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SENSING DNA WITH ALTERNATING CURRENTS USING A NANOGAP SENSOR EMBEDDED IN A NANOCHANNEL DEVICE

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Abstract

We report an integrated nanochannel/nanoelectrode sensor for the detection of DNA using alternating currents. We find that DNA can be detected using platinum as the metal for the detecting electrodes, with a signal to noise ratio exceeding 10. We argue that the signal is at least in part electrochemical in nature, thus holds the promise to yield a sequence-dependent signal. However, we also find that for large voltages, DNA attaches irreversibly to the driving electrodes.

Keywords

DNA sequencing; nanochannels; nanoelectrodes

1. Introduction

The emerging availability of DNA sequencing for diagnostic use is the outstanding technological revolution of the past decade.¹ However, current technology focuses on the sequencing of a relatively large number of molecules, and even emergent single-cell analysis techniques usually are based on amplification of the genetic material.^{2,3} Third-generation approaches attempt to read single DNA molecules, which becomes possible with a direct electrical signal. In this group of techniques, nanopore sequencing is the most mature technique.⁴ While nanopores mostly sense the ionic current around the molecule, a second family of detectors attempts to use local electrodes to directly probe the electronic signature of different bases. Local analyses based on tunneling have produced a DNA-specific signal,⁵⁻⁷ but have yet to demonstrate the detection of a sequence of an extended polymer. Other electronic analyses have attempted to use the modification in a field-effect device to probe the passing DNA strand.^{8,9} Devices based on the capacitive charging current of DNA

in an a.c. electric field can detect DNA, while it is not directly apparent whether they would be sequence-sensitive unless sequence-specific probes are provided.¹⁰

Here we attempt a third option, that of voltammetric detection at a pair of electrodes using an alternating driving current. In prior work, DNA molecules were directly detected using both direct¹¹ and alternating¹² currents. We will attempt to follow these publications in testing DNA in solution, and not the binding of DNA to an electrode.¹³ From the bulk electrochemical measurement, we can be assured of a sequence-specific signal should a DNA-specific signal be detected at a potential beyond the threshold for electrochemical activity.¹⁴ By using an alternating current, we potentially amplify the signal of a single molecule if we can measure multiple, repeated half-cell reactions on the same molecule.

Our device geometry will be that of a nanoelectrode pair coupled to a long nanofluidic channel. If the nanochannel width and height are approximately on the order of persistence length of the polyelectrolyte (~50 nm for dsDNA), DNA stretches such that a location along the channel axis can be attributed to a location along the genome.¹⁵ Thus the channel enforces the processivity that is required for the ordered reading of a DNA sequence. It further introduces a vastly increased control over the translocation of DNA through the sensor.^{16–18} The control stems from decoupling the process of translocation and the process of reducing the entropy of a free polymer coil during localization and confinement to a sensor volume. Note that our detection mechanism is fundamentally different from current blockage devices^{19,20} which share an apparently similar geometry.

2. Methods

2.1. Nanofabrication

The device fabrication process is illustrated in Fig. 1. Briefly, the device is built on a fused silica wafer. Nanoelectrodes are fabricated by electron beam lithography and evaporation of 50 nm platinum on a 5 nm titanium adhesion layer. The electrodes are connected to microelectrodes. The nanochannels and microchannels are prepared following ideas and discussions with John Mannion of Harold Craighead's group at Cornell University. Templates for nano and microchannels are formed by a chrome structure that is formed by electron beam and photolithography. The device was then covered by a silicon nitride/silicon oxide/silicon nitride stack to close the access holes that were used to increase the removal rate of chrome. The chrome was removed using an aqueous chrome etchant that entered the device through reactive ion-etched access holes. The access holes were sealed through deposition of another silicon nitride/silicon oxide/silicon nitride stack, and the final reservoirs were formed by a final reactive ion etch step.

Since the active region of the device is not accessible after the insulator deposition, we imaged the active region of the device after electrodes and channel templates are finished (see Fig. 2). In the completed device, two 50-nm tall electrodes are separated by about 20 nm. The effective channel length, defined by the lateral overlap of the slightly offset nanoelectrodes, is about 70 nm. The sides of the electrodes are exposed to the solution, thus introducing a surface of about 0.01 μm^2 not directly part of the gap. The nanochannel confining the DNA is about 50 nm tall and 200 nm wide.

2.2. Device layout and detection setup

The fluidic system follows the familiar H-pattern in which two microfluidic channels are linked by a nanofluidic region^{15,21} [see Fig. 3(a)]. Because the sensing concept demands two electrodes on opposing sides of the nanochannel, we are only able to provide one nanochannel on a strictly planar device. We attached six evenly spaced electrodes along the channel. The leads to each of the nanoelectrodes were protected by planar shields that were

held at ground potential [see Fig. 3(b)]. Due to the insulating substrate, the device is extremely electrostatically sensitive. Thus, all nanoelectrode leads were initially tied to ground. The ties were cut using a pulsed nitrogen laser only after the device was filled with liquid and all probe leads were attached. The experiment was carried out within a shielded enclosure.

As illustrated in Fig. 4, we applied a common sinusoidal voltage to each electrode pair and monitored the current through each electrode pair using a current to voltage converter ($5 \times 10^7 \text{ V/A}$, 100 kHz bandwidth, FEMTO Messtechnik GmbH). The current was either recorded directly (using an oscilloscope), or using a lock-in amplifier (Signal Recovery 7225). Since we were concerned about stray capacitances impairing our measurement, we implemented an analog subtractor circuit that formed the difference between the preamplified signals of two independent gaps along the same channel that were spaced by $60 \mu\text{m}$.

We used DNA samples that are well characterized by previous nanofluidic experiments. In particular, we used $1 \mu\text{g/mL}$ of λ -DNA suspended in 2x TBE buffer, titrated to pH 7.8, to which 0.2% by weight PVP was added to suppress electroendosmosis. The quality of DNA was confirmed by staining with an intercalating dye at a ratio of 1 dye per 50 bp and imaging in a fluorescence microscope. The device was initially wet using a DNA-free solution that was otherwise identical to the final solution.

3. Results and Discussion

3.1. Characterization of devices

We determined the current characteristics of the device by directly monitoring the current through a single nanoelectrode pair as a sinusoidal driving voltage was applied (see Fig. 5). We observe a noise floor of at most 10 pA for the signal at a bandwidth of 100 kHz (5 pA without liquid). In an a.c. experiment it is essential that the detected current is sensitive to the local electrodes, not just stray capacitance. In order to assess the point at which the ohmic current becomes leading, we swept the amplitude of the driving voltage. At low driving amplitudes, the signal was sinusoidal with the characteristic phase shift of a capacitor. As the driving voltage passed about 1.4 V (rms), we observed the emergence of a second, in-phase peak characteristic of ohmic conduction. This is consistent with the onset of hydrolysis, and supports our interpretation that we measure a fundamentally electrochemical signal. The current through electrode at driving voltages below 2 V was stable throughout the 1-h test.

In Fig. 6, we show a circuit simulation (ANSYS HSPICE) of the equivalent circuit in which a single gap is replaced by a circuit comprising two diodes that represent the oxidation and reduction reactions which occur during forward and backward bias, respectively. We find good qualitative agreement between our model (see Fig. 6) and the observed data (see Fig. 5). We thus believe that the device indeed is sensitive to the local environment, and that stray capacitance in the form described by us in an earlier publication²² is not limiting in any case.

At low driving currents, where no electrochemical currents can be excited and the current is purely capacitive (see Fig. 5), we can determine the effective capacitance of the device to be 60 fF. The estimation of the theoretical capacitance based on the device layout is complicated by two principal factors: (i) our gap geometry is somewhat complicated, (ii) the Debye length is a significant fraction of the gap and the exact effective dielectric constant strongly depends on the interface between electrode and liquid. We estimate from geometric considerations that the capacitance should be on the order of 1 fF. Thus, we conclude that a

sizable fraction of the capacitance is comprised of stray capacitance. However, the bridge configuration allows us to remove the stray capacitance which is not associated directly with the junction.

In an electrochemical device, the ohmic leakage current in absence of the analyte is the second source of background signal. In examining the work by Chou's group,¹⁹ we notice a relatively large current in the absence of DNA and bias smaller than the hydrolysis threshold. For their nontunneling geometry (18 nm gap), an effective electrode area of 35 nm × 35 nm and a driving voltage they measured 1 nA at 10 mV driving bias. That is a current density of ~80 A/cm². In comparison, at the same driving voltage our excessive capacitive current density that masks the entire ohmic contribution is at most 1:2 A/cm². The ohmic component is much smaller, at most 10% of the capacitive current. The difference is even more remarkable in light that we used a higher strength buffer. One possible difference between the reports by those authors and here is our ensurance that no light hits the device. We found that illumination with blue light, and in particular fluorescent light bulbs, leads to a large background current. The phenomenon is well described in the literature, and photolysis of water at TiO₂/Pt compounds is a source of hydrogen.²³

The current through the nanoelectrode pair was monitored over multiple hours without and with a d.c. voltage applied between the microchannels. We did not observe a change in the current as long as the voltage was kept within the window of 0 V to 2 V.

3.2. Detection of dsDNA

We now switched our setup to the differential, lock-in amplifier-based setup described in the Methods section. By slightly detuning the difference, only about 90% of the common signal was rejected. Full matching would lead to a second harmonic signal and peculiar phase behavior at low voltages. We characterized the electrode pair in absence of DNA through impedance measurements at 5 kHz to 15 kHz. The device mostly shows a capacitive behavior below 1.4 V [see Figs. 7(a) and 7(c)]. The small dissipative onset at 0.75 V is possibly attributable to hydrolysis of the Ti underlayer that was used to anchor the Pt electrodes to the wafer, or the buffer chemistry.

We then added DNA-bearing solution, set the exciting voltage at 0.75 V, and applied a nano-channel voltage of 2 V between the microchannels to bring DNA molecule into the electrode gap. After approximately 1 min, the detected current dramatically increased, and we removed the 2 V bias between the microchannels. We repeated the impedance characterization [see Figs. 7(c) and 7(d)]. We observe three shoulders in the I/V diagram. The onset of charge injection is now shifted to approximately 0.4 V. This injection is very prominent in the phase curve. Further shoulders are located at ~0.9 V, and ~1.4 V. We believe the ~1.4 V feature is fundamentally the same as in the DNA-free solution.

The interpretation of our results is somewhat complicated because we do not utilize a three-electrode cell that is commonly utilized in electrochemical measurements. Our nanogap sensor is a two-electrode cell and lacks the reference electrode. While it might appear possible to insert a reference electrode into the microchannel, simulations similar to the ones above showed that the high resistance of the nanochannel would require an exquisitely sensitive and impossibly fast circuit to sense and compensate the current through the reference. Thus, we face the problem that we are effectively measuring the voltage and current between the counterelectrode and the working electrode, which would under normal circumstances be considered a poor strategy. However, it is our intent to detect DNA and potentially identify DNA, and not to perform precision cyclic voltammetry. The same thought leads us to use a sinusoidal driving voltage. A square voltage similar to that used in differential pulse polarography would yield a less ambiguous electrochemical interpretation

of the current, but would then not perform as well in the phase detection mode. However, the phase may be a better probe if the quantity of DNA is varying while the nature of the current stays constant since the current magnitude likely scales with the amount of DNA, while the phase at which the current rises within each cycle stays constant.

We can nevertheless explore whether the signal is predominantly due to effects of ions introduced into the electrode gap with the DNA, or whether it is the specific electrochemical interaction of DNA with the surface. In order to investigate this question and reassure us that we capture a DNA-specific signal, we repeated our experiment in a macroscopic cell in which two platinum wires with 127 μm diameter and 5 mm submerged length had been inserted at a distance of 2 mm to 3 mm (see Fig. 8). Because of the increased dimensions, we had to decrease the driving frequency to values around 1000 Hz in order to observe the voltage-dependent impedance that is indicative of an electrochemical signature. We first note that the current signal is in general higher in the presence of DNA. We attribute this to the ionic contribution of DNA to the conductivity, and it mirrors the data by Tsutsui.¹¹ At about 0.8 V we observe an increase in the current in the presence of DNA, but not in pure buffer. A second increase in current around 1.2–1.4 V is present in both cases. The onset points of current increase are also visible in the phase channels. Comparing macroscale and microscale experiments, we are confident that the increased signal at 0.8 V is a feature unique to DNA-bearing solutions. An electrochemical mechanism appears as the most likely candidate for the feature.

We note that our electrode material (Pt) is commonly used in fabrication of a standard hydrogen electrodes. We further expect that the anode is the electrode that interacts with free DNA in solution, based on the negative charge of the phosphate backbone and the connected repulsion from the cathode. Thus, the cathode should be unaffected (to the first order) by DNA, and should be approximately tied to the reference potential while little current flows through it (that is before the onset of the full current at 1.4 V. While it is naturally speculative in nature, we believe that it is informative to make the assumption that the cathodic Pt electrode is approximately at the potential of the standard electrode, and attempt to identify features that correspond in our published data. For the reasons above, published data on DNA voltammetry typically probes the oxidative current.¹⁴ The potential reported for oxidation of guanine is typically on the order of 0.7–0.8 versus Ag/AgCl, meaning they should be detected at about 0.5–0.6 V against a standard hydrogen electrode.²⁴ The adenine peak is reported at about 1.1 V versus Ag/AgCl, leading us to expect a peak at approximately 0.9 V. Since the peak voltage at our 0.4 V injection point is about 0.55 V, we identify this feature with guanine oxidation. The feature at 0.9 V then can be attributed to a combination of adenine, thymidine and cytidine oxidation.

The conclusions essentially hold for all frequencies in Figs. 7(c) and 7(d), with a small migration to higher transition values with increasing frequency (from 5 kHz to 15 kHz). We attribute this to the ionic relaxation time of the structure. Briefly, the bandwidth of the entire device should be limited by the ionic relaxation within the gap. For an effective gap of 50 nm and an assumed approximate diffusivity of $\sim 10^{-6} \text{ cm}^2/\text{s}$, we anticipate a critical frequency of $\sim 10^4$ Hz. If the gap is driven faster than this frequency, the ions within it cannot fully polarize. Indeed, in Fig. 7(d) we see that the phase peak at 1 V is only fully formed at 5 kHz. The driving frequency could be increased if the gap size was decreased.

The detection concept of our device had hypothesized that a *reversible* electrochemical detection would see a DNA molecule scanned past the detector. The reversibility is required to (1) measure the same base multiple times, thus increasing the signal and (2) prevent sticking to the electrode. From prior publications it was known that oxidized bases would not spontaneously reduce, but rather would stay in their oxidized form. However,

application of a large negative bias (-1 V versus Ag/AgCl) was reported to release DNA from gold.²⁵

In our experiment, we did not observe a return of the signal level to a virgin signal once a DNA signal had been detected and a nanochannel bias had been applied to flush the DNA out. The impedance curve remained unchanged under application of a renewed 2 V bias between the microchannels. We thus conclude that the oxidized species tethers DNA at the electrode after the initial cycle. We attribute this to our two-electrode configuration, which did not allow us a sufficiently high cathode potential of DNA dissociation without a very rapid simultaneous oxidation reaction at the anode. Note that initial tests to run the electrode pair at biases above 3 V lead to I–V curves with delayed spikes (trailing the voltage) that indicated a possible hydrogen–oxygen reaction in the gap, and concomitant degradation of the electrode. This is likely a unique effect of nanoscale electrodes, diffusion of gases in a small volume and very high current densities. A third electrode could not be used in our device since the impedance of the nanochannel would limit any electrode kinetics of the reference.

However, we can speculate whether using a local reference could lead to the cyclic operation we seek. The literature indicates that denatured bases are permanently oxidized in a d.c. current, but that a.c. currents above 1 kHz or so are not as damaging.²⁶ DNA *in vivo* also is hypothesized to oxidize and reduce reversibly without permanent damage.²⁷ We thus believe that a properly solvated, double stranded molecule indeed can be probed cyclically, but that a reverse bias has to be provided.

We did not observe a decay of the electrochemical signal within the first few minutes of DNA capture. We believe this may be due to a very high initial concentration of DNA due to an a.c. collapse and concentration effect in dsDNA,^{28,29} and the diffusion of base oxidation products away from the sensor after base degradation.

4. Conclusions

We have presented an integrated nanofluidic/nanoelectric device, analyzed its electrical properties in a typical buffer solution and demonstrated the detection of DNA using an voltammetric sensing mechanism. The main shortcoming of the presented work is that using the described electrochemical detection mechanism leads to an irreversible attachment of DNA to the electrodes of the sample.

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References

1. Lander ES. *Nature*. 2011; 470:18797.
2. Marcy Y, Ouverney C, Bik EM, et al. *Proc. Natl. Acad. Sci. USA*. 2007; 104:1188994.
3. Zong C, Lu S, Chapman AR, et al. *Science*. 2012; 338:1622. [PubMed: 23258894]
4. Maitra RD, Kim J, Dunbar WB. *Electrophoresis*. 2012; 33:341828.
5. Tanaka H, Kawai T. *Nat. Nanotechnol.* 2009; 4:51822.
6. Huang S, He J, Chang S, et al. *Nat. Nanotechnol.* 2010; 5:86873.
7. Tsutsui M, Matsubara K, Ohshiro T, et al. *J. Am. Chem. Soc.* 2011; 133:91248.
8. Fritz J, Cooper EB, Gaudet S, et al. *Proc. Natl. Acad. Sci. USA*. 2002; 99:141426.
9. Gokirmak A, Tiwari S. *Proc. SPIE*. 2005; 6008:600818.

10. Berggren C, Stålhandske P, Brundell J, et al. *Electroanalysis*. 1999; 11:156160.
11. Tsutsui M, He Y, Furuhashi M, et al. *Sci. Rep.* 2012; 2:394. [PubMed: 22558512]
12. Galloway M, Soper SA. *Electrophoresis*. 2002; 23:37608.
13. Levine PM, Gong P, Levicky R, et al. *Biosens. Bioelectron.* 2009; 24:19952001.
14. Boussicault F, Robert M. *Chem. Rev.* 2008; 108:262245.
15. Tegenfeldt JO, Prinz C, Cao H, et al. *Proc. Natl. Acad. Sci. USA.* 2004; 101:1097983.
16. Carpenter JH, Karpusenko A, Pan J, et al. *Appl. Phys. Lett.* 2011; 98:253704. [PubMed: 21772582]
17. Karpusenko A, Carpenter JH, Zhou C, et al. *J. Appl. Phys.* 2012; 111:24701247018.
18. Wang Y, Reinhart WF, Tree DR, et al. *Biomicrofluidics*. 2012; 6:14101141019.
19. Liang X, Chou SY. *Nano Lett.* 2008; 8:14726.
20. Menard LD, Mair CE, Woodson ME, et al. *ACS Nano*. 2012; 6:908794.
21. Riehn R, Austin RH, Sturm JC. *Nano Lett.* 2006; 6:19736.
22. Tung C-K, Riehn R, Austin RH. *Biomicrofluidics*. 2009; 3:31101. [PubMed: 20216956]
23. An W-J, Wang W-N, Ramalingam B, et al. *Langmuir*. 2012; 28:752834.
24. Oliveira-Brett AM, Piedade JAP, Silva LA, et al. *Anal. Biochem.* 2004; 332:3219.
25. Wang J. *Electroanalysis*. 2001; 13:635638.
26. Suzuki T, Yano Y, Yokoyama J, et al. *Genes Environ.* 2012; 34:136139.
27. Genereux JC, Boal AK, Barton JK. *J. Am. Chem. Soc.* 2010; 132:891905.
28. Zhou C, Reisner W, Staunton R, et al. *Phys. Rev. Lett.* 2011; 106:248103. [PubMed: 21770604]
29. Tang J, Du N, Doyle PS. *Proc. Natl. Acad. Sci. USA.* 2011; 108:161538.

Fig. 1. Schematic of fabrication procedure. (a), (e) Electrodes are deposited. (b), (f) Channel template is formed. (c), (g) The channel is sealed. (h) An access hole is established. (d), (i) The channel template is removed.

Fig. 2.
SEM of a single nanoelectrode pair with nanochannel template.

Fig. 3. Optical micrographs of device layout. (a) The device consists of two microchannels (left and right, vertical) linked by a nanochannel (center, horizontal). Six pairs of nanoelectrodes contact the channel and are attached to a microelectrode infrastructure (vertical). (b) Details of layout in the vicinity of a nanoelectrode after channel template is removed.

Fig. 4. Detections scheme. Two independent junctions along the same channel are driven with the same signal, and the independent currents are detected. The converted signals are then mixed to remove the bulk of the parasitic capacitance, before the signal is detected using a lock-in amplifier. The signal is also monitored using an oscilloscope.

Fig. 5. Single-electrode gap traces of current under harmonic driving [(a) 0.75 V rms, (b) 1.5 V rms].

Fig. 6. Simulated current profiles for equivalent circuit containing one forward and one reverse reaction at each electrode. (a) Schematic of equivalent circuit. (b) Current (red, broken) and voltage (green, solid) at 0.75 V driving amplitude. (c) Current and voltage at 2 V driving amplitude.

Fig. 7. Current-voltage curves of devices in lock-in detection. (a) and (c) are for a device before DNA was added, while (b) and (d) are with DNA. (a) and (b) are current amplitudes while (c) and (d) are the phase between current and driving voltage.

Fig. 8. Current–voltage characteristics in a macroscopic cell with DNA at 10 mg/L in 0.5x TBE. (a) Current without DNA. (b) Current with DNA. (c) Phase without DNA. (d) Phase with DNA. (e) Current component attributed to DNA. (f) Phase of the current attributed to DNA. In all graphs red, green and blue curves are for 500 Hz, 1000 Hz and 1500 Hz, respectively. Arrows positions are discussed in text.