Real-time single-molecule dynamics observed with a nanoscale transistor.

L.C. Brousseau, III

Quantum Logic Devices, 111 Cooperative Way, Suite 220, Georgetown, TX 78626,
lou@quantumlogicdevices.com

ABSTRACT

Direct observation of single molecular reactions and motions are impossible by optical techniques due to diffraction limits. However, ensemble measurements of binding dynamics in biological systems offer limited understanding of individual actors in metabolic and reproductive pathways. Questions of protein folding and mis-folding are one area where a more complete view is needed, as over 50% of human cancers and many other diseases are linked to subtle changes in protein conformation that lead to alterations of their function. Here we report the direct observation of molecular reactions and dynamics at sub-millisecond timescales by coupling the reaction state of antibodies and other proteins to the conductivity of a nanscale single electron transistor (SET). By preparing quantum-dot SETs that are the same size (7nm) as the biomolecules under study, the sensitivity of the devices can be utilized to measure the subtle fluctuations in molecular charge associated with *single* binding events. This "molecular gating" effect is capable of sensing the small electric dipole fluctuations associated with protein folding even in the absence of charge creation/annihilation.

Keywords: molecular dynamics, single electron transistors, antibodies, nanosensors, real-time detection

1 BACKGROUND

Direct observation of molecular reaction dynamics in real time. This capability is desired for many applications, such as drug design, medical device surface engineering, and disease research (transitional protein complexes, protein folding, etc.). Optical techniques are limited to the study of ensembles of molecules and the light beams interact with everything in their path, which complicates elucidation of specific molecular processes. Ensemble averaging is also a limitation in the case of surface plasmon resonance (SPR), and the signal is sensitive to any dielectric change on the surface of the metal film, including non-specifically bound species. Advanced optical methods, such as fluorescent Förster Resonant Energy Transfer (FRET) are able to observe single-molecule molecular processes, but with observation times (~ 100μs - 1 ms) limited by molecular diffusion through small optical detection volumes and interaction distances limited to donor-acceptor fluorophore distances of up to 70Å. Electronic means of detecting biomolecular interactions using electrochemical reporters, coated nanowires, or chemically sensitive field effect transistors (ChemFETs) for single molecule dynamic studies have suffered from ensemble averaging, non-specific binding effects, and slow measurement times. Nanowire FETs have shown single-molecule sensitivity, but the large surface area of a micron length wire is subject to non-specific binding effects, since any adsorbate can cause a change in conductivity due to electron scattering.

This paper describes efforts to exploit the sensitivity of another nanoscale device, the single electron transistor, that overcomes the limitations of other methods by measuring the impedance of the SET as a function of antibody binding state. Likewise, nanoparticle-based single electron transistors have also been shown to be highly sensitive detectors of biomolecular binding events [1]. Because the quantum dot of the SET is the same size as the single IgG antibody "gate" molecule, non-specific binding effects are eliminated (see Figure 1). Although the antibody electrostatic surface is highly complex, the net dipole of the charged functional groups of the antibody still acts upon the SET conductivity through capacitive coupling. Measurement of the device impedance thus provide a direct indication of the antibody binding state.

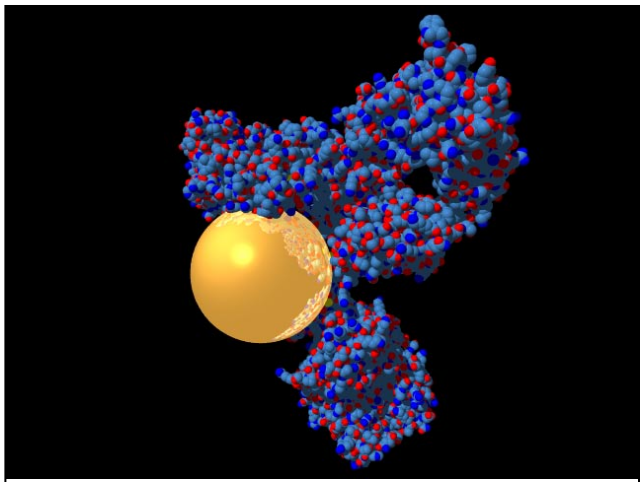

Figure 1 – illustration of a single IgG antibody conjugated to a 5nm gold nanoparticle quantum dot of a single electron transistor. Electrodes are omitted for clarity.

2 EXPERIMENT

The integration of traditionally "dry" semiconductor technologies and "wet" biological/chemical methods has attracted considerable interest recently, through cross-disciplinary collaborations and increased availability of

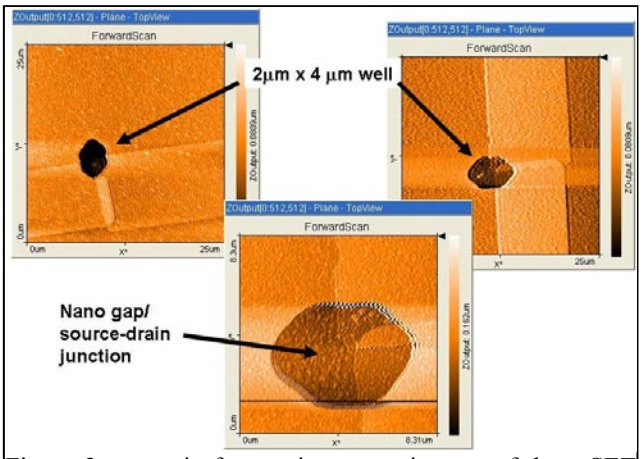

Figure 2 – atomic force microscope images of three SET junctions after RIE to expose sidewall nanogap.

specialized resources, such as nucleic acid synthesizers and nanofabrication facilities (e.g. National Nanotechnology Infrastructure Network). The work described here was performed at the nanobiointegration laboratory of Quantum Logic Devices and the Microelectronic research Center fabrication facility at the University of Texas (Austin) J.J. Pickle Campus.

2.1 Materials

Antibiotin and streptavidin (SA) conjugated to 5nm gold colloids were purchased from Kirkegaard & Perry Labs (KPL, Gaithersburg, MD) and used as the molecular sensing element in these experiments. These particles were prepared from affinity-purified anti-biotin or streptavidin and fractionated by column chromatography to have a single antibody on each colloid. Bovine Serum Albumin (BSA) was purchased from Sigma-Life Science and used as a blocking agent for the electrode surface that was not occupied by the colloidal gold conjugates. Stock solutions of all reagents were prepared from nuclease-free doubly-deionized water (18MΩ ddH_2O) or absolute ethanol, stored at 4°C, and syringe filtered through 0.22micron membranes prior to use. 1,6-hexanedithiol, 1,8-octanedithiol, dodecanethiol, and other chemicals were purchased from Sigma and used as received. <100> oriented phosphorous-doped silicon wafers were purchased from Virginia Semiconductor. MicroChem AZ5214e photoresist was used for positive and negative (reversal) imaging, and developed with AZ726 metal-ion-free developer. All solvents (ethanol, isopropyl alcohol, acetone, methanol) were electronics grade and purchased from Fisher Scientific. Purified water (18MΩ) and all other process chemicals were provided by the UT fabrication facility.

2.2 Device Fabrication

Virgin wafers were cleaned with hot 3:1 piranha solution for 8 minutes [CAUTION!]. Wafers were then prepared with 1250 Angstroms of thermal oxide by steam oxidation. Blanket deposition of 5nm chromium/10nm gold/2nm chromium was accomplished by e-beam evaporation of the pure metals. Gold drain electrodes were fabricated using contact lithography along with wet etching with selective Chromium etchant and Gold etchant (Transene) to make 8 micron wide wires. An optional "spacer oxide" was deposited by SiH_4/N_20 PECVD to a thickness of 15nm to minimize the effects of the electrode overlap. The nanogap between the source and drain electrodes was defined by depositing a blanket 2.5nm SiO_2 thin film using PECVD. Overlapping source electrodes were then fabricated via lift-off process of 2nm chromium/10nm gold. A top oxide of approximately 25nm thickness was deposited by PECVD over the entire wafer, followed by masking and plasma-assisted reactive ion etching (CF_4) to etch a 2 μm x 4 μm microwell that exposed the sidewall of the device stack for nanoparticle assembly. Film thicknesses and device dimensions at each step were measured with atomic force microscopy (Figure 2) to ensure compliance with design specifications. Wet-phase chemical self-assembly of a thiol-terminated anchoring layer to capture gold colloids from aqueous sol was then performed for each whole wafer. Briefly, 100ml of 2mM solutions of octane dithiol were freshly prepared and the wafer incubated for 3 hours at room temperature resulting in an anchoring layer selectively deposited on the gold electrodes (but long enough to span the gap oxide with a 5nm gold particle). After rinsing with ethanol followed by DI H_2O, wafers were blown dry under a stream of nitrogen then immediately placed into a glass dish and covered with biotin-gold conjugate sol for 12 hours at room temperature. AFM images of the device junctions confirmed the presence of nanoparticles at the gap, as did the presence of

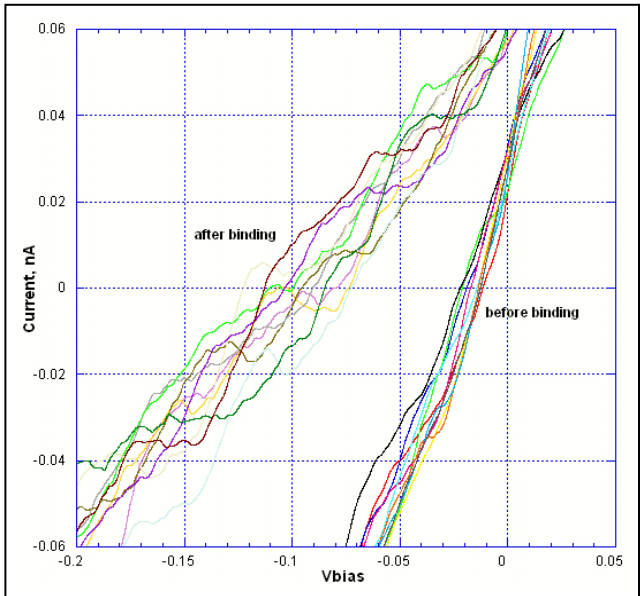

Figure 3 – Current vs. Voltage data for biotin binding to antibiotin antibody coupled to an SET illustrating the negative shift in the point of zero charge (PZC, i.e. bias at zero current) of the nanoparticle due to binding of biotin at 2 μM concentration.

NSTI-Nanotech 2009, www.nsti.org, ISBN 978-1-4398-1783-4 Vol. 2, 2009

the characteristic Coulomb staircase in the I-V traces. Formation of a gate oxide and gate electrode by PECVD and e-beam evaporation/liftoff, respectively, completed the single electron transistor.

2.3 Molecular Binding Assay

Briefly, the prepared SETs were placed under 20 µl of 10 mM PBS buffer (pH 7.4) in a fluidic well and connections made to a Keithley 2400 Sourcemeter with TestPoint software application captures 8 scans per minute for 12 to 20 minutes for each experiment. The baseline response (current) of the device corresponding to the un-bound antibody state was measured by collecting four sweeps from –1 V to +1 V at a sweep rate of 100 mV/sec. An aliquot of the target or control solution is then injected and the device response measured. The baseline response signal was recorded for voltage sweeps (0 to 1 V, relative to ground) or steps and continued at 5-second intervals after injection of a one-microliter aliquot of antigen (1 µM in 10 mM PBS).

2.4 Results

Changes in the point of zero charge of the nanoparticle were determined directly from the DC voltage sweep data, as shown in Figure 3. A measure of the device impedance was calculated by taking the FFT of the first derivative of the current vs. voltage data [2]. A plot of the relevant spectral window is shown in Figure 4 for a typical device. In all devices tested, a positive shift in the peak frequency of the impedance magnitude is observed after the injection of biotin. Control experiments demonstrate that no response is seen for changes in ionic strength or 2-indanone solution as a control molecule that is similarly-sized to biotin, indicating that the frequency shift observed is due to a change in nanoparticle capacitance induced by the binding of the biotin to the antibiotin probe.

Figure 5 plots the device change in PZC (ΔPZC) over time to a 2nM solution of biotin. Two large changes are seen within a few minutes of the injection of biotin to the assay droplet. No further changes are observed up to 12 minutes. The changes are almost identical in magnitude and correspond to the two binding sites of the IgG capture antibody.

3 DISCUSSION

A closer inspection of the data in Figure 5 suggest that the antibody-coupled SET can provide insight into the binding dynamics of the antibody-antigen pair. The diffusion of biotin to the SET from the edge of the microwell covers a distance of approximately 1.5mm (in about 2 minutes, enabling a rough calculation of the diffusion coefficient for biotin < 50 µm/s^2, which is reasonable based on size of the molecule. The timescale of the abrupt changes observed upon binding, however, is

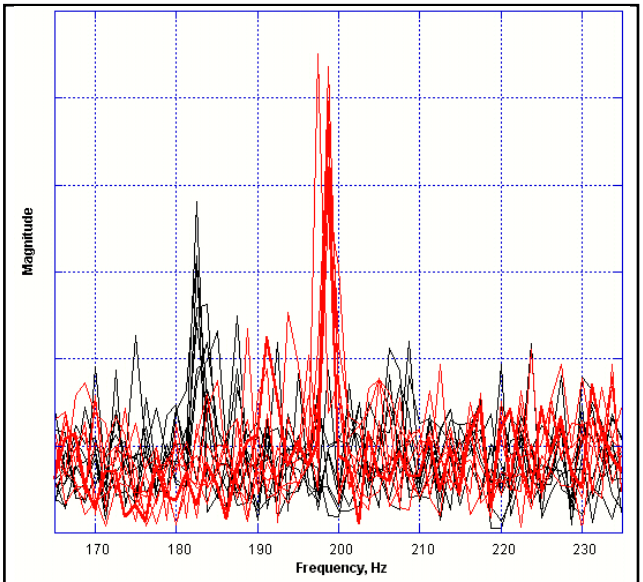

Figure 4 – (left) Calculated impedance response of a nanoparticle SET with a single anti-biotin antibody probe before (black traces) and after (red traces) injection of biotin to the sample cell (final concentration = 2 µM).

slower than expected by several orders of magnitude. This is likely due to the steric hindrance of the binding pockets on the immobilized antibody, and could also reflect the resistance to conformational changes that occur preceding and during binding. The smaller relative magnitude of the second change – 3.5Hz vs 4.5Hz – suggests that the second binding results in a smaller change in the net dipole of the antibody, or is possibly further away from the SET quantum dot resulting in a weaker effect. These possibilities

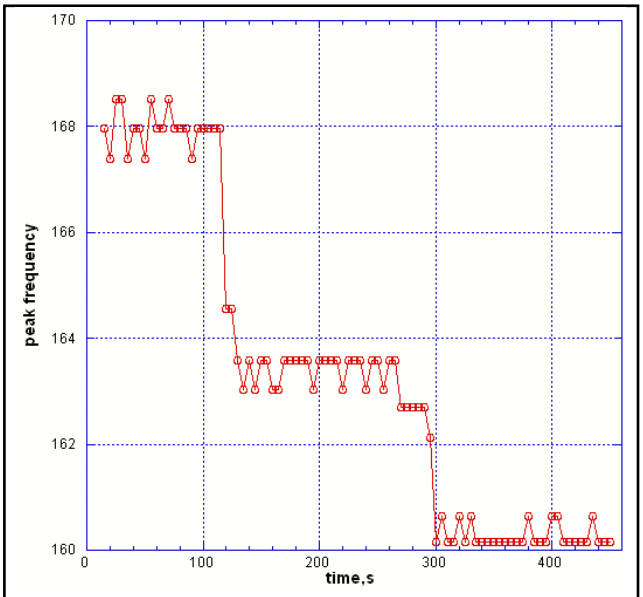

Figure 5 – shift in SET impedance frequency versus time for binding of 4nM solution of biotin. A single monoclonal antibiotin IgG antibody is attached to an SET. The two shifts in frequency correspond to the two binding sites of the anibody.

are under further investigation with large ensembles of device to generate usable statistical quantities.

4 CONCLUSION

By coupling a single monoclonal antibody to the quantum dot of a single electron transistor (SET), a highly sensitive measurement of the antibody binding state is possible. The direct, real-time observation of binding dynamics is also afforded, which could provide valuable information in the fields of drug discovery, systems biology, and for highly selective diagnostic platforms.

5 ACKNOWLEDGEMENTS

This work was supported by grants from the Texas Emerging Technology Fund and NASA contract NNJ06JA22C.

REFERENCES

[1] Louis C. Brousseau, III, Qi Zhao, David A. Shultz and Daniel L. Feldheim. *pH-Gated Single Electron Tunneling in Chemically Modified Au Nanoclusters*. J. Amer. Chem. Soc., **1998**, *120*, 7645.

[2] Yoo, J.-S. and Park, S.-M. *An Electrochemical Impedance Measurement Technique Employing Fourier Transform*. Analytical Chemistry **2000**, *72*, 2035.