Design of Biosensors with Extended Linear Response and Binary-Type Sigmoid Output Using Multiple Enzymes

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Abstract — **We review results on theoretical modeling of biochemical systems in support of the studies of several types of biosensors and systems for "biocomputing" information processing. The developed theoretical techniques have been utilized to investigate performance and optimize a novel approach to biosensors with extended linear response for lysine detection and also to study a "binary" response of a metabolic "branch point effect" system for detection of glucose. We study the experimental data for the flow-injection amperometric biosensor based on the action of Lysine-2-monooxygenase and L-Lysine-alpha-Oxidase. Lysine is a homotropic substrate for both enzymes. Parameter values are identified for an extended linear range of response. For a "branch point effect" system, we demonstrate that an "intensity filter" mechanism can yield a sigmoid response useful in biochemical signal processing and enzyme-based biosensing applications.**

Keywords *– enzyme biosensor; linear response; differential sensitivity; intensity filter*

I. INTRODUCTION

We report our recent results in the field of biosensing and biocomputing aimed at investigating the control of several types of biochemical responses for lysine [1], Section II, and glucose detection [2], Section III. These investigations have been motivated by interest in novel diagnostic applications [3-8] involving sensing, information and signal processing in chemical [9-11] and biochemical [12-15] systems, including those based on enzyme-catalyzed reactions [16-18]. Such systems use enzyme-catalyzed reaction cascades with properties optimized for the information/signal processing in biocomputing systems [19-24] and for controlling the input/output signal response to improve detection in biosensors of different types [8,25-28]. New applications have been considered for the multi-enzyme biosensors with response/actuation of the threshold-type, "digital" nature [1,25,28,29].

A key requirement in design of biocomputing systems has been the avoidance of noise amplification by biochemical processes used as network elements [7,30]. In this regard, the most promising approach has been to transform a typical convex shape signal in a biocatalytic process to a sigmoid-shape response, similar to natural systems [31,32]. Such transformation of the response can yield "biomolecular filters." These filters are usually based on different mechanisms such as self-promoter enzyme properties [33], pH control by buffering [23,34], redox transformations [35], or utilization of competing enzymatic processes [28]. Their action is based on realizing a sigmoid response of the output as a function of the input [2,7,22-24,36-38]. This can be achieved, for instance, by designing systems with "intensity filtering" in which a fraction of the output signal is neutralized by adding an additional (bio)chemical process [7,20,28,35]. When the added filtering reaction practically stops (once the added reactant is consumed), the intensity of the output signal increases, resulting in a sigmoid shape of the response [22-24,36]. Such a response allows a clear differentiation of the levels of the "binary" logic-**0** and logic-**1** output signal levels, as well as avoids noise amplification from the input signal to the output. We consider an example of such a system in Section III.

However, in many biosensor applications the level of the output signal must be approximately proportional to the concentration of the detected "input-signal" substance [27,39-42]. This enables easy calibration in response to the variations of the enzyme activity from batch to batch and during the extended times of biosensor use. Optimization of such biochemical sensing systems should be aimed at achieving a high degree of linearity [1]. We illustrate design of biosensors with extended linear response, by utilizing a combined function of more than a single enzyme, in Section II.

II. BIOSENSORS WITH EXTENDED LINEAR RESPONSE

The experimental study and analysis of the systems for lysine detection have shown that a typical enzymatic process can have a good linear sensitivity only at lower concentrations of lysine [27,40-42]. Another allosteric enzymatic process has an approximately linear response to the same substrate, but only for a range of larger input concentration. Incorporation of this allosteric enzyme in the biochemical system leads to the effect of increasing the linear range of the signal [1,40], but also in some cases to a certain decrease in its intensity. Control of the proportions of the two enzymes in the system can optimize the linearity of this bi-enzymatic biosensor signal [1,39-42]. Many experimental studies of bi-enzymatic biosensors have been recently reported [8,43-46]. Therefore, theoretical analysis of

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their performance optimization is warranted [1]. We consider a particular system for detection of lysine that was measured amperometrically by oxygen consumption in a flow-through analyzer [1,27,40-42].

The first enzymatic process is catalyzed by L-Lysine-alpha-Oxidase (LO):

$$\text{L-Lysine} + O_2 \xrightarrow{\text{LO}} \text{α-keto-ε-aminocaproate} + H_2O_2 + NH_3 \quad (1)$$

The output signal of this enzymatic process was measured by the consumption of oxygen. Its shape is linear in a small range of input lysine concentration [27], and it saturates for larger concentrations. The second, allosteric enzymatic process was catalyzed by Lysine-2-monooxygenase (LMO):

$$\text{L-Lysine} + O_2 \xrightarrow{\text{LMO}} \text{δ-aminovaleramide} + CO_2 + H_2O \quad (2)$$

The shape of the output signal for this process is not linear for small inputs. It has a self-promoter sigmoid form (homotropic response) [27,40]. However, when both enzymes LO and LMO are present then the resulting response signal can be made linear with a good degree of linearity. LMO was added approximately in multiples of the LO concentration. With the proper choice of the process parameters, the linearity of the signal will be considerably extended without significantly reducing the output signal intensity [1,27,40-42].

For this biosensor system we propose a modeling approach [1] to quantify the sensitivity and degree of linearity of the resulting signal. It is not always possible to model a system's response in detail, because kinetic equations for biochemical processes involve many pathways and rates. Therefore, our numerical modeling has focused on few key parameters sufficient to quantify the signal processing. The reason for this has been that the available experimental data are usually limited and frequently very noisy.

Most enzymes have mechanisms of action that are quite complex, with many pathways, involving a lot of adjustable rate parameters. These rate constants are normally enzyme-batch dependent and typically change considerably with the chemical and physical conditions of the system. Therefore, we use a simplified kinetic consideration for few key processes in terms of rate constants. This approach allows finding an approximate representation of the shape of the system's response curve or surface with few adjustable rate parameters.

For the first, non-allosteric enzyme, LO, we use the standard Michaelis-Menten (MM) description:

$$E_1 + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C_1 \quad (3)$$

$$C_1 \xrightarrow{k_2} E_1 + P \quad (4)$$

The first enzyme, LO, of concentration E_1, consumes the substrate (L-Lysine), the concentration of which is denoted as S, and produces the complex, C_1. This complex later transforms to the product, P, while restoring the enzyme E_1. All the concentrations here are time dependent. The first step of the reaction is naturally reversible. For biosensor and information processing applications this system is typically driven by the forward reaction. Thus, we assume that $k_{-1} \approx 0$. Therefore, there are only two adjustable parameters, $k_{1,2}$, for an approximate description of the LO-catalyzed kinetics.

Note that the real experimental data for this biosensor were obtained for flow systems at certain "measurement time," $t = t_m$, here $t_m = 120$ sec [27,40], as function of the input concentration, S. Since both enzymatic processes (LO and LMO) were in their steady states, we can use a representative product concentration, $P(t)$, calculated as a function of time, ignoring spatial variation of the product concentration along the flow.

The actual measurements [27,40-42] were made amperometrically. Therefore, the signal measured, to be denoted V, is not one of the concentrations of the product chemicals, but is rather proportional to the oxygen consumption rate in the system. The conversion factor of the product concentration P to the actually measured signal V is not known, and it can depend on the chemical conditions, specifically, the pH [40], and on the enzyme batch. Thus, the conversion factor, γ, was taken as another adjustable parameter:

$$V = \gamma P(t_m) \quad (5)$$

It is now possible to write down a set of differential rate equations and carry out numerical simulations to analyze the available experimental data. These equations are shown below, once the second enzyme, LMO, kinetics is included. Figure 1 illustrates the results of such modeling. The data for the LO-only experiment were fitted assuming the initial values $E_1(0) = 0.26$ μM, and $S(0)$ varying from 0 up to 60 mM. The parameters were $k_1 = 2.1 \times 10^{-3}$ $\text{mM}^{-1}\text{sec}^{-1}$, $k_2 = 1.0 \times 10^{-5}$ sec^{-1}, $\gamma = 145$ mV/mM.

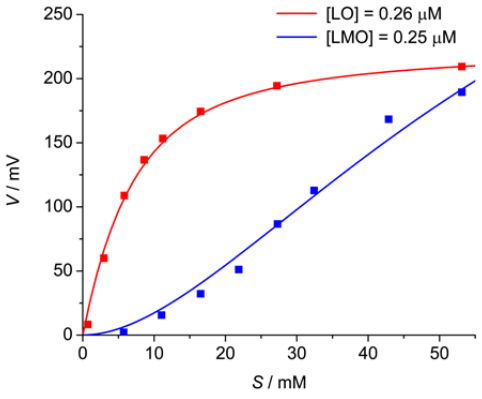

Figure 1. Illustration of data fitting [1] for the LO-only (red) and LMO-only (blue) experiments [40].

For the second added enzyme (LMO), $E_2(t)$, we should take into account the fact that L-Lysine has a self-promoter substrate property (Figure 1). Apparently, LMO is an allosteric enzyme, but the detailed scheme of its functioning has not been studied in the literature. We apply a simplified version of the model [1,47-49] of conventional self-promoter allostericity, which explains sigmoid properties:

$$E_2 + S \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} E_2^a \tag{6}$$

$$E_2^a + S \underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} C_2 \tag{7}$$

$$C_2 \overset{k_5}{\rightarrow} E_2^a + P \tag{8}$$

For this scheme, we assume that some amount of substrate is used to transform LMO into a more active form, E_2^a, where this active form of enzyme works according to the MM scheme similar to Eq. (3-4). This description ignores many possible kinetic pathways connecting them into effective single steps allowing to limit the number of adjustable parameters, and assuming that the conversion factor, γ, is the same. Note the product of the reaction (8) differs from that in (4), for LO. We can use the notation *P* in both cases, because the two processes consume oxygen—which is the actual measured signal—identically. The initial value was $E_2(0) = 0.25$ μM, and the fitted constants were $k_3 = 0.39 \times 10^{-3}$ mM^{-1}sec^{-1}, $k_4 = 0.47 \times 10^{-3}$ mM^{-1}sec^{-1}, $k_5 = 1.2 \times 10^{-3}$ sec^{-1}. The results of our modeling for an LMO-only system are shown in the Figure 1.

Figure 2 illustrates the data vs. model with the two enzymes, with a good degree of consistency for all the initial LMO:LO ratios [1,27,40-42]. The functioning of the enzymes is interconnected via the substrate concentration, which enters in the rate equations:

$$\frac{dE_1(t)}{dt} = -k_1 S E_1 + k_2 C_1 \tag{9}$$

$$\frac{dS(t)}{dt} = -k_1 S E_1 - k_3 S E_2 - k_4 S E_2^a \tag{10}$$

$$\frac{dE_2(t)}{dt} = -k_3 S E_2 \tag{11}$$

$$\frac{dE_2^a(t)}{dt} = k_3 S E_2 - k_4 S E_2^a + k_5 C_2 \tag{12}$$

$$\frac{dV(t)}{dt} = \gamma(k_2 C_1 + k_5 C_2) \tag{13}$$

where $V(t)$ is the measured signal at time $t = t_m$.

The initial values were $E_1(0) = 0.29$ μM for LO, and $E_2(0) = r \times 0.26$ μM for LMO, with $r = 1, 8, 12, 29$. These parameters were obtained for the data from the original experimental works [27,40]. For $r = 1$, the amounts of the enzymes per unit mass of the gel on which they were immobilized were identical [42].

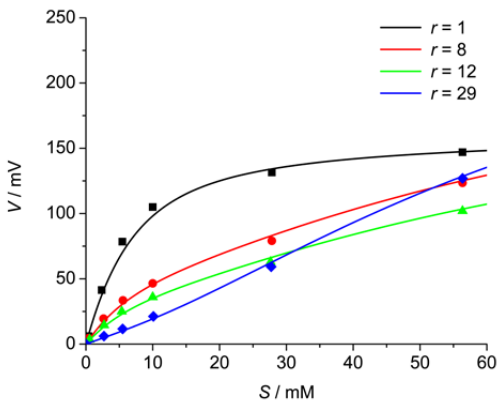

Figure 2. Data fitting [1] for LMO:LO initially present at ratios $r = 1, 8, 12, 29$, see [1,40].

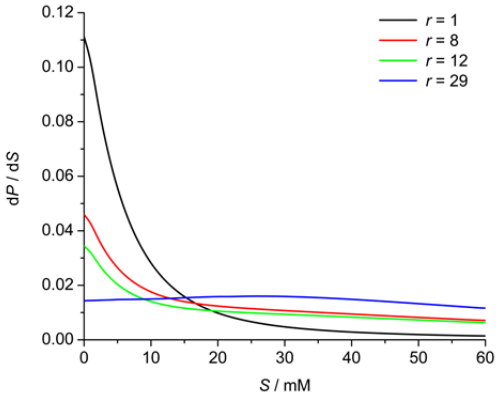

Figure 3. Differential sensitivity calculated over the desired input range, 0 to 60 mM, for various values of the ratio, *r*.

The bi-enzymatic biosensor can be optimized by selection of the parameters, here exemplified by *r*, to get an approximately linear response in the desired input regime. In order to evaluate the properties of the output signal we use the differential sensitivity of the system over the input range, *S*(0) = 0 to 60 mM, see Figure 3:

$$\frac{dP}{dS} = \frac{dP(t_m)}{dS(0)} \tag{14}$$

We aim at finding parameters minimizing this function over the desired input range. Figure 1 shows that for $r = 0$, LO-only, gives us an almost linear signal only for the range of inputs between 0 and 5 mM. However, when $r = 29$ (Figure 2), the response has an approximately linear shape in the extended input range from *S*(0) = 0 to 60 mM, as confirmed by the differential sensitivity plotted in Figure 3.

However, the overall biosensor sensitivity drops due to such modifications. Therefore, other quality measures must be also defined and considered, in addition to "linearity," as detailed in [1], but not reviewed here. Each of such quality measures can be formally defined and modeled. For example, the linearity of the response can be characterized

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by a measure of the deviation of the differential sensitivity over the input signal range from the average slope:

$$\Delta = \frac{\left(\int_0^{S_{max}} \left[\frac{dP(S)}{dS} - \frac{P(S_{max}) - P(0)}{S_{max}} \right]^2 dS \right)^{\frac{1}{2}}}{\frac{|P(S_{max}) - P(0)|}{S_{max}}} \quad (15)$$

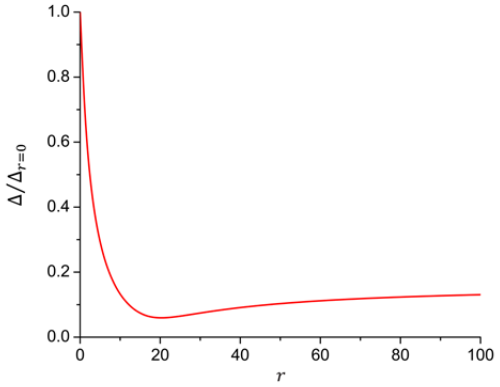

Figure 4. A measure of the response linearity for various values of *r*.

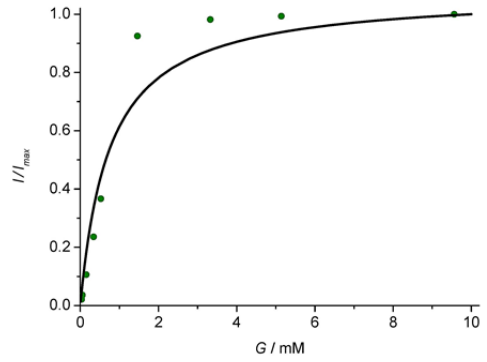

Figure 5. Experimental data [28] (circles) for the normalized current and numerical model [2] (line) without the filtering process.

For the best quality of response's linearity we have to minimize $\Delta/\Delta_{r=0}$, plotted as a function of r in Figure 4,which indicates that values from 14 to 32 represent an optimal range for the parameter *r* selection based on the "linearity" criterion. The actual minimum of the linearity measure (Figure 4) is close to r = 20, consistent with the experimental results [40].

III. BIOSENSORS WITH BINARY-TYPE SIGMOID RESPONSE

We consider an enzymatic biosensor with the binary-type sigmoid response [2,28]. The input in this system is glucose, G, with the initial concentration from 0 mM to 10 mM. These values are assumed as logic-**0** and logic-**1** respectively used to define the YES/NO signals. Electrode-immobilized glucose oxidase (GOx) consumes glucose [2,28]. The output signal $I(t_g)$ was measured at the "gate time" $t_g = 180$ sec as the current due to transfer of two elementary units of charge per reaction cycle [28]. The usual response shape for such biocatalytic reactions is convex, see Figure 5. However, for "binary" information processing it is useful to have a signal of a sigmoid shape [37]. This was accomplished [28] by consuming a fraction of the input (glucose) directly in solution, by a competing enzymatic process. The second enzyme, hexokinase (HK), H, has been added, consuming glucose and adenosine triphosphate (ATP), A, resulting in a biochemical "filtering" effect, see Figure 6.

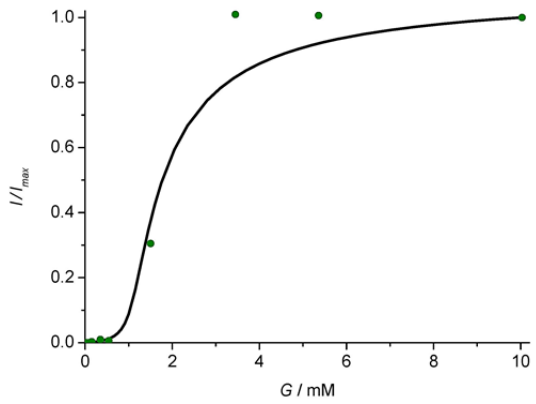

Figure 6. Experimental data [28] (circles) for the normalized current and numerical model [2] (line) with the "filter" process.

We developed a model [2] based on a rate-equation modeling of the key steps of the enzymatic processes in this biosensor system, using a limited number of adjustable parameters. The GOx enzymatic process is modeled as:

$$E + G \overset{k_1}{\rightarrow} C \overset{k_2}{\rightarrow} E + \cdots \quad (16)$$

The intermediate complex C is gluconolactone, for the kinetics of which we have:

$$\frac{dC(t)}{dt} = k_1 G(t) E(t) - k_2 C(t) \quad (17)$$

The current $I(t)$ is proportional to the rate of the second reaction in Eq. (16); $I(t_g) \propto k_2 C(t_g)$. Assuming that the oxygen concentration was constant, it was absorbed in k_2. Equation (17) is exactly solvable:

$$C(t) = \frac{k_1 E(0) G}{k_1 G + k_2} \left[1 - e^{-(k_1 G + k_2)t} \right] \quad (18)$$

The input G has value from 0 to G_{max} = 10 mM. Without the filtering process, least-squares fit of experimental data from [28] gives us the estimates $k_1 \cong 80$ mM^{-1}s^{-1}, $k_2 \cong 60$ s^{-1}.

With the added filtering process, we consider the pathway of the HK biocatalytic process in which glucose transforms into the complex *D*:

$$H + G \overset{k_3}{\rightarrow} D + \cdots \quad (19)$$

$$D + A \overset{k_4}{\rightarrow} H + \cdots \quad (20)$$

This approach uses two adjustable parameters, $k_{3,4}$:

$$\frac{dG}{dt} = -k_3 HG \quad (21)$$

$$\frac{dH}{dt} = -k_3 HG + k_4 DA \quad (22)$$

$$\frac{dD}{dt} = k_3 HG - k_4 DA \quad (23)$$

$$\frac{dA}{dt} = -k_4 DA \quad (24)$$

The results of a numerical solution and fitting of the available data (see Figure 6) allow us to estimate $k_3 = 14.3 \pm 0.7 \text{mM}^{-1}\text{s}^{-1}$, $k_4 = 8.1 \pm 0.4\ \text{mM}^{-1}\text{s}^{-1}$ [2].

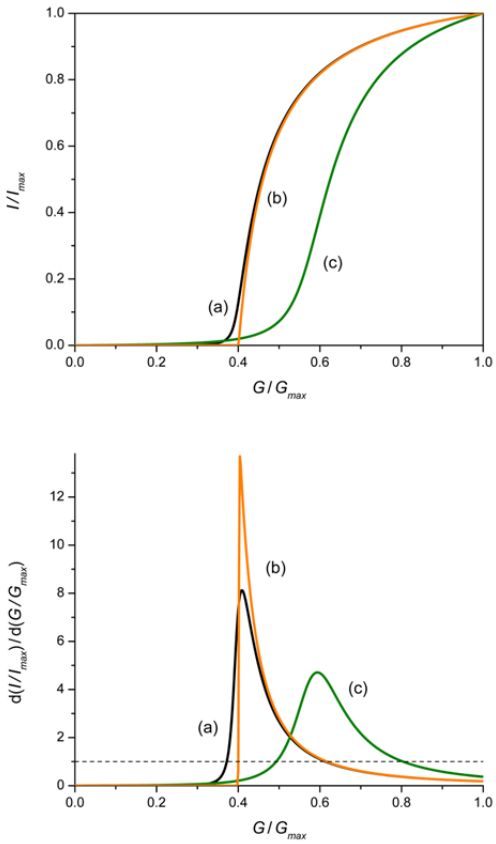

Figure 7. Examples of sigmoid curves (top panel) and their derivatives (bottom panel) for three different selections of the parameters used to control the response: (a) HK = 4 μM and ATP = 4 mM; (b) HK = 8 μM and ATP = 4 mM; (c) HK = 3 μM and ATP = 6 mM.

In order to minimize noise amplification in the output signal, the biosensor parameters must be chosen properly. There are usually several sources of noise. The main is the fluctuations in the input signal and the transmission of this noise from input to output. This transmission could amplify or suppress the noise [3,7,37]. Avoiding this "analog noise" amplification during signal processing is paramount for network stabilization [9,30]. Noise reduction can be achieved by modifying the system's response to sigmoid [2,22-24,34-38,50,51].

Note that a sigmoid curve has a peaked derivative. In order to achieve the response curve as symmetric as possible we consider the position and width of this peak, aiming at finding parameter values for which it is narrow and centrally located. Examples are given in Figure 7, and details of the optimization for the present system are presented in [2].

IV. CONCLUSION

In summary, we reported approaches [1,2] allowing optimization of biosensors based on functioning of multiple enzymes with different kinetics, yielding a binary-type sigmoid or an extended linear response. Kinetic modeling allowed us to evaluate the effects of varying system parameters that can be adjusted to control the response.

V. ACKNOWLEDGEMENT

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