Label-free direct detection of biomolecular analytes from optically inaccessible samples.

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ABSTRACT

Fluorescent optical methods for biomolecular detection are widely used for research and diagnostic applications. However, these techniques are useful only in sample matrices that are optically transparent non-scattering, which precludes most biologically relevant fluids, such as blood, urine, and saliva. Errors are introduced by the manipulations and purification steps necessary to prepare biological samples for analysis, as well as by the labeling and amplification steps required for low-abundance analytes. As a result, almost all diagnostic procedures must be performed in resource-intensive laboratories with highly-trained technicians. This limits not only wide-spread access to many tests, but also the capacity of the healthcare infrastructure to accommodate epidemics. For this reason, many efforts have been made over the past few decades to develop label-free, non-optical methods for performing biological testing. We report here a nanoelectronic transistor platform that overcomes limitations of optical techniques by eliminating labels, as well as surpasses other electrochemical and electronic technologies with respect to "noise." By coupling analyte-specific biological receptors to single-electron transistor (SET) arrays, a simple, label-free, real-time, non-optical platform is created that can analyze biological samples quantitatively from real-world samples, such as blood, urine, or saliva. Dynamic range is scalable by increasing the number of transistors coupled to the same type of receptor. Binding-induced electrostatic fluctuations in the molecular probe molecule serve to "gate" the conductivity of an SET affording a direct measure of the molecular binding state. Quantitative measurement of analyte concentration is achieved by counting the ratio of bound to unbound receptors as a function of time. The measurement of binding rate can also be used as a means of distinguishing specific versus non-specific cross-reactivity of similar molecules from complex mixtures.

Keywords: label-free assay, real-time binding, single electron transistor, dna, antibodies

1 BACKGROUND

Easy rapid technologies for biomolecular detection and analysis are recognized as highly desirable. Research in genomics, proteomics, and systems biology would benefit from high-throughput, adaptable platforms that would also accelerate the pace of sequencing, gene association studies, and analysis of molecular structure-function relationships. The discovery of new biomarkers and research into biological complexity would benefit from technologies that would enable high multiplexing and facile correlation of disparate data sets. For applications of these growing bodies of knowledge, sensitive yet easy to use diagnostics are needed at low cost for deployment at point of care and even home-based settings.

Various label-free approaches have been tried, including electrochemical, field-effect transistors, surface plasmon resonance, and nanowires conductivity, yet none has emerged as a clear winner in the market. Here we describe a nanoelectronic device, the single electron transistor [1] that overcomes some of the shortcomings of other approaches and promises a scalable, inexpensive, and adaptable platform that could be useful in a variety of applications.

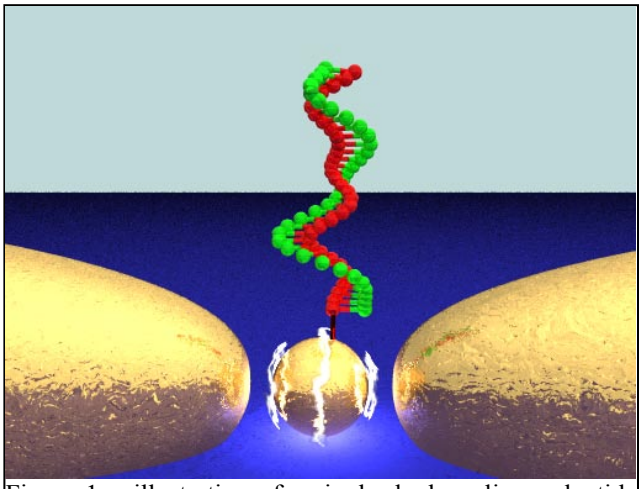

Figure 1 – illustration of a single duplex oligonucleotide coupled to the 5nm gold nanoparticle quantum dot of a single electron transistor. Hybridization causes measureable changes in conductivity in the device, even for single molecules.

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 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 Single electron transistor arrays

Two orthogonal wire grids (i.e. crossbar geometry) were fabricated to address individual SETs as follows. 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 2 micron wide wires (nominally 5 micron pitch). The nanogap between the source and drain electrodes was defined by depositing a blanket 2.5nm SiO_2 thin film using SiH_4/N_20 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 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.

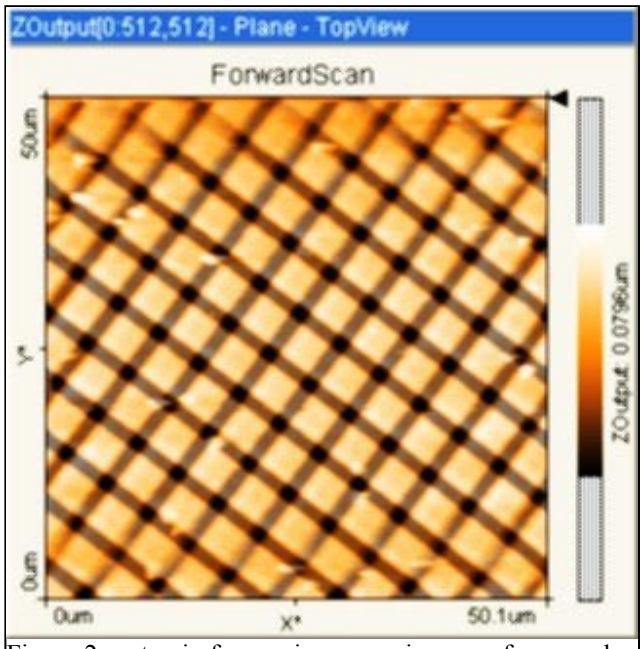

Figure 2 – atomic force microscope images of a cross-bar array of SETs illustrating the high –density of devices fabricated prior to coating with oxide and opening of microwells.

Arrays of SETs were addressed in parallel for binding studies, which involved measuring the aggregate response of all devices in the array as a function of target analyte concentration. The array current at a fixed voltage was monitored by a custom data acquisition system that converted the raw current versus time signal into a digitized response through a 10-bit ADC. The data stream was then captured onto a computer for further analysis.

2.3 Binding assay protocols

Briefly, the baseline response (current) of the device corresponding to the un-bound antibody state was measured by placing a 10μl drop of 1X phosphate buffered saline

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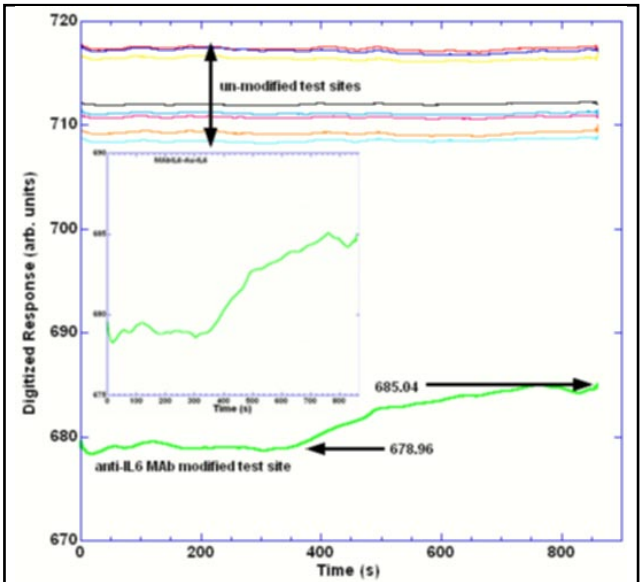

Figure 4 – SET array current versus time plot for IL6 binding to monoclonal anti-IL-6 antibody capture probe (shown in green) at bottom of plot. Top 8 traces are for on-modified SET arrays without capture antibodies. Inset shows expanded y-axis of positive capture spot response.

(PBS) to cover the SET array. The bias was held at 0.5 V and a sampling rate of approximately 13 sec^{-1} was used. A 1 µl injection of the target or control solution began the binding experiment. Data were recorded until a steady-state was observed (i.e. the current stopped changing).

2.4 Results

Figure 3 illustrates the time-based response of the current of a 10,000 element SET array to a solution of 2ng/ml Interleukin 6 (IL-6). At the top of the plot are negative control spots that had no monoclonal anti-IL-6 antibody attached to the SET arrays. The inset shows the response on an expanded y axis. The graph shows the digitized value of the array current, which gradually rises after injection of the target IL-6 and reaches a steady-state value within 5 minutes of the onset of binding. Figure 4 shows a calibration plot of SET arrays response versus IL-6 concentration. The line fits a Langmuir isotherm to the data and allows calculation of a binding coefficient of 2.36e-9 M, in reasonable agreement with literature values (~4e-9 M).

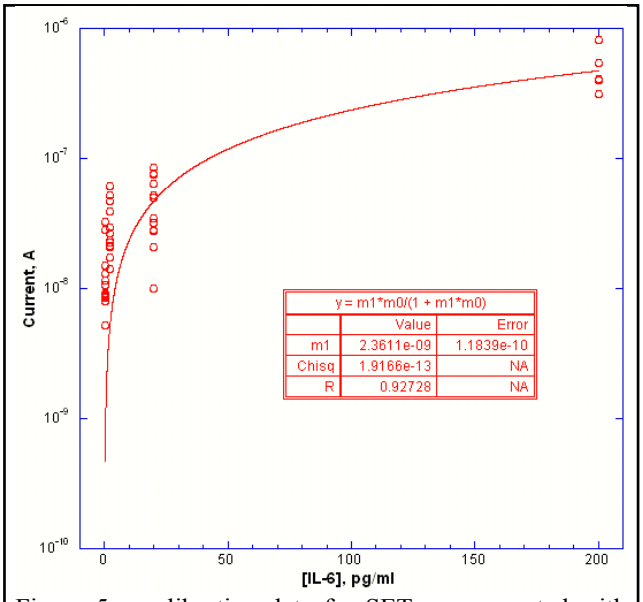

Figure 5 – calibration data for SET arrays coated with monoclonal IL-6 antibody. The calculated binding coefficient is 2.36e-9.

3 DISCUSSION

Experiments performed for oligonucleotides and antibody-based assays showed similar results. Table 1 summarizes the analytes tested and the range of concentrations used. Additionally, experiments were also performed in serum and guanidinium thiocyanate lysis solution to exploit the non-optical nature of the platform.

Table 1

Analyte	Concentration range
25-mer oligonucleotide	fM - µM
35-mer oligonucleotide	pM - µM
IL-6	200 fg/ml – 200 pg/ml
TNF-a	0.1 pg/ml - 1 ng/ml
IGF-1	2 pg/ml - 2 ng/ml
Erythrpoietin	0.4 pg/ml – 4 ng/ml

4 CONCLUSIONS

A rapid, label free method of detecting and quantifying biomolecular analytes without labels or complex protocols has been demonstrated. By coupling the reaction of a single biomolecule to a single electron transistor, a sensitive, real-time platform is created for biological analyses. Elimination of labels and optical detection also affords low cost, adaptable electronic microarrays that can be deployed in point of care and even home-based settings.

5 ACKNOWLEDGEMENTS

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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.