Ion Channel Biosensors—Part II: Dynamic Modeling, Analysis, and Statistical Signal Processing

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Abstract—This paper deals with the dynamic modeling, analysis, and statistical signal processing of the ion channel switch biosensor. The electrical dynamics are described by a second-order linear system. The chemical kinetics of the biosensor response to analyte concentration in the reaction-rate-limited regime are modeled by a two-timescale 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 mixture of Neumann and Dirichlet boundary conditions. By using the theory of singular perturbation, we analyze the model so as to 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.

Index Terms

I. INTRODUCTION

THE COMPANION paper (Part I) described the construction and experimental studies of the ion channel switch (ICS) biosensor. The ICS biosensor provides an interesting example of engineering at the nanoscale [1]. Its functionality 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 min. Our modeling and analysis in this paper will capture these salient features. Because the biosensor can detect analyte concentrations smaller than 1 picomolar, mass transfer of analyte over the electrodes becomes the dominant design criterion. This requires careful modeling of the chemical kinetics (how the analyte molecules interact with the binding sites), together with the mass transport dynamics of fluid flow (how analyte molecules flow onto the electrodes). Finally, the intrinsically digital output from the biosensor permits the use of sophisticated statistical signal processing algorithms to estimate the type and concentration of analyte.

A. Main Results

The following are our main results.

1) Modeling of Biosensor Electrical Response and Chemical Kinetics: In Section II, we give a complete model description of the electrical response and chemical kinetics of the biosensor. The electrical dynamics of the ICS biosensor are described by an equivalent second-order linear system. The chemical kinetics detail how the biosensor responds to analyte molecules—from analyte molecules binding to the receptors to the eventual disruption of the ability of gramicidin molecules to form dimers. Thus, we convert the qualitative description of the biosensor operation given in the companion paper into mathematical equations for a dynamical system.

2) Analyte Flow and Analysis of Biosensor Dynamics: In Section III, we analyze the biosensor dynamics. We show via eigen decomposition that the biosensor response to analyte concentration has a two-timescale behavior. This permits analysis of the chemical kinetics as a singularly perturbed system [2], [3]. One of the highlights of this analysis is that it mathematically justifies the experimentally observed response of the biosensor to analyte concentration. Another highlight of our modeling and analysis is that we can predict the biosensor performance at very low analyte concentrations (e.g., picomolar to femtomolar). In such cases, it is necessary to consider the analyte flow and its interaction with the receptors at the biosensor electrode. We model the analyte flow as a diffusion-type partial differential equation, which interacts with the chemical kinetics when analyte molecules interact with the biosensor. This results in a two-timescale behavior. This permits analysis of the chemical kinetics as a singularly perturbed system [2], [3]. One of the highlights of this analysis is that it mathematically justifies the experimentally observed response of the biosensor to analyte concentration. Another highlight of our modeling and analysis is that we can predict the biosensor performance at very low analyte concentrations (e.g., picomolar to femtomolar). In such cases, it is necessary to consider the analyte flow and its interaction with the receptors at the biosensor electrode.

3) Statistical Signal Processing: A further goal of detailed modeling and analysis of the biosensor is to design sophisticated statistical signal processing algorithms that exploit these model dynamics to classify analytes and estimate their concentrations.

In Section IV, we illustrate how statistical signal processing algorithms can be used to detect the presence of analyte.

B. Related Work

The companion paper provided a literature review of biosensors involving ion channels and tethered lipid membranes. Here, we outline related work in modeling, analysis, and statistical signal processing involving biosensors. The equivalent electrical model we introduce in Section II-A for the lipid membrane, interfacial capacitance, and electrolyte resistance is similar to that used in electrophysiological models of cell membranes, see [7] for a textbook treatment. The conceptual idea behind electrophysiological models originates from the work of Cole, who pioneered the notion that cell membranes could be likened to an electronic circuit [8]. The chemical kinetics discussed in Section II-B result in a system of nonlinear ordinary differential equations. The work of Lauffenburger and...
Linderman [9] is an excellent example of such chemical kinetics and binding. Similar models have been adopted in a lateral flow bioreactor in [10]. The singular perturbation methods we use in Section III-A are well known in nonlinear systems theory, see [2]. More sophisticated stochastic singular perturbation methods are studied in [11]. The mass transport dynamics (partial differential equation) coupled with chemical kinetics discussed in Section III-B results in a diffusion partial differential equation with Neumann and Dirchlet boundary conditions [4]–[6]. Similar formulations for binding and dissociation between a soluble analyte and an immobilized ligand are studied in [12]. Mass transport dynamics are formulated in [4] and [13] for a two-compartment model where analyte molecules move between the two compartments. Goldstein et al. [6] discuss the accuracy and theoretical basis of different models for mass transport effects in the binding of analytes. Dehghan [14] discusses several different finite-difference methods to solve the advection–diffusion equation and the stability of the numerical methods. Finally, the statistical signal processing of ion channel currents (Section IV) is an active area of research with several papers published since the 1990s. Hidden Markov models (HMMs) have been widely used, see [15] and [16] and references therein.

II. MODELING THE DYNAMICS OF THE ICS BIOSensor

This section constructs mathematical models for the ICS biosensor’s response to analyte molecules. In Section II-A and II-B, white-box models for the electrical response and chemical kinetics are formulated. Finally, a black-box model for the biosensor response based on experimental observations is given in Section II-C. This sets the stage for Section III where the white-box models are analyzed, resulting in a mathematical justification of the black-box model.

A. Electrical Dynamics of the Biosensor

The ICS biosensor can be viewed as a biological transistor. Fig. 1(a) illustrates the equivalent circuit of the biosensor before and after the detection of analyte. Fig. 1(b) details the components of the equivalent circuit. The resistor $1/G$ 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 in solution, while 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 (which, as with biological systems, operates on ion flow) and the electron flow of 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 return path in the bathing sample solution. In a flow chamber, the dimensions of the return path can be sufficiently small so that $R_2$ becomes significant. The values of $C_1$, $C_2$, $R_1$, and $R_2$ are functions of electrode area. Typical values for 2009 generation of ICS biosensors are listed in Table I.

Let $V_{out}$ denote the external applied potential and $i$ denote the output current as depicted in Fig. 1(b). With $V_{out}(s)$ and $I(s)$ denoting the Laplace transforms, the admittance transfer function of the equivalent circuit parameterized by $G$ is

$$H(s) = \frac{I(s)}{V_{out}(s)} = \frac{s^2 + s a G}{s^2 R_2 + s (b_2 + b_1 G) + b_2 G}.$$  \hspace{1cm} (1)

The constants in $H(s)$ are $a = 1/C_1$, $b_1 = R_2/C_1$, $b_2 = 1/C_1 + 1/C_2$, and $b_3 = 1/C_2 C_2$.

Electrodes in the 2009 generation ICS biosensors have an area of 0.03 cm$^2$. The resistance of the biosensor when no analyte is present is approximately 60 kΩ. This can be reconciled with the 10$^{11}$ Ω per channel resistance of gramicidin A as follows. Since there are 10$^5$ gramicidin channels per centimeter square, each electrode of area 0.03 cm$^2$ contains approximately 3 × 10$^6$ channels with approximately half of them dimerized. So, the

![Figure 1](image.png)

**Fig. 1.** ICS biosensor comprises an ion channel switch. (a) (Top) Switched-on state when the ion channels are conducting and (bottom) switched-off state when the ion channels are not conducting. The equivalent electrical circuit shown in Fig. 1(b) results in a second-order transfer function.

**TABLE I**

<table>
<thead>
<tr>
<th>Element</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane Capacitance $C_1$</td>
<td>0.5μF/cm$^2$</td>
</tr>
<tr>
<td>Interfacial Capacitance $C_2$</td>
<td>3.5μF/cm$^2$</td>
</tr>
<tr>
<td>Biosensor Resistance $R_1$</td>
<td>$144$ kΩ–$600$ kΩ</td>
</tr>
<tr>
<td>Electrolyte Resistance $R_2$</td>
<td>$200$ Ω</td>
</tr>
</tbody>
</table>

The area of the electrode is 0.03 cm$^2$. The resistance of the biosensor when no analyte is present is approximately 60 kΩ. This can be reconciled with the 10$^{11}$ Ω per channel resistance of gramicidin A as follows. Since there are 10$^5$ gramicidin channels per centimeter square, each electrode of area 0.03 cm$^2$ contains approximately 3 × 10$^6$ channels with approximately half of them dimerized. So, the...
effective resistance of all the dimerized ion channels (which act as parallel resistors) is approximately 60 kΩ. The measured current is the average effect of the formation and dissociation of thousands of dimers, and is approximately continuous valued.

B. Chemical Kinetics of the Biosensor

This section formulates the dynamics of the chemical reactions in the biosensor with the goal of modeling how the biosensor conductance $G$ in (1) evolves. Recall that $G$ decreases with time if analyte molecules are present due to chemical reactions that inhibit the formation of gramicidin dimers.

The reactions involved in the ICS biosensor stem from the binding of analyte molecules to the binding sites on the membrane followed by cross linking of the mobile ion channels to these bound analytes. The species involved in these reactions are separated into primary species and complexes. The primary species are analyte $A$ with concentration $x$, binding sites $B$, free moving monomeric ion channels $c$ with concentration $C$, and tethered monomeric ion channels $s$ with concentration $S$. The complexes denoted as $d$, $w$, $x$, $y$, and $z$ with concentrations, $D$, $W$, $X$, $Y$, and $Z$ are formed according to the following chemical reactions:

$$
a + b \rightleftharpoons f_{11} a + c \rightleftharpoons f_{12} w \quad w + c \rightleftharpoons f_{13} y \quad x + b \rightleftharpoons f_{21} x + c \rightleftharpoons f_{22} d \quad a + d \rightleftharpoons f_{31} z \quad x + s \rightleftharpoons f_{32} z. \tag{2}
$$

In (2), $f_i$ and $r_i$, for $i = \{1, 2, 3, 4, 5, 6, 7\}$, respectively, denote the forward and backward reaction rate constants. For reactions occurring in 3-D space, such as binding of analyte with binding sites, the forward reaction rate constants $f_i$ have units of $M^{-1} \cdot s^{-1}$ (M denotes molar concentration, i.e., moles per liter). For reactions occurring in 2-D space, such as dimerization of the ion channel, $f_i$, have units of centimeter square-per second per molecule. The backward reaction rate constants $r_i$ have units of per seconds.

The chemical reactions in (2) give a complete symbolic description of the operation of the ICS biosensor that was qualitatively described in Section II of the companion paper. The forward part of the first equation reports on an analyte molecule $a$ being captured by a binding site $b$ and the resulting complex $w$ is denoted by $w$. The third equation states that a free moving gramicidin monomer $c$ in the outer leaflet of the bilayer lipid membrane (BLM) can bind to the complex $w$, thus producing another complex, denoted by $y$. An analyte molecule can also be captured by the binding site linked to the freely diffusing monomer $c$. The second equation states that this results in the production of the complex $x$. The complex $x$ can still diffuse on the outer leaflet of the BLM, and hence can move toward a tethered binding site $b$ and bind to it, resulting in the complex $y$ (fourth equation). On the other hand, the complex $x$ can diffuse on top of the tethered ion channel monomer $s$, which results in the production of complex $z$ (seventh equation). The event that determines the biosensor conductance (and thus the current flowing through the biosensor) is the binding of the free moving ion channel monomer $c$ and the tethered ion channel monomer $s$. This results in the formation of a dimer $d$ (fifth equation).

Indeed, the biosensor conductance is proportional to the dimer concentration, i.e., $G(t) = constant \times D(t)$. Finally, an analyte molecule can also bind to an already formed dimer, which again produces the complex $z$ (sixth equation).

We are now ready to formulate the chemical kinetics of the ICS biosensor. The total reaction rates are straightforwardly obtained from (2) as

$$
R_1 = f_1 AB - r_1 W \quad R_2 = f_2 AC - r_2 X
R_3 = f_3 WC - r_3 Y \quad R_4 = f_4 XB - r_4 Y
R_5 = f_5 CS - r_5 D \quad R_6 = f_6 AD - r_6 Z
R_7 = f_7 XS - r_7 Z. \tag{3}
$$

Define $u = \{B, C, D, S, W, X, Y, Z\}^T$ 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 (3). Then the nonlinear ordinary differential equation describing the dynamics of the chemical species is

$$
\frac{du}{dt} = Mr(u(t)) \tag{4}
$$

where $M =$

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

The initial concentrations of the primary species in $u(0)$, namely, $A^*, B(0), C(0), S(0)$, and $D(0)$, are given in Table II. The initial concentrations of the secondary species are zero. Note that in Table II, $S(0)$ denotes the initial concentration of the tethered gramicidin monomers. Also, $S \approx S(0)$ during the experiment.

The previous system of equations is obtained as follows. Consider, for example, the primary species $b$ in (2). According to the first and fourth equations in (2), $b$ is consumed when it binds to $a$ and $x$, and is produced when $w$ and $y$ decompose. So the change in the concentration of $b$ can be expressed as

$$dB/dt = -R_1 - R_4. \tag{22}
$$

This yields the first equation in the system (4). The evolution of other species is derived similarly, yielding (4).

Equation (4) is a complete representation of the chemical kinetics of the biosensor. Recall the biosensor conductance $G(t) = constant \times D(t)$, when $D(t)$ denotes the dimer concentration.
concentration as a function of time and is obtained by solving (4). However, note that (4) does not model the dynamics of the analyte concentration \(A\). The dynamics of \(A(x_1, x_2, x_3, t)\) over space \((x_1, x_2, x_3)\) and time \(t\) are given by the mass transport partial differential equation, see Section III-B.

Example: Table III gives the typical forward and backward reaction rate constants \((f_i, r_i)\) for two important examples. The first example deals with protein–receptor interaction such as the detection of the protein streptavidin by using biotin as the binding site. The second example in Table III deals with antigen–antibody interaction such as the detection of the pregnancy hormone [human chorionic gonadotropin (hCG)] by using the antibody immunoglobulin \(G\) (IgG) as the binding sites of the biosensor. We will use these values in Section III-A when identifying the fast and slow dynamics of (4).

C. Black-Box Model for Biosensor Response to Analyte

The final step in this modeling section is to describe the input/output behavior of the biosensor. This can be viewed as a "black-box" model in comparison to the previous two sections where physical/chemical laws were used to construct a "white-box" model. Let \(n = 0, 1, \ldots\) denote discrete time (with typical sampling interval of 1 s) and \(A\) denote the concentration of analyte. As described earlier, the presence of analyte results in a decrease of the biosensor conductance \(G\) in (1). Detailed experimental analysis of the biosensor response show that \(G\) evolves in discrete time according to one of the three different concentration modes \(M\):

\[
G_{n+1} = f^M(G_n, A) + w_n
\]

\[
M = \begin{cases} 
1, & A \text{ is low: } f^1(G_n, A) = G_n + \kappa_0 \\
2, & A \text{ is medium: } f^2(G_n, A) = \kappa_1 G_n + \kappa_2 \\
3, & A \text{ is high: } f^3(G_n, A) = \kappa_3 G_n + \kappa_4 
\end{cases}
\]

where \(\kappa_0, \kappa_1, \kappa_2, \kappa_3, \) and \(\kappa_4\) are constants, with \(|\kappa_1|, |\kappa_3| < 1\) to ensure stability of the system (5). The variable \(w_n\) is a noise process that models our uncertainty in the evolution of \(G\). The function \(f^M\) models the fact that the biosensor conductance \(G_n\) decreases according to one of the three distinct modes depending on the analyte concentration \(A\). For low (or no) analyte present \((M = 1)\), the conductance decreases linearly. For medium and high concentrations \((M = 2, M = 3)\), the decrease in conductance is exponential with different decay rates. High analyte concentration refers to \((A \geq 10^{-6} \text{ M})\) and medium analyte concentration refers to \((A \geq 10^{-3} \text{ M})\).

III. Analyte Flow and Biosensor Dynamics

Having formulated models for the electrical response and chemical kinetics, we are now ready to analyze these models to predict the ICS biosensor’s response. We will determine how the chemical kinetics (4) interact with the partial differential equation of mass transport of analyte \(A(x_1, x_2, x_3, t)\). Here, \(x_1, x_2,\) and \(x_3\), respectively, denote the \(x, y,\) and \(z\) spatial axes, \(t\) denotes continuous time. Substantial insight is gained by considering the following two important subcases.

Case 1 (Reaction-rate-limited kinetics): In the reaction-rate-limited kinetics regime, large analyte flow rates, high analyte concentration, or low binding site densities (e.g., millileter per minute flow rate, micromolar concentration, or less than \(10^9\) binding sites per centimeter square) compensate for the depletion of analyte molecules due to rapid reaction at the biomimetic
surface populated by the binding sites. In this regime, it is reasonable to assume that the analyte concentration is approximately constant over space and time, i.e., \( A(x_1, x_2, x_3, t) = A^* \), where \( A^* \) denotes a constant analyte concentration. We consider this case in Section III-A, where we will mathematically justify the black-box model (5) by applying singular perturbation analysis to the white-box model developed earlier. We will derive the empirically observed black-box model (5).

Case 2 (Mass-transport-influenced kinetics): Here, the biosensor chemical kinetics are influenced by both mass transport and reaction rates. Therefore, the local concentration of analyte \( A(x_1, x_2, x_3, t) \) varies over space and time. In Section III-B, we model the change in analyte concentration over time and space by a boundary value partial differential equation.

Why do analyte mass transport kinetics matter? We start with the following motivating example. Fig. 3 shows quantitative predictions of the change in the biosensor resistance per unit time for various binding site densities and sample flow rates for high analyte concentrations (100 pM) and low analyte concentrations (10 fM). As shown in Fig. 3, at high analyte concentrations, the biosensor response is insensitive to flow rate. This corresponds to the reaction-rate-limited kinetics (case 1). However, for low analyte concentration and high binding site density, a high flow rate is required to achieve a measurable response. This corresponds to mass-transport-influenced kinetics (case 2), [19]. It is also apparent from Fig. 3 that a high binding site density is essential for high sensitivity. With high binding site density, target molecules collide more frequently with receptors, and are thus captured more quickly. The greater the ratio of binding site density to analyte concentration, the faster the response of the biosensor.

A. Case 1: Reaction-Rate-Limited Kinetics

In this section, the chemical kinetics in the reaction-rate-limited regime (constant analyte concentration \( A^* > 1 \mu M \)) are analyzed as a two-timescale dynamical system. We will use singular perturbation theory to approximate the time evolution of dimer concentration.

To determine the slow and fast modes of the nonlinear chemical kinetics depicted in (3) and (4), we compute the eigenvalues \( \lambda_i \) of the linearized version of (4). For typical parameter values of the ICS biosensor in Tables II and III, \( |\lambda_{7,8}| \gg |\lambda_{1,2,3,4,5,6}| \). Therefore, species \( Y \) and \( Z \) decay at a rate much faster than the other species. Accordingly define the fast species \( \beta = \{Y, Z\} \) and slow species \( \alpha = \{A, B, C, D, S, W, X\} \). Let \( g(\alpha, \beta) \) denote the vector field of the fast variables and \( f(\alpha, \beta) \) the vector field of the slow variables. Equations (3) and (4) can now be expressed as a two-timescale system [initial conditions \( \alpha(0) \) and \( \beta(0) \) are specified next (4)]

\[
\frac{d\alpha}{dt} = f(\alpha, \beta) \quad \epsilon \frac{d\beta}{dt} = g(\alpha, \beta) \quad (6)
\]

where \( \epsilon \approx 1/|\lambda_7| = 10^{-2} \) is chosen as the smallest time constant of the governing differential equations [2].

The following theorem uses basic singular perturbation theory, specifically Tikhonov’s theorem, [2, Sec. 11.1], as well as the approximate relation \( S \approx S(0) \) to simplify the aforementioned two-timescale nonlinear system. The resulting simplified system yields the evolution of biosensor conductance versus analyte concentration according to the modes described in Section II-C, namely, linear and exponential decays.

Theorem 3.1: Consider the chemical species dynamics depicted by the two-timescale system (6). Then, as \( \epsilon \to 0 \), the dimer concentration \( D(t) \) converges to the trajectory \( \bar{D}(t) \) defined by the following system:

\[
\frac{d\bar{D}}{dt} = -\bar{D}(r_5 + f_6 A^*) + (f_5 C + \frac{r_6 f_7 X}{r_6 + r_7}) S(0). \quad (7)
\]

More specifically, suppose the initial dimer concentration \( \bar{D}(0) \) at time \( t = 0 \) is within an \( O(\epsilon) \) neighborhood of \( \beta = h(\alpha) \), where \( h(\alpha) \) denotes the solution of the algebraic equation \( g(\alpha, \beta) = 0 \) in (6). Then, for all time \( t \in [0, T] \), \( |\bar{D}(t) - \bar{D}(t)| = O(\epsilon) \), where \( T > 0 \) denotes a finite time horizon.

Theorem 3.1 exploits the two-timescale nature of the chemical kinetics (6) to arrive at an approximate equation (7) for the dimer concentration \( D(t) \), which is within \( O(\epsilon) \) of the true solution. The proof follows from verifying the conditions of Theorem 11.1 in [2, Sec.11.1], and is omitted due to lack of
space. However, here is some intuition. It can be shown that as 
\( \epsilon \downarrow 0 \), the fast dynamics approach the quasi steady state \( h(\alpha) \) 
defined in the theorem. This quasi steady state \( h(\alpha) \) of the 
fast variables \( \beta \) is then substituted in the slow dynamics in 
(6), which results in the following approximate dynamics for 
the slow species: \( d\tilde{\alpha}/dt = f(\tilde{\alpha}, h(\tilde{\alpha})) \). We are interested in a 
specific component of \( \tilde{\alpha} \), namely, the approximate dimer concentration \( \tilde{D} \), which can be shown to evolve according to (7).

We can now analyze the response of the biosensor to differ-
ent analyte concentrations \( A^* \). Discretizing (7) using Euler’s 
method with step size \( h > 0 \) yields

\[
D_{n+1} = \left( 1 - \left( r_5 + f_6 A^* \right) h \right) D_n \\
+ h \left( S(0) \left( \frac{f_5 C (r_6 + r_7) + r_6 f_7 X}{r_6 + r_7} \right) \right). 
\]  
\( (8) \)

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

\[
\mathcal{M} = \begin{cases} 
1, & A^* \text{ is low: } f^1(D_n, A^*) = D_n + \kappa_0 \\
2, & A^* \text{ medium: } f^2(D_n, A^*) = \kappa_1 D_n + \kappa_2 \\
3, & A^* \text{ high: } f^3(D_n, A^*) = \kappa_3 D_n + \kappa_4 
\end{cases} 
\]  
\( (9) \)

where the noise \( w_n \) is defined similarly to (5). The constants \( \kappa_i \) 
for \( i \in \{1, 2, 3, 4\} \) are computed as

\[
\kappa_1 = \kappa_3 = (1 - (r_5 + f_6 A^*) h) \\
\kappa_2 = \kappa_4 = h \left( S(0) \left( \frac{f_5 C (r_6 + r_7) + r_6 f_7 X}{r_6 + r_7} \right) \right). 
\]  
\( (10) \)

Summary: We have shown that the dimer concentration 
evolves according to three distinct modes that depend on the 
analyte concentration. Using (9), since the conductance \( G \) 
is directly proportional to dimer concentration \( D \), we arrive at the 
black-box model (5) of Section II-C, which was based on ex-
perimental observations.

B. Case 2: Mass Transport Kinetics

Here, we consider the second subcase where the ratio of 
analyte concentration to binding site density is small [e.g., 
\( A/B = 1 \ \text{pM}/10^9 = 10^{-21} \), see (2)]. Then mass transport ef-
facts become the dominant criterion in achieving an acceptable 
response rate of the biosensor to analyte. The analyte concentra-
tion is no longer a constant \( A^* \), but a function of time and space 
\( A(x_1, x_2, x_3, t) \). In this section, the partial differential equation 
governing the mass transport of analyte molecules is derived.

Analyte is transported to the reacting surface of the ICS 
biosensor, by diffusion and flow, where it reacts with the immo-
bilized receptors. The flow chamber used for the biosensor has 
a rectangular cross section, and is illustrated in Fig. 4, where 
the height of the chamber along the \( x_3 \)-axis is \( h = 0.1 \text{ mm} \). The 
length of the chamber along the \( x_1 \)-axis is \( L = 6 \text{ mm} \) and the 
width of the chamber along the \( x_2 \)-axis is \( W = 2 \text{ mm} \).

It is shown in [20] that when the aspect ratio \( h/W \) is small 
(e.g., less than 0.1), the variations in analyte concentration along

the width of a flow chamber are negligible. In the case of ICS 
biosensor with flow chamber shown in Fig. 4, the aspect ratio is 
0.05. So we can ignore the variations along the \( x_2 \)-axis and the 
analyte concentration is equal to the injection concentration.

The solution containing the analyte enters at \( x_1 = 0 \) and flows along the \( x_1 \)-axis.

C. Model Evaluation

How good are the aforementioned models in predicting the 
ICS biosensor response? We conducted experiments on the ICS 
biosensor for detecting streptavidin at concentrations of 10 and 
100 fM, and 10 pM. Fig. 5 illustrates the highly sensitive re-
sponse of the biosensor and how this response depends on the
analyte flow rate. Fig. 5(a) shows the experimentally observed increase in biosensor resistance for flow rate $\nu = 150 \mu\text{L/min}$. It shows that as the analyte concentration is increased, the biosensor exhibits faster response. It is remarkable that the biosensor responds to concentrations as low as 10 fM. To validate this experimental response, several tests were conducted in which a bolus of streptavidin at 10 fM, 100 fM, or 10 pM was added to the feed line, requiring 20 min to reach the sensor. The line was operated at a constant flow rate of 150 $\mu\text{L/min}$. We computed the "purge time" required at this flow rate for the bolus to purge the residual buffer from the line. Our experiments show that at this "purge time," the resistance started to increase. When the flow was stopped, the resistance increase stopped, as depicted by the arrows in Fig. 5(a). Restarting the flow caused the resistance rise to recommence.

The aforementioned experimentally measured response rate of the biosensor was compared with that predicted by our model. We computed the predicted response as follows. The mass transport effects were computed by solving (11) subject to boundary conditions (12)–(14) via the finite-element method on the rectangular flow cell shown in Fig. 4. The chemical kinetics were computed by solving (4) numerically. This yields the dimer concentration $D(t)$ in (4) and also the biosensor conductance $G$. Fig. 5(b) shows the experimentally measured response rate (black dots) to 10 fM streptavidin at various flow rates from 0 to 200 $\mu\text{L/min}$. Also shown (in purple) is the theoretical response rate predicted by our model. As can be seen by eyeballing the plots, the predicted performance of the biosensor closely matches the experimental performance. Fig. 5(b) shows the experimental and predicted response rate in the reaction-rate-limited region of operation. Finally, Fig. 5(c) shows the predicted and experimental response rate in the range of 0–100 fM streptavidin. It confirms that the biosensor response rate increases with analyte concentration.

**IV. SIGNAL PROCESSING WITH BIOSENSOR**

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. Today's generation of ICS biosensor has electrodes of 1 mm radius comprising millions of individual gramicidin channels. The measured current is of the order of microamperes. 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 (5). It is a future goal to miniaturize these electrodes. Electrode sizes of 1 $\mu\text{m}$ radius comprise only a few ion channels. 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 III-B for measurements at multiple electrodes resulting in enhanced sensitivity. The noise levels are substantial and careful modeling of the noise distribution is required.

Construction of ICS biosensors with microelectrodes is the subject of our on-going research. Here, we provide a proof of concept of the signal processing capabilities by using a simpler biosensor setup, see [21] for experimental details. For experimental convenience, we used a covalently linked dimer of gramicidin ion channels (called bisgramicidin A) incorporated into an untethered bilayer membrane excised from a giant lipid vesicle seen in Fig. 6. The bilayer is supported over the 1 $\mu\text{m}$ diameter opening of a micropipette as shown in Fig. 7.

With the biosensor setup shown in Fig. 7, the contact area between the micropipette and liposome contains only 2–5 bis-
granomicidin channels. So the measured current is of the order of tens of picoamperes and measurement noise becomes a significant issue. The combined response of these channels yields a finite-state signal that can be modeled as a finite-state Markov chain (see [21] for details). The current pulses that generate this finite-state signal are thought to arise from conformational interconversion in the bisgramicidin A secondary structure [22]. The measured biosensor signal can be modeled as a noisy finite-state Markov chain, i.e., a hidden Markov model (HMM) [15]. Modeling the noise is a challenging task. It arises from thermal noise, the antialiasing effect from sampling, and an open channel noise with its power proportional to the inverse of frequency. Fig. 8 shows the power spectral density of a typical sequence of biosensor recordings, and 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 bisgramicidin A ion channels, see [23]. To model this correlated noise process, we used an autoregressive (AR) Gaussian process that comprises white Gaussian noise process $W_t$ filtered by an all-pole filter, see [21] for the use of Ljung-box test for model adequacy.

Having verified the adequacy of the HMM for representing the biosensor current, it is straightforward to construct an HMM maximum-likelihood classifier to detect analyte molecules. We refer to [21] for details of the HMM classifier equations and performance of the biosensor on experimental data.

V. CONCLUSIONS AND EXTENSIONS

In this paper, we constructed models for the electrical, chemical, and analyte mass transport dynamics of the ICS biosensor. The chemical kinetics of the biosensor were modeled as a two-timescale nonlinear dynamical system in the reaction-rate-limited case. Using singular perturbation theory, we explained mathematically the experimentally observed behavior of the biosensor to analyte concentration. For low analyte concentrations, mass transport dynamics became the dominant design constraint. By comparing with the experimental response, we showed that the mass transport flow model coupled with chemical kinetics accurately predict the biosensor response. Finally, for micro-sized electrodes, we described how statistical signal processing algorithms can be used to classify the analyte concentration.

When employing antibodies or other well-defined receptors, stochastic detection in conjunction with spatial analysis across an electrode array can yield improved sensitivity in the biosensor. An extension of this study is to examine the coherence of channel noise across such an array of electrodes. We anticipate an improvement in detection threshold proportional to $N^{-1/2}$ rather than $\sqrt{N}$, where $N$ is the number of independently read electrodes in the array. Such enhanced versions of the biosensor will yield performance closer to the capabilities of antennae in moths and the olfaction receptor epithelia in dogs.

REFERENCES


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