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Biochemistry and Semiconductor Electronics – The next Big Hit for Silicon?

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Abstract

Two recent developments portend a new era for silicon electronics in biomedical applications. Firstly, highly specific chemical recognition and massively parallel sample preparation techniques are being combined with VLSI to make new kinds of analytical chips. Secondly, critical dimensions are beginning to approach the size of biomolecules, opening new pathways for physical interactions between molecules and semiconductor structures. Future generations of hybrid chemical-CMOS devices could revolutionize diagnosis and make personalized medicine cheap enough to become widespread.

Introduction

When one thinks of chemistry and electronics, CHEMFETs or ISFETs (ion-sensitive FETs) come to mind. A 2004 review of the field lamented that, despite hundreds of papers published in the preceding decade, “It is therefore quite remarkable that there has been no significant commercialization of these sensors”.¹ That situation has just changed, and yet more rapid change is expected. There are two drivers for these changes. The first driver is the realization that massively parallel biochemical sample preparation techniques can be used on (and analyzed with) VLSI devices. By combining the exquisite chemical specificity of DNA polymerase with the rather old technology of the ion-sensing FET (ISFET),² Ion Torrent is selling what appears to be currently the cheapest solution for whole genome sequencing.³ Doing this required combining the technology for massively parallel assembly of the clonal colonies of the millions of small fragments of the genome (required for whole genome sequencing) with the manufacture of VLSI arrays of sensors. The second driver (of the next phase of development) lies with the fact that extreme UV lithography enables mass production of electronic devices with critical dimensions that approach molecular length scales.⁴ Filling-in of micron sized channels using ALD allows construction of fluidic devices with diameters of a few nm.⁵ Thus mass production of both electronics and fluidics on molecular length scales is becoming possible. This brief review gives an overview of some of this technology. I begin with the Ion Torrent method for DNA sequencing. Despite its impact, there is essentially no description of it, beyond the brief outline posted on the company’s website and descriptions contained in published patent applications. However, its potential applications clearly go beyond DNA sequencing, and, as an example, I propose here another combination of ISFET arrays with massively parallel sample preparation for the purpose of analyzing kinase activity, a central problem in biological signaling. The second driver, exploiting molecular length-scales in electronics, is illustrated with two ambitious projects that may be commercialized in a few years. One is the DNA transistor⁶ designed by IBM for atomic-scale control of DNA motion. The other is a project to read the

sequence of DNA by means of electron tunneling.^{7,8} In addition, a common feature of the readout mechanisms discussed here is the use of chemical recognition combined with electronic readout, a point I will return to at the end of this review. The age of robust chemical interfaces with CMOS is here, and many exciting developments will surely occur in the near future.

DNA sequencing with ISFETs

Dramatic reductions in the cost of whole genome sequencing could lead to the identification of rare mutations or re-arrangements as molecular markers of the molecular phenotype of disease. This will allow treatments to be chosen that are known to target the correct molecules, and will also allow monitoring of the progress of diseases at the molecular level. All DNA sequencing techniques used to date rely on the copying of a target DNA strand by DNA polymerase (but see the section below on nanopore sequencing). The basic chemical reaction is illustrated in Figure 1. The DNA chain is extended from the 3' end (referring to the carbons in the sugar ring, as marked on the figure) and only at the junction where a single strand meets a double strand (the opposite strand has the sugars oriented the opposite way so copying it is much more complicated because polymerase only works in a 5' to 3' direction). Thus, if the 3' end of the chain shown in Fig. 1 has an unpaired T as the next base in the single strand, DNA polymerase will add an A, using adenosinetriphosphate (in general, we refer to any of the four base triphosphates as nucleotide triphosphates – NTPs). The 3' OH on the end of the chain attacks the α phosphate (with the help of the polymerase) to add another A to the chain as shown in the reaction products on the lower part of Figure 1. One of the phosphates is incorporated into the new chain, which is again terminated in a 3' OH group. The γ and β phosphates remain as a pyrophosphate ion with 4 negative charges, and the hydrogen that was bonded to the previous 3' OH group is released as a proton (H^+). Any of the steps in this process can be used for sequencing. For example, Sanger sequencing relies on “doping” the NTPs with a small amount of NTP that lacks the needed OH group so as to cause stops in the replication. 454 Life Sciences exploits the release of the energetic pyrophosphate ion to drive an enzyme that generates light flashes as an NTP reacts with the extending chain. Pacific Biosciences uses NTPs (actually quadruphosphates) that have dyes attached to the part that is cleaved off during the extension reaction, with a different color for each base, so that the incorporation of bases can be followed in real time on single molecules. Remarkably, the obvious proton product (H^+) seems to have been ignored until quite recently. Pourmand et al. reported on the direct electrochemical detection of these protons in 2006.⁹ Addition of a large number of nucleotides in a small volume will result in a local fall in pH as the protons are released, and Ion Torrent uses an ISFET to detect this. This has several advantages. No reagents (beyond the polymerase and NTPs) are needed, and this reduces costs dramatically. The detection is purely electronic – no optics are required. And the detection scheme is compatible with VLSI techniques, so potentially millions of devices can be placed on a chip. The layout and function of a device is shown in Fig. 2. An ISFET (Fig. 2A) has a gate electrode that is connected only to a passivation layer (usually glass). The interfacial potential of the glass is controlled with respect to the source by means of a reference electrode placed in contact with the electrolyte above the passivation layer. The glass surface changes its surface charge in response to the local pH by means of two competing reactions involving silanol groups on the surface:

The ISFETs are arranged in an array under wells, each containing beads with clonal colonies of DNA to be sequenced (Fig 2B). The genome is split into small fragments, each one of which is replicated thousands of times on each bead, and coupled to a primer to start the process of polymerization (so the chains are shown as part double stranded with a single strand overhang). Fig. 2C shows the sequence of events as each of the nucleotide triphosphates (NTPs) is added and then washed out in turn. In the example in the figure, the next base in the single strand is an A, so there is no reaction with dATP. However, adding dTTP results in the addition of T to the double strand, with a consequent fall in pH around the bead containing copies of this particular sequence. The result is a spike in signal out of the ISFET. The signal is transient because of the buffering action of the surface and solution bring the local pH back into equilibrium. The remaining "C" requires a dGTP, so no signal is generated until dGTP is added. Thus the spikes signal which base is being added. If a homopolymer run occurs (e.g., TTT) the size of the signal is increased accordingly.

ISFETs, by themselves, are not particularly selective, so their use as pH sensors is affected by the presence of other chemicals in solution that might interact with the glass passivation layer. The beauty of the Ion Torrent device lies in using the exquisitely sensitive DNA polymerase as the local generator of protons.

The ISFET itself can be made chemically-specific. An Intel team have recently synthesized a specific chelator for the pyrophosphate ion (Fig. 1) so that, bound to an ISFET surface, pyrophosphate production can be read out as a signal of nucleotide addition.¹⁰

Kinase Kinetics via ISFET measurement

Protein kinases are enzymes that modify neutral amino acid residues on target proteins to add a charged phosphate group. Kinase activity represents probably the most important and ubiquitous signaling pathway in cells. For example, many proteins get imported into the cell nucleus once they are phosphorylated. One possibility is that they become part of an ion current flux through the nuclear pores as a result of the added charge. Once inside the nucleus, they can then act as transcription factors, regulating the expression of their target genes. Fundamental issues are: at what rate a given kinase marks a given target (or even what its targets are); and what drugs do to alter kinase activity. Kinase inhibitors are one of the largest classes of drugs on the market. The action of a kinase is illustrated in Fig. 3 (where the target is shown as a single isolated and neutral serine – in practice it would be incorporated into a protein via amide bonds). Here, the reaction catalyzed by the kinase is the degradation of adenosinetriphosphate into adenosinediphosphate, and the replacement of the H in the hydroxyl group of the serine residue with a charged phosphate. And, again, a proton is released in the reaction, so an ISFET can be used to monitor the course of the reaction.¹¹ In order to exploit the potential parallelism of VLSI, a means is need to reliably "print" the entire human proteome onto a chip, one colony of identical proteins above each ISFET. If this were done, then it would be possible to address the entire proteome to ask which proteins were phosphorylated by a given kinase, and then to ask how various drugs affect the rate of particular kinase-protein interactions. Happily, a means to "print the proteome" on a chip does exist. Nucleic Acid Programmable Protein Arrays (NAPPA) are produced by printing the genes for each protein onto a chip. Each gene is modified to contain the code for trapping of the protein product by a capture element that is also programmed into the array. The double stranded DNA is stable indefinitely. When a protein chip is required, the chip is flooded with cell extract. The genes are translated into RNA and the RNA translated into proteins by ribosomes in the cell extract. Each protein is confined to the well in which its gene was printed because of the capture reagent coded into it.¹² Building protein arrays on top of ISFET arrays will enable massively parallel assays of kinase activity and inhibition.

The DNA transistor

An IBM research team have proposed a remarkable device, the DNA transistor, for controlling the movement of a charged polymer. The proposal is all the more remarkable in coming from a lab that, as an industry leader, knows well the constraints of manufacturing, so it give us a glimpse into what the industry believes may be possible. Figure 4 shows the layout of the device schematically. Single stranded DNA is stretched out in a nanopore (by an externally applied force in the computer in this case, but using electrophoresis in practice) so that the base-to-base spacing is 0.74 nm. The charges on the backbone phosphates are shown by the “-“ signs in the circles. The DNA is shown passing through three planar metal electrodes, separated by dielectric layers. Metal layers of twice the base spacing, and dielectric layers of 2.5 times the base spacing result in optimal trapping. Molecular dynamics simulations carried out on the Bluegene super computer show that, with opposing electric fields between the electrode pairs of $\sim 1\text{V/nm}$, the DNA becomes trapped, but is readily ratcheted through the device base-by-base by an adequate external force. The goal here is to control the dwell time of each base inside the nanopore with the eventual goal of reading DNA sequence. Control of the dwell-time is a major challenge for nanopore sequencing.⁷ Whether or not this approach is needed or will work in practice remains to be seen, but the proposal itself is a strong statement of the industry’s confidence in its ability to manufacture devices with critical dimensions on the molecular scale.

Recognition Sequencing

How might one read DNA sequence as DNA is passed through a nanopore as described above? One proposal is to read the ion current passed by pore as it is blockaded to differing degrees by the various bases.⁷ This approach requires an atomically-thin pore to achieve single base resolution and this has not been achieved to date. One solution is to hold a single base at the narrowest part of the pore using a double stranded region to trap it.¹³ An alternative is to use a polymerase as ratcheting device, stalling it by depriving it of the needed NTPs. However, one approach that is completely physical, requiring essentially no biochemistry, is to use electron tunneling as a probe of the chemistry of the DNA base located between two closely spaced electrodes. Calculations by Zwolak and Diventra¹⁴ suggest that this is indeed possible. The great benefit is that even quite “blunt” electrodes appear much sharper when viewed through the exponential matrix elements that control tunneling rates. Thus, it should be possible to pick out an individual base embedded within a DNA polymer. However, this exponential sensitivity also presents the main challenge – how to line the target DNA up with sub Angstrom precision and how to remove the effects of water, ions and hydrocarbon contamination on the electrodes? In fact, DNA bases will bind to gold,¹⁵ so that in a small enough tunnel junction (one that can be spanned by a single base, i.e., less than 1 nm) it is possible to obtain tunneling signals characteristic of the bases.¹⁶ However, the current distributions are very wide (owing to lack of specificity in the interactions) and the gap is too small to pass single-stranded DNA through readily.

In order to circumvent these issues, we use a technique we call recognition tunneling.¹⁷ It is illustrated in Figure 5. Fig. 5A shows two tunneling electrodes, each one of which is functionalized with a “recognition molecule” (“R” in the figure). The molecule, R, is designed to form strong bonds with the electrode, but only weak, non-covalent bonds with the target molecules (DNA bases). The tunnel gap is set to be large enough that the R molecule on one electrode will not interact with the R molecule on the second, so the tunnel current background is essentially free of features. When target molecules are injected into the solution in which the tunnel gap is immersed, transient spikes in current occur as target molecules span the tunnel gap. If the target molecules (a and b in Figure 5B) form different kinds of non-covalent bonds with the two R molecules, each will generate characteristic

signals (I_a and I_b in Fig. 5B). Our first realization of this approach was carried out in an organic solvent (trichlorobenzene) where simple ideas about hydrogen bonding may well apply, and Fig. 5C shows the predicted hydrogen bonding patterns whereby the four DNA bases are trapped by the recognition molecule (mercaptobenzoic acid in this case) in the predicted order of increasing electronic conductance ($T < G < C < A$).¹⁸ The measured distribution of spike heights for each of the four nucleosides (Fig. 5D) follows this predicted order. The technique also works in aqueous solution with the appropriate reader molecule, and we have shown that a single base can be identified in a DNA polymer, surrounded by different bases in the chain, thus realizing the predicted promise of tunneling.⁸

Unfortunately, theory and experiment no longer agree for these experiments in aqueous electrolyte, most likely because of the role that water plays in non-bonded interactions (the theoretical calculations are carried out in vacuum). We have recently synthesized a third-generation reader molecule (imideazole-2-carbamide¹⁹) and this give larger signals with better discrimination between the bases. Using it, we have scanned a functionalized probe over DNA molecules trapped on a functionalized surface (Fig. 5E). Occasionally, we are able to track the probe along a few bases sequentially arranged in a polymer and Fig. 5F shows signals from an alternating AC polymer (top) and an alternating C-5-methyl-C polymer (bottom - ^mC stands for 5-methyl-C). 5-methyl-C is an important marker for silencing of gene expression. These results demonstrate how the recognition complex can form rapidly in the tunnel junction, so that each base in turn is recorded. Note how the signals last for a significant fraction of a second for the data shown here (this time depends on the scan speed). This implies that the intrinsic lifetime of the complex at zero pulling force is long (on the order of seconds). This long lifetime was confirmed for the second generation reader molecule. We used force spectroscopy to characterize both the intrinsic lifetime of a recognition complex and the parameters that control dissociation under an applied force.⁸ The results suggest that a combination of trapping by a recognition complex and an externally applied electrophoretic force might be adequate to achieve the desired degree of control of the speed of DNA translocation through a nanopore.

Fabrication of an array of reading devices (Fig. 5G) should be no more (and perhaps less) complex than that of the DNA transistor discussed in the last section.

Finally, since no polymerase is required, the technique may be applicable to any heteropolymer. The 20 amino acid residues present many motifs for non-covalent bonding, and while unraveling 20 signatures for the amino acids will be much more of a challenge than unraveling 5 (four DNA bases plus ^mC) it may not be impossible to sequence proteins this way.

Summary and Outlook

Three common themes underlie the developments reviewed here: 1) Biochemical methods for massively parallel production of sample libraries can be combined with the large-scale parallelism of VLSI to turn complex and massive sets of chemical data directly into digital electronic data. 2) New devices physics is enabled when device dimensions approach the molecular length-scale and this will extend massively-parallel analysis to the single molecule level and remove some of the constraints of a purely biochemical approaches. 3) The lack of chemical specificity of conventional electronic sensors is readily solved by finding the right kind of coupling between highly specific molecular sensors and electronic devices. In the case of the Ion Torrent sequencer the recognition is done by the polymerase and the released proton couples to the electronic readout. In the case of recognition sequencing, the recognition molecules form specific bonds with the target analyte, and electron tunneling couples the chemical and electronic systems.

Challenges remain. Surprisingly, there is little effort on surface chemistry for materials that are compatible with VLSI. Silicon surface chemistry has been studied extensively²⁰ but most of the work on metals has focused on gold, a material that is not compatible with CMOS fabrication facilities, though there has been some study of monolayers on the more suitable Pd.²¹

Nonetheless, the age of large-scale bioelectronic measurements is already with us, as attested to by the commercial success of Ion Torrent in the DNA sequencing arena. Devices like the DNA transistor will extend applications to the single molecule level, cutting out the expensive chemistry needed to generate libraries and to produce large quantities of DNA by polymerase chain reaction (PCR). Single molecule analysis will be even more important for proteomics, were there is no equivalent of PCR for producing large quantities of sample. The ability to work with tiny amounts of material will simplify diagnosis, eventually enabling “molecular biopsies” on patients.

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Figure 1.

The chemistry of adding a base to DNA: Bases are added using a nucleotide triphosphate (Adenosine triphosphate is shown here) that Watson-Crick pairs with the first base on an overhanging single strand at a single-strand-double-strand junction. The positioning of the base and covalent linking of the backbone is carried out by DNA polymerase, which operates only along the chain in a direction specified by the asymmetry of the sugar molecules as from the 5' carbon to the 3' carbon in the sugar ring (as marked on the Figure). The OH at the 3' end of the chain attacks the bond between the α and β phosphates in the triphosphate, leaving an extended chain, a pyrophosphate ion and a proton as reaction products. The protons produced by adding a nucleotide to each of the many molecules in a clonal colony of DNA molecules can be detected by an ISFET.

Figure 2.

How the Ion Torrent system works. A) An ISFET detects changes in the gate potential owing to changes in the surface charge on a passivation layer as the local pH changes. The interfacial potential is stabilized relative to a reference electrode in the solution above the passivation layer. B) Each ISFET sits under a well containing a unique clonal colony of DNA molecules to be sequenced (each molecule is hybridized to a primer to form a double stranded region to initiate polymerization). In this way, a very large number of fragments can be sequenced in parallel. C) How the signal is generated: the change in gate potential on addition of a nucleotide is transient as the surface and solution buffer the local pH. A spike is produced only when the correct complementary NTP is flushed into the solution. The sequence here shows the addition of a T followed by a G (so that the original unknown sequence is read as AC).

Figure 3.

Kinase activity: Kinases add charged phosphate groups to otherwise neutral amino acid residues, illustrated here for a serine. This is one of the most important steps in signaling, usually marking a protein for transport into the cell nucleus. Here, the ATP is used as a source of phosphate, but the reaction is very similar to the nucleotide addition shown in Figure 1. Once again, a proton is an electronically detectable reaction product.

Figure 4.

The DNA transistor: Metal electrodes are sandwiched between dielectric spacers (each 2.5 times the phosphate to phosphate distance along DNA) and the DNA is driven by electrophoresis through a nanopore drilled through the device. The electrophoretic field produces the force labeled as F . Opposing large electric fields within the device ($+E$ and $-E$) serve to trap the DNA, so that it can be ratcheted through the device one base at a time.

Figure 5.

Recognition tunneling for readout of DNA sequence: A) Two electrodes are functionalized with molecules (“R”) that form non-covalent bonds with a target. The tunnel gap is bigger than twice the length of R so that no signals are generated until a target molecule (“a” or “b”) bridges the gap via non-covalent interactions with R. B) Differences in the noncovalent interactions between a and b allow discrimination between types of molecules using the same R molecules. C) Proposed hydrogen bonding patterns between the DNA bases and mercaptobenzoic acid as the “R” molecule (in organic solvent). The order $T < G < C < A$ is the predicted order of electronic conductance. D) Experimentally measured distribution of tunnel current spike heights showing the predicted order of conductances. E) Arrangement for reading sequence by sweeping a probe functionalized with imideazole-2-carbamide, a reagent that reads sequence in aqueous buffered electrolyte. F) Examples of sequence reads for two alternating polymers. Note that each base generates a burst of tunneling noise with distinct characteristics. C generates large spikes of short duration. A generates smaller spikes of much longer duration and lower frequency. 5-methyl C generates high frequency bursts of small spikes. G) Proposed recognition sequencing chip. Each pore passes through a pair of planar electrodes separated by the tunneling distance (of about 2.4 nm) and each pair of electrodes is separately addressable and functionalized with reader molecules. DNA is driven through the pores electrophoretically.