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From Sensors to Systems: CMOS-Integrated Electrochemical Biosensors

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ABSTRACT Novel electronic detection techniques are being increasingly sought as components of highly scalable technologies for high-throughput biosensing applications. Among the techniques being considered, electrochemical detection offers an attractive alternative. Advancement in nanoscale electrochemistry makes this an opportune moment to consider the prospects of its integration with CMOS processes. This paper focuses on the new properties and challenges that emerge from the downscaling of electrode dimensions, focusing, in particular, on redox-cycling-based approaches to nanoscale electrochemical devices. We explore the possibilities of interfacing arrays of such devices with CMOS process technology to create highly parallelized integrated platforms. We cite selective examples to provide a qualitative overview of the general design constraints that attend any system-level integration process. We also discuss several challenges that limit the scalability of such platforms and that need to be overcome to create reliable and robust CMOS-integrated electrochemical biosensing platforms.

INDEX TERMS Electrochemistry, nanoelectrochemistry, CMOS integration, biosensing, nanobiosensors, DNA sequencing, redox cycling.

I. INTRODUCTION

Nanobiosensing - the use of nanoscale tools for biological or biochemical sensing - is an intensely active area of contemporary research. Much of this activity emanates from two inter-related expectations: 1) from a fundamental perspective the hope is that probing biological systems on the smallest length scales using nanoscale tools might eventually reveal new and exciting properties in biological systems, just as it revealed new behavior in physical systems and materials; 2) from an application point of view, an analogy is drawn with microelectronics. Indeed, nothing is more emblematic of the power of ''nano'' than the image of billions of transistors packed onto a microprocessor chip. The immense computing power that such massive parallelization affords, elicits the question whether something similar can be attained for biosensing platforms. Pushing sensors into the ''nano'' domain opens up avenues for highly miniaturized, dense and multiplexed arrays with a potential to significantly advance biochemical sensing and diagnostics. Indeed, if such platforms can be integrated with microelectronic technology, then economies of scale in large-scale silicon manufacturing can further ensure that such platforms are cost-effective. This allure of scalability is one of the main factors driving the search for novel *all-electrical* detection platforms, obviating the need for optics-based systems which are typically more cumbersome and expensive. A striking illustration of this power of scalability is the sequencing of the full human genome. Ion Torrent's pH-based sequencer consisted of millions of micron-scale wells coupled to underlying metal-oxide-semiconductor field-effect transistors (MOSFETs), which could detect millions of bases in a few hours [1]. Similarly high-throughput sensors are sought for applications in proteomics [2] and cell-based assays [3], with a potential to broadly impact biomedical science, ranging from disease diagnostics to screening drugs for potential efficacy or toxicity [4].

In the present paper we explore the prospects for developing highly scalable CMOS-integrated *electrochemical* sensing platforms. Because they convert chemical information directly into an electrical signal (current or voltage), electrochemical techniques and CMOS technology form a complementary pair. CMOS process technology is the bedrock of modern integrated circuit technology and underpins the most common electronic components such as microprocessors, logic or memory elements. The advantages that CMOS integration offers in terms of performance, cost and high throughput has made it a key component in a very diverse set of sensing systems ranging from MEMS-based physical sensors [5] to affinity-based biosensors [6]. Its conjugation with electrochemistry can offer a powerful and versatile alternative to other promising integrated bioelectronic detec-

tion schemes that utilize field-effect transistors (FETs), or nanopores [7], [8]. Recent advancements in creating electrochemical systems on the nanoscale offer the possibility of creating massively dense sensor arrays on chip. It is thus an opportune moment to assess the potential as well as the challenges associated with integration of nanoelectrochemical arrays with CMOS process technology.

We begin the paper with a brief overview of basic biosensing principles. We then move to electrochemical systems, paying particular attention to the advantages and novel features of nanoelectrode systems as well as discuss some unique physical features that typify those scales. Of the different approaches to accessing nanoscale effects in electrochemical detection, we focus especially on redox cycling. Then we address system-level integration, as we discuss the interfacing of electrochemical transduction with CMOS circuitry. Through selective examples of our own work we offer the reader a glimpse of the constraints that emerge when designing fully integrated systems. We conclude by highlighting key impediments to the realization of fully functional integrated systems, and how they might be overcome. Apart from the physical challenge of "mixing silicon and water", issues of scale, cost and performance optimization raise additional questions. Overall, our aim is not to review the literature on CMOS-integrated electrochemical sensors, but rather to cite selective examples that can give the reader a feel for the challenges associated with designing highly scalable electrochemical platforms. We hope to provide a roadmap to researchers interested in transitioning from thinking just about the nanoelectrochemical transducers and their unique capabilities to envisioning fully integrated systems.

II. ELECTROCHEMICAL SENSING

Electrochemical sensing encompasses a large variety of techniques. For example, field-effect transistors, nanopores, impedometric sensors can all be thought of as electrochemical in some sense. Here, however, we focus on one particular type of electrochemical detection scheme that relies on *faradaic* reactions, i.e., the direct exchange of electrons between molecular entities in solution and an electrode. This is the most common type of detection scheme. On the basis of the quantity being measured, faradaic electrochemistry can be classified into voltammetric, amperometric or impedance-based. The first two techniques primarily measure current as a function of constant or changing electrode potential. Impedance-based sensors typically measure impedance at the electrode-solution interface in response to an AC signal superimposed on a constant DC bias. They are an important sub-class of electrochemical sensors, especially in the context of "label-free" sensing [9]. However, our focus in the present paper will mainly be on amperometric sensors, and unless otherwise specified, electrochemical sensing is considered to mean amperometric sensing.

A unique advantage of electrochemical detection is that chemical selectivity is, to a great extent, inbuilt in the transduction mechanism itself. Different redox-active molecules typically have different standard redox potentials, and this can serve as a unique marker for individual molecule types. Thus by analogy to fluorescence assays, where different "colors" can be used, in electrochemistry different redox-active "tags" which exchange electrons with an electrode at differing redox potentials can be employed. In addition to this thermodynamic handle, the dependency of electron-transfer kinetics (and thus, current) on applied electrode potential, provides another lever for selectivity, albeit with less control. Another advantage is that electrolytes comprising of highly dissociated salts in solution, which are necessary for most biological samples, are not only compatible with electrochemical measurements but are in fact usually a necessity. These advantages are especially apparent when contrasted with other comparable "label-free" electrical detection techniques. Because FETs rely solely on electrostatic interactions, they are often challenging for biological samples because of limitations due to Debye screening from the supporting salts and media which are needed for the samples. Likewise, nanopore-based schemes rely on analytes being charged in order for them to be driven across the pore.

Ultimately, the three most important metrics to evaluate sensor performance, namely, sensitivity, selectivity and response times, depend on a complex interplay between the assay that converts biological information to chemical information and the transduction mechanism which converts chemical information into an electrochemical signal (Fig. 1). These can all be addressed at the sensor level and we briefly review each of these sub-components below:

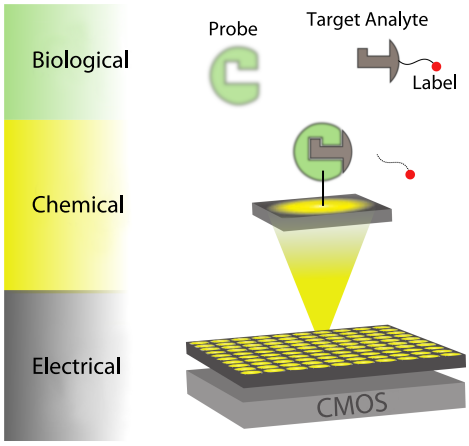

FIGURE 1. Information cascade in a biosensing platform.

A. ASSAY

At the heart of most biosensing assays, lie two simple processes: a molecular recognition event followed by its transduction into a measurable signal. The recognition event in an assay (antibody-antigen binding, DNA hybridization, enzyme-substrate binding etc.) occurs between a "probe" or "receptor" that is functionalized on the sur-

face of the sensor and a target analyte that is present in the sample. This event is usually configured to culminate in either a change in some physico-chemical property (mass, index of refraction, impedance, etc.) or in the release of a signaling molecule usually referred to as a ''label'' (fluorophore, redox-active tag etc.) which can be detected by an appropriate sensor. In many cases this label need not be necessarily released in order to be detected. Both these approaches confer advantages and disadvantages.

The ''label-free'' approach can save valuable amounts of time and cost required to label analytes, which is not insignificant in real applications. But it is prone to the problem of nonspecific binding, wherein a non-target molecule adventitiously binds to the probe giving rise to a false signal. Labeling schemes can circumvent this problem by engineering the release of the label only upon the required binding. In addition, the ability to employ multiple labels can enhance multiplexing by allowing the detection of multiple targets in the same biological sample. However, labeling schemes entail collection of the labels at the sensor surface, and thus longer integration times, which may not always be efficient. Both these transduction schemes can be further augmented to enhance selectivity or sensitivity. Enzyme-linked immunosorbent assay (ELISA) is a classic example where an additional probe (labeled or unlabeled) is required for signal transduction. Alternatively, schemes using enzymes can be designed such that multiple labels are released from individual binding events, thus leading to an intrinsic chemical amplification of the signal. In the context of electrochemistry, in most (but not all) cases impedance-based methods are used in conjunction with label-free assays, while labeled analytes are detected using amperometric or voltammetric techniques.

A key point not apparent in Fig. 1 is that the response time of the sensor is determined not just by the kinetics of the binding event, but also by the transport of analytes to the probe. This competition results in two regimes, one where mass transport is relatively slower than the binding kinetics, such that the response is governed by the growth of the ''depletion'' layer (the length scale a target analyte has to traverse) around the sensor. The other where analyte transport is rapid and a slower surface binding reaction determines the overall response time. Mass transport, whether via diffusion or convection (e.g., pressure-driven or electrokinetic), is intimately linked to the geometry of the sensor and its surroundings (e.g., the size of microfluidic channel that an electrode is embedded in) and needs to be carefully optimized depending upon the application. For example, a situation where the sample is plentiful, and target analytes can be constantly furnished to accomplish binding with probes, will require a different optimization of mass transport, compared to a situation where a sample is scarce, and an immediate tradeoff needs to be made between amount captured and the time taken. In labeled systems, the transport of the released label (after the binding event) to the sensor is another step in the process, and it is well known that diffusion can impose fundamental limits on the response of ultra-sensitive electrochemical detectors [10]. Mass transport must therefore be carefully considered while designing assays and quick scaling-type evaluations can offer useful insights [11]–[13]. For more on this, we refer the reader to the excellent review by Squires et. al. [14]. In most cases the precise nature and limitations of the assay, especially at the surface-binding reaction level, are independent of the following signal transduction mechanism, be it optical, electrostatic, mechanical, electrochemical etc. Very often the same assay (with minor modifications) can be coupled to diverse transduction schemes. We now turn our focus to the latter process in the information cascade, i.e., the transduction of chemical information into a measurable signal, in particular an electrochemical signal.

B. NANOSCALE ELECTROCHEMISTRY

Systems that have integrated CMOS circuitry with electrochemical detection have typically utilized micron-scale electrodes. The integration of truly nanoscale electrochemical systems is rare. One of the reasons is that the push towards exploring the ''nano'' dimension in electrochemistry has been relatively recent [15]. Even so, majority of efforts in designing and exploring the nanoscale regime in electrochemistry has relied on developing tip-based nanoelectrodes. These methods typically involve variants of micro-pipet pulling techniques, wherein, electrochemically-etched metal wire is embedded in an insulator and then mechanically or chemically polished to expose a nanoscale tip. These methods while simple and quick, suffer from the twin disabilities of lack of reproducibility and uncertainty with regard to the actual geometry of the nanoelectrode [16]. By contrast, the use of microfabrication methods to fabricate chip-based nanoelectrochemical systems, which has seen much progress in recent years, circumvent both these problems to a great extent [17]. More importantly, such an approach is inherently amenable to CMOS integration.

The downscaling of electrode dimensions brings several benefits. For example, for a disk electrode of radius r the double layer capacitance, C_{dl}, of the electrode scales with the area (r^2) of the electrode and the resistance, R, with r^{-1}. Therefore the RC_{dl} response time of the electrode scales with the dimension r of the electrode. Thus nanoelectrodes enable very rapid control of the interfacial potential at the working electrode. Further, because of the very small currents flowing in the system, the potential drop between the working and reference electrodes, the so-called iR effects are minimized, thereby enabling accurate measurements even in highly resistive media. The small sizes also ensure that radial components to diffusion enhance mass transport leading to very high current densities.

Besides the dimension of the electrode itself, two other length scales that directly impact the response of a nano-electrochemical system must be kept in mind. The Debye length, κ^{-1} is the length over which counter-ions shield the

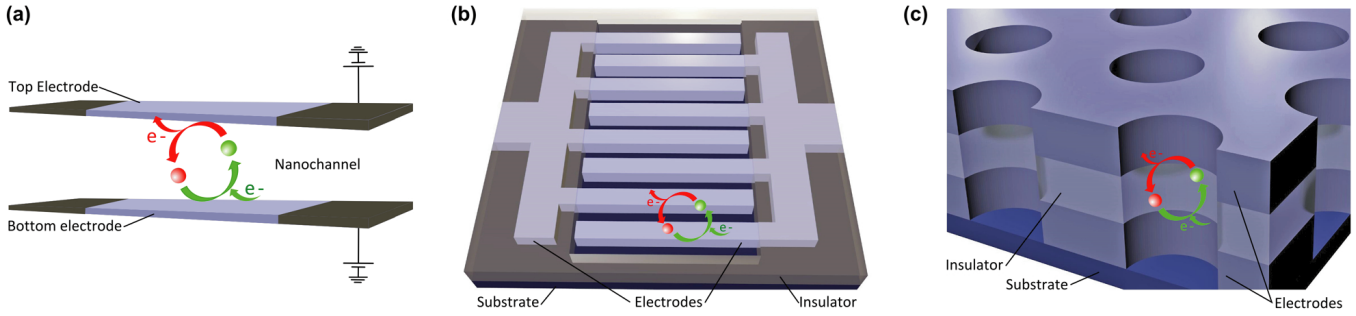

FIGURE 2. Redox cycling. Molecules diffusing between electrodes that enclose a tiny volume, are repeatedly oxidized at one electrode and reduced at the other, thereby amplifying the electrochemical current. The figure shows realizations of redox cycling in different electrode geometries: (a) Nanocavity (b) Interdigitated electrodes, and (c) Pore-based.

charge on an electrode. For 1 mM of a monovalent electrolyte, $\kappa^{-1} \sim 10$ nm [18]. The diffusion length, $\delta \sim (Dt)^{1/2}$, on the other hand is the length over which molecules diffuse from the bulk solution to the electrode surface. Here, t is time and D is the diffusion coefficient. For a hemispherical electrode of radius r_0 the concentration of the analyte in the vicinity of the electrode varies as $1/r$, where r is the distance from the surface of the electrode. Although, there is no characteristic length scale for this process, typically at a distance of $\delta \sim 10r_0$ from the electrode surface, bulk concentration of the analyte is recovered [18]. Therefore, limit where κ^{-1} is comparable to δ is more easily realizable for nanoscale electrodes. This can have very interesting consequences for the electrochemical response. The motion of molecules within the diffusion length can be electrostatically influenced by the electrode and the commonly held assumption of purely diffusive mass transport breaks down in this regime. Another key consequence of reducing electrode dimensions, is that the number of molecules being probed by the electrode decreases. Thus mean-field theories of transport are no longer adequate to describe the motion of molecules, and the discreteness of particles and stochastic nature of molecular motion must be manifestly accounted for.

The steady state current for an electrode is given by $i_{ss} = nFACm_o$ where F is Faraday's constant, A is the area of the electrode, C is the bulk concentration of the analyte, and m_0 is the mass transfer coefficient. m_0 varies depending on the electrode geometry. For example, for a hemispherical electrode it is D/r_0, where r_0 is the radius of the electrode. Thus, while the current densities increase, the overall steady state current declines as the critical dimension of the electrode is downscaled. For nanoelectrodes, measuring such low currents (< pA) requires the use of sensitive current-measurement circuitry and a places a simultaneous constraint on the measurement bandwidth. More commonly, therefore, nanoelectrodes are used in arrays or as parts of an ensemble that are connected in parallel. This leads to an amplification of the overall current, but the ability to multiplex via individually addressable electrodes is lost. The protocols to fabricate nanoelectrode arrays of a variety of geometries (disks, bands etc.) as well as with various electrode materials has been reviewed extensively, and so we shall not elaborate on this any further [19]–[21].

C. REDOX CYCLING

An attractive technique that allows the harnessing of truly nanoscale phenomena while at the same time allowing for signal amplification is redox cycling. Redox cycling is an established scheme for signal amplifcation in electrochemistry, with its earliest realization dating back to the 1960s. It has also been used extensively in scanning electrochemical microscopy (SECM) [22]. This approach relies not so much on downscaling the dimensions of the electrodes themselves, but rather on using multiple electrodes to enclose nanoscale volumes. Its simplest realization involves having two closely spaced electrodes that are biased such that one of them is oxidizing while the other is reducing (Fig. 2a). Any electrochemically active molecule in the vicinity of this electrode pair can thus be oxidized at one electrode and reduced at the other. A molecule diffusing in such a nanocavity is serially oxidized and reduced multiple times. This cycling allows it to shuttle multiple electrons across an external circuit (and not just one, as in the case of a single electrode), thus leading to an intrinsic amplification of the electrochemical signal.

In the limit where the electrodes are biased such that the current is limited purely by diffusion (by having high salt concentrations and high ''overpotential''), the current that each individual molecule contributes is inversely proportional to the time taken to diffuse between the two electrodes, $i_p = eD/z^2$, where $-e$ is the charge on an electron, D is the diffusion constant and z the spacing between the two electrodes. The average current measured is then directly proportional to the average number of molecules in the cavity, $\langle I \rangle = \langle N \rangle i_p = \langle N \rangle eD/z^2$, where $\langle N \rangle$ is the average number of molecules in the cavity at a given time. Thus, as the spacing decreases the current increases quadratically. The ability to fabricate electrode pairs with ever smaller spacings results in significant amplification of the current. For example, a nanocavity with $z = 50$ nm, is capable of generating a current ~ 20 pA from as little as 300 molecules! (assuming

$D = 10^{-9}$ m^2/s). This concept has therefore been used to attain the ultimate level of sensitivity - the detection of single molecules [10], [23], [24].

An alternative mechanism by which redox cycling can be used was proposed by Zhu et. al. [25] and Zhu and Ahn [26]. Here, instead of measuring redox cycling current, the voltage on one of the electrodes is monitored. Initially redox cycling is enabled by biasing the electrodes at oxidizing and reducing potentials respectively. After a short time, one of the electrodes is disconnected. The charge stored on this electrode via the double-layer capacitance is consumed by redox cycling, thereby causing a decay in the potential of this floating electrode. The rate of ''discharge'' is proportional to the concentration of redox species in solution, and thus provides the sensitivity. A key advantage compared to the amperometric mode of redox cycling is that the device now outputs a voltage, which can greatly simplify the underlying CMOS circuitry and reduce its footprint.

The use of redox cycling for signal amplification has two advantages. Firstly, because current density depends on the spacing, z, and the total current depends on the area of overlap between the two electrodes, A, these two parameters can be tuned independently. Secondly, the platform is scalable. In other words, shrinking the lateral area of the two electrodes does not compromise the sensitivity of the sensor and consequently, minimizing sensor dimensions to increase density of the sensor array is feasible. In fact, by lowering A, C_{dl} is lowered, thereby reducing the overall background current. This is an interesting contrast to MOSFETs, where the noise increases proportionally with decreasing gate area. But it's worth emphasizing that this scalability is true only in the case where the molecules can be trapped in the device. For sensors that are diffusively coupled to a bulk reservoir, the signal scales linearly with A.

Redox cycling is not without its own set of constraints. Firstly, the redox molecule must be chemically stable. Both the reduced and oxidized forms of the molecule must not disintegrate on the experimental timescale. In addition, there must not be alternative reactive pathways by which they are consumed. Ideally, the molecules should diffuse rapidly, and have a high heterogeneous electron-transfer rate constant, so that the most efficient cycling can be achieved at low overpotentials.

Electrode geometries for redox cycling can be configured in at least three ways: nanocavities, pore-based and interdigitated electrodes (IDEs) [27]. Nanocavities are outlined in Fig. 2a; two plane parallel electrode are embedded in a nanofluidic channel that is coupled to a bulk reservoir. The electrodes don't necessarily have to be linear, but can also be circular or any other shape. Pore-based devices consist of a vertical stack of two or more electrodes that are separated by a passivation layer or dielectric material (Fig. 2c). Coupling to the bulk reservoir is achieved by etching small pores in this stack to expose the multiple electrode layers, each of which can be independently biased for redox cycling. This geometry allows for very good coupling to the bulk fluid and also an easy fabrication route to high density arrays. This has been harnessed to fabricate chips which comprise nearly a billion nanopore sensors in an area of 9 mm^2 [28]. A slightly different variant of this geometry - recessed ring disk nanoelectrode arrays - involves the bottom most layer of the stack to be an electrode which upon exposure results in a disk like electrode at the base of the stack [29], [30]. Unlike the first two geometries, IDEs consist of co-planar electrodes that are arranged in an interdigitated comb like geometry (Fig. 2b). While much easier to fabricate compared to the other two approaches, they are usually the least sensitive because the inter-electrode spacings are larger due the resolution limits imposed by conventional lithographic techniques. Geometry can impact not only the sensitivity but also noise characteristics of redox cycling sensors. In the low-frequency regime the noise spectral density for nanocavity-type sensors is dominated by so-called ''diffusion'' noise (with a characteristic $f^{-3/2}$ scaling), that arises from the stochastic fluctuations of molecules in and out of the active sensor area. These fluctuations are also affected by the coupling to the bulk reservoir as well as adsorption [31]–[33].

III. SYSTEMS LEVEL PERSPECTIVE

We now turn our attention to the interfacing of electrode systems described above with CMOS process technology to create fully integrated systems. Our purpose is not to lay bare the anatomy of a typical CMOS integration process, for which we direct the reader to the literature on integration of electrochemical biosensor arrays with CMOS [26], [34]–[40]. Instead, we hope to give a broad overview of the system-level design thinking that must motivate the creation of scalable CMOS-integrated electrochemical arrays.

Conceptualizing a fully functional integrated system is challenging, not only because of the sheer number of components that need to be individually optimized for performance, but also because these components interact with one another and their mutual impacts need to be well understood. For example, choosing an electrode material impacts not only the chemistry that will be required for surface functionalization, but also the interaction of the redox molecule, the double-layer capacitance as well as the background noise levels. Likewise, the dimensions of the electrode are intimately linked to problems of mass transport of the analyte as well as to the sensitivity requirements of the current-measurement circuitry in CMOS.

The first step in system-level design thinking is clear identification of needs. With CMOS integration, one naturally presumes that a need for multiplexing and parallelization exists. However, why parallelization is required, on what scales, what it can enable and at what cost are questions that need to be clearly answered before proceeding with integration. In general it is well known that multiplexed scaling through sensor arrays can enable efficiency and massive throughput where large amount of biological information needs to be evaluated

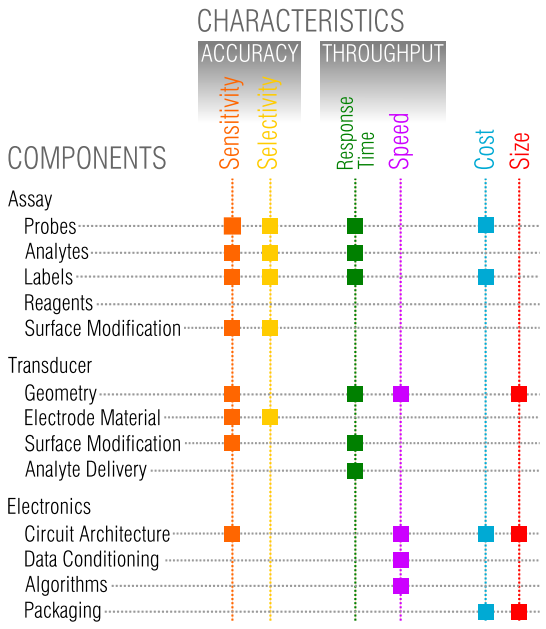

FIGURE 3. Components of an integrated electrochemical biosensing system and the features they impact.

(e.g., genomics). Additionally, it can enable cross-comparative analysis (between sensors), allow for redundancies (as insurance against failed sensors), or a wide dynamic range (with different sensors operating at different sensitivities) [41]. However, as outlined earlier, the response times, sensitivity and selectivity, are invariably determined at the assay and sensor level. Apart from specialized situations such as orthogonal sensing or in cases where parasitics affect performance and need to be minimized, CMOS can do little to improve the intrinsic performance of the sensors. CMOS integration becomes truly useful when large-scale parallelization through sensor arrays is required, and its utility must be evaluated on the basis of what it offers for modularity, ease of data processing, accuracy, and cost (Fig. 3). Even so, depending on the application, many of these same features can be obtained on smaller scales with board-level integration. Potentiostats needed for electrochemical measurements can be easily implemented on printed circuit boards (PCBs), at very low cost. Of course, it becomes challenging to increase the array density at the sensor level since routing a large number of wires from the array to the external world becomes correspondingly difficult for reasons of space. In fact, well before limitations due to space are reached, the impact of parasitics and cross-talk between the wires pre-empts further downscaling. In addition to reducing parasitic capacitance, CMOS integration may allow for denser pixel integration, improve the portability, reduce the form factor, and allow for a lower cost system. Nonetheless, unless *massive* parallelization is warranted, board-level electronics can be a viable, low-cost and effective alternative for most biosensing applications, especially where cheap and portable diagnostics are required.

Another consideration in an integrated electrochemical sensor system is the integration methodology. Depending on the specific application, the optimal method of integration may change. Either the CMOS electronics can be on the same wafer as the sensor array (''monolithic'') or be fabricated on a separate wafer and bonded. This decision will be determined to a great extent by the density of the sensor array as well as the underlying circuit topology for the desired functionality, and the comparative ease or difficulty of monolithic integration.

A large pixel size at the sensor level allows chip-to-chip bonding of the device layer to the CMOS layer. Being able to fabricate two separate chips and then bonding, requires the connection leads to be sparse enough (typically >10 μm) so that connections can be made at a higher metal layer. There are multiple advantages of being able to fabricate the CMOS and sensor chip separately. From a functional perspective this allows for the fabrication of devices in separate foundries with different set of tools and wafer sizes which is not possible with a monolithic integration strategy. It may not be widely appreciated that typically, foundries processing CMOS lack the necessary tools to fabricate sensors which are more MEMS like or involve common electrode materials. Hence, it is usually necessary to continue processing at another foundry equipped with the appropriate tools and materials. Differences in acceptable sizes for the two wafers may necessitate wafer coring to convert the wafer size, thereby increasing the processing complexity and potentially reducing the yield of the process. However, processing the two wafers separately allows for a greater thermal budget on the sensor wafer, which in turn relaxes the overall requirements of the process, which is otherwise not possible with monolithic integration. It also makes it easier to undertake surface functionalization steps on the electrodes without the risk of damaging any underlying CMOS components.

For systems where post processing is relatively simple, or pixel pitches are too dense (on the order of 1 μm) a monolithic integration scheme can be chosen. CMOS wafers can simply continue to be processed to build on the sensor layers.

IV. CMOS-INTEGRATED ELECTROCHEMICAL SENSORS

Here, we briefly review the key decisions that need to be made when designing CMOS electronics for such integrated electrochemical sensors. Most of these center around issues of sensing modality (current vs. voltage), detection limits, signal bandwidth, device area and noise sources. The specific architectures we highlight below are chosen to address these areas. We will give two examples from work at the Integrated Biosystems Lab at Intel Labs related to redox-cycling based DNA sequencing applications. DNA sequencing is a good fit for CMOS integrated technologies because of the enormous data throughput required as well as the high volume manufacturing that can justify costs. We merely wish to illustrate through these examples some of the design choices and qualitative tradeoffs that need to be made at the CMOS

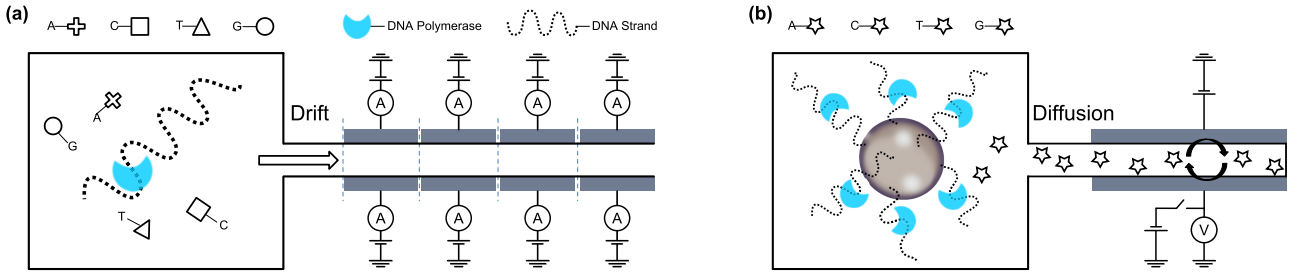

FIGURE 4. Two redox-cycling based schemes for DNA sequencing. (a) Unique electrochemical tags are released when complementary labeled nucleotides are incorporated by a polymerase. These tags are detected downstream at the single-molecule level by nanocavity devices. (b) Multiple copies of the target DNA are functionalized on a bead. Base incorporation reactions result in the release of multilple copies of the same redox-active tag, which can be detected by redox cycling.

electronics level, given the constraints imposed by the desired overall system function.

Both examples employ a biological to chemical information transduction scheme that involve nucleotide analogs with redox tags. A probe functionalized on the chip consists of a DNA strand which needs to be sequenced and which is loaded with a DNA polymerase. Upon encountering a complementary nucleotide, the polymerase incorporates the nucleotide into the strand and in the process releases the redox tag. The tag can only become redox active after nucleotide incorporation and has no activity while bound to the freely diffusing nucleotide [42].

The first example is designed to read out the sequence of DNA in real time (Fig. 4a) [43]. In this scheme after the immobilized polymerase incorporates nucleotide analogs in real time, an electrochemical single-molecule detection unit located downstream detects and identifies the single redox tag molecule that is produced as a by-product of the incorporation reaction. The single-molecule detection unit can be a redox cycling nanocavity as described previously. Such devices have been shown to generate currents of $\sim$20 fA, corresponding to single, individual molecules [23]. Forming an array of these sensors can allow for high throughput sequencing. Measuring such low currents implies that the CMOS electronics for this sensor needs to be designed with a very high gain and low noise. With a high-gain system, bandwidth of the detection electronics will be low due to the fundamental tradeoffs between sensitivity and bandwidth. The area that is needed for the detection electronics also gets larger as the signal to be detected becomes smaller. This is because in case of weak signals, a larger gain is needed at the first stages of the signal amplification. At the same time flicker noise, which is inversely proportional to the gate area, needs to be minimized. While circuit techniques such as chopper stabilization and correlated double sampling can mitigate $1/f$ noise, typically such a detection modality requires a process node with favorable flicker noise characteristics and adequate gain (0.13 μm or above). Larger nodes also have the advantage of having lower gate leakages. Circuitry needed for such an application is estimated to be on the order of 100 μm^2 per sensor pixel (with multiple channels multiplexed in each pixel). In this specific example parasitic capacitance is the largest contribution to the noise, hence it is critical that the dominant capacitance in the system is minimized. With the sensor part of the system attached on the CMOS die, the parasitic capacitance can be minimized. However, the double layer capacitance of the electrodes dominates and the dominant noise source is the electrode. Hence, it is critical that the system is constructed with all the components co-optimized and the electrode area minimized. Having the input amplification on-chip is crucial in this scheme due to the ultra-low currents being measured. Once the signal is sufficiently amplified, it can be digitized on chip or off chip depending on the real estate available. To resolve minute differences in signal, typically high precision Analog to Digital converters (ADCs) are needed. Often it may be more effective to have the ADC off chip for such applications, so that the area is better utilized by including the required transimpedance amplifiers on chip.

The second example pertains to an alternative scheme for DNA sequencing, which involves having multiple copies of DNA per pixel (on the order of 1000s) and introduce the nucleotides one at a time for readout (Fig. 4b). In this system the signal is relatively stronger and consequently, the detection circuitry can be much simpler and occupy a smaller space [44]. Using the double-layer capacitance inherent to the electrode-electrolyte interface, current resulting from redox cycling of the tag can be integrated on the double-layer capacitance. The resulting voltage decrease (or increase) can be read out without disturbing the voltage on the capacitor through a source-follower configuration [45]. This allows one to build the electronics that can be scaled down so that pixel sizes below 1 μm^2 can be realized without compromising the signal integrity. Further, the pixels can be read out without the need of sampling at more than 1 second per pixel. In this situation the flicker noise characteristics of the transistors used are not as important. Hence, any process node with acceptable leakage currents can be used, thus allowing the pixel size to be scaled by adopting a more advanced process node. This enables the realization of massively parallel arrays by 1) being able to scale the circuitry, 2) reducing the number of times that the signal needs to be read out per unit time, and 3) reducing the amount of data that needs to be transferred from the chip so that I/O does not become a bottleneck in the

process. In this specific application, shared column ADCs are incorporated (similar to an imaging chip) and this requires relatively low real-estate overhead on chip.

V. CHALLENGES

The design and development of fully integrated biosensing platforms is complex owing to the diverse functionalities that need to be bridged efficiently. Here we address areas that pose important challenges, both practically and conceptually, in the design of these platforms. We categorize these as device-level challenges and system-level challenges.

A. DEVICE-LEVEL CHALLENGES

1) REFERENCE ELECTRODE

Any electrochemical assay relies on being able to control the interfacial potential at the working electrode very precisely and accurately. This is usually accomplished by employing a second, so-called reference electrode in solution, whose own potential remains invariant with any current passing through it [18], [46]. As an example let us consider a commonly used reference electrode – the silver-silver chloride (Ag/AgCl) electrode. In a conventional large scale reference electrode, a silver wire coated with silver cholride is embedded in a highly concentrated solution of chloride ions. This excess of chloride ensures that the potential of the electrode is unchanged upon the passage of current through it (via the Nernst equilibrium for the reaction $AgCl + e^- \rightleftharpoons Ag + Cl^-$). This situation is not easily realizable on chip, where patterned and deposited features are dominated by high surface-to-volume ratios. It is therefore very challenging to deposit sufficiently large volumes of AgCl and package them in chloride ions such that the dissolution of AgCl can be avoided or slowed. This inability compromises both the stability as well as the lifetime of the reference electrode. Alternatives like using only Ag (as a so-called *pseudo-reference* electrode) are very prone to fluctuations and drift due to surface reactions on the metal. Another alternative is to minimize the current flowing through the reference by having a separate counter electrode which sinks or sources most of the current. However, this costs valuable space on the chip. Additionally it must be ensured that the products of the electrochemical reaction at the counter electrode are not transported back to the active sensor array. This is easily achieved in conventional ''bulk'' electrochemistry, where the counter electrode is far away from the working electrode, but may be more challenging on chip. Ways to microfabricate stable, drift-free and durable reference electrodes on miniaturized chip-scale electrochemical platforms has typically relied on methods such as electroplating to create more porous AgCl [47] or embedment in porous polymer matrices [48]. Recent work on modeling the behavior of redox-cycling based nanogap sensors in the absence of any reference electrode, suggests that background parasitic electrochemical currents determine the solution potential [49]. Interestingly however it was found that asymmetry in the electrode geometry by having one electrode from the pair more exposed to the bulk solution, can enable that electrode to pin the solution potential and serve as a (pseudo)-reference electrode.

2) ADSORPTION

Electrode systems on the nanoscale are characterized by high surface-to-volume ratios. This implies that analyte molecules which need to be transported and detected have a greater probability of being non-specifically adsorbed on either the transport paths (channels, cavities, etc.) or the detection element. In electrochemical systems, depending on the nature of adsorption, this can lead to an increase in noise, electrode passivation, and attenuation and eventual loss of signal. Two possible ways of mitigating this are by way of choosing different electrode materials, or by surface modification of electrode or channel surfaces. For example, it is well known that carbon electrodes (graphitic or diamond-like) are less susceptible to adsorption of redox active molecules compared to noble metal electrodes like gold (Au) and platinum (Pt) [50]. Another alternative is to functionalize the surface with self-assembled monolayers, with suitable end-groups that can prevent adsorption [32]. A key challenge here is to use low-thickness coatings, since the electron-transfer rate constant depends exponentially upon the distance from the electrode, and this could lead to lowering the overall current. On the other hand, good, pin-hole free coatings typically require long-chain hydrocarbons to maximize hydrophobic interactions and thus provide stability and order.

3) SURFACE FUNCTIONALIZATION

Almost all on-chip biosensing platforms require the functionalization of the sensor arrays with a bio-recognition element (antibodies, *ss*DNA, enzyme etc.). Further, the throughput depends crucially on having as many distinct sensor elements in an array as possible. Therefore, as the senor array becomes dense, it becomes very challenging to implement site-specific coupling reactions to uniquely functionalize elements in an array. A simple way around this problem is to use external elements that can be independently functionalized off-chip, e.g., beads [1]. While not easy, this greatly reduces the complexity of the task by offering a greater ease of biochemical handling during functionalization steps. In some cases, the entire sensor array may need to be functionalized with a common coating, say, to prevent adsorption of redox tags on electrode surfaces as described above. This is somewhat easier but by no means trivial. It requires careful analysis of the interactions between the tags, electrode surfaces, constituents of the solution and the conditions of the experiment.

B. SYSTEM-LEVEL CHALLENGES

1) PACKAGING

Another challenge in realizing integrated sensor systems is the packaging. Making electrical connections to chips is a fairly standardized and mature process. However, there is no established and standard way of making fluidic and electrical

connections on the same device. Most of the system needs to be protected from fluid, and surfaces which have fluid contact need to be appropriately passivated to prevent the diffusion of unwanted fluid or salts. One way of addressing this is to make a standard electrical package with wire-bonding, then bonding a separate polymeric part to form a fluid cell in which fluids can be introduced to the chip surface [1]. The polymeric material in turn needs to be chemically inert and unreactive towards samples. Various materials like SU8, polyimide and parylene have been employed [39]. While this approach allows for a reliable and repeatable connection of the device, it also significantly increases the packaging costs. Typically, packaging costs can almost be as high as the cost of the silicon in a standard semiconductor product; the added fluidic integration may push the packaging costs higher. Another consideration with sensor systems is to accommodate any surface modification that may need to be made on the chip, placing additional constraints on the post-modification handling of the chip and the storage conditions. It is usually preferred that any surface modification that is temperature and environment sensitive be made as the last step of the packaging process. Alternatively, schemes which involve no packaging can be established [38], because usually such devices are designed for one time use only, relaxing the requirements on the reliability and the working lifetime of the device. For comparison, conventional packaging for microelectronics aims for reliability over several years.

2) SIGNAL CONFINEMENT

The time taken for a redox tag to diffuse from a site localized near one sensor element to the neighboring scales with the square of the distance between them ($\tau = \Delta x^2/2D$). As the sensor array density increases, and the pitch decreases, one has to contend with an increased probability of signaling molecules diffusing into neighboring sensors. This ''cross-talk'' can significantly deteriorate the quality of data obtained and complicate data analysis [51]. Molecules that diffuse away also attenuate the signal of the sensors in situations where the molecules need to be trapped so that the signal can be integrated over the number of molecules or time. This results in a lowering of the detection limit. High rates of sampling may not always be feasible due to limitations of the assay, I/O or bandwidth of the electronics. Thus, physical methods of confining the signal in small volumes are required and may indeed be indispensable to avoid bottlenecks in the scaling of CMOS-based arrays. One solution may consist of having the functionalized bead itself trap the molecules by blocking the access holes in a micro-well [52]. Microfluidic trapping may offer another alternative solution. For example, the ''Dimple machine'' - a pneumatically actuated PDMS lid was shown to seal fluorescent molecules in nanoscale wells in fused silica [53]. Remarkably, the lid could be reversibly operated for over 200 cycles.

3) COST VS. PERFORMANCE

CMOS integration can enable high performance through large-scale multiplexing, but this often comes at a significant cost. And although CMOS integration is commonly invoked in the context of cheap, miniaturized, portable, point-of-care devices, CMOS processing itself is far from cheap. With biosensing platforms, additional costs of biochemical materials as well as analysis of the enormous amounts of data generated is also substantial. Large scale integration can surely lower the cost-per-data, as well as other materials and handling costs, but only a high volume production of CMOS-integrated sensors can lower cost-per-data-per-chip. Simply put, the cost of CMOS processing is linked to scaling of volume production. This is a salient issue as we move from prototype to envisioning products. Prototypes are often made because they *can* be made. Deployable products usually require more compelling reasons to be realized. In this sense, CMOS-integration is ideally suited for areas that require high data throughput *and* large volume production. Needless to say, there can be niche applications where CMOS-integration is irreplaceable and where costs may only be a minor consideration.

It is well worth reiterating that we have only considered limitations at the transduction level here, and not dealt with any challenges in the assay. A robust, reliable and reproducible assay is by no means trivial and in most biosensing systems is the most critical component that determines overall performance. It is also the most complex component, in that the parameter space within which assays have to be optimized is much wider and much less well-defined than for any other component of the integrated system. A common bottleneck in assay development is that many assays which are typically developed in solution might not be as efficient when transferred to the chip surface. Thus assay development is often an iterative process between development in solution where parameters like buffer strength, binding kinetics, lifetimes etc. can be more easily optimized and the chip surface where these parameters may change. Independent methods to verify assay efficiency on chip can ease or speed up this process. Other effects like non-specific binding that leads to spurious signals, degradation or denaturation of labels or enzymes, surface fouling etc. are all issues that are frequently encountered in assays and can be (partly) mitigated by careful and application-specific chemical modifications.

VI. CONCLUSION

In this article, we have tried to provide a broad overview of the unique physical and chemical features that characterize nanoscale electrochemical sensing systems, in particular those based on redox-cycling. In addition, we have offered a glimpse of several design features that need to be considered to integrate these systems with CMOS processes. The

advancement in nanoscale electrochemical tools is enabling biosensing and biological experimentation in new regimes. To cite only a few examples, nanoscale electrodes have now been used for several exciting fundamental studies such as mapping metabolite distribution in single cells, [54] studying enzymatic turnover of fewer than 50 molecules, [55] or highly localized measurement of action potentials from individual electrogenic cells [56]. In some instances the combination with CMOS integration can enable progress into newer areas, hitherto inaccessible. A recent study used a CMOS-integrated electrochemical array to map the distribution of metabolites in a bacterial biofilm colony [57]. Dense nanoscopic electrodes with a high spatial resolution could offer a more fine-grained view of metabolite distribution in heterogeneous environments. Such arrays can be used similarly in neuroelectrochemical studies to detect neurotransmitters such as dopamine that are electrochemically active. Areas in which large-scale parallelization is required, such as genomics and proteomics, will remain a promising area for CMOS-integrated electrochemical sensing.

Biosensing platforms are also transitioning from being portable to being wearable. The current surge in interest in wearable health and fitness devices is bringing renewed focus on integration of biological and chemical sensors with low-power IC design [58]. The development of wearable platforms raise several interesting problems around efficient sampling, reusability of sensors, data processing and communication, and power optimization. Additionally, hopes to construct these systems on conformable, flexible substrates, will require other problems of interconnects, passivation and packaging to be solved. CMOS-integrated electrochemical sensing has much to offer in these new areas, provided the rationale for integration is clearly articulated and trade-offs between cost, scale and performance carefully considered.

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