Nanometer-scale Gene Chip

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The convergence of biology, information science and integrated circuit (IC) fabrication technology is poised to profoundly affect human nature. A compelling example is the recent success in genomics, which has been based as much on IC fabrication technology and computing as on biology. The data generated from genomic studies could not be acquired without micro-fabricated DNA micro-arrays or "gene chips", and it could not be analyzed without the enormous computational power made available through ICs. A gene chip uses optical fluorescence to measure simultaneously the hybridization of labeled, single-stranded DNA to many thousands of partial or whole gene sequences immobilized on a glass surface (the 'chip'), but its capabilities are limited in scope. While the computational performance of the IC used in the microprocessor of a computer has relentlessly doubled every 18 months for the last thirty years because of the miniaturization of the circuit components, the gene chip does not scale simply to smaller volumes of DNA; nor is it easily scaled to permit the simultaneous analysis of the expression of millions of genes instead of thousands; and it cannot be readily adapted for use outside the lab.

Consequently, we are exploring the prospects for using silicon-compatible, artificial nanopores to supersede conventional microarrays for sequencing nucleic acids.[1,2] In particular, we plan to produce a revolutionary type of silicon integrated circuit that incorporates Metal-Oxide-Semiconductor Field Effect Transistor (MOSFET) technology with an on-chip nano-pore mechanism for probing the electrical activity of single molecules such as *DNA* directly. Figure 1(a) is a schematic illustration of a cross-section through the integrated nanopore/nanometer-scale MOSFET mechanism that we plan to fabricate, and Figure 1(b) is a simulation of the voltage signal that develops on the gate electrode of the MOSFET as a polyanion (like DNA) transits the nanopore. The simulation indicates that it may be possible to rapidly and uniquely

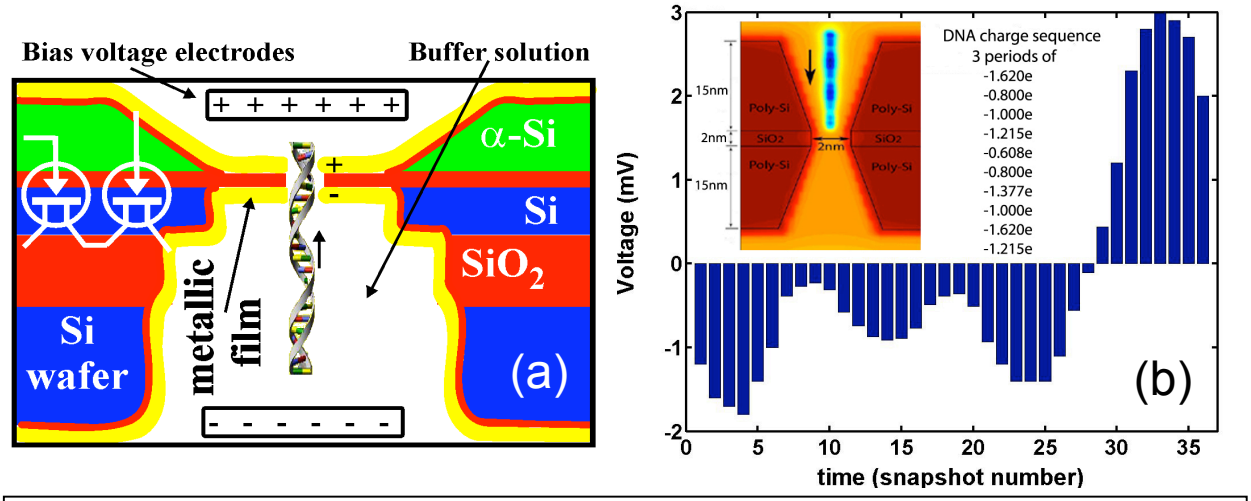

FIGURE 1. (a) Schematic illustration (not to scale) of the proposed integrated nanopore/nanotransistor. The nanopore is etched through a membrane formed from a MOS capacitor consisting of a gate oxide (red), a polycrystalline (green) and *SOI* layer (blue) and thin metal layers (yellow) on a Silicon-On-Insulator (*SOI*) wafer. The fully processed SOI wafer, represented by the *poly*/SiO_2/*Si* layers in the lower half of the figure, incorporates the gate oxide and the thin silicon layer immediately beneath it into the gate electrode of MOSFET. The nanopore spans two compartments of electrolyte (white) that contains the molecule. With a voltage applied across the electrolyte, the molecule traverses the pore and an electrical signal is measured between the two electrodes. The small size of the pore assures that only one *DNA* molecule transits the pore at a time. (b) A simulation of the difference voltage between the electrodes corresponding to the translocation of a single *DNA* molecule across a capacitor membrane. The time-dependent signal corresponds is an electrical signature of the charge sequence in the *DNA* and the osciallatory nature reflects the periodicity in the charge sequence.

identify groups of nucleotides or base-pairing using this mechanism, provided the amplifier has sufficient bandwidth and the background noise and parasitics can be reduced. The electronic detection of biological analytes could have several advantages over the conventional fluorescent microscopy, which is used so prevalently in biology to discriminate the experimental outcomes. If each analyte has a characteristic signature, then an electronic biosensor could facilitate the analysis of the data by eliminating the need for sensitive dyes, thereby improving the dynamic range for detection. An electron bio-sensor might identify the analyte and measure the concentration at the same time. Moreover, several analytes might be identified concurrently using a single sensor.

A recent advance in semiconductor nanotechnology has facilitated the development of this novel molecular sensor. Following other innovations in inorganic nanopores,[2,3] we have discovered a way to produce nanometer-diameter pores with sub-nanometer precision in robust MOS-compatible membranes by using a tightly focused, bright, high-energy electron beam. This lithography strategy is superior to schemes that use focused ion beam milling[3] or ion-tracks in conjunction with a deposition to produce pores because of the tight focus, high resolution and brightness available with an electron beam. Using electron-beam sputtering in conjunction with high integrity, ultra-thin (<10nm) MOS-compatible films suitable for membranes, we have already produced 1-10nm diameter pores, which are comparable in diameter to *DNA*, through membranes ~5-50nm thick, which range over the *DNA* persistence length.

Subsequently, we have begun testing the efficacy of using an artificial nanopore like a molecular Coulter counter for detecting *DNA*. According to this strategy for detection, *DNA* in the electrolytic solution that surrounds the membrane is transported through the nanopore by applying a voltage across the membrane. As the molecule translocates across the membrane, the electrolytic current through the pore is temporarily blocked; the magnitude and duration of the blocking transient provide a signature that is used to discriminate different nucleic acid polymers. Figure 2(a) is an illustration of one variation of the observed current traces, showing a transient associated with 50-mer poly (dT) (poldeoxythymidylate) single-stranded *DNA* molecule (*ssDNA*) blocking the ionic current through a 0.9±0.2nm diameter pore in a ~10nm thick Si_3N_4 membrane. Poly(*dT*) is supposed to exhibit a single helix structure with the bases turned out

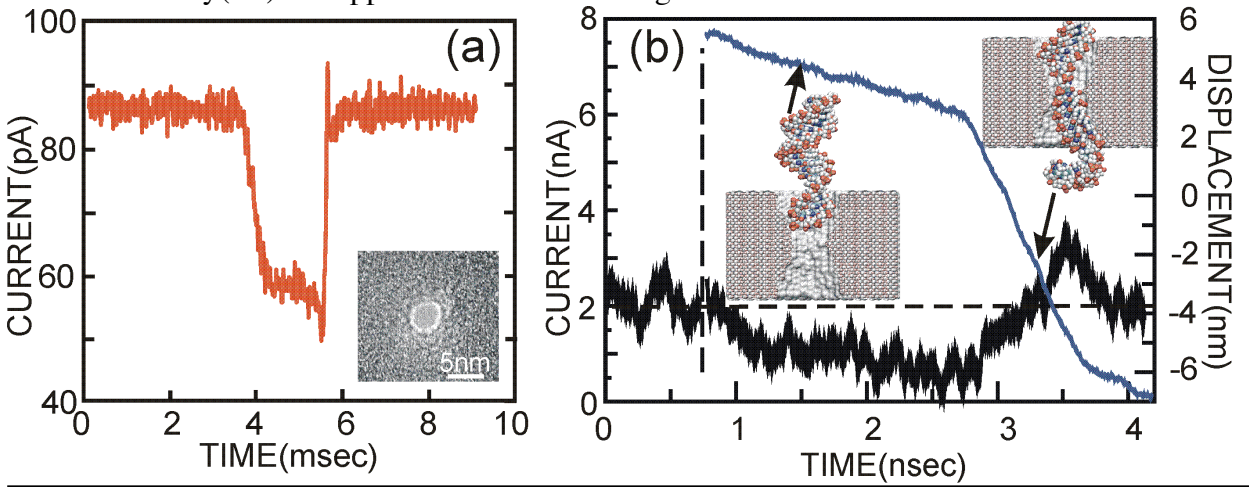

FIGURE 2. Transients are observed in the ionic current through the nanopore associated with a blockade of the electrolytic current by a single *DNA* molecule. Figure 2(a) shows a transient observed in the current through a ~1nm diameter pore in a ~10nm thick Si_3N_4 membrane that is associated with the translocation of a single 50mer poly(dT) *ssDNA* molecule. Corresponding to an applied voltage of 120mV, the open current through the pore is 87pA, but near 4msec the current is reduced to 56pA ostensibly due to a single *ssDNA* molecule blockading the pore. The current remains near 56pA for about 1.5msec and then, following a sharp spike the current returns to the open value. A *TEM* image of these the pore is shown at 0° tilt in the inset. Figure 2(b) shows a molecular dynamics simulation of a electrolytic blocking current signature (black) of a translocation through a 2.4nm diameter nanopore. Superimposed on the blockade current is a plot of the displacement of the center-of-mass of the *DNA* (blue) and two insets illustrating the molecular configuration a particular times during the event.

protruding at the periphery and it is tilted with respect to the helix axis.[21] Because of the low concentration of the *ssDNA* in the electrolytic solution, and the small volume of the pore (~6nm^3), we suppose that this electrical signature is indicative of a single molecule interacting with the pore. This current trace, measured at 120mV, is reminiscent of the blockades reported by Kasianowicz et al.[2] induced by the same molecule using a □-hemolysin pore, although the open current is lower (87pA compared with 120pA for □-hemolysin) for the same applied voltage, presumably because of a smaller pore diameter and different charge in the pore.

Notice that the 87pA open current due to the ionic flux through the pore is abruptly reduced near 4msec to a level of 56pA ostensibly because a single DNA molecule enters and effectively blocks ionic current through the pore. We observed that the blockaded current is relatively constant for about 1.5ms and then near 6ms, following an abrupt spike that occurs on a shorter time scale than the instrument resolution, and the open current level is restored. Using molecular dynamics, we investigated *DNA* translocation through the nanopore at atomic resolution. To simulate the *DNA*/nanopore system, we adapted the methodology of microscopic simulations developed for membrane proteins[1]. A molecular force-field describing water, ions and nucleic acids[2] was combined with the MSXX force-field developed for silicon nitride. The latter force-field was transcribed in terms of potential functions used for bio-molecular modeling. Fig. 2(b) illustrates a simulated system that includes a patch of a silicon nitride membrane dividing an aqueous solution of potassium chloride into two compartments connected by the nanopore. In a typical simulation, a *DNA* molecule is placed in front of the pore, and an constant applied electrical field acting on all atoms of the simulated system draws the molecule into the pore. (We also observed migration of K^+ and Cl^- through the pore.) According to these simulations, the transit time for *DNA* is less than a microsecond, which is does not account for all of the transients observed experimentally. In a 50ns simulation, we observed a translocation of a short double-stranded *DNA* d(polyC)$_{20}$ through a pore of 2.4±0.2 nm diameter in a silicon nitride membrane 5.2 nm thick, driven by an electrical field of 2.6□ 10^8V/m, which is ten times higher than the experimental value. Figure 2(b) illustrates a translocation process, depicting the position of the *DNA* center of mass (blue) and the electrolytic current through the pore (black). Within the first few nanoseconds of the simulation, the electrical field captures four pairs of nucleotides nearest to the pore aperture and drives them into the pore. The rest of the molecule then moves down the pore following the charged backbone of the first nucleotides. After 5ns, the *DNA* reaches the narrowest part of the pore and slows down. At the end of the simulation, the *DNA* double helical structure remains intact with the exception of the six base-pairs that first entered the pore.

Since control of the molecular transit time through the pore is a key aspect of the proposed sensor, we are currently pursuing a two-pronged approach to obtain more accurate appraisal of it. First, we are developing nanopores with integrated electrodes like those shown in Figure 1(b) that show reduce parasitics which should facilitate high frequency (~1GHz) measurements of the voltage or current associated with a translocation event. And second, we are examining the effects of electrostatic charge in the pore and the configuration of the molecule at the entrance aperture on the translocation time.

References

[1] For further information about this project email **<gtimp@uiuc.edu>**

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