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Research Focus

Integrated electronic detection of biomolecules

Babak A. Parviz

Department of Electrical Engineering, Campus Box 352500, University of Washington, Seattle, WA 98195, USA

Electronics offers several unique opportunities for the detection and characterization of biomolecules such as oligonucleotides and proteins. Solid-state microfabrication technology, similar to that used to make integrated circuits, can be employed to make integrated electronic sensing systems that are capable of simultaneously detecting multiple molecules. Here, we review some of the capabilities afforded by electronics for rapid and sensitive detection of biomolecules and discuss a recent demonstration of a multi-marker electronic sensing system for detection of uropathogens in clinical samples.

Electronic sensing of biomolecules

The rapid progress of the semiconductor industry in recent years has enabled the construction of several advanced engineered devices and systems. This progress has also generated several tools and unique capabilities that can be used in biological sciences for the detection of biomolecules at extremely low concentrations in a cost-effective, expedient and multiplexed fashion. In this article, we briefly review some of the capabilities offered by electronics for the detection of biomolecules. We discuss recent work that demonstrates how multiplexed electronic sensing can be robustly deployed for the analysis of clinical samples [1] and conclude by mentioning some of the promising areas in electronic sensing of biomolecules for exploration in the near future.

Tools from the semiconductor sector

Modern semiconductor technology has made access to sophisticated components and systems possible in a cost-effective fashion. Integrated circuits such as microprocessors routinely employ hundreds of millions of devices with feature sizes smaller than 65 nm (which is on the same size scale as a virus) and such systems can integrate multiple functionalities including signal amplification and computing in a single platform. Electronic systems can detect small signals with a wide dynamic range; for example, meters that measure currents in the range 100 aA to 1 A are in regular use (this is a dynamic range of 16 orders of magnitude).

Advanced electronic fabrication has made high performance components readily available at a low cost. In a prototype demonstration costing only a few dollars [2], commercially available electronic components such as detectors used in photocopy machines and laser diodes used in laser pointers were used to construct a system to measure rabbit IgG at picomolar concentrations and anti-HIV-1 antibodies in human-patient sera. This semiconductor technology offers cost-effective integration of multiple functionalities, nanoscale functional devices, interconnection of millions of devices to make systems and advanced signal detection and processing techniques. An important question is how to leverage the existing capabilities of this trillion-dollar industry to advance the biological sciences.

Microanalytical systems for manipulation and detection of biomolecules have taken full advantage of the advances in the semiconductor industry in recent years. The advent of microfabrication technologies for integrated circuits and their application in microelectromechanical systems has generated exponential growth in the development of microanalytical systems. The primary advantages acquired by miniaturization and integration of components of an analytical system on a chip are: (i) lowering the reagent cost by using small volumes; (ii) processing many samples in parallel; (iii) integrating functionalities such as electronic detection or data processing into a single system; (iv) constructing portable systems – an ability that is important in point-of-care analysis devices [3] and field forensics; and (v) taking advantage of new scale-dependent physical phenomena to improve the device performance. Examples of such effects include increasing the local concentration of a reagent by binding it to a surface in microchannels and using fast diffusion times across small sample volumes to speed-up reactions. Another scale-dependent phenomenon is the change of effective conductance of a nanoscale semiconducting structure due to binding of molecules on its surface. This has recently been used for direct nanoscale electronic detection of small-molecule (ATP)–protein (tyrosine kinase) interactions [4].

The most successful devices to date that take advantage of microfabrication are DNA arrays [5]. Arrays have been used for sequencing and, recently, in a new format to determine gene expression using post-transcription RNA interference [6]. Several chips have been demonstrated that are capable of performing PCR with analysis volumes as low as 3 nl [7]. For detection of molecules in microanalytical systems, techniques such as change in mechanical resonance or stress of microfabricated cantilevers [8], amperometric, voltametric and conductometric electrical detection [9] have been demonstrated. Although these methods provide facile ways for integration of detection in a complete system, their detection limit has lagged behind optical methods [10]. Some of the best results to date have been acquired using fluorescence detection with on-chip capillary electrophoresis to reach femtomolar concentrations for nucleic acid analysis [11,12]. Integration of sample preparation, proper microfluidic interfacing with the macroscale world, increasing the detection

Corresponding author: Parviz, B.A. (parviz@u.washington.edu)
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sensitivity and selectivity, improving chip reliability and facile handling of large datasets generated by parallel microfabricated systems are some of the issues hindering the transition of many laboratory-on-a-chip systems from prototypes to the actual research laboratory and field use.

A multi-marker electronic sensor in practice

Despite the hurdles mentioned above, a few pioneering works have made substantial progress in the transition of integrated electronic sensing systems to clinical applications. In a recent study, Liao *et al*. [1] showed how electronic sensing methods can be used for the analysis of clinical samples to detect uropathogens. Their system consisted of 16 sets of microfabricated gold electrodes. Each electrode set was functionalized with a specific capture probe for detection of the 16S rRNA of a bacterium of interest. The molecular functionalization scheme was crucial for the sensor operation and was performed as follows. After the microfabrication of electrodes on a substrate, a biotinylated self-assembled monolayer was formed on the metal surfaces [13]. Streptavidin was used as an intermediate to position biotinylated capture probes on the substrate. Independently, bacterial samples were lysed and the corresponding rRNA was allowed to hybridize with a detector probe attached to fluorescein. This rRNA–detector probe complex hybridized with the capture probe on the metal electrodes. To perform the amperometric detection, anti-fluorescein antibody conjugated with horseradish peroxidase (HRP) was attached to the molecular construct on the electrode surface and was exposed to a solution containing 3,3′,5,5′-tetramethylbenzidine (TMB) [14]. The enzymatic activity of HRP with TMB as the redox intermediate was detected by monitoring the current between electrodes at a constant potential. The magnitude of the current was directly proportional to the number of enzymes present and, hence, dependent on the number of rRNA molecules attached to the electrode. The magnitude of the current provided a direct measure of the number of bacteria present in the original sample.

Liao *et al*. [1] used a chip with 16 electrochemical chambers in which the electrodes were functionalized with seven different capture probes for simultaneous measurement of six different targets (*Escherichia coli, Proteus mirabilis, Klebsiella* and *Enterobacter* spp*., Pseudomonas aeruginosa, Enterococcus* spp*., Enterobacteriaceae*) and a universal control probe. The sensing system was capable of detection and differentiation with remarkable robustness when used to analyze clinical urine samples with varied pH, cellular debris and blood cell count. The system was able to detect 16S rRNA at femtomolar concentrations and at a corresponding bacterial count of 40 000 cells per ml. The system did not require amplification of the genomic content using PCR and generated reliable read-out within only 40 minutes of commencing bacterial lysis. This work, the first to validate multiplexed electrical uropathogen detection from clinical samples, paves the way for the development of more advanced systems in this area. Although the microfluidic handling was not automated or miniaturized, it is expected that this task can be readily accomplished. Also, electrochemical desorption of self-assembled thiolates can be used to program the molecular construct attached to each electrode and increase the number of distinct capture probes in the system.

Two issues remain crucial for the operation of the system. One is the design of the capture and detector probes. These probes determine the selectivity and, to a large extent, the sensitivity of the device. The other important parameter is the transduction mechanism used to generate an electronic signal from the molecular recognition and binding event. In the example described here [1], an enzymatic reaction is taken advantage of to amplify the molecular binding event and generate a measurable signal. Although the molecular binding event is indeed amplified, it requires multiple molecular assembly steps and labels. Transduction mechanisms that do not require labels and can generate an electronic signal directly from the molecular binding event are potentially more robust; however, the sensitivity of such techniques remains in question.

Concluding remarks and future perspectives

The exploration of synergy between electronics and molecular biology is at its early stages. Transduction mechanisms that can directly convert molecular recognition and binding events to electronic signals will have a crucial role in bridging the two subjects. Many transduction mechanisms, such as the one mentioned earlier, use molecular assemblies on electrodes and labels. Label-free transduction methods such as those that rely on quantum mechanical tunneling current through a portion of a molecule of interest, on ionic current through a nanopore modulated by a biomolecule [15] or on current through a nanoscale semiconductor structure altered by the electric fields of bound molecules [4] will have a substantial impact on the development of minimally-invasive, sensitive and arrayed electronic detection systems.

Another important issue is multiplexing the sensing system. Although it is possible to use optical techniques such as fluorescent microscopy to detect multiple molecules at once, spectral overlap limits the utility of the photonic methods to the measurement of only a few molecules at a time in confined spaces. Electronic detection methods offer the only feasible route to detect a large number of biomolecules (tens to hundreds of different molecules) at once over an area comparable to the size of bacteria.

Data from inputs with signal-to-noise ratios much smaller than one are regularly extracted in electronic circuits such as those in satellite telecommunication systems. This signal extraction is performed by the proper use of coding schemes for the transmitted signals. These coding schemes, or schemes closely related to them, can potentially be used to improve the signal-to-noise ratio of electronic detection systems by encoding the probe signals. This approach is currently unexplored in electronic sensing but holds great promise in pushing the limits of detection for electronic biomolecular detection systems.

Another area of substantial promise is the time resolution of molecular binding events. It is interesting to note that many biochemical processes occur in timescales that are considered long in electronics. For example, DNA polymerase typically takes a few milliseconds (corresponding to kHz bandwidth) to add a new nucleotide to a strand of DNA. High bandwidth (GHz) and sensitive

electronic read-out techniques enable ultra-fast resolution of the molecular binding events in time. A further intriguing opportunity is the use of electronic detection methods for closed-loop intervention in a biochemical process at the nanoscale. The process can be monitored electronically and, at a relevant timescale, an event such as the application of an electric field or the release of a new molecule through nanofluidic channels can be initiated. This mode of operation opens a new landscape in engineering biochemical processes using close electronic control at the molecular level.

In recent years, electrical engineering has made long strides in developing advanced technologies with extremely high sensitivity, high speed, wide dynamic range, multifunction integration and cost-effectiveness. Bringing these capabilities into the biological sciences will have a major impact on the state-of-the-art of tools that are available for biology and medicine. The research mentioned here has already paved the way for more advanced systems to arrive in the near future.

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Multidrug-resistant genes are associated with an 86-kb island in *Acinetobacter baumannii*

Marilyn C. Roberts

Department of Pathobiology, School of Public Health and Community Medicine, University of Washington, Seattle, WA 98195, USA

A strain of multidrug-resistant *Acinetobacter baumannii* that caused a 26% mortality rate in a French epidemic was characterized and compared with an antibiotic-susceptible strain of *A. baumannii*. The multiresistant strain carries many of its antibiotic-resistance genes on an 86-kb region, whereas the susceptible strain lacks these genes in a homologous region. Characterization of this multidrug-resistant *A. baumannii* strain highlights the limited options for current therapy and raises concerns for future treatment options in an era in which few novel antibiotics are being developed.

Multidrug resistance

Widespread antibiotic use has accelerated the development and spread of antibiotic and multidrug-resistant (MDR) bacteria in humans and the environment. Resistant bacteria are more difficult and more costly to treat than antibiotic-susceptible bacteria and resistant infections often have reduced therapeutic options [1]. Clinically significant antibiotic resistance is frequently caused by the acquisition of foreign genes as a result of horizontal gene transfer of mobile elements. These elements can rapidly disseminate between bacterial populations in different ecosystems [2]. Horizontal gene transfer provides bacteria with the flexibility to adapt to their environment, as illustrated by a sequence comparison between an epidemic methicillin-resistant *Staphylococcus aureus* (MRSA) and the sequences of other published *S. aureus* genomes [2]: the MRSA isolate carried ~6% novel genes when compared with methicillin-susceptible *S. aureus*. Most of the novel genes were mobile elements carrying virulence and antibiotic-resistance genes. Recently, the genome of an MDR *Acinetobacter baumannii* strain that caused a French epidemic was compared with the genome of

Corresponding author: Roberts, M.C. (marilynr@u.washington.edu)
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