J|A|C|S
COMMUNICATIONS

Published on Web 08/12/2006

Label-Free "Digital Detection" of Single-Molecule DNA Hybridization with a Single Electron Transistor

Louis C. Brousseau, III

Quantum Logic Devices, Building D, Suite 98, 7801 North Lamar, Austin, Texas 78752.

Received May 15, 2006; E-mail: lou@quantumlogicdevices.com

Recent developments in proteomics,[1] RNA interference,[2] and systems biology[3] have underscored the large payoff to human health achievable through a deeper understanding of complex biomolecular processes. A significant obstacle to realization of this vision is the complexity, variability, and expense of conducting biomolecular assays using optical labeling and detection.[4] Here we present a novel assay that eliminates fluorescent labels and enables observation of single-oligonucleotide hybridization in complex matrixes with greatly simplified protocols. Electronic coupling of single oligonucleotide probes to the quantum dot (QD) of a single electron transistor (SET) affords direct observation of duplex formation in real-time via "molecular gating" of current through the device (illustrated in Figure 1).

The SET consists of a QD coupled to source, drain, and gate electrodes through thin tunnel barriers. The charge of a single electron on the gate electrode is sufficient to modulate device current. The classical theory[5] for operation of SETs has been well developed and demonstrated in both lithographic[6] and nanoparticle-based[7] examples (see Supporting Information (SI)). In 1997 Brousseau and Feldheim demonstrated that the tunneling current through a gold colloid QD could be modulated by the reaction of attached ligands.[8] In the present work, a nanoparticle-based SET was formed by immobilizing a 5 nm gold colloid-oligonucleotide conjugate between two electrodes with tunneling barriers defined by monolayers of alkanethiol.[9] Hybridization of single 24-mer or 36-mer oligonucleotides probes[10] (see Table S2, SI) to their complements produced measurable shifts of the QD charging voltage. Negative controls, including pH changes, ionic strength changes, and mismatched targets, generated no measurable response.

In a typical experiment, a baseline conductance was first recorded for the device in a microwell under 19 μL of 0.1X SSC buffer at room temperature. A 1 μL aliquot of solution containing target molecules was injected at the edge of the well to initiate the experiment. The source-drain bias voltage, V_{sd}, (at 1 V/s) was swept from -500 to $+500$ mV at an interval of five seconds for 30 min. The charging voltage, ΔV, of the device was measured as the width of the current plateaus in the plot of I versus V_{sd}, and/or corresponding peaks in the (conductance) G versus V_{sd} plot.[8] (See Figure S1 and S2 for representative data.)

Figure 2 shows results of hybridization experiments for various concentrations of matched 24-mer or 36-mer oligonucleotide. In each case, a reduction of ca. 50% in the charging voltage was observed indicating capture of the target oligo. The magnitude and direction of the change in ΔV is consistent with V_{offset} being altered by an increase in negative charge ($\sim$0.2 e) on the oligonucleotide probe.[11] Variance of the initial value of ΔV for each device is due to the size variation of the QDs used. Successful detection was achieved at concentrations as low as femtomolar and in various matrixes: distilled water, 0.1X citrate-stabilized saline (SSC), 0.1 M phosphate-buffered saline (PBS), quanidinium thiocyanate lysis buffer, and human serum).

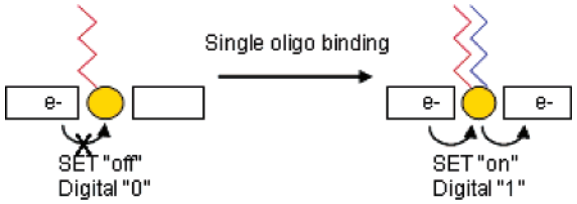

Figure 1. Illustration of the concept of "digital detection" of oligonucleotide hybridization.

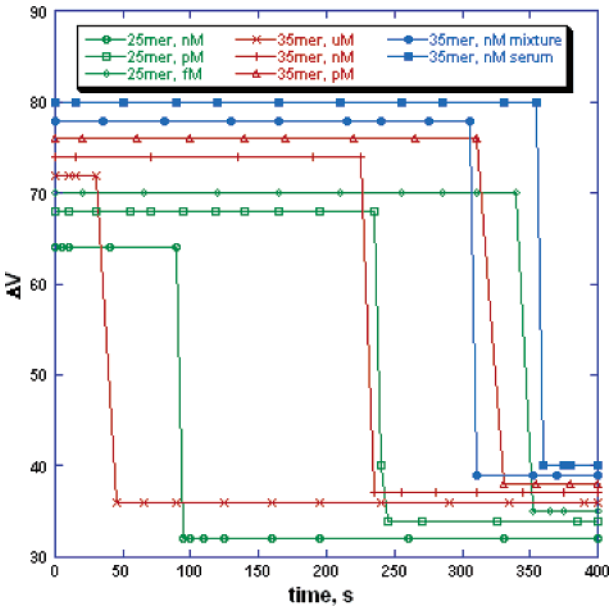

Figure 2. SET response to addition of various concentrations of matched target oligos in PBS, illustrating decrease in ΔVth upon binding.

A series of experiments were performed with 24-mer oligonucleotide probes to determine if the probe sensing ability of the SET could be regenerated by melting the oligonucleotide duplex ($T_m = 58$ °C). After first recording the capture of the matched target at nanomolar concentration, the microwell was rinsed several times with 65 °C 1X SSC. Injection of a fresh aliquot of target oligo resulted in detection of the binding event after several minutes. This sequence was repeated five times with the same device, with successful regeneration and detection recorded for every experiment. Table S2 lists the results of all hybridization experiments performed. No false positives were observed for the more than 50 experiments performed.

The diffusion coefficients of the 24- and 36-mers were calculated for comparison to literature values. The stochastic nature of molecular diffusion at low concentrations should be evident for diffusion to a single point detector (i.e., the SET). A plot of $1/N_o$ versus $1/\sqrt{t}$, where N_o is the number of introduced targets at the edge of the well and t is the average measured detection time for

10.1021/ja063022f CCC: $33.50

each concentration, affords calculation of the diffusion coefficient (D) after fitting to an exponential function of inverse time (see Figure S3 and SI for derivation and discussion). On the basis of this treatment, D of the 36-mer was found to be equal to 59 $\mu m^2/s$, while that of the 24-mer was found to be equal to 92 $\mu m^2/s$. These values are a bit higher than reported measurements ($D = 24$ $\mu m^2/s$) of single 30-mer ssDNA[12] but within the range bounded by the Zimm model, which gives $D = 117$ $\mu m^2/s$, or calculated according to the Kirkwood and Riseman treatment, $D = 51$ $\mu m^2/s$ (both calculations for the 36-mer).

Interestingly, capture of target strands containing mismatches was not seen within 30 min for nanomolar solutions (Table S2). This high specificity is the likely result of several factors that effect the nucleation and stability of hybrid formation as well as the distance dependence of the molecular gating effect. From fluorescent studies on surface immobilized oligonucleotides it is known that hybrid stability and formation is influenced by the surface properties and melting temperatures can be different than for oligos in solution.[13] Hybridization proceeds by first forming a nucleation complex of a few base pairs, then a "zippering" of additional base pairs along the strand. At each step, the process either proceeds or dissolves, depending on the stability of the incomplete duplex. The presence of a mismatch in the early stages of duplex formation can result in dissolution of the hybrid. It has been reported that formation of stable surface-tethered duplexes is also affected by nucleotide composition and length, hybridization conditions (stringency), steric effects from the substrate and adjacent strands, and surface charge density.[14] In the present case, the sequences are relatively short, salt concentrations are low, and the density of probes on the surface is low. The short hexanethiol tether accentuates the steric destabilization of duplex formation by the nanoparticle surface (Southern found that 40 atoms were the optimum tether length for hybrid yields) as do negatively charged citrate and/or chloride ions adsorbed to the nanoparticle surface not covered by thiols. Access to the probe is also restricted by two hydrophobic surfaces (PE coating on the Pt/IR electrode and octanethiol SAM on the gold bead electrode) which form a channel approximately 7 nm wide around the probe oligo (Figure S4). (Charge on these two polymer surfaces might also play a role in the observed behavior.) We hypothesize that base mismatches prevent duplex completion by early termination of hybrid formation. The most likely location of the nucleation complex is near the distal end of the probe. For the (centrally located) single-mismatched target, the longest matched sequence is only 18 bases, which has a melting temperature below the experimental temperature in the 0.1X SSC used (T_m (half-probe) = 24 °C, see Table S1). As a result, only perfectly matched strands would form complete hybrids (T_m = 50 °C). Because the capacitive coupling of the molecular gate to the SET drops off rapidly with distance, only the few base-pairs closest to the nanoparticle induce enough charge on the QD to modulate the current. Incomplete duplex formation is thus not observed. Additional experiments are being performed to test the sensitivity of the SET to tether length, spacer sequences at the proximal end of the probe, and to understand the impact of static surface charges on hybrid formation and stability. A new platform of chip-based planar SETs is being developed that will provide better control of surface properties (charge and hydrophobicity) and steric occlusion of the capture probe.

In summary, a novel platform has been demonstrated for label-free direct detection of nucleic acid binding, coupling the molecular reaction state to the conductivity of a nanoscale transistor. By replacing the gate electrode of a single electron transistor with an oligonucleotide probe molecule, low concentrations of matching target can be detected without optical labels or associated experimental restrictions. The resulting assay is simple and highly selective, even in complex matrixes. Parallel fluorescent spotting assays confirm these observations (Figure S5). It is expected that advances in nanofabrication will allow large-scale arrays of SET sensors to be fabricated to provide enhanced sensitivity and dynamic range. Such arrays would be suitable for integration into a number of electronic formats for accelerating genomic research and diagnostic applications.

Acknowledgment. The author wishes to thank D. Mentzer, E. Miles, D. Feldheim, and C. Gorman for their helpful discussions. Portions of this work were funded by the National Institutes of Health (Grant R43CA93116-01) and the U.S. Army Medical Research and Materiel Command (Grant DAMD17-03-C-0041).

Supporting Information Available: Details of the SET microwell assembly are provided, along with protocols for molecular gate attachment and binding experiments. Diffusion analysis and confirmatory fluorescent spotting experiments performed on immobilized gold nanoparticles on glass slides is presented to validate the binding reactions performed on the SET. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Graves, P. R., Haystead, T. A. J. *Microbiol. Mol. Biol. Rev.* **2002**, *66* (1), 39−63.
(2) Pasquinelli, A. E.; Hunter, S.; Bracht, J. *Curr. Opin. Genet. Dev.* **2005**, *15* (2), 200−205.
(3) Heath, J. R.; Phelps, M. E.; Hood, L. *Mol. Imaging Biol.* **2003**, *5* (5), 312−325.
(4) Kothapalli, R.; Yoder, S. J.; Mane, S.; Loughran, T. P., Jr. *BMC Bioinformatics* **2002**, *3*, 22.
(5) Mullen, K.; Ben-Jacobs, E.; Jaklevic, R. C.; Schuss, Z. *Phys. Rev. B Condens Matter Mater. Phys.* **1988**, *37* (1), 98.
(6) Schoelkopf, R. J.; Wahlgren, P.; Kozhevnikov, A. A.; Delsing, P.; Prober, D. E. *Science* **1998**, *280*, 1238.
(7) Dorogi, M.; Gomez, J.; Osifchin, R.; Andres, R. P.; Reifenberger, R. *Phys. Rev. B: Condens Matter Mater. Phys.* **1995**, *52* (12), 9071.
(8) Brousseau, L. C., III; Zhao, Q.; Shultz, D. A.; Feldheim, D. L. *J. Am. Chem. Soc.* **1998**, *120*, 7645−7646.
(9) Electrochemically etched Pt/Ir and gold wires with <50 nm pointed tips were brought into tunneling distance using a home-built current-feedback control system with piezoelectric actuation. Electrodes were coated with a thin (<micron) layer of polyethylene by spray coating from a 1% solution in toluene, followed by drying at 65 °C for 30 min. The Bard method was used to open a nanopore ca. $(100\ nm)^2$ at the end of the electrode and 5 nm gold colloid-oligonucleotide conjugates (i.e., the SET quantum dots) were attached using 1,8-octanedithiol. Although multiple particles are likely present on the electrode, conductance signatures indicate that only one is selected for tunneling.
(10) Single molecular gates were achieved by either of two methods: (1) purchase of nanoparticle-molecule conjugates from bioreagent supplier (BBI, R&DSystems, Sigma-Genosys, etc.) with gel chromatographic separation of products to obtain only one ligand per nanoparticle or (2) stericly limited self-assembly of ligands onto immobilized QDs via incubation in 1 mM solutions for 12 h (see SI).
(11) Fritz, J.; Cooper, E. B.; Gaudet, S.; Sorger, P. K.; Manalis, S. R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, 99 (*22*), 14142−14146.
(12) Xu, X.-H.; Yeung, E. S. *Science* **1997**, *275*, 1106−1109.
(13) Southern, E.; Mir, K.; Shchepinov, M. *Nat. Genet.* **1999**, *21*, 5−9.
(14) Shchepinov, M. S.; Case-Green, S. C.; Southern, E. M. *Nucleic Acids Res.* **1997**, *25* (6), 1155−1161.

JA063022F