Proceedings of the 26th Annual International Conference of the IEEE EMBS
San Francisco, CA, USA • September 1-5, 2004

Effects of Scaling on the SNR and Speed of Biosensors

Arjang Hassibi, Thomas H. Lee, Reza Navid, Robert W. Dutton, and Sina Zahedi
Department of Electrical Engineering, Stanford University, Stanford, CA, United States

Abstract— **This paper presents a stochastic model for the observed signal of biosensors, a model that predicts the signal fluctuation of the system and the SNR associated with it using a Markov chain process. In the process, transition probabilities are derived from the target and probe binding kinetics in view of statistical motion and random walk events. Based on this model, we are able to estimate the settling time, power-spectral density (PSD), and signal to noise ratio (SNR) of general affinity-based biosensors. The effects of scaling from macroscopic to microscopic regimes are also studied, which indicate a fundamental tradeoff between settling time (speed) and signal fluctuation (noise). The model is also applied to analyze the behavior of a DNA hybridization electronic detector.**

Keywords—**Biosensor, noise, scaling, DNA hybridization**

I. Introduction

Sensors by definition are devices that respond to a physical stimulus, such as thermal, electromagnetic, or mechanical energy by producing a signal, usually of electrical nature. The quantities that conventional sensors measure typically corresponds to various macroscopic definitions of matter and energy (e.g. pressure, conductance, temperature, etc.); yet without an exception, independent of the application or the sensing mechanism, the detectable signal in all of the existing sensory systems is generated by the aggregate contribution of individual incidents (e.g. collision, absorption, emission, etc.), originating from probabilistic microscopic systems. When such systems are isomorphically reduced in size, i.e. scaled down with all dimensions of the system decreased uniformly, the changes in length, area, volume ratios and number of microscopic detectable particles alter the performance of the detection [1]. With the current trend towards the implementation of small-scale micro-fabricated electrochemical and biological sensors [2], a comprehensive understanding and statistical model of the observed signal and the uncertainty associated with it in terms of signal to noise ratio (SNR) has become more essential.

In this paper, we investigate the intrinsic signal fluctuation of affinity-based biosensors with emphasis on effects of excessive scaling. The unique feature of scaled down biological and chemical sensors compared to other types of traditional sensors is their high susceptibility to signal fluctuation. This is mainly due to the relatively large physical dimensions of the particles (e.g. large polymer molecules, proteins, nucleic acids, cells, etc.) compared to the micro- and nano-fabricated sensing elements. This characteristic potentially drives the biosensors well into the mesoscopic regimes, where little statistical signal averaging is present. It is imperative to understand that the overall fidelity of the sensor is not only a function of the intrinsic signal fluctuation, but also the specificity of the recognition entities (e.g. specificity of DNA probes in Microarrays [3]); nonetheless, we analytically show that independent of their relative contribution, the inherent signal fluctuation eventually dictates the measurement's uncertainty in excessively-scaled sensory systems.

To fully explore the effects of signal fluctuation on the SNR of biosensors, we introduce a Markov chain stochastic model for the affinity-based biosensors in Section II. This model takes into account the biochemical reaction kinetics and each particle's diffusion and drift processes. In Section III, we derive the closed-from solutions of the sensor's settling time as well as the observed signal power spectral density (PSD), which demonstrates the tradeoff between the response time (speed) and SNR (noise). In Section IV, as a practical example, we implement the derived model to examine the behavior of an ISFET device for electronic detection of DNA hybridization [4]. The presented model in this paper not only introduces a detection limitation for biosensors, but also provides design-oriented insight into the fabrication of low-noise micro- and nano-scaled transducers.

II. Model

The aim of a biosensor is to produce either discrete or continuous electronic signals which are correlated to the presence or concentration level of a single analyte or a group of analytes (i.e. target species). These sensory systems take advantage of the selectivity of various bimolecular interactions (e.g. antibody-antigen affinity). The processes which govern the microscopic interaction between such molecule pairs are probabilistic and founded on statistical mechanics. Microscopically speaking, for such reactions to occur, the molecules have to collide (i.e. have intimate proximity), and then react prior to moving away. To better understand the behavior of biosensors, it is necessary to characterize their probabilistic motion in view of

This research was in part supported by NSF in support of the Network for Computational Nanotechnology (NCN).

0-7803-8439-3/04/$20.00©2004 IEEE

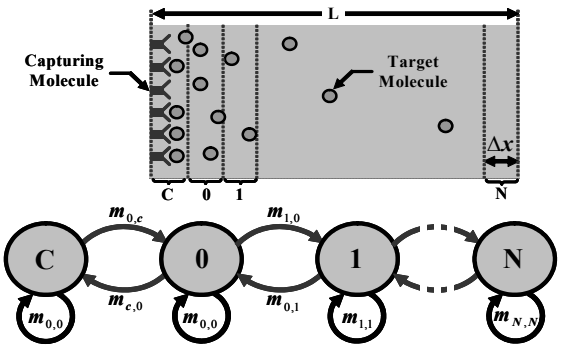

Fig. 1. Markov chain model of a one-dimensional affinity-based biosensor. Different states corresponds to different locations within the reaction chamber.

interactions. In the following, we present a stochastic model for both processes.

A. Molecular-Level Motion

Molecules immersed in the aqueous reaction chambers of biosensors are subject to thermal-fluctuation and perhaps on certain sensory platforms to electromagnetic or mechanical forces [5]. While mechanical movement (e.g. convection) and electromagnetic forces (e.g. electrophoresis) are deterministic, thermal fluctuation movement is not. Thermal fluctuation of a particle from a microscopic point of view follows the characteristics of typical random walk process (i.e. Brownian motion), which transpire into a diffusive spreading in macroscopic systems.

Statistically following the motion of molecules in the general case is challenging; yet, we can apply a Markov chain process [6] to model the particle motion within the reaction chamber (Fig.1). In this model, each state of the chain corresponds to a set of fixed coordinates within the reaction chamber. The transition probabilities are subsequently defined as probability of molecules moving from each coordinate to the next. Hence, for the target molecule X, with diffusion coefficient D and position $x(t)$ at time t, the transition probability between two adjacent coordinate pairs (states) i and $i+1$ in time interval Δt, defined by $m_{i+1,i}$ is

$$\begin{aligned} m_{i+1,i} &= \text{Prob}\left(x(t+\Delta t) = i+1 \middle| x(t) = i\right) \\ &= D\Delta t\Delta x^{-2} + \upsilon(i+1,i) \end{aligned}, \tag{1}$$

where $\upsilon(i+1,i)$ is the contribution of drift and convection. The transition matrix $M \in R^{N\times N}$ (N number of states), can then be applied to find the probability distribution of the system $\underline{x}(t) \in R^N$ at an arbitrary time t, given initial distribution of $\underline{x}(0)$ [6]. If M has an eigenvalue equal to one, then the system has an equilibrium distribution given that all other eigenvalues are less than or equal to one.

B. Interaction

Molecular collision results in different possible outcomes (reactive, elastic, and inelastic collision [7]), hence we can use a probabilistic model to predict the specific binding of target X molecule to Y molecules.

Assume that molecule Y is confined within a specific coordinate (e.g. state zero in Fig.1), then meaningful reactions only occur when $x(t) = 0$ (i.e. molecule X is in intimate proximity of Y). If the bulk-phase reaction between these species has the association rate k_1, and disassociation rate of k_{-1}, such that (symbol [ ] indicates concentration)

$$\begin{cases} X + Y \xleftrightarrow{k_1, k_{-1}} XY \\ \dfrac{d[XY]}{dt} = k_1[X][Y] - k_{-1}[XY] \end{cases}, \tag{2}$$

we can apply the following approximation to find the transition probabilities between captured state c, and collided state 0.

$$\begin{aligned} m_{c,0} &= k_1[Y_m]\Delta t \\ m_{0,c} &= k_{-1}\Delta t \end{aligned} \tag{3}$$

The physical justification for (3) comes from the fact that in the saturation concentration of Y, defined by $[Y_m]$, the system ensures that all X molecules are effectively in close proximity of a Y molecule, a condition which is similar to when $x(t) = 0$. Hence, for $x(t) = 0$ in biosensors and $[Y_m]$ in bulk-phase reaction, we expect the same reaction kinetics, k_{-1} and $k_1[Y_m]$.

Now based on (3) we can derive the new transition matrix $M \in R^{(N+1)\times(N+1)}$, which also includes molecular binding at the surface as a new state. It is imperative to understand that although the example illustrated in Fig.1 is one-dimensional, our derivations are generally applicable to multi-dimensional systems with more complicated boundary conditions.

III. Stochastic Analysis

A. Equilibrium and Settling Time

Most biosensors quantify the amount of captured molecules in chemical equilibrium, which is commonly independent of the initial condition of the system. Depending on the assay procedure, these systems can observe either a single sample of biochemical process (e.g. Microarrays or ELISA based Immunoassays [8]) or ongoing chemical reactions (e.g. ISFET-based biosensors). As mentioned in the previous section, the steady state

distribution ρ, can be derived by analyzing the eigenvalues and eigenvectors of the homogenous transition matrix. If the stability criteria exists, the equilibrium distribution of n molecules simply becomes $n \times \rho$.

The next quantity which we need to evaluate is the necessary time for the system to reach chemical equilibrium. This parameter is in fact a function of system dynamics in view of the initial condition; yet a pessimistic approximation for the worst-case time constant of the system τ_T can be obtained by simply adding all time constants associated with all N eigenvalues which are less than one. This is similar to the same approach as the open circuit time constant method in linear circuits [9] along with its deficiencies. The closed-form expression for τ_T can be analytically derived for the one-dimensional system in Fig.1 as

$$\tau_T = \frac{1}{k_{-1}} + \frac{1}{k_1[Y_m] + 4D[Y_m]^{2/3}} + \frac{L}{4D[Y_m]^{1/3}}, \quad (4)$$

where $L = N \times \Delta x$ is the actual dimension of the reaction chamber. It can also be shown that the settling time approximation has always a component proportional to the reaction chamber size (i.e. length, area and volume for one, two, and three-dimensions respectively). Equation (4) also indicates that the settling time has a component inversely proportional to the intrinsic kinetic rates of the reaction and the diffusion coefficient.

B. Signal Power Spectral Density

In order to find the power spectral density of the observed signal, we need to evaluate the autocorrelation function of X being in state c. For this purpose we initially derive the transition probabilities between state 0 and c (the relevant states for detection in equilibrium), summarized by the following two-state transition matrix

$$M' = \begin{bmatrix} 1-\hat{k}_1\Delta t & k_{-1}\Delta t \\ \hat{k}_1\Delta t & 1-k_{-1}\Delta t \end{bmatrix}, \quad (5)$$

where $\hat{k}_1 = k_1[Y_m]\rho_0/(1-\rho_c)$. ρ_0 and ρ_c are the steady state distribution probabilities of state 0 and c derived from ρ, respectively. Next, we calculate the closed-form autocorrelation function for state c, $R_C(\tau)$, from the correlation matrix as

$$R_C(\tau) = \alpha + \beta \exp\left[-(\hat{k}_1 + k_{-1}) \times |\tau|\right]. \quad (6)$$

The values of α and β are $2\rho_c\hat{k}_1/(\hat{k}_1 + k_{-1})$ and $\rho_c(k_{-1} - \hat{k}_1)/(k_1 + k_{-1})$ respectively. Now, we can determine the Fourier transform of (6) to find the power spectral density $S_C(\omega)$, which has the Lorentzian profile.

$$S_C(\omega) = \alpha\delta(\omega) + \frac{\beta}{1 + (\hat{k}_1 + k_{-1})^2\omega^2} \quad (7)$$

C. SNR

The overall noise power in a single measurement σ_S^2, is equal to $(\alpha + \beta) - \rho_c^2$; hence for a system consisting of n independent particles and transducer noise power of σ_T^2, we have

$$SNR = \frac{n^2\rho_c^2}{\sigma_T^2 + n\rho_c(1-\rho_c)}. \quad (8)$$

IV. Implementation

Now as an example, we analyze the stochastic characteristics of an ISFET device for electronic detection of DNA hybridization. The structure of this specific biosensor consists of a planar field-effect sensing element at the bottom of the cubic reaction chamber with dimensions L [4]. The sensor responds to the surface potential changes which arise from the selective binding of charged molecules to the surface (e.g. DNA molecules bind to complementary probes). In this platform, diffusion is the only source of molecular motion. The diffusion coefficient of the target molecule, a single strand 20 base-pair DNA, defined by D_t, was estimated to be 1.5×10^{-6}cm^2/s, whereas $[X]$ is set to be 0.1nM ($\approx 6\times10^{11}$ molecules/cm^3). The reaction rates as well as the simulation specifications are mentioned in Table I.

TABLE I
DNA Hybridization Sensor Specifications

Quantity	Value
DNA diffusion coefficient (D_t)	1.5×10^{-6}cm^2/s
DNA concentration ($[X]$)	0.1nM
Forward binding rate (k_1)	3×10^7M^{-1}s^{-1}
Reverse rate (k_{-1})	5s^{-1}
Probe saturation concentration ($[Y_m]$)	1μM
Transduction noise Power (σ_T^2)	1Molecule^2
Simulation lattice size (Δx)	60nm
Simulation time increment (Δt)	4.7μs

As shown in Fig.2a, the settling time approximation calculated from (4) gives a reasonable upper and lower bound for the transient responses of the system. The responses within these boundaries correspond to the progression of captured state probability toward its asymptotic value, given different random initial distributions. The size of the reaction chamber here is 10^3μm^3. In Fig.2b, we show the effects of isomorphical

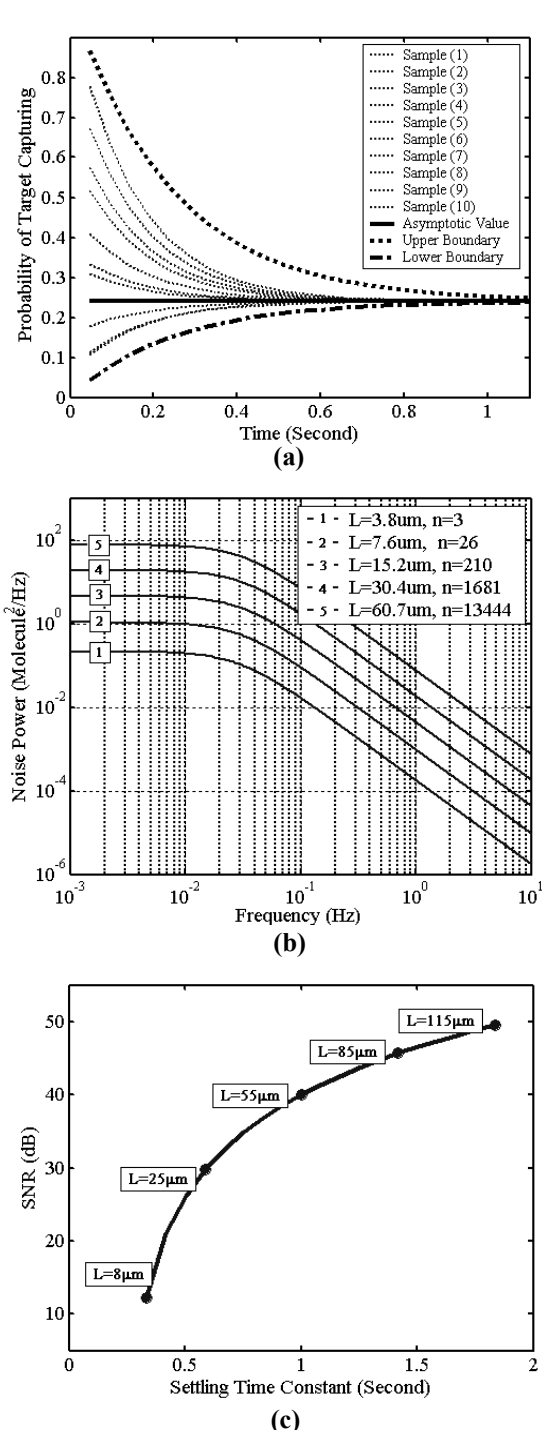

Fig. 2. Simulation results of a biosensor for DNA hybridization detection. In (a), the settling time of 10 random initial distributions is compared to the estimated worst-case settling time in (4). In (b), the power spectral density in view of isomorphical scaling of the detector is plotted. The plot in (c) shows the tradeoff between system settling time (speed) and SNR.

scaling on the PSD profile of the captured DNA molecules. As the size of the chamber and the number of molecules become larger, the noise power grows, but the 3dB bandwidth remains constant. Ultimately in Fig.2c, for the same scaling regime as in Fig.2b, SNR vs. settling time is plotted which shows the fundamental tradeoff between noise and speed of biosensors.

V. Conclusion

The observed signal in affinity-based biosensor is a function of captured molecules by the recognition sites. The probabilistic motion and interaction of molecules results in a random signal fluctuation (noise) which is generally observed in addition to the transducer noise. We have shown that this noise component has a Lorentzian power spectral density and its amplitude is proportional to the target concentration. The observed signal to noise ratio of these systems also decreases as the system is isomorphically scaled down, while the system's speed increases. The methods presented in this paper, can be applied to the design of various sensory systems, specifically low-noise biochemical detectors with micro and nano-scaled transducer structures. Based on these models, one can also derive a variety of estimation techniques to better detect analytes in biosensors [10].

Acknowledgment

The authors want to thank Sam Kavusi, and Moon-Jung Kim for insightful technical feedback.

References

[1] M. J. Madou and R. Cubicciotti, "Scaling issues on chemical and biological sensors," *Proceedings of the IEEE*, vol. 91, no. 6, pp. 830-838, June 2003.

[2] G. A. T. Kovacs, *Micromachined Transducers Sourcebook*. New York, NY: McGraw-Hill, 1998, pp. 687-761.

[3] M. Schena, *Microarray Analysis*. Hoboken, NJ: Wiley 2002, pp. 121-158.

[4] J. Fritz, E.B. Cooper, S. Gaudet, P.K. Sorger, and S.R. Manalis, "Electronic detection of DNA by its intrinsic molecular charge," *PNAS*, vol. 99, no. 22, pp. 14142-14146, Oct. 2002.

[5] H. C. Berg, *Random Walks in Biology*. Princeton, NJ: Princeton University Press, 1983, pp. 48-62.

[6] S. Karlin and H. M. Taylor, *A First Course in Stochastic Processes*. San Diego, CA: Academic Press, 1975, pp. 45-166.

[7] R. S. Berry, S. A. Rice, and J. Ross, *Physical Chemistry, 2nd Edition*. New York, NY: Oxford University Press, 2000, pp.805-818.

[8] E. P. Diamandis and T. K. Christopoulos, *Immunoassay*. San Diego, CA: Academic Press, 1996, pp. 25-49.

[9] T. H. Lee, *The Design of CMOS Radio-Frequency Integrated Circuits, 2nd Edition*. New York, NY: Cambridge University Press, 2004, pp.234-254.

[10] S. Zahedi, R. Navid , and A. Hassibi, "Statistical modeling of biochemical detection systems," unpublished.