

Ion-Channel Biosensors: Construction and Dynamic Modeling

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Abstract—This paper deals with the construction and dynamic modeling of a novel biosensor that exploits the selective conductivity of biological ion channels. The biosensor comprises gramicidin A channels embedded in a synthetic tethered lipid bilayer. It provides highly sensitive and rapid detection of a wide variety of analytes. In this paper we outline the principle of operation of this ion channel biosensor including the fabrication of biochip arrays and reproducibility/stability issues. Then the electrical dynamics and chemical dynamics of the biosensor are modeled. The electrical dynamics are modeled by a second order linear system. The chemical dynamics of the biosensor response to analyte concentration in the reaction-rate-limited regime are modeled by a two-time scale nonlinear system of differential equations. Also the analyte concentration in the mass-transport-influenced regime is modeled by a partial differential equation subject to a mix of Neumann and Dirichlet boundary conditions. By using the theory of singular perturbation, we develop models that predict the performance of the biosensor in transient and steady-state regimes. Finally, we outline the use of statistical signal processing algorithms that exploit the biosensor dynamics to classify analyte concentration.

Glossary

- ICS—Ion Channel Switch
- hCG—Human chorionic gonadotropin (glycoprotein hormone is produced during pregnancy and can be used for early detection).
- IgG—Immunoglobulin G, the most common human immunoglobulin
- β hCG— β subunit of hCG gonadotropin
- PVP—Polyvinylpyrrolidone
- ELISA—Enzyme Linked Immunosorbent Assay (a biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample)
- BLM—Bilayer lipid membrane
- X $\equiv$ aminocaproyl group
- 4X $\equiv$ four aminocaproyl groups
- 4XB $\equiv$ four aminocaproyl-linked biotin
- MSL—Membrane spanning lipid
- MSL4XB—Membrane spanning lipid with four aminocaproyl-linked biotin

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- SAM—Self assembled monolayer
- DI—Deionized water
- gA—Gramicidin A ion channel
- gAglyB—Gramicidin with a glycine group linker to Biotin
- gA5XB—Gramicidin with five aminocaproyl group linker
- Fab—Fragment antigen binding portion of antibody

I. INTRODUCTION

Biological ion channels are water-filled sub-nano-sized pores formed by protein molecules in the membranes of all living cells [17], [5]. Ion channels play a crucial role in living organisms by selectively regulating the flow of ions into and out of a cell thereby controlling the cell's electrical and biochemical activities. This paper deals with modeling the dynamics of a novel biosensor that exploits the selective conductivity of ion channels. Such ion channel based biosensors can detect target molecular species of interest across a wide range of applications. These include medical diagnostics, environmental monitoring and general bio-hazard detection.

In [7], a novel biosensor which incorporates gramicidin A ion channels into a tethered synthetic cell membrane was developed by our coauthor and published in *Nature*. Over the last few years, [48], [8], [6], [41], many novel functionalities have been added to successive generations of this biosensor. Throughout this paper, we refer to this ion channel biosensor as the *Ion Channel Switch (ICS) biosensor*.

CHECK Biological sensory systems function by registering changes in the electrical conductivity of specialized cell membranes [2]. A well documented example is the acetylcholine triggered cation channel involved in cross synaptic nerve conduction [36]. An important feature of this mechanism is its inherent amplification with a single molecule potentially triggering the passage of up to a million ions per second across an otherwise impermeable membrane. In [24] a light sensitive bacterial proton pump, bacteriorhodopsin, was incorporated into liposomes, which was stabilized with polymerisable phosphatidylcholines. These were observed to pump protons upon being photocycled. CHECK

The ICS biosensor provides an interesting example of engineering at the nano-scale. It is significant that the

functionality of the device depends on approximately 100 lipids, and a single ion channel modulating the flow of billions of ions in a typical sensing event of approximately 5 minutes. Since the gramicidin channels (each with conducting pore of diameter 0.4 nm and length 2.8 nm) move randomly in the outer lipid leaflet of the membrane (1.4 nm thick), we can view the biosensor as a fully functioning nano-machine with moving parts. Indeed, each individual gramicidin channel diffuses randomly in the order of 1 micron per second. Furthermore, the 4 nm thick lipid bilayer is tethered 4 nm away from the gold surface by hydrophilic spaces thereby allowing ions to diffuse between the membrane and gold. This permits a flux in excess of 10^6 ions per second to traverse each channel.

A. Main Results

This paper describes the construction and operation of the ICS biosensor along with signal processing of its measured signals. The ICS biosensor has several interesting properties (from a fabrication and modeling point of view) that make it a worthwhile case study.

1. *Construction and Operation of the Biosensor:* The ICS biosensor incorporates a self assembled monolayer providing enhanced stability (see literature review below for a comparison with other schemes). The tethered bilayer permits two-dimensional diffusion of gramicidin channels which provides a remarkable gating mechanism. Since gramicidin has an inherent chemistry that permits attachment of a wide range of receptors, the biosensor can be used to detect many different analytes. The ICS sensing mechanism does not require washing (unlike ELISA (see Glossary)), provides large amplification (millions of ions for every channel dimerization) and sensitivity since a single channel can diffuse and identify analyte molecules bound to capture sites, and provides an objective electrical readout which is intrinsically digital. The digital output permits the use of sophisticated statistical signal processing algorithms to estimate the type and concentration of analyte.

In Section II, the construction of the biosensor is discussed in detail. The interface between the biochemical part of the biosensor and the electrical measurement system is provided via a gold surface to which the lipid bilayer containing the ion channels is tethered and a silver coated return electrode which is immersed in the electrolyte above the biomimetic surface. We also discuss how the biosensor can be readily adapted to miniaturization and multiplexing (multiple electrodes).

2. *Dynamical Response of Biosensor:* In modeling the performance of the ICS biosensor, it is necessary

to consider the chemical and mass transfer dynamics to predict performance. Because the sensor can detect analyte concentrations smaller than 1 pico-molar, mass transfer of analyte over the electrodes becomes the dominant design criterion. This in turn requires careful modeling of the chemical dynamics (typically modeled by a two-time scale system of ordinary differential equations) together with the mass transport dynamics (partial differential equations of fluid flow). In Section III the electrical dynamics of the ion channel biosensor are modeled by an equivalent second order linear system, see [48],[41]. We then formulate the biosensor response to analyte concentration as a two-time scale nonlinear dynamical system. The presence of analyte decreases the concentration of the *dimers* formed in the biosensor, thus decreasing the channel admittance. In order to study the evolution of the channel admittance in response to the introduction of analyte, the chemical kinetics of the biosensor are modeled as a *singularly perturbed* system [20], [9]. The analyte concentration in the mass-transport-influenced region of operation of the biosensor is modeled by a partial differential equation subject to a mix of Neumann and Dirichlet boundary conditions, [27], [10], [15]. Analysis of the chemical kinetics of the biosensor as a Singularly Perturbed system leads to the result that the channel conduction evolves according to three regimes depending on the concentration of the analyte in the system.

3. *Analyte Concentration Classification:* The eventual goal of detailed modeling of the biosensor is to design sophisticated statistical signal processing algorithms that can exploit these model dynamics to rapidly classify analytes and estimate their concentrations. Using the derived equations describing the evolution of channel conductance as a function of analyte concentration, we illustrate how multi-hypothesis classifiers (via Kalman and Hidden Markov Model filters) can be used to classify the concentration of the analyte present in the system.

B. Related Work

The literature on biosensors is vast. We refer the reader to [32] for an interesting overview of the interface between ion channels and microelectronics. Several companies/research groups have developed biosensors based on synthetic lipid monolayers and bilayers. For example OhmX Corporation is currently developing a reagentless biosensor system using self assembled monolayers (SAMs) tethered to a gold surface for the electronic detection of biomarkers in clinical samples [13]. Also Bayley's group at Oxford has made substantial contributions in ion channel biosensors [33],[19]. We

focus on the ICS biosensor developed by AMBRI in this paper as one example of an ion channel based biosensor

Below we provide a highly focused review of related work in biosensors involving ion channels and tethered lipid membranes. The first attempt at developing a practical membrane-based biosensor device was reported in [24]. The poor stability of the receptor-membrane complex limited the range of applications of the device. An active cytochrome C has also been incorporated into a tethered membrane in [30] and is one of the first examples of a functionally active biomimetic surface.

The stabilization [23] of the *bilayer lipid membrane* (BLM) has been a central theme in the development of ion channel biosensors. Many strategies have been developed, but these primarily focus on physisorbing or chemically attaching a layer of hydrocarbon to a silicon [16], hydrogel [26], polymer [29] or metal surface [43]. Subsequently a second layer of mobile lipids is fused onto the tethered monolayer to form a tethered bilayer lipid membrane. The papers [38] and [34] review earlier works on BLM stabilization. In [28] peptide nanotubes have been fabricated within supported SAMs. The ICS biosensor employs an alkane disulphide bond to stabilize the bilayer lipid membrane at the electrode surface.

Attempts to engineer receptor-based gated ion channels have also been reported, see [41]. Mechanisms range from anti-channel antibodies that disrupt ion transport [4], to molecular plugs that block the channel entrance [25]. Ompf porin channels from E coli, were incorporated into a tethered BLM and their conduction altered using the channel blocker colicin [44]. The detection of single gramicidin channel currents in a tethered membrane is described in [13]. All mechanisms proposed so far have had a very limited range of application and require re-engineering for each new analyte. The ICS, whilst using ion channel transduction provides a mechanism that may be adapted to many different classes of target.

Miniaturization and patterning are two major opportunities for tethered membrane technologies [12], [42]. The functionalities which may be brought to tethered bilayers are becoming extensive. Topographical templates for chemiselective ligation of antigenic peptides to self assembled monolayers have been fabricated in [40].

to fix: ICS biosensor advances: covalent linkage of Fab to ion channel, use of flow cells, miniaturization of electrode dimension from 1 mm to 20 microns.

The remainder of this paper is organized as follows. Section II explains the details of the fabrication and operation of the biosensor in single and multi-array formats. Section III describes the electrical modeling

of the biosensor. Section IV deals with modeling the chemical dynamics and analyte mass transport in the biosensor. Finally, Section V presents an overview of statistical signal processing algorithms for detecting the presence of analyte given noisy measurements from the biosensor. It is hoped that these three aspects, namely construction, modeling, and signal processing, provide the reader with an interesting case-study that can be suitably modified to other types of biosensors.

II. CONSTRUCTION AND OPERATION OF ICS BIOSENSOR

In this section we discuss the fabrication and operation of the ICS biosensor. This discussion forms the basis for modeling the biosensor dynamics in subsequent sections. This section is organized as follows. First the gating mechanism caused by random movement of gramicidin channels is explained. Then we discuss how the sensor operation depends on the analyte size. Fabrication of arrays of such sensors is then outlined along with multi-analyte detection. Finally we discuss scalability issues of the biosensor in terms of reproducibility of sensor performance and stability/storage.

A. Principle of Operation

The low molecular weight bacterial ion channel gramicidin ([18], [47]) has been used, (see [7], [49], see also [6] for a review), as the basis of a biosensor platform with a range of applications for the detection of low molecular weight drugs, large proteins and micro-organisms. The ICS biosensor employs a lipid monolayer tethered via a disulphide group to a gold surface. The membrane is separated from the gold surface by an ethylene glycol spacer which provides a reservoir for ions permeating through the membrane. The transduction mechanism depends on the properties of gramicidin A within a BLM. gramicidin monomers diffuse within the individual monolayers of the BLM. The flow of ions through gramicidin only occurs when two non-conducting monomers align to form a conducting *dimer*. The gramicidins within the tethered inner leaflet of the lipid bilayer are also tethered. Also attached to the gold surface as part of the inner leaflet are membrane spanning lipids. The arrival of analyte cross-links antibodies attached to the mobile outer layer channels, to those attached to membrane spanning lipid tethers. Due to the low density of tethered channels within the inner membrane leaflet, this anchors them distant, on average, from their immobilized inner layer channel partners. Gramicidin *dimer* conduction is thus prevented and the

ionic admittance of the membrane decreases. Applying a small alternating potential between the gold substrate and a reference electrode in the test solution generates a charge at the gold surface which causes electrons to flow in an external circuit.

The membrane stability is primarily enhanced by tethering the inner membrane leaflet to the gold surface. However additional stability is achieved by substituting a major fraction of the tethered lipids with archaeobacterial lipids. These are lipids modeled on constituents found in bacteria capable of surviving extremes of temperature and hostile chemical environments. Characteristics of these lipids are that the hydrocarbon chains span the entire membrane, [22] and that all ester linkages are replaced with ethers [37]. BLM films have previously been formed from archaeobacterial lipids [14] and resulted in membranes that are stable to temperatures in excess of 90°C. A stable membrane incorporating ion channels can be self-assembled [31] on a clean, smooth gold surface using a combination of sulphur-gold chemistry and physisorption. Most studies of the ICS biosensor have used antibody Fab (see Glossary) fragments as the receptor; however, the approach has also been demonstrated to operate using oligonucleotide probes, heavy metal chelates and cell surface receptors.

B. Large and Small Analyte detection

Large analytes include proteins, hormones, polypeptides, microorganisms, oligonucleotides, DNA segments and polymers. In the same manner that an ELISA sandwich assay may be developed based on a complementary antibody pair, the ICS biosensor may be adapted to the detection of any antigenic target for which a suitable antibody pair is available. The bacterial ion channel gramicidin A is assembled into a tethered lipid membrane and coupled to an antibody targeting a compound of diagnostic interest. The binding of the target molecule causes the conformation of the gramicidin channels to switch from predominantly conducting *dimer* to predominantly non-conducting monomers as shown in Fig. 1. For target analytes with low molecular weights such as therapeutic drugs where the target is too small to use a two site sandwich assay, a competitive adaptation of the ICS is available. This is shown in Fig. 2.

C. Biochip arrays

Sensor arrays have been fabricated using silicon nitride, silicon carbide and glass substrates. Using this format a multi-analyte detection capability is demonstrated. Multi-analyte detection is an advantage as it

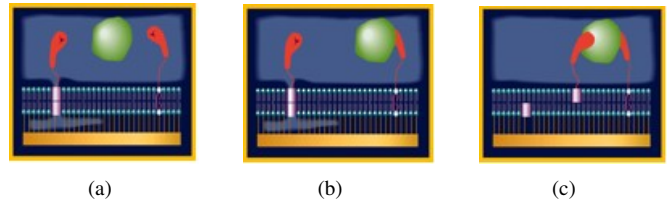


Fig. 1. Large analyte transduction mechanism. The binding of analyte (green) to the antibody fragments (Fab) (red) causes the conformation of gramicidin A to shift from conductive dimers to non-conductive monomers. This causes a loss of conduction of ions across the membrane. The scale can be visualized by the fact that the tethered lipid bilayer is 4nm thick.

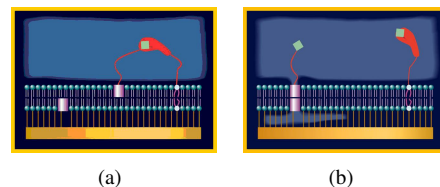


Fig. 2. Small analyte transduction mechanism. In the absence of analyte, the mobile channels cross link to anti-target Fabs anchored at the tether sites. Dimer formation is prevented and the conductance of the membrane falls. The introduction of analyte competes off the hapten (target analogue) and increases the membrane conductance

permits on board calibration to correct for systematic variations which can occur across an electrode array and to correct for electrode to electrode variation between different sensors. A novel element in the design of these arrays is the use of a titanium oxide ring at the perimeter of the electrode opening. The titanium ring is designed to provide a mechanical seal for the outer leaflet preventing it from diffusing beyond the area of the tethered inner leaflet lipids. In addition, the titanium seal retains water during the patterning of antibodies, and during the dry down process for storage. Examples of electrode arrays are shown in Fig. 3, recorded by both optical and scanning electron microscopy.

In Fig. 3(a) a 4 x 4, 16 element array of 150µm diameter electrodes is shown and Fig. 3(b) a test array of four electrodes ranging from 150µm to 20µm diameter. A consequence of reducing the electrode diameter from 150µm to 20µm is a reduction of the membrane capacitance and an increase in the membrane resistance. Both measures scale with membrane area, the capacitance linearly and the impedance inversely.

Although the impedance of these electrodes is dependent on area, the time constant of the response is independent of area. A dependence is expected, however, when the spacing of the ion channels or antibodies are comparable to the electrode dimensions. In the present

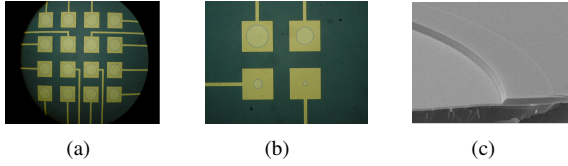


Fig. 3. (a) Optical microscopy image of a 16 element sensor array with $150\mu\text{m}$ diameter electrodes. The apparently square geometry of the sensor elements arises from the gold being patterned as rectangles and the silicon nitride openings being round. The thin (100 nm) and transparent silicon nitride allows the gold to be viewed through the nitride layer. Also visible is the light grey $2\mu\text{m}$ wide Titanium oxide ring. (b) Optical microscopy image of a test array with four electrode elements of $150\mu\text{m}$, $100\mu\text{m}$, $50\mu\text{m}$ and $20\mu\text{m}$ diameter. (c) Scanning electron microscopy image of the edge of one of the $150\mu\text{m}$ electrode openings showing the Titanium oxide ring and the gold to silicon nitride interface. The etch angle at the gold to silicon nitride edge is 65° .

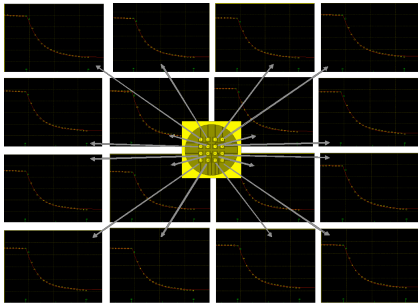


Fig. 4. Change of admittance versus time over an array of 16 electrodes. The abscissa (time) axis spans 8 min and the admittance axis spans a FSD of 70ns . The schematic of the array in the center of the trace shows the electrodes from which each trace was recorded. The CVs for the amplitude of the response across the 16 electrodes is 2.8% and to the τ of an exponential fit to the decay is 2.1%.

case these spacings are approximately $0.1\mu\text{m}$ - $1\mu\text{m}$, far smaller than the smallest $20\mu\text{m}$ diameter electrode reported here. One consequence of using an electrode array to measure the target species concentration rather than a single electrode of comparable area is the improvement in the quality of estimating the response rate. This is shown in Fig. 4. An exponential fit to the admittance decay curve across the 16 electrodes yields coefficients of variation (standard-deviation/mean) of well below 10% for a strong response. This indicates that the silicon chip fabrication procedures can provide a highly reproducible electrode geometry and structure. The distributed sensing array possesses a statistical improvement of 16 independent measures rather than one.

D. Multi-analyte detection

Sensor arrays also have the ability to measure multiple target concentrations from a single sample addition to

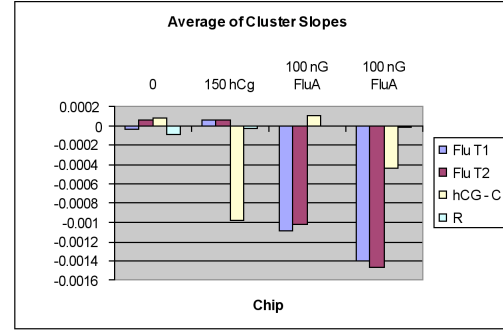


Fig. 6. From left to right, the first set of bars correspond to electrode with no receptors, second set correspond to electrodes with hCG receptors, third and fourth set contain influenza A receptors. Chip challenged with single sample possessing 150mIU/ml hCG and 100ng/mL influenza A virus.

the sensor. A key problem when fabricating an array capable of multi-analyte detection is the site specific decoration of the chip with different antibodies. The approach used here is shown in Fig. 5. A fluid handling spotter (sciFlexarrayer leased from Scienion AG Berlin) was loaded with the appropriate antibody solution and directed to a chip surface that had been partially dried from glycerol, trehalose, PVP or their combinations.

The lower size limit of the electrodes was determined by the resolution achievable by the spotter-surface characteristics and not the constraints of the electrode-membrane characteristics. The limiting dimension set by the spotter-surface combination was $80\mu\text{m}$ diameter whereas the limit set by the electrode-membrane combination was $10\mu\text{m}$. Fig. 5(a & b) shows an example of decorating a chip array with four arbitrary antibody receptors. That is, one quadrant, contains four electrodes, with the pregnancy hormone (human chorionic gonadotrophin – hCG), two quadrants have antibody receptors for influenza A and a further quadrant uses the reference receptor to a target not in the test sample. Figure 6 shows the response to four samples containing either 150mIU/ml hCG, 100ng/L influenza A virus or neither. The reference electrode cluster yielded a null result to all three challenges; the β hCG cluster yielded a positive response (reduction in admittance read as a negative slope) to the 150mIU/ml hCG sample but zero to the influenza A challenge, whereas the influenza A clusters yielded a positive result to challenge with influenza A but zero to β hCG. These data show the ability of an electrode array to detect multiple target species in one sample addition. In this case the sample volumes used were $100\mu\text{L}$ but these can be reduced to $10 - 20\mu\text{L}$ using a coplanar return electrode.

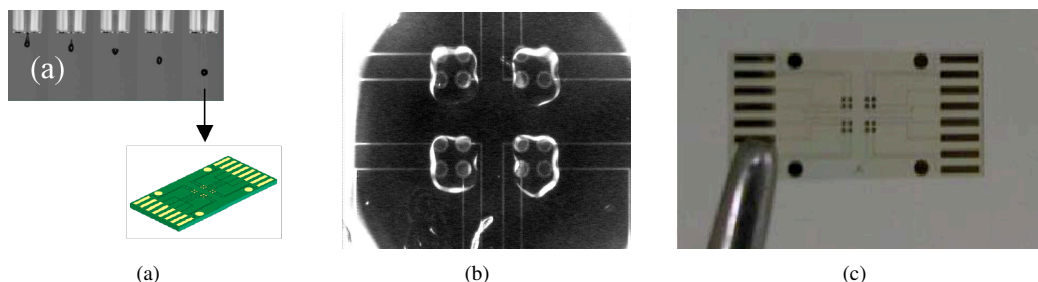


Fig. 5. (a) Site specific coating was achieved by directing a metered volume of biotinylated antibody Fab onto an electrode element ($230\mu\text{m}$ diameter) in the array. The electrode surface was pretreated with 2-5% trehalose solution and dried prior to spotting the surface. Prior to spotting the membranes had been assembled to a common structure across all electrodes, including a streptavidin linker to the ion channels and membrane spanning lipids. (b) The sciFlexarrayer (Scienion AG Berlin) provided a stream of 20pL volume drop. Typically 15 drops were applied to each electrode resulting in 12nL per quadrant of four electrodes. Each quadrant received a different biotinylated antibody fragment. (c) The chip used here was a cluster of four 2×2 electrode arrays - each $230\mu\text{m}$ diameter on either a glass or silicon substrate.

E. Scale-up issues

Reproducibility of results is essential for calibrating a sensor. Also stability and storage are critical issues for biosensors. We discuss these issues below.

1) *Reproducibility*: Variation in the sensor performance occurs between test elements within a single chip and between chip to chip. Custom fabricated chips were supplied by Micralyne, Edmonton, Canada. The line resolution of standard silicon foundry techniques substantially exceeds the requirements of the BioChips used here. Silicon was chosen as a platform for scaling up sensor production since it was viewed as a mature technology that operated within a highly controlled, clean environment. However, an unexpected problem arose in the adaptation of standard photolithographic approaches to the fabrication of patterned surfaces for use in Au-alkanethiol based sensors. The etch procedures employed to expose the patterned gold seriously contaminates the gold surface in a manner that we have been unable to totally reverse. Cleaning solutions such as Piranha followed by deionised (DI) water and ethanol could achieve a functioning device but the longer term stability was seriously compromised. High energy Argon Ion milling of the surface was necessary immediately prior to coating the thiol or disulphide species regardless of the wet cleaning process employed. The optimal approach is to vary the lithographic process steps to permit a deposit gold last sequence, which although requiring a final masking step, achieves the best results.

Monitoring sensor performance over many batches provides an estimate of the batch to batch variation in performance. Figure 7 shows a one month record of response to 60mIU hCG, run without calibrators or any internal corrections for an array of sixteen measures

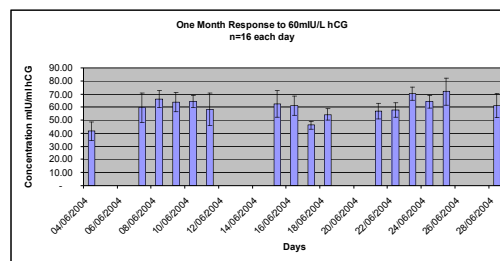


Fig. 7. The daily means of sixteen estimates of $60\text{mIU}/\text{ml}$ hCG in PBS at 30°C over a one month period. Similar results were obtained over a twelve month period.

on a plastic slide. Each day represents an average of sixteen measures from one array and over the month from sixteen separate arrays. A batch is complicated to define as a new batch is initiated when any of the electrodes, chemistries or biochemistries is altered. The sample challenge was a standard solution of $60\text{mIU}/\text{L}$ hCG. The average daily CV over 16 measures was 12% and the CV of the daily means over the 16 arrays measured during the month was 4.7%. These measures were performed in PBS at 30°C on sensors made that day.

A major step towards achieving a quantitative test is to provide an on board calibrator to correct for daily variation. This is particularly important when measurements are made from serum or blood where matrix variations cause substantial changes to the standard curve. In the absence of a calibrator the variation essentially eliminates the possibility of all but semi-quantitative measurements. Figure 8(a & b) show an example of the potential improvement in reproducibility that can

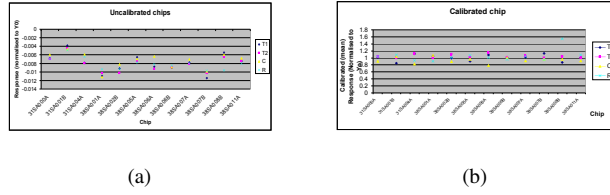


Fig. 8. Improvement in reproducibility that can be achieved by employing a calibrator (a) Uncorrected response rate to a challenge by analyte. T_1 and T_2 are two tests of the target concentration, C is a calibration measure and R is a reference channel that permits a differential measure. The latter is used in blood or serum where matrix artifacts are minimized through the use of a differential reading between the test and reference electrode. The data have been normalized to the gating amplitude only. (b) Response to challenge by analyte in which the calibrator response has been used to normalize the test responses. The effect of normalizing to the calibrator values is to reduce the CVs of T_1 and T_2 from 27% and 22% to 15% and 14% respectively.

be achieved by employing a calibrator. The approach depends on a multi-analyte capability. Figure 8(a) shows a series of individual chip arrays. The horizontal scale describes particular chip arrays. The vertical scale is a measure of the response rate to a challenge of analyte. In Fig. 8(a) the response rate has been normalised to the gating amplitude of each measure. This is equivalent to reporting on the inverse of the time constant of an exponential fit to the response. In Fig. 8(b) the same data is processed differently. Calibration data is now used to scale the test data resulting in a substantial improvement in reproducibility. The provision of a calibrator channel is a significant benefit brought by simultaneous Multi-Analyte Detection.

2) *Stability and Storage*: A key requirement for a diagnostic technology is an ability to be stored for extended periods with minimal degradation in performance. A serendipitous observation has permitted insights into some of the mechanisms that cause age dependent changes in the ion channel switch sensor. Figure 9(a) shows a schematic of a sensor containing the biotinylated gramicidin analogue, gaglyB, being gated by the multivalent protein, Streptavidin (MW 60kDa). It was found that if the linker attaching the biotins to the ion channel was reduced in length to a glycine group, then the streptavidin on rate to the biotin was ineffective in achieving gating on the 5-10 minute timescale. However, if as shown in Fig. 9(b) the membrane spanning lipid, MSL4XB is present, possessing a far longer linker between the biotin and the tethered lipid, a strong rapid gating occurs. The experimental evidence for this is shown in Fig. 10(a and b). This suggests that streptavidin binds to the biotin attached by the longer linker on

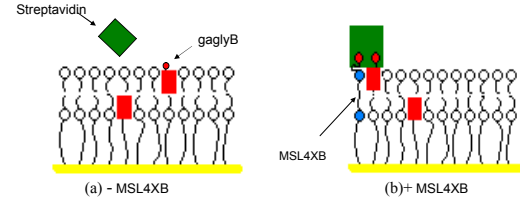


Fig. 9. Mechanism for understanding a major source of change in performance upon storage (a) Schematic of a sensor membrane containing gaglyB, the short linker biotinylated gramicidin ion channel and no biotinylated membrane spanning lipid MSL4XB. (b) The same construct containing MSL4XB.

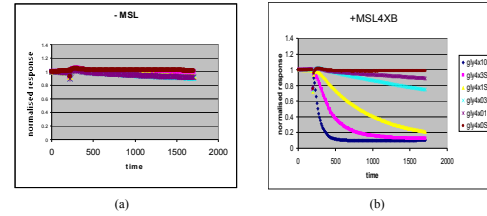


Fig. 10. Experimental gating result for the membrane challenged by 0, 0.1nM, 0.3nM, 1nM, 3nM or 10nM streptavidin. (a) with no MSL4XB, little response is seen at all streptavidin concentrations. (b) with MSL4XB with 0, 0.1, 0.3, 1.0, 3.0 and 10nM Streptavidin causes a progressively larger faster response.

the MSL4XB but not to the biotin attached by the far shorter linker on the gaglyB. However, once bound to MSL4XB, the streptavidin is presented in a way to permit gating of the gaglyB. It is proposed that gating of the gaglyB only occurs from the gaglyB diffusing to a site where streptavidin has already bound to the MSL4XB. This provides a method of determining the average diffusion distance of the ion channel in the membrane and a means of probing whether this distance alters on storage. By titrating the gating reaction rate to the concentration of the streptavidin challenge, the on rate or f_1 of streptavidin to the MSL4XB is determined. This may be contrasted with the far slower on rate, f_1 of the streptavidin to the gaglyB. Comparing these f_1 values with those obtained from directly cross linking ion channels possessing longer 5XB linkers it is possible to determine the diffusion distance. The various combinations of f_1 are given in Table I. In Table I it is evident that when using the longer 5XB linkers the dependence on the MSL4XB is almost eliminated.

Taking the ratio of the second and third entries of Table I provides a measure of the reaction rate f_1 of the mobile channel, gA5XB density to the density

Case	Configuration	Slope = f_1 ($M^{-1}s^{-1}$)
1	gA5XB + MSL4XB	7.40×10^6
2	gAglyB + MSL4XB	1.19×10^6
3	gA5XB No MSL4XB	6.90×10^6
4	gAglyB No MSL4XB	0.43×10^6

TABLE I
EXPERIMENTALLY DETERMINED f_1 VALUES FOR THE VARIOUS
COMBINATIONS OF ION CHANNEL AND MSL4XB DESCRIBED
ABOVE.

of the tethered membrane spanning, MSL4XB sites. The ratio is 0.17 meaning that the density of mobile channels is greater than the density of the tethered membrane spanning lipid MSL4XB capture sites. This is necessary if Streptavidin is used as a linker species linking the channel or the tethered lipid to the biotinylated antibodies. Higher levels of MSL4XB results in too great a cross linking of the gA5XB to MSL4XB by the Streptavidin. This problem is eliminated when using a covalent linkage to the antibody fragment and larger densities of MSL4XB are possible resulting in significantly higher sensitivities. However, in the present study, using the density of channels added in the mobile layer as 3×10^8 molecules/cm² we may determine the MSL4XB density as 6.7×10^9 molecules/cm². Varying the MSL4XB density relative to this estimate now permits a measure of the diffusion distance of the ion channels. This is seen in Fig. 11 in which the MSL4XB density is plotted relative to the value used above. This value is described as the Standard (Stnd) value. It can be seen that when the sensors are fresh, the gating amplitude rises from a very low value at zero MSL4XB to a maximal value at approximately the standard density of MSL4XB. This indicates a diffusion distance of the channels of approximately $(6.7 \times 10^8) - 0.5 = 0.5 \mu m$. This further suggests a complex structure to the membrane surface restricting the diffusion distance. If the diffusion were unrestricted a diffusion distance $\times 10$ this value would be expected based on reported values of the self diffusion coefficient of gramicidin in lipid bilayers. The alternative explanation that the diffusion coefficient is slowed is not supported by the kinetics of the gating response. When the sensors are dried and stored, the diffusion distance is reduced by 40% suggesting the drying and storage have further restricted the average diffusion distance (Fig. 11).

To maintain a gating response for long periods of storage requires increasing the density of MSL4XB. Figure 12 shows storage times at 20°C for 28 days. After 3 months at 20°C, the titration response deviates

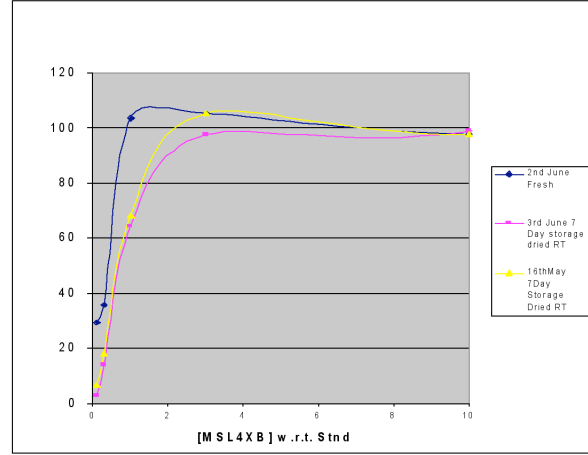


Fig. 11. Plot of gating magnitude versus MSL4XB density relative to the standard density of 6.7×10^8 MSL4XB/cm². Drying and storage for two weeks at 20°C causes a need for higher densities of MSL4XB to achieve the same gating magnitude. Increasing capture site density improves both storage and sensitivity of the sensor.

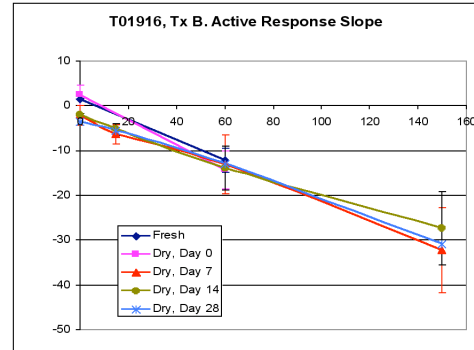


Fig. 12. Plot of titration gating rate versus hCG concentration for a sensor stored at 20°C dry, for up to one month.

outside acceptable limits. Storage at 4°C significantly extends the acceptable titration response and can survive beyond six months. Factors causing failure appear to be associated with the diffusion of the mobile species. In all cases the loss of responsiveness is at high target concentrations, leaving the low concentration region of the standard curve substantially unaltered. This further suggests a diffusion mechanism as an important cause of performance loss and more work is required to understand and control these effects. Whether or not the slope of the titration curve is lost, the membrane integrity does not fail. When stored dry, the membrane metrics indicate that the membranes remain sealed or conducting close to their initial value over extended periods of time.

Element	Value
Membrane Capacitance C_1	$0.5\mu\text{F}/\text{cm}^2$
Interfacial Capacitance C_2	$3.5\mu\text{F}/\text{cm}^2$
Ion Channel Resistance C_1	$60\text{k}\Omega\text{--}600\text{k}\Omega$
Electrolyte Resistance R_2	200Ω

TABLE II

TYPICAL VALUES FOR THE COMPONENTS OF THE EQUIVALENT ELECTRICAL SYSTEM OF THE BIOSENSOR, DEPICTED IN FIG. 13. THE VALUES ARE GIVEN IN TERMS OF PER AREA UNITS AND THE AREA OF THE ELECTRODE IS 0.03cm^2 .

III. MODELING THE DYNAMICS OF THE ION CHANNEL BIOSENSOR

With the above description of the operation and construction of the ICS biosensor, this section and the next section deal with constructing mathematical models for the biosensor's electrical response and chemical dynamics. Obtaining an accurate mathematical model of the chemical kinetics and electrical response of the biosensor is important in the design, operation and analysis of the biosensor. For example, an accurate model of the biosensor dynamics allows us to determine the flow rate for various analyte concentrations to optimize the sensor response, see Section IV-A. Also detailed modeling/analysis of the chemical kinetics of the biosensor can be exploited in designing sophisticated statistical signal processing algorithms for rapidly classifying analytes, see Section V.

A. Electrical Dynamics of the Biosensor

The gramicidin A Ion channel biosensor can be modeled as a biological transistor. Fig.13(a) illustrates the equivalent circuit of the switch before and after the detection of analyte. Fig.13(b) details the components of the equivalent circuit. The resistor $R = 1/G$ models the biosensor resistance and increases with the presence of analyte. C_1 denotes the capacitance of the membrane while C_2 denotes the interfacial capacitance of the gold substrate. Note that one face of the capacitor C_2 is charged due to ions, the other face is due to electrons that form the output current of the biosensor. Thus C_2 provides the interface between the biological sensor and the electrical instrumentation. R_2 denotes the resistance of the electrolyte and its value varies depending on the type of electrolyte and the dimensions of the flow chamber. Finally, R_3 denotes the input resistance of the amplifier at the next stage (which is ideally very large). Value of the equivalent electrical system components are functions of electrode area and flow chamber dimensions. Typical values are listed in Table II.

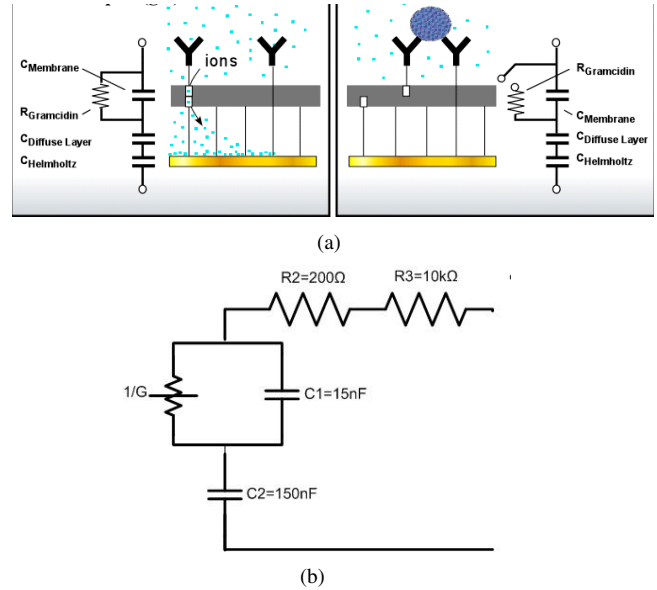


Fig. 13. The ICS biosensor comprises of an ion channel switch that acts like a biological transistor. Fig.13(a) denotes the switched-on state when the ion channels are conducting. Fig.13(b) denotes the switched-off state when the channels are not conducting. The equivalent electrical circuit shown in Fig.13(b) results in a second order system.

The admittance transfer function of the equivalent circuit parameterized by G is (where s represents Laplace transform variable)

$$H(s; G) = \frac{I(s)}{V_{out}(s)} = \frac{s^2 + s a G}{s^2 R_2 + s(b_2 + b_1 G) + b_3 G} \quad (1)$$

The constants in $H(s)$ above are $a = \frac{1}{C_1}$, $b_1 = \frac{R_2}{C_1}$, $b_3 = \frac{1}{C_1 C_2}$, $b_2 = \frac{1}{C_1} + \frac{1}{C_2}$.

Before proceeding we remark that the biosensor we consider below comprises of electrodes with area 0.03cm^2 . Each such electrode contains several millions of ion channels. Therefore the measured current is the average effect of the formation and dis-association of thousands of dimers and is approximately continuous-valued. Specifically each channel dimer possesses a resistance to sodium ion flow of $10^{11}\Omega$. In the present data, electrodes of 2mm diameters result in an area of 0.03cm^2 , which for 10^8 channels per cm^2 results in 3×10^6 channels per electrode. It is worth noting that the density of the mobile and tethered channels is such that approximately 50% of the channels are dimerized. Taking the parallel resistance of all the ion channels into consideration the ion channel resistance is approximately $60\text{k}\Omega$. In contrast, for micro-size electrodes of area 10^{-10}m^2 (or smaller), the current would be a finite state

process since only a few *dimers* would form and dis-associate; such micro-sized electrodes are studied in our recent work [21].

B. Dynamics of Biosensor Response to Analyte

From an abstract point of view, we can describe the biosensor response to an analyte by a two time scale stochastic dynamical system: Let $k = 1, 2, \dots$ denote the fast time scale. Typically, with a sampling frequency of 3kHz, k evolves on the milli-second time scale. Let $n = 1, 2, \dots$ denote the slow time scale. We will refer to n as batch number. So for fixed large positive integer T , the n -th batch on the slow time scale corresponds to times $k \in [(n-1)T+1, \dots, nT]$ in the fast time scale. Typically, T is chosen in the order of a few seconds.

Let A denote the concentration of analyte. From detailed experimental analysis of the biosensor and reaction rate dynamics analysis presented in Section IV, it is known that the conductance G of the biosensor evolves on the slow time scale according to one of 3 different concentration modes; $\mathcal{M}$:

$$G_{n+1} = f^{\mathcal{M}}(G_n, A) + w_n, \quad (2)$$

$$\mathcal{M} = \begin{cases} 1 & A = 0 : f^{\mathcal{M}}(G_n, A) = G_n \\ 2 & A_{\text{medium}} : f^{\mathcal{M}}(G_n, A) = \kappa_1 G_n + \kappa_2 \\ 3 & A_{\text{high}} : f^{\mathcal{M}}(G_n, A) = \kappa_3 G_n + \kappa_4 \end{cases}$$

Here $\kappa_1, \kappa_2, \kappa_3, \kappa_4$ are constants, with $|\kappa_2| < 1$. w_n is a zero mean white Gaussian noise process with small variance and models our uncertainty in the evolution of G . The function $f^{\mathcal{M}}$ models the fact that the admittance G_n of the membrane decreases according to one of 3 distinct modes depending on the concentration A of the analyte. For no analyte present ($\mathcal{M} = 1$), the admittance remains constant. As described earlier, when analyte is present the admittance G in (1) decreases due to analyte molecules binding to the antibodies in the biosensor. For medium and high concentration ($\mathcal{M} = 2, \mathcal{M} = 3$), the decrease in admittance is exponential with different decay rates. High analyte concentration refers to ($A \geq 10^{-8}\text{M}$) and medium analyte concentration refers to ($A \geq 10^{-9}\text{M}$). Change in channel conduction as a function of analyte concentration is discussed in more detail in section IV-B.

Fig.14 shows examples of the biosensor response to Streptavidin with different concentrations; see [6], [48] for details. Using Streptavidin as the protein antigen and biotin as a low molecular weight receptor provides a rapid and clear demonstration of the different kinetic regimes of the sensor function. The Streptavidin-biotin binding pair is one of the strongest and best characterized

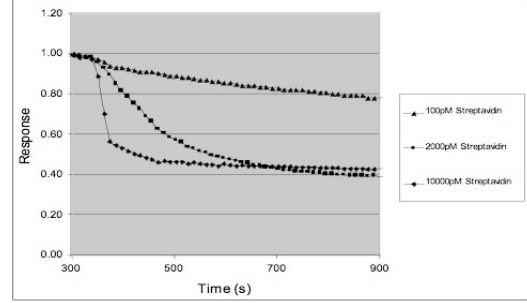


Fig. 14. Biosensor response to Streptavidin; see [6], [48] for details

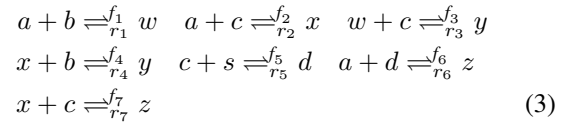
interactions available today and is used as a model system in Fig.14 to permit full characterization of the sensor.

In the next section we will derive models according to which the channel conductance evolves for various analyte concentrations, by analyzing in detail the chemical dynamics of the biosensor.

IV. CHEMICAL DYNAMICS OF BIOSENSOR

As described in the previous section, the resistance of the biosensor channel is determined by the concentration of the gramicidin A *dimers* formed in the biosensor. As the concentration of the *dimers* decreases the channel admittance decreases. Therefore to study the evolution of the channel conductance as a function of time we need to analyze the evolution of the *dimer* concentration in the biosensor. Since the dynamics of the biosensor including the *dimer* concentration are governed by many chemical species and their reactions, we analyze the dynamics of these reactions.

The reactions involved in the biosensor mainly stem from the binding of the primary species and the secondary species or complexes. The primary species are Analyte, a , of concentration A , binding site b of concentration B , free moving monomeric ion channel c of concentration C , and tethered monomeric ion channel s of concentration S . While the complexes are w, x, y and z with concentrations, W, X, Y and Z and are formed according to the following equations;



where, f_i denote the forward reaction rate constants with units of ($\text{M}^{-1}\text{s}^{-1}$) in case of 3D reactions, such as binding of analyte with capture sites, and (cm^2s^{-1}) in case of 2D reactions, such as dimerization of the

Initial Concentration of Species	Value
Mobile gramicidin A monomers $C(0)$	$10^8 \text{ molecules/cm}^2$
Tethered gramicidin A monomers $S(0)$	$10^9 \text{ molecules/cm}^2$
Tethered Capture Site $B(0)$	$10^9/\text{cm}^2 - 10^{11}/\text{cm}^2$
Analyte A^*	$\mu\text{M-fM}$

TABLE III
TYPICAL CONCENTRATIONS OF PRIMARY SPECIES IN THE ICS
BIOSENSOR.

Reaction Rate	Streptavidin-Biotin	hCG-IgG
$f_1 = f_2 = f_6 = f_7$	$7 \times 10^6 \text{ (M}^{-1}\text{s}^{-1}\text{)}$	$5 \times 10^5 \text{ (M}^{-1}\text{s}^{-1}\text{)}$
$f_3 = f_4$	$10^{-10} \text{ (cm}^2\text{s}^{-1}\text{)}$	$5 \times 10^{-11} \text{ (cm}^2\text{s}^{-1}\text{)}$
f_5	$10^{-11} \text{ (cm}^2\text{s}^{-1}\text{)}$	$10^{-11} \text{ (cm}^2\text{s}^{-1}\text{)}$
$r_1 = r_2 = r_6 = r_7$	$10^{-6} \text{ (s}^{-1}\text{)}$	$10^{-4} \text{ (s}^{-1}\text{)}$
$r_3 = r_4$	$10^{-6} \text{ (s}^{-1}\text{)}$	$10^{-4} \text{ (s}^{-1}\text{)}$
r_5	$10^{-2} \text{ (s}^{-1}\text{)}$	$10^{-2} \text{ (s}^{-1}\text{)}$

TABLE IV
TYPICAL VALUES OF THE REACTION RATES FOR
ANTIGEN-ANTIBODY PAIR hCG-IgG AND STREPTAVIDIN-BIOTIN
PAIR. REFER TO (3) TO UNDERSTAND THE MEANING OF EACH
REACTION RATE.

ion channel, and r_i for $i = \{1, 2, 3, 4, 5, 6, 7\}$ denote backward reaction rate constants with units of (s^{-1}) . The total reaction rates can be computed as below,

$$\begin{aligned}
 R_1 &= f_1 AB - r_1 W & R_2 &= f_2 AC - r_2 X \\
 R_3 &= f_3 WC - r_3 Y & R_4 &= f_4 XB - r_4 Y \\
 R_5 &= f_5 CS - r_5 D & R_6 &= f_6 AD - r_6 Z \\
 R_7 &= f_7 XS - r_7 Z
 \end{aligned} \quad (4)$$

The initial concentrations of the primary species, A^* , $B(0)$, $C(0)$, and $S(0)$, are given in Table III. Note that in Table III, $S(0)$ denotes the initial concentration of the tethered gramicidin A monomers, and also that $S \approx S(0)$ through out the experiment.

As mentioned before the ICS biosensor converts a biological binding event to a decrease in channel conductance, in case of large analyte molecules. So as suggested by the dynamics of the sensor, the forward and backward reaction rates, denoted above as f_i and r_i , depend on the type of analyte and capture sites used in the biosensor. An example of antigen-antibody detection is the detection of hCG, using IgG as the capture site (hCG and IgG are defined in Glossary). The biosensor can be used to detect specific types of proteins such as Streptavidin as well. In case of streptavidin, Biotin is used as the capture site. Typical values of f_i and r_i for these two cases are shown in Table IV.

The analyte concentration A is generally a function of both space and time. In a flow cell Diffusion and advection carry analyte molecules to the biomimetic

surface where binding occurs according to the chemical kinetics defined in (3). Three operating regimes for the system can be distinguished: (i) reaction-rate-limited kinetics, (ii) mass-transport-limited kinetics, or (iii) kinetics influenced by both mass-transport and reaction-rate [46].

In reaction-rate-limited kinetics regime, the depletion of analyte molecules due to reaction at the biomimetic surface is compensated for rapidly. Therefore for analyte concentrations in the order of μM it is reasonable to assume, $A(x_1, x_2, x_3, t) = A^*$, where A^* denotes a constant analyte concentration and x_1 , x_2 and x_3 respectively denote the x, y and z coordinate axes. In this region depletion of analyte molecules is compensated for by either large flow rates with low analyte concentration or average flow rates with high analyte concentration.

In (ii) and (iii) where chemical kinetics are influenced by both mass-transport and reaction-rates, the local concentration of analyte varies due to the chemical reactions between the analyte and the binding sites, thus $A(x_1, x_2, x_3, t)$. At very low analyte concentrations very high flow rates are required to get the highest response rate of the system. Dependence of response rate on flow rate and analyte concentration is discussed in more detail in section IV-A. The modeling of the chemical dynamics of the system are explained in the following sections.

A. Comprised between Capture Density and Analyte Flow Rate

A high capture density is essential for high sensitivity. With high capture density, target molecules collide more frequently with receptors and are thus captured more quickly. The greater the ratio of capture sites to diffusing ion channels, the faster the increase in resistance.

To illustrate the requirements for modeling both the reaction-rate limited kinetics and mass-transport limited kinetics, we first illustrate these effects via numerical simulation.

Fig. 15 shows quantitative predictions of the change in the resistance per unit time for various capture site densities and sample flow rates, for high analyte concentrations (100pM) and low analyte concentrations (10fM). As apparent in this figure at high concentrations the response is insensitive to flow rate, whereas at low concentrations of analyte and high capture densities a high flow rate is required to achieve a measurable response.

The reaction-rate-limited chemical kinetics are modeled in the next section. In the reaction-rate-limited regime of operation, as explained above mass transport effects are negligible.

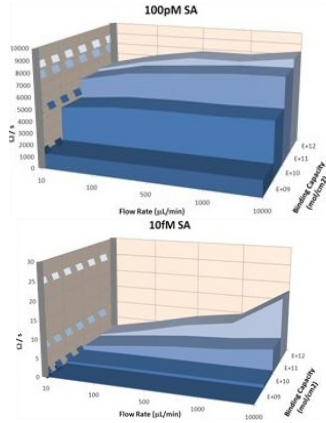


Fig. 15. Simulations of the response to 100pM and 10fM Streptavidin for various capture site to ion channel densities and flow rates. At 100pM and low capture densities (nearest trace) increasing flow rate (left to right) has little effect. At 10fM and high capture densities (rear trace), increasing flow rate has a big effect. High flow limits are shown as dashed lines at the left.

B. Reaction-Rate-Limited Detection Kinetics

In this section the chemical kinetics in the reaction-rate-limited regime are modeled as a two time scale system. Linearizing the nonlinear system of differential equations describing the chemical dynamics of the system, the system is rewritten in terms of fast and slow dynamics. Then using Theorem 4.1, an equation describing the evolution of *dimer* concentration with time is derived. Therefore it is found that the *dimer* concentration evolves according to three modes depending on the analyte concentration. Keeping in mind that the channel conductance is directly proportional to the *dimer* concentration in the system, we arrive at (2), in section III-B, describing the evolution of the channel conductance as a function of time and analyte concentration.

As explained in the previous section, in the reaction-rate-limited regime, ($A^* > 1\mu\text{M}$), analyte concentration is constant. We will denote the constant analyte concentration as A^* .

Defining $u = \{B, C, D, S, W, X, Y, Z\}^T$, and $r(u(t)) = \{R_1, R_2, R_3, R_4, R_5, R_6, R_7\}^T$, where T denotes transpose and R_i are defined in (4), we can write the nonlinear differential equation describing the evolution of the chemical species as;

$$\frac{d}{dt}(u) = Mr(u(t)) \quad (5)$$

where M is a matrix defined by;

$$M = \begin{pmatrix} -1 & 0 & 0 & -1 & 0 & 0 & 0 \\ 0 & -1 & -1 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 0 & -1 & 0 & -1 \\ 1 & 0 & -1 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & -1 & 0 & 0 & -1 \\ 0 & 0 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 & 1 \end{pmatrix}$$

Clearly the system of differential equations given by (5,4), is solveable numerically. However, in order to make further design and statistical signal analysis of the system possible, analytical equations describing the evolution of the system state are required. In this section we will try to analyze the system of differential equations describing the chemical kinetics of the biosensor, in order to arrive at these analytical equations.

Analyzing the linearized version of (5) and estimating the eigenvalues with the typical parameter values for the biosensor given in table IV and table III, it is found that $|\lambda_{7,8}| \gg |\lambda_{1,2,3,4,5,6}|$. Therefore the eigenvalues associated with the species Y and Z are larger in absolute value and it can be concluded that these species decay at a rate much faster than the rest and thus it is reasonable to place these species in the “fast” group and the rest in the “slow” group.

Define, $\beta = \{Y, Z\}$, and $\alpha = \{B, C, D, S, W, X\}$ and let $g(\alpha, \beta)$ denote the vector field of the fast variables and $f(\alpha, \beta)$ the vector field of slow variables. Equations (4),(5) can be expressed as a two-time scale system;

$$\dot{\alpha} = f(\alpha, \beta) \quad (6)$$

$$\epsilon \dot{\beta} = g(\alpha, \beta) \quad (7)$$

Here $\epsilon > 0$ and as indicated in [20] is chosen to be the smallest time constant of the governing differential equations, therefore $\epsilon \approx \frac{1}{|\lambda_7|} = 10^{-2}$.

The following theorem uses basic singular perturbation theory, specifically Tikhonov’s theorem, [20, Sec.11.1], as well as the approximate relation, $S \approx S(0)$, to simplify the above two-time scale nonlinear system. The resulting simplified system yields the evolution of the admittance of the biosensor versus analyte concentration according to the two modes described in Section III-B, namely linear, and exponential decay.

Theorem 4.1: Consider the chemical species dynamics depicted by the two time scale system (6),(7). Then as $\epsilon \rightarrow 0$, the fast time-scale variables arrive at the quasi-steady state and the dimer concentration, $D(t)$,

converges to the trajectory of the following system:

$$\frac{d}{dt}(D(t)) = -D(r_5 + f_6 A^*) + \left(f_5 C + \frac{r_6 f_7 X}{r_6 + r_7} \right) S(0) \quad (8)$$

Specifically the trajectories that start in an $O(\epsilon)$ neighborhood of $\beta = h(\alpha)$, where $h(\alpha)$ denotes the solution of the algebraic equation $g(\alpha, \beta) = 0$ satisfy;

$$|D(t) - \bar{D}(t)| = O(\epsilon), \quad |z(t) - \bar{z}| = O(\epsilon) \quad (9)$$

for all $t \in [0, T]$ where T denotes a finite time horizon.

Eq. 8 can be solved using Euler's method with step size h to arrive at;

$$D_{n+1} = (1 - (r_5 + f_6 A^*)h) D_n + h \left(S(0) \left(\frac{f_5 C(r_6 + r_7) + r_6 f_7 X}{r_6 + r_7} \right) \right) \quad (10)$$

The proof of Theorem 2.1 is in the appendix.

Therefore the *dimer* concentration of the biosensor, D , evolves according to one of the following three modes, depending on the concentration of the analyte, A^* ;

$$D_{n+1} = f^{\mathcal{M}}(D_n, A^*) + w_n, \quad (11)$$

$$\mathcal{M} = \begin{cases} 1 & A^* = 0 : f^{\mathcal{M}}(D_n, A^*) = D_n \\ 2 & A^* \text{ medium} : f^{\mathcal{M}}(D_n, A^*) = \kappa_1 D_n + \kappa_2 \\ 3 & A^* \text{ high} : f^{\mathcal{M}}(D_n, A^*) = \kappa_3 D_n + \kappa_4 \end{cases}$$

where w_n is as defined in (2) and the constants κ_i for $i \in \{1, 2, 3, 4\}$ are calculated from the following equations using medium A^* for κ_1 and high A^* for κ_3 ;

$$\kappa_{1,3} = (1 - (r_5 + f_6 A^*)h),$$

$$\kappa_{2,4} = h \left(S(0) \left(\frac{f_5 C(r_6 + r_7) + r_6 f_7 X}{r_6 + r_7} \right) \right) \quad (12)$$

Note that the *dimer* concentration evolves according to three modes defined in (11), depending on the analyte concentration A^* which is constant in the reaction-rate-limited regime of operation. Also recall that the biosensor is in the reaction-rate-limited regime of operation when $A^* > 1\mu\text{M}$.

Using (11) and noting that the channel conductance is directly proportional to *dimer* concentration, we can derive equations explaining the evolution of the channel conductance on the slow time scale.

C. Mass Transport Influenced Detection Kinetics

As explained in Section IV-A, when the ratio of analyte concentration to binding sites is small (e.g. 10^{-11} moles per 10^{10} binding sites per cm^2) then mass

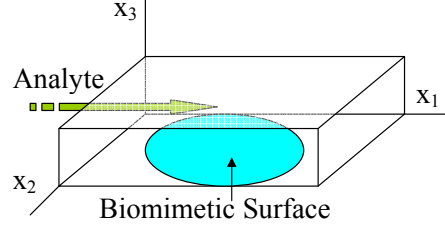


Fig. 16. A schematic of the flow chamber. The reactive surface is located at $x_3 = 0$. The solution containing the analyte molecules enters at $x_1 = 0$ and flows along the x_1 -axis

transport effects in the ICS biosensor become dominant. In this section the partial differential equation governing the mass transport of analyte molecules is derived. In the mass transport influenced region of operation, the analyte concentration is no longer a constant A^* but is a function of time and space, $A(x_1, x_2, x_3, t)$.

Analyte is transported to the reacting surface of the ICS biosensor, by diffusion and flow, where it reacts with the immobilized receptors. The flow chamber has a rectangular cross section. A schematic of the flow chamber is shown in Fig. 16, where the height of the chamber, along the z -axis, is $h = 0.1\text{mm}$, the length of the chamber along the x -axis is $L = 6\text{mm}$ and the width of the chamber along the y -axis is $W = 2\text{mm}$.

In [3] it is shown that for aspect ratio $h/W = 0.1$ and in general small, the variations along the width of a flow chamber are not noticeable. In the case of this biosensor with flow chamber geometry as denoted in Fig. 16, the aspect ratio is 0.05, hence we can ignore the variations along the y -axis and the analyte concentration is $A(x_1, x_3, t)$. In the flow chamber the analyte concentration $A(x_1, x_3, t)$ is governed by the following reaction-diffusion partial differential equation [10]

$$\frac{\partial A}{\partial t} = \gamma \left(\frac{\partial^2 A}{\partial x_1^2} + \frac{\partial^2 A}{\partial x_3^2} \right) + v \frac{\partial A}{\partial x_1} \quad (13)$$

Here γ is the diffusivity constant of the analyte (e.g., $\gamma \approx 10^{-6}\text{cm}^2\text{s}^{-1}$ for streptavidin or hCG). There are four boundary conditions that need to be considered:

(i) The chamber boundary at $x_3 = h$ is reflective and the mass flux must equal zero. This results in the boundary condition:

$$\frac{\partial A}{\partial x_3} \Big|_{(x_1, x_3=h, t)} = 0 \quad (14)$$

(ii) At the biomimetic surface $x_3 = 0$, the mass flux must equal the time rate of change of the concentration

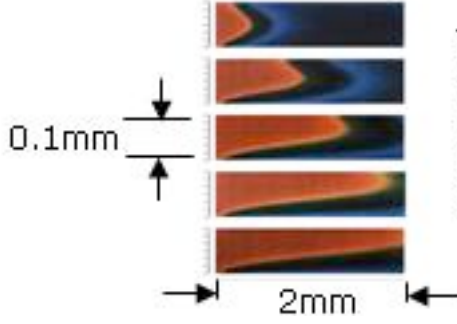


Fig. 17. Finite element solution of flow snapped at five time intervals.

of the species that combine with a . This results in the boundary condition

$$\frac{\partial A}{\partial x_3} \Big|_{(x_1, x_3=0, t)} = \frac{\partial X}{\partial t} + \frac{\partial W}{\partial t} \quad (15)$$

where, $\frac{\partial X}{\partial t}$ and $\frac{\partial W}{\partial t}$ are nonlinear functions defined in (5).

(iii) At the entry to the flow cell $x_1 = 0$, the analyte concentration is equal to the injection concentration, $\bar{A}$ and at $x_1 = L$, where the analyte exits, the mass flux is zero. That yields the third and fourth boundary conditions

$$A(x_1 = 0, x_3, t) = \bar{A}, \quad \frac{\partial A}{\partial x_1} \Big|_{(x_1=L, x_3, t)} = 0 \quad (16)$$

The analyte concentration $A(x_1, x_3, t)$ is therefore found by solving (13) subject to boundary conditions (14-16).

To simulate mass transport effects, we solved (13) subject to boundary conditions (14-16) via the finite element method on the rectangular flow cell shown in Fig.16. Fig.17 shows a typical solution obtained from the finite element analysis. A bolus of sample flowing left to right, shown at five time intervals. The bottom of the flow cell has a high capture density of receptors. Whereas at the top surface the sample is retarded by the usual flow hydrodynamics at a wall. At the bottom surface sample is depleted by binding to the capture sites delaying the passage of the sample.

Fig.18(a) shows the increase in resistance of the channel (decrease in channel conductance) as the protein streptavidin is captured on the membrane for a sample flow of $100\mu\text{L}/\text{min}$. It is significant that when the flow is stopped (shown by arrows in the figure), the resistance change ceases. This arises from flow dependent analyte depletion at the electrode surface. Fig. 18(b) shows the superposition of the resistance increase resulting from a challenge of 10fM streptavidin at various flow rates from $0\mu\text{L}/\text{min}$ to $100\mu\text{L}/\text{min}$. Also shown is the predicted

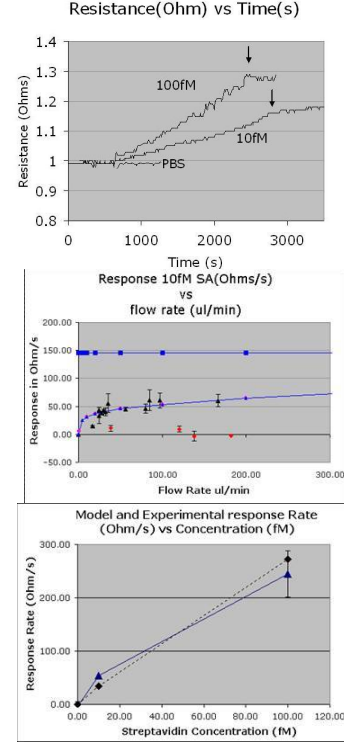


Fig. 18. (a) Response of ICS sensor for the concentrations shown and $100\mu\text{L}/\text{min}$ flow rate. Note that when the flow stops the response stops at all concentrations. This is due to analyte depletion at the sensor surface. (b) Dependency of response of sensor to flow rate (black dots). The lines (purple) through the data points are predicted by the model. The straight line at 150 is the high flow limit. The red diamonds are PBS controls. (c) Predicted and experimental titration curves for the ICS sensor response to Streptavidin in the range 1fM - 100fM at $150\mu\text{L}/\text{min}$. The triangles are experimental data and the diamonds are from the model.

high flow resistance change which is three times the magnitude of change experienced at even $100\mu\text{L}/\text{min}$. This difference shows the utility of sample flow when operating at high capture densities and low analyte concentrations. Fig. 18(c) shows a superposition of the predictive and experimental titration curve in the range of 0fM to 100fM streptavidin.

V. STATISTICAL SIGNAL PROCESSING WITH BIOSENSOR

With the construction and modeling of the ICS biosensor described above, our goal in this section is to describe how the measurements from the biosensor can be used to detect the presence and concentration of analytes. Based on the size of the sensing electrodes, there are two possible cases:

a) *Medium-sized electrodes*: The current generation ICS biosensor has electrode sizes of 1 mm radius. Thus the sensor response is dependent on the net current changes from millions of individual gramicidin channels. The measured current is in the order of micro amperes. The measurement noise is insignificant (apart from a slow baseline drift). So for the ICS biosensor, the concentration of the analyte can be determined straightforwardly from the three dynamic modes described in (2). In future generation ICS biosensors, the electrodes will be miniaturized. Electrode sizes of $10\ \mu\text{m}$ radius comprise of a few hundred gramicidin channels. By central limit theorem arguments, the net current can be approximated by a Gaussian probability distribution. In this case, the measurement noise can not be ignored. Because of the linear Gaussian dynamics of the underlying current, a Kalman filter based algorithm [1] can be derived to classify the concentration of the analyte in the system as medium, high or null.

b) *Micro-electrodes*: Electrode sizes of radius $1\ \mu\text{m}$ comprise of only a few ion channels. In this case, the current pulses from individual channels can be resolved and the biosensor records a finite state “digital” output. The arrival of individual analyte molecules can then be detected at individual electrodes. This allows for exploitation of the analyte flow equations in Section IV-C for measurements at multiple electrodes thereby resulting in enhanced sensitivity. The biosensor output can be modeled as a noisy finite state Markov chain, i.e., a Hidden Markov Model [11]. The noise levels are substantial in this case and careful modeling of the noise distribution is required.

Construction of ICS biosensors with such small electrodes is the subject of our current research. Here we provide a simple proof-of-concept of the signal processing capabilities by using a simpler biosensor setup, see [21] for experimental details. We use a covalently linked dimer of gramicidin ion channels (called bis-gramicidin A) incorporated into an untethered bilayer membrane excised from a giant lipid vesicle seen in Figure 19. The bilayer is supported over the $1\ \mu\text{m}$ diameter opening of a micropipette as shown Figure 20. For illustrative purposes we used a low molecular weight analyte methylbenzthonium chloride (MBC) shown in Figure 21 that blocks conduction of the gramicidin channel.

The construction of the HMM detector for MBC was done as follows. First maximum likelihood model parameters of the transition probability, state levels of the Markov chain and parameters of noise distribution are estimated from the data. This is done in the presence or

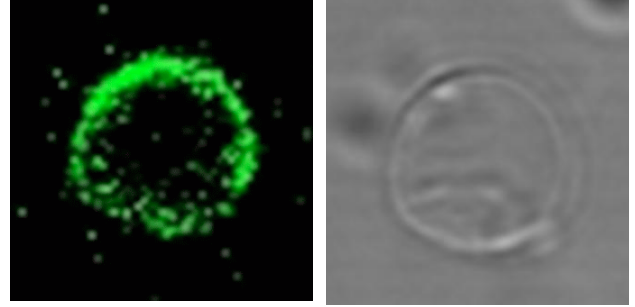


Fig. 19. Fluorescence image (left) of biosensor’s horizontal optical section shows the bis-gramicidin A channels labelled using FITC and identified by the green color. Phase-contrast image (right) of the same horizontal slice shows the overall shape of the biosensor

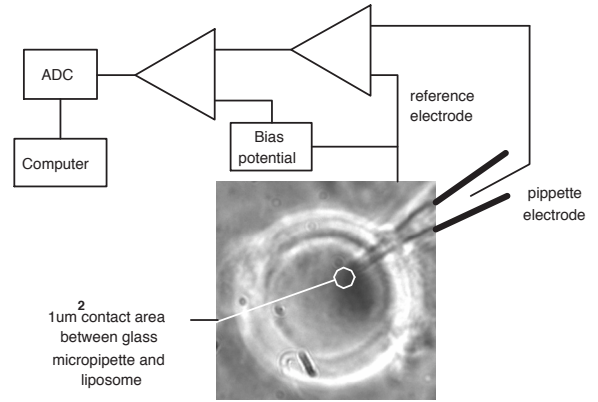


Fig. 20. Photo of glass micropipette and liposome, with block diagram of the electrical detection scheme. The solution in the recording pipette was $0.5\ \text{M KCl}$ with the liposomes suspended in a $0.5\ \text{M NaCl}$ solution. The conductance of bis-gramicidin channels under these conditions is approximately $20\ \text{pS}$.

absence of the channel blocker MBC. The detection of the channel blocker is now reduced to a classification problem. Modeling the noise was a challenging task. This arises from thermal noise, the anti-aliasing effect from sampling and an open channel noise with its power proportional to the inverse of frequency. Figure 22 shows the power spectral density of a typical sequence of biosensor recordings and clearly shows that the power decreases at a rate of $-10\ \text{dB/dec}$ at low frequencies indicating the presence of $1/f$ noise. This $1/f$ noise is discussed in other studies of bis-gramicidin A ion channels, see [39]. To model this correlated noise process, we used an auto-regressive (AR) Gaussian process that comprises white Gaussian noise process W_k filtered by an all-pole filter, see [21] for the Ljung-Box test used as a portmanteau lack of fit test for model adequacy.

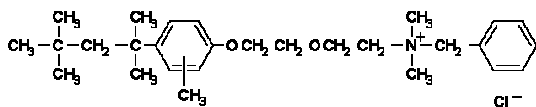


Fig. 21. Chemical Structure of Methylbenzthonium Chloride (MBC)

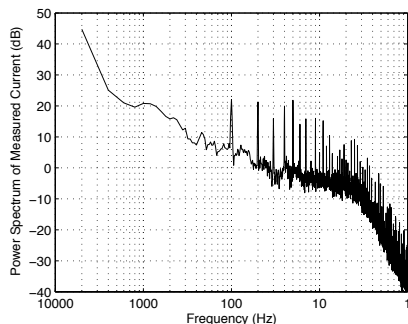


Fig. 22. Power spectral density of biosensor response clearly shows the $1/f$ open channel noise and the anti-aliasing effect in the samples.

Figure 23(a) shows the output of the biosensor before and after the analyte MBC is added. A Hidden Markov Model (HMM) maximum likelihood classifier is then used to detect the presence of the target molecules, see Figure 23(b). While it is difficult to identify the transition in the recordings in 23(a), the detection algorithm detected a model change shortly after MBC was added as shown in Figure 23(b). The detection trace in Figure 23(b) indicates a switch in the most likely model from θ_1 (no MBC present) to θ_2 (MBC present) at $k = 59.865$ seconds, approximately 0.2 seconds after MBC is added. We refer the reader to [21] for a complete description.

Remark: The current pulses arising from these channels are a consequence of a different mechanism to that of monomeric gramicidin diffusing in the inner and outer bilayer membrane of the ICS biosensor. Current fluctuations have been reported by [?] for bis-gramicidin. These were thought to arise from conformational inter-conversion in the bis-gramicidin A secondary structure. The mechanism generating the current fluctuations is unimportant for our description here.

VI. CONCLUSIONS AND EXTENSIONS

This paper began with a description of construction and operation of an ion channel switch (ICS) biosensor. The electrical dynamics of the biosensor were modeled by a second order linear system. Furthermore, the chemical kinetics of the biosensor were modeled as a two-timescale nonlinear dynamical system in case of

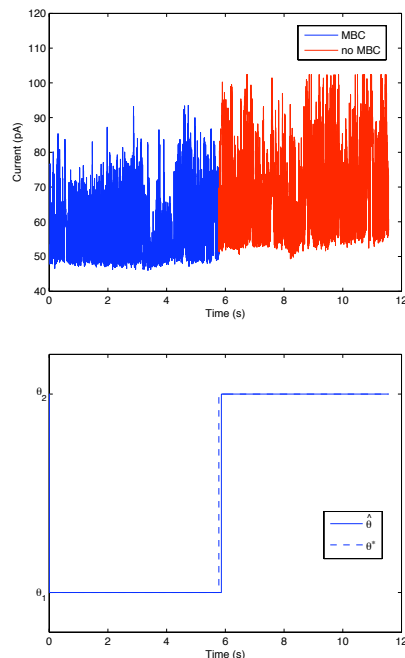


Fig. 23. (a) Current pulse recordings on an expanded scale before (blue) and after (red) channel blocker MBC. (b) Maximum likelihood detection generated using Hidden Markov Model estimation algorithm. While it is difficult to identify the transition by eyeballing the recordings in Figure 23(a), the detection algorithm detected a model change shortly after MBC was added.

reaction-rate limited dynamics. Using Singular Perturbation theory, an analytical equation expressing the evolution of the *dimer* concentration with time as a function of analyte concentration was derived. A partial differential equation describing the evolution of the analyte concentration in time and space in case of mass transport influenced chemical kinetics was derived. Finally, for the case of micro-sized electrodes, we described how statistical signal processing algorithms can be derived for detecting the presence of analyte and classifying the analyte concentration.

There are several extensions that we are currently considering. When employing antibodies or other well defined receptors much of the sensitivity advantages of stochastic sensing, occurs when using stochastic detection in conjunction with the spatial analysis across an electrode arrays. Exploiting the arrival statistics of a target molecule on the array, in addition to the stochastic ion current properties within each element permits a rapid estimate of the spontaneous concentration. When using a large number of channels the benefit of measurement redundancy in an array is the classical $\sqrt{N}$, where

N is the number of independently read electrodes in the array. It is worthwhile examining if the dynamics of the fluid flow (given by a partial differential equation) of the analyte can be exploited to devise a more efficient detector for the analyte and estimator for its concentration.

APPENDIX

A. Singular Perturbation Modeling of the Biosensor

The presence of “fast” and “slow” species in the nonlinear set of equations describing the evolution of the chemical species in the biosensor system, depicted in (5), allows us to rewrite the system dynamics in a multi-time scale setting as done in Section IV-B and denoted in (6,7).

Setting $\epsilon = 0$ in (7), we can solve for the quasi steady state as follows;

$$g(\alpha, \beta) = 0 \Rightarrow \beta = h(\alpha) = \left(\frac{f_4 X B + f_3 W C}{f_7 X \bar{S} + f_6 A^* D} \right)$$

Replacing β in (6) by $h(\alpha)$, we arrive at the reduced model;

$$\dot{\alpha} = f(\alpha, h(\alpha)) \quad (17)$$

Let $\bar{\alpha}$ denote the solution to (17), and define $y = \beta - h(\alpha)$. Then letting $dy/d\tau = \epsilon dy/dt$ and noting that setting $\epsilon = 0$ freezes the α variables at t_0 which has now been shifted to the origin we arrive at the boundary-layer model which as explained in detail in [20] is a shifted version of the quasi-steady-state solution of the fast dynamics $h(\alpha)$;

$$\begin{aligned} \frac{d}{d\tau}(y) &= g(\alpha(t_0), y + h(\alpha(t_0))) \\ &= \begin{pmatrix} 0 & -(r_3 + r_4) \\ -(r_6 + r_7) & 0 \end{pmatrix} \begin{pmatrix} y_1 \\ y_2 \end{pmatrix} \end{aligned} \quad (18)$$

If the conditions of Theorem 11.1 in [20, Sec.11.1] are satisfied, we can conclude the proof of Theorem 4.1 in Section IV. Next we will show that these conditions are in fact satisfied for the biosensor model.

- In (6),(7) the functions f, g , their first partial derivatives with respect to (α, β) and $\partial g/\partial t$ are continuous; the function $h(\alpha)$, and the Jacobian $[\partial g(\alpha, \beta)/\partial(\beta)]$ have continuous first partial derivatives with respect to their arguments.
- The component functions of the reduced model in (17) and their partial derivatives with respect to the components of the functions are each continuous on $\mathbb{R}$, therefore the component functions are each locally Lipschitz in α on $\mathbb{R}$. Thus the vector function

$f(\alpha, h(\alpha))$ is Lipschitz continuous in α on $\mathbb{R}$, and has a unique solution $\bar{\alpha}$ on $\mathbb{R}$.

- The origin is an exponentially stable equilibrium point of the boundary-layer model. To analyze the stability of the origin for the boundary-layer model we look at the eigenvalues of the matrix defined in (18). The eigenvalues are found to be $\lambda_1 = -0.011$ and $\lambda_2 = -0.002$. Therefore the origin is an exponentially stable equilibrium point of the boundary-layer model.

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