Equilibrium and dynamic design principles for binding molecules engineered for reagentless biosensors

Seymour de Picciotto [a], Barbara Imperiali [b,c], Linda G. Griffith [a,d], K. Dane Wittrup [a,d,e,*]

[a] Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[b] Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[c] Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[d] Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[e] Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

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ABSTRACT

Reagentless biosensors rely on the interaction of a binding partner and its target to generate a change in fluorescent signal using an environment-sensitive fluorophore or Förster resonance energy transfer. Binding affinity can exert a significant influence on both the equilibrium and the dynamic response characteristics of such a biosensor. We here develop a kinetic model for the dynamic performance of a reagentless biosensor. Using a sinusoidal signal for ligand concentration, our findings suggest that it is optimal to use a binding moiety whose equilibrium dissociation constant matches that of the average predicted input signal, while maximizing both the association rate constant and the dissociation rate constant at the necessary ratio to create the desired equilibrium constant. Although practical limitations constrain the attainment of these objectives, the derivation of these design principles provides guidance for improved reagentless biosensor performance and metrics for quality standards in the development of biosensors. These concepts are broadly relevant to reagentless biosensor modalities.

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The field of biosensors has seen in the last decade a multitude of new approaches for the application of reagentless sensors. The overall strategy is the combination of a recognition unit and a signal-transducing unit into one molecular entity. The most commonly used signal is change in sensor fluorescence, arising either from fluorescence resonance energy transfer (FRET) or from solvatochromism. FRET was first described over half a century ago, and its application in biology has grown with design and implementation of myriad biosensors (reviewed in [1]). Solvatochromism is a more recent development, but is becoming more widely used as new scaffolds (affinity molecules) and dyes (environmentally sensitive fluorophores) are developed and become available (reviewed in [2]). Several groups have successfully developed solvatochromic-based biosensors using DNA aptamers [3–5], native protein receptors [6,7], peptides [8,9], or engineered binders using protein scaffolds [10–15]. However, to our knowledge, none of these groups have purposely engineered binders with affinities specified for optimal performance as a sensor, relying instead on previously described proteins. Selecting an existing binder with an affinity

above the detection threshold is likely an adequate approach for categorical detection of the presence or absence of an analyte. However in a complex biological system, analyte concentrations may vary rapidly on the time scale of seconds to minutes. For dynamic measurement of time-varying analyte levels, the biophysical characteristics of the binding event can significantly impact biosensor accuracy and sensitivity. Given the availability of directed evolution protein engineering methodology to create binding molecules of almost arbitrary affinity and widely varying association and dissociation rates [16,17], these variables are available degrees of freedom for improvement of biosensor performance. Recently Haugh developed a reaction-diffusion model to investigate biosensor signal interpretation in live cell imaging, with an emphasis on capturing intracellular and membrane-localized phenomena [18]. This analysis resulted in the identification of an important trade-off between robust signal and perturbation of the biological system or signal saturation. Here, we perform a theoretical analysis of biosensor dynamics, delineating time and length scales important in observation of intracellular as well as extracellular phenomena (e.g., detecting autocrine loops). Using a sinusoidal signal as an input ligand concentration, as biological signals do vary, we present new important considerations for the appropriate implementation of a biosensor. Further, we propose

* Corresponding author at: Building 76-261, 500 Main Street, Cambridge, MA 02139, USA.
E-mail address: wittrup@MIT.EDU (K.D. Wittrup).

metrics for quality standards in the development of biosensors by direct comparison between the input signal and the measured signal and thereby derive design criteria for improved performance.

Model formulation

The system consists of three state variables: the concentrations of ligand (L), unbound sensor (S_F) and bound sensor (S_B). By virtue of mass balance, the sum of the concentration of unbound and bound sensor is always equal to the total sensor concentration constant (S_{Tot}). A linear correlation between the bound sensor and the output signal intensity is assumed. The two rate constants governing this process are the association (k_{on}) and the dissociation (k_{off}) rate constant.

The mathematical description of this interaction, a reversible bimolecular reaction, is well documented from the perspective of dynamic steady-state equilibrium; however, it has generally been investigated in an environment of constant ligand concentration [19–21]. To determine the optimal design criteria in a dynamic system where the input (i.e., L) is time varying, we apply a frequency response approach by sinusoidally varying the analyte input, L, and characterizing the dynamic fluorescence intensity response of the sensor, which is proportional to the concentration of bound sensor S_B. A range of physiological behaviors can be modeled by systematic variation of the mean (L_0), amplitude (A_L) and period (T) of the time-variant ligand concentration. With these parameter definitions, the input function L is defined as

$$[L] = L_0 + A_L \sin(2\pi/T t). \tag{1}$$

To score a given set of design parameters of a sensor, we choose three signal properties: mean signal intensity (M), normalized amplitude (A), and phase delay (Φ), as defined in

$$M = (S_B^{max,eq} + S_B^{min,eq})/2S_{Tot} \tag{2}$$

$$A = (S_B^{max,eq} - S_B^{min,eq})/S_{Tot} \tag{3}$$

$$\Phi = (t(S_B = S_B^{max,eq}) - t(S_B = S_B^{min,eq}))/T. \tag{4}$$

We assume that the system is reaction limited. Indeed the Damkohler number, defined as the ratio between the characteristic time for diffusion and that of reaction (complex formation in this context, see Eq. (5)), will be much smaller than 1 for all relevant k_{on}, k_{off}, ligand concentration ([L]), diffusion coefficient (D) so long as the distance (r_b) over which the measurement must be spatially resolved is less than 1 μm (see Fig. S1).

$$Damkohler\# = \tau_{diff}/\tau_{rxn} = (r_b^2/D)/(k_{on}[L] + k_{off})^{-1}). \tag{5}$$

The described system is now fully governed by the three differential equations:

$$d[L]/dt = 2\pi/T A_L \cos(2\pi/T t) - k_{on}[L][S_F] + k_{off}[S_B] \tag{6}$$

$$d[S_F]/dt = -k_{on}[L][S_F] + k_{off}[S_B] \tag{7}$$

$$d[S_B]/dt = +k_{on}[L][S_F] - k_{off}[S_B]. \tag{8}$$

To simplify this system further, we assume that the ligand is in excess. Of course, as shown in the analysis by Haugh [18], this is a constraint that must be calculated for any real system since this assumption can often break down. Systematic use of a sensor concentration of one-twentieth that of the minimum ligand signal guarantees excess ligand concentration. By substituting Eq. (1) into (8), and using conservation of mass for the sensor species, we obtain the 1-D governing equation:

$$d[S_B]/dt = k_{on}(S_{Tot} - S_B)(L_0 + A_L \sin(2\pi/T t)) - k_{off}[S_B]. \tag{9}$$

A convenient analytical solution to this system is not available. Therefore, we solved this equation for a variety of parameter conditions by numerical Euler integration (performed in MatLab). The results are shown in the next section.

Results

Dynamic consideration reveals the crucial importance of kinetic rates optimization

Intuitively, a sensor that has a very high affinity for its ligand might be expected to perform as a weak dynamic sensor since the characteristic time for complex dissociation would likely be much greater than the period of the signal. Relevant input signal conditions depend greatly on the system under study. In Fig. 1 we show approximate concentrations and time scales for concentration variation for various classes of biological events. Many physiological processes result in great variation of analyte concentration such as cell-cycle-related proteins, signaling cascades, immune response activation, among many others. Mathematically, the sinusoidal function is a benchmark for representing time-variant signals. For example the well-known Bode plot uses a sinusoidal signal to characterize a system's frequency response [22]. We first investigated how output signal differs with varying the dissociation rate constant (k_{off}). As an initial input signal, we chose a mean ligand concentration of 3 nM with sinusoidal oscillation between 1 and 5 nM with a period of 100 min. This signal is shown as a solid gray curve on Fig. 2a. In black are shown four different sensors with varying k_{off} but identical association rate constant ($k_{on} = 10^5 M^{-1} s^{-1}$). In this first approach, we show the signal for the first 4 periods (400 min). An initial condition corresponding to $S_B = 0$ was chosen for the analysis depicted in Fig. 2a, hence an initial transient in signal response is observed .The signals progress toward their dynamic steady state, where higher signal intensities are reached with decreasing k_{off} as expected given the equation for complex concentration under the pseudo-first-order approximation.

$$[S_B] = S_{Tot}L_0/(L_0 + k_{off}/k_{on}). \tag{10}$$

This transient behavior is followed by a dynamic steady state characterized by a constant value of the mean signal. The equilibrium half time ($t_{1/2}$) is defined as

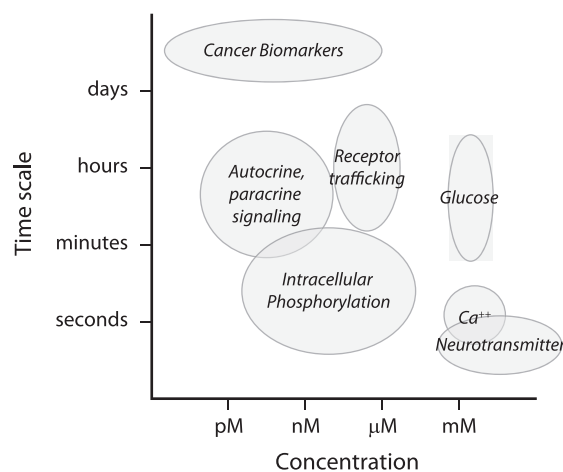$$\tau_{1/2} = \ln(2)/(k_{on}L_0 + k_{off}). \tag{11}$$

Fig.1. Various biologically relevant molecules and processes are depicted in this figure. Typical mean concentration is shown on the horizontal axis ranging from picomolar (pM) to millimolar (mM) against expected time scales for variation in the ligand concentrations.