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Label-free “Digital Detection” of Single-Molecule DNA Hybridization with a Single Electron Transistor

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Recent developments in proteomics,¹ RNA interference,² and systems biology³ 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 matrices 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 theory⁵ for operation of SETs has been well developed and demonstrated in both lithographic⁶ and nanoparticle-based⁷ examples (see Supplemental Information). In 1997 Brousseau and Feldheim demonstrated that the tunnelling current through a gold colloid QD could be modulated by reaction of attached ligands.⁸ In the present work, a nanoparticle-based SET was formed by immobilizing a 5nm 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 probes¹⁰ (see Table S2 – Supplemental Information) 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 μ l of 0.1 $\times$ SSC buffer at room temperature. A 1 μ l 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 1V/s) was swept from –500 mV to +500 mV at an interval of five seconds for 30 minutes. 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 a 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 ($\sim 0.2e$) 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 matrices: distilled water, 0.1 $\times$ citrate-stabilized saline (SSC), 0.1M phosphate-buffered saline (PBS), guanidinium thiocyanate lysis buffer, and human serum).

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 ($T_m=58^\circ\text{C}$). After first recording the capture of the matched target at nanomolar concentration, the microwell was rinsed several times with $65^\circ\text{C } 1\times\text{SSC}$. 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 (Supplemental Information) 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 $1/N_0$ versus $1/\sqrt{t}$, where N_0 is the number of introduced targets at the edge of the well and t is the average measured detection time for each concentration, affords calculation of the diffusion coefficient (D) after fitting to an exponential function of inverse time (see Figure S3 and Supplemental Info for derivation and discussion). Based on this treatment, D of the 36-mer was found to be $D = 59 \mu\text{m}^2/\text{s}$, while that of the 24-mer was found to be $D = 92 \mu\text{m}^2/\text{s}$. These values are a bit higher than reported measurements ($D = 24 \mu\text{m}^2/\text{s}$) of single 30-mer ssDNA¹² but within the range bounded by the Zimm model, which gives $D = 117 \mu\text{m}^2/\text{s}$, or calculated according to the Kirkwood and Riseman treatment, $D = 51 \mu\text{m}^2/\text{s}$ (both calculations for the 36-mer).

Interestingly, capture of target strands containing mismatches was not seen within 30 minutes 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 was 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 surface 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 $0.1\times\text{SSC}$ used (T_m (half-probe) = 24°C , see Table S1). As a result, only perfectly matched strands would form complete hybrids ($T_m = 50^\circ\text{C}$). 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 matrices. 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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9. Electrochemically etched Pt/Ir and gold wires with <50nm pointed tips were brought into tunnelling 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 minutes. The Bard method was used to open a nanopore ca. (100 nm)² at the end of the electrode and 5nm 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 tunnelling.
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) sterically-limited self-assembly of ligands onto immobilized QDs via incubation in 1mM solutions for 12 hours (see Supplemental Info).
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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 ΔV_{th} upon binding.