34 for 9, 12, 15, and 18 bp long DNA molecules, respectively. Then, the calculations are carried out using the Gaussian 16⁵⁹ software package with the B3LYP exchange–correlation function and 6-31G(d,p) basis set which provides a balance between memory requirements, computational time, and accuracy.^{60,61} Previous studies have demonstrated that the electronic structure of biomolecules in vacuum exhibits a vanishing HOMO–LUMO gap.⁶² As a result, the utilization of implicit or explicit solvation models is necessary. In this work, we assumed that the only effect of the solvent is to modify the equilibrium electronic structure of the DNA molecules; thus we used the implicit solvation method PCM (polarizable continuum model), within the Gaussian 16 software.⁵⁹ Molecular orbitals are generated using the Avogadro software.⁶³

Density of States Calculations. We obtain Fock (H_0) and overlap (S_0) matrices from DFT calculations and employ a Löwdin transformation⁶⁴ to convert H_0 into a Hamiltonian, H , in an orthogonal basis set via the following equation:

$$H = S_0^{(-0.5)} H_0 S_0^{(-0.5)} \quad (1)$$

The diagonal elements of the H matrix account for the energy levels of each atomic orbital, and the off-diagonal elements represent the coupling between these atomic orbitals.

The density of states (DOS) along the molecule in a given energy is calculated using Green's function method. First, we compute the retarded Green's function (G^r) as shown in eq 2.

$$[E - (H + i\eta)]G^r = I \quad (2)$$

where E is the energy and H is the Hamiltonian defined in eq 1.

Next, we calculate DOS for each atom (m) by extracting the corresponding diagonal elements of the retarded Green's function as follows:

$$\text{DOS}(m, E) = \frac{\text{Im}(\text{diag}(G_m^r(E)))}{\pi} \quad (3)$$

For the 2D DOS plots, we sum the DOS values of each atom for the corresponding nucleobase and energy.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsnano.3c10844>.

List of DNA sequences used for experiments; Figure S1 showing fluorescent microscopy images of self-aligning mCNT-DNA-mCNT nanohybrids; Figure S2 showing AFM image of a mCNT-DNA-mCNT nanohybrid; Figure S3 showing analysis of Au-mCNT-Au device; Figure S4 showing analysis of mCNT-DNA-mCNT devices of different lengths (9, 15, 18 bp duplexes); Figure S5 showing two-dimensional DOS plots and corresponding isoplots for alternating G:C triplets; Figure S6 showing conductance vs time plot of 614G probe hybridized to 614D target; Figure S7 showing conductance vs time plot of 614G probe hybridized to 614G target (PDF)

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Author Contributions

Bo Liu, fabricated devices, performed experiments, analyzed the results, and wrote the manuscript. Busra Demir performed simulations and edited the manuscript. Caglanaz Akin Gultakti performed the simulations and edited the manuscript. Jonathan Marrs fabricated the devices and edited the manuscript. Yichen Gong performed I - V characterization. Ruihao Li performed AFM scanning. Ersin Emre Oren oversaw and analyzed the simulations and edited the manuscript. Joshua Hihath conceived the experiments, analyzed the results, and wrote the manuscript.

Notes

A preprint of this manuscript is available on ChemRxiv: Liu, B.; Demir, B.; Gultakti, C. A.; Marrs, J.; Oren, E. E.; Hihath, J. Self-Aligning Nanojunctions for Integrated Single-Molecule Circuits. *ChemRxiv* 2023, 10.26434/chemrxiv-2023-tk0m2 (Jul 6, 2023).

The authors declare no competing financial interest.

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