ENZYMATIC LOGIC GATES WITH NOISE-REDUCING SIGMOID RESPONSE

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Introduction

Biochemical computing is an emerging field of unconventional computing that attempts to process information with biomolecules and biological objects using Boolean logic. In this work electrode-immobilized glucose-6-phosphate dehydrogenase enzyme catalyzed a reaction which carries out the Boolean AND logic gate. We report the first experimental realization of a sigmoid shape response in one of the gate inputs which is a desirable shape for biocomputing application as it allows reduction of the analog noise. A kinetic model is also developed and used to evaluate the extent to which the experimentally realized gate is close to optimal.

Recently there has been significant interest in biochemical [1] information processing, including that based on enzyme reactions [2]. Enzymatic reactions have been shown to mimic digital logic gate functions [3] and elementary arithmetic operations [4] as well as “networked” in Boolean logic circuits [5].

Here we consider the AND logic function based on electrode-immobilized enzyme glucose-6-phosphate dehydrogenase (G6PDH). The biocatalytic reaction

\[ G6P + NAD^+ \overset{G6PDH}{\longrightarrow} \text{NADH} + \cdots \]  

(1)

has two inputs: glucose-6 phosphate (G6P) and cofactor nicotinamide adenine dinucleotide (NAD+), and one output: the reduced cofactor (NADH); the other product (\( \cdots \)) in aqueous solution is 6-phosphogluconate. G6PDH is used in biosensors [6] for biomedical applications.

Cyclic voltammetry measurements

The activity of the G6PDH-modified electrode was followed by cyclic voltammetry measurements in the presence of G6P and NAD+ in solution. The reaction (1) with the immobilized enzyme resulted in the production of the reduced cofactor NADH which was re-oxidized electrochemically. The oxidation of NADH was observed as the anodic current peak on the voltammograms and was taken as the output signal of the enzyme-based AND logic gate.

Gate response function

To map out the response function of the logic gate, the output signal should be measured for inputs not only at the logic-0 and 1 but also for intermediate values of the reduced concentration variables; these are defined as

\[ x = [\text{NAD}^+](t = 0)/[\text{NAD}^+]_{\text{max}}, \]  

(2)

\[ y = [\text{G6P}](t = 0)/[\text{G6P}]_{\text{max}}, \]  

(3)

\[ z = [\text{NADH}](t = t_{\text{gate}})/[\text{NADH}]_{\text{max}}. \]  

(4)

Here the notation \([\cdots]_{\text{max}}\) is used to denote the maximum concentrations corresponding to the logic-1 input values at \( t = 0 \), and the output at the time of the voltammogram taking, \( t = t_{\text{gate}} \). Thus, if we scan inputs between 0 and the maximum, and record the corresponding output, we can map out the gate response surface, \( z = F(x, y) \).

As described in our recent works [7,8], networking of a gate in a larger “circuit” for biochemical logic applications requires control of the noise buildup. The level of the noise largely depends on the gate’s environment. However, the degree of analog noise amplification should be kept in check to ensure stable, scalable operation of increasingly complex networks. To this end, we would like to have a “sigmoid” response function \( F(x, y) \) that has small gradients at all the logic points. With the largest gradient less than 1, such a gate would actually offer analog noise spread reduction, i.e., incorporate a filtering feature in its function. While such functions are encountered in some natural processes [9], they have been elusive in simple (bio)chemical reactions and have not thus far been realized experimentally. Here we consider an interesting option: a response “sigmoid” in only one of the two inputs; see Fig. 1.

Rate equations for the biocatalytic reaction

The biocatalytic process in (1) is not a direct reaction. It involves several steps and possible competing pathways, the precise kinetics of which is not fully understood, especially for the electrode-immobilized enzyme as the biocatalyst. We use a phenomenological rate equation approach within a simplified, few-parameter description, which bypasses...
the kinetic issues of the experimentally observed “self-promoter” property [9] of G6P. The rate equation for G6P, denoted by $[G6P](t) = G(t)$, is

$$dG/dt = -[\alpha + \beta (G_0 - G)]GM.$$  \hspace{1cm} (5)

We assume that the dominant reaction pathway is the one corresponding to the substrate being captured by the enzyme, of concentration $[G6PDH](t) = M(t)$. The reaction rate is initially proportional to $\alpha G(t)M(t)$, while at later times the rate increases proportionately to the amount of the consumed substrate (the “self-promoting” effect), $\alpha \rightarrow \alpha + \beta[G_0 - G(t)]$, where $G_0 = G(0)$ is the initial concentration of the input G6P.

The resulting complex of concentration $C(t)$, then combines with NAD$^+$, of concentration $[NAD^+](t) = N(t)$ to yield the product $[NADH](t) = P(t)$, as well as to restore the biocatalyst. We will use this rather simplified description to write the remaining rate equations for our system:

$$dC/dt = [\alpha + \beta(G_0 - G)]GM - \gamma NC,$$ \hspace{1cm} (6)

$$dM/dt = -[\alpha + \beta(G_0 - G)]GM + \gamma NC,$$ \hspace{1cm} (7)

$$dP/dt = -dN/dt = \gamma NC.$$ \hspace{1cm} (8)

Here $\alpha$, $\beta$ and $\gamma$ are adjustable parameters (rates) which are used to fit experimental data and to explore the noise-amplification properties of our biocatalytic system.

![Fig. 2: Maximum gradient of the response surface vs. the reaction time and initial value of [G6PDH].](image)

Data analysis and gate-function properties

The experimental response surface is shown in Fig. 1; the fitting of these data with the rate equations (5)-(8) is also shown. Since the experimental response function is rather noisy, the fitting was performed as a weighted non-linear least-squares fit of the data with emphasis in the region of small [G6P], for which the self-promoter property is observed. This yielded $\alpha = 0.03 \text{mM}^{-1}\text{s}^{-1}$, $\beta = 42 \text{mM}^{-2}\text{s}^{-1}$, $\gamma = 1.05 \text{mM}^{-1}\text{s}^{-1}$.

We then computed [9] the gradients at the four logic points as functions of the effective enzyme concentration and reaction time. The maximum of these gradients in the parameter range covered, happens to be at the logic-01 point. Interestingly, our randomly selected (for experimental convenience) values of the parameters yield the noise amplification factor 1.16, which is already better than those potentially achievable (but requiring dramatic variation of parameter values) in earlier studied enzymatic systems [7].

Within our model, we can also “optimize” the gate “machinery” by changing the enzyme activity and/or the gate time, while keeping all the other parameters fixed. This is shown in Fig. 2: There is a broad region corresponding to reaction times longer than ~200s and to enzyme activities comparable or larger than in our system, which yields practically no noise amplification, i.e., the largest gradient was very close to 1, with values as low as ~1.05 within experimental reach.

In summary, we reported [9] the first realization and performance analysis of an enzymatic AND gate with a noise-reducing “sigmoid” response in one of the inputs. The studied enzymatic reaction was found to have a relatively small degree of analog noise amplification for the selected experimental parameters, which turned out to be in the theoretically calculated optimal regime.

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