Mass Action Modeling of Catalysis Through Reaction Flux Estimation

Ethan Stancliffe
University of Iowa

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MASS ACTION MODELING OF CATALYSIS THROUGH REACTION FLUX ESTIMATION

by

Ethan Stancliffe

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Michael A. Mackey
Thesis Mentor

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David G. Wilder
Biomedical Engineering Honors Advisor

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Mass Action Modeling of Catalysis
Through Reaction Flux Estimation

Author: Ethan Stancliffe, Biomedical Engineering and Mathematics
Faculty Mentor: Dr. Michael Mackey, Department of Biomedical Engineering
Honors Advisor: Dr. David Wilder, Department of Biomedical Engineering
Abstract

The complexity of biochemical networks necessitates the use of computational and mathematical frameworks to accurately characterize and study these systems. However, modern frameworks developed for this task have inadequacies that limit their accuracy or scalability. In this report, a mathematical model of the canonical enzyme substrate binding network is developed, and, using estimated true and maximal reaction rates, a methodology utilizing principles of flux balance analysis is developed to deduce the individual reaction rate constants in the network. It is then shown that these two reaction rates are not sufficient to unambiguously define a mass action kinetic model of this network. Nevertheless, the methodology developed greatly reduces the degrees of freedom of the system, and, as a result, the solution space of the network can be examined computationally and analytically revealing several non-intuitive sensitivities.

Introduction

Biochemical networks have long fascinated biophysicists and, recently, systems biologists. The biological networks that govern cellular decision making, metabolism, etc. are often complex and highly nonlinear; as such, there is a need to study and model these networks in silico to systematically compile biological information and predict the often non-intuitive behavior of these networks under a multitude of environmental and structural conditions. In so doing, the time and expense required by traditional biochemical experimentation to characterize these networks can be reduced, and, further, the elucidation of unobservable or immeasurable phenomena will be possible.
One approach to modeling metabolic networks has been bolstered through the development of flux balance analysis or FBA (Gianchandani, Chavali, & Papin, 2010). This computational tool utilizes linear programming and steady state conservation relations to find optimal reaction rates, or fluxes, for each reaction within the metabolic model. A stoichiometric matrix, $S$, is created that encapsulates the stoichiometry of each reaction. In this matrix, each column represents a reaction and each row a chemical species. With this matrix, conservation of mass can be employed such that, at steady state, the following relation exists, where $v$ is a vector containing the flux value for each reaction.

\[ S \cdot v = 0 \] (1)

Unfortunately, for most networks, this system is underdetermined and before the analysis can be completed, an objective function must be formulated such that linear programming can find an optimal solution. This objective function generally consists of a single reaction containing a multitude of chemical species that are thought to represent a biomass-forming reaction (Feist & Palsson, 2010). Thus, the linear program searches the solution space for the largest flux value of the biomass forming reaction thereby optimizing the system for growth (Gianchandani et al., 2010).

The power of these models lie in their ability to produce quantitative predictions without the necessity for knowing biophysical constants that are necessary to parametrize alternative modeling approaches, such as an ordinary differential equations (ODE) based mass action model. Flux balance analysis allows for the behavior of a system to be estimated under well-defined perturbations. For example, in genome scale metabolic modeling, metabolic genes are identified and linked together with logical AND/OR relationships that connect to a reaction (Haggart, Bartell, Saucerman, & Papin, 2011). From these relationships it is then possible to determine the behavior of the network when a gene is inactivated in silico by examining the predicted biomass
reaction flux and pathway utilizations (Haggart et al., 2011). The perturbations that can be explored with these models are discreet by nature. For example, decreased enzyme affinity or increased competition cannot be simulated using these models. The components are binary: on or off, and although they allow for a reduction in computational expense and negate the need for explicit rate constant definition, subtle perturbation simulations cannot be achieved. Ideally, these models could be enhanced through the addition of kinetics.

The principle of mass action asserts that under infinitely well stirred, isothermal, conditions the rate of a reaction is proportional to the concentrations of the reactants raised to their respective stoichiometric powers, shown in Figure 1. This assertion allows for an ODE model to be constructed to capture the nonlinear complexity of biological networks. However, to make such a model functional, rate constants need to be determined for each elementary reaction within the network.

These biophysical constants are often difficult to measure experimentally or predict theoretically. Although it is possible to deduce these values through modern structural protein modeling and simulation, the computation time is still too large to deduce the constants required for large scale biochemical modeling (Gräter & Li, 2015). Experimentally, Michaelis-Menten analysis (Goudar, Sonnad, & Duggleby, 1999) has been used to deduce approximate values of rate constants through measuring the rate of an enzymatic reaction as a function of substrate concentration. However, many of the assumptions employed in the formal derivation of the Michaelis Equation (Michaelis, Menten, Johnson, & Goody, 2011) are not valid under

\[
\frac{dC_F}{dt} = k_c A^x B^y
\]
physiological conditions (Schnell, 2014), (Grima & Schnell, 2006), (Zhou, Rivas, & Minton, 2008). Additionally, this analysis does not produce all of the constants needed to parametrize a mass action kinetic model. Ideally, the lumped reaction rate, found through applying flux balance analysis to a biochemical network, and an experimentally estimated maximal rate would provide sufficient constraint to deduce closed form solutions for the rate constants of a mass action mathematical model. This would allow for the kinetics of the network to be studied without a significant increase in modeling complexity.

However, before a mass action parametrization paradigm could be implemented within an entire genome scale metabolic model, the validity of this approach must be shown for a simple network. This investigation studies the canonical enzyme substrate binding model that is utilized in Michaelis-Menten analysis (Michaelis et al., 2011), shown in Figure 2. Although this biochemical network is simple, its vast use in enzymatic study provides the motivation for its examination.

\[
E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \rightarrow E + P
\]

Figure 2: Simple reaction network utilized in Michaelis-Menten Analysis representing uninhibited enzyme substrate binding.

In this report, a mathematical model of this network is created, and, using estimated true and maximal reaction rates, a method utilizing principles of flux balance analysis is developed to deduce the individual reaction rate constants in the network. In the Methodology, this method is mathematically derived and rigorously examined for a general case. Following, the Results and Discussion section apply this methodology with a specific true and maximal reaction rate to create a constrained mass action kinetic model. This model is then examined analytically and
computationally to expose the non-intuitive behavior and sensitivities of this network. Finally, in the Conclusion, an experimental protocol will be proposed to calculate the values of these rate constants.
**Methodology**

*Development of the Biochemical Test Network*

To test the validity of defining a mass action biochemical model of a simple reaction network from a lumped reaction rate and a maximal rate, the enzyme substrate binding model shown in Figure 3 was used. In this network, substrate enters the system continuously at a fixed rate $V_{in}$ and product flows out of the system at the lumped reaction rate, $V_{out}$, during steady state. The enzyme, E, and substrate, S, combine to form the intermediate complex, ES, where the product, P, is produced and the enzyme is released. For this system it is assumed that the substrate concentration remains constant throughout the simulation as substrate is pumped into the system at the same rate at which it is consumed.

\[
E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} E + P
\]

\[
V_{in} \Rightarrow S \quad V_{out} \Leftarrow P
\]

Figure 3: Reaction network under investigation. This network involves uninhibited enzyme substrate binding with the substrate flowing into the network at a constant rate $V_{in}$ and product diffusing out in proportion to product concentration eventually leading to a steady state rate of $V_{out}$. Each elementary reaction in this network is given a rate constant $k_i$, which is unknown.

**Mathematical Modeling**

Using the principle of mass action (Érdi, 1989), a mathematical model of this network was constructed and is shown below. However, for this model to be functional, closed form solutions for the rate constants, $k_1$, $k_2$, and $k_3$, need to be derived from the elementary steady state reaction rates of the network and initial conditions. For completeness, a fourth rate constant, $k_0$, must be defined to represent the coefficient of product diffusion. However, since the
concentration of product does not appear in any term of equation (2) that relates to enzyme, substrate, or enzyme-substrate complex concentrations, the product concentration is trivial for this analysis, as such, for simplicity \( k_0 = 1 \).

\[
\frac{dC_E(t)}{dt} = -k_1 C_E(t)C_S(t) + k_{-1} C_{ES}(t) + k_2 C_{ES}(t)
\]

\[
\frac{dC_S(t)}{dt} = -k_1 C_E(t)C_S(t) + k_{-1} C_{ES}(t) + V_{in}
\]  \( (2) \)

\[
\frac{dC_{ES}(t)}{dt} = k_1 C_E(t)C_S(t) - k_{-1} C_{ES}(t) - k_2 C_{ES}(t)
\]

\[
\frac{dC_P(t)}{dt} = k_2 C_{ES}(t) - k_0 C_P(t)
\]

To find the steady state reaction rates for each elementary step in the network, FBA can be employed to systematically deduce these values. To form the linear program, it is necessary to develop a stoichiometric S matrix shown below in equation (3).

\[
S \text{ Matrix: } \begin{array}{cccccccc}
E + S & \rightarrow & ES & & & & & \\
E & & & & & & & \\
S & & & & & & & \\
ES & & & & & & & \\
P & & & & & & & \\
\end{array}
\]

\[
\begin{array}{ccccccc}
E & -1 & 1 & 1 & 0 & 0 & \\
S & -1 & 1 & 0 & 1 & 0 & \\
ES & 1 & -1 & -1 & 0 & 0 & \\
P & 0 & 0 & 1 & 0 & -1 & \\
\end{array}
\]  \( (3) \)

Additionally, the domain of flux values must be established. As discussed above, for this network, the rate of product removal at steady state is \( V_{out} \), and the maximal possible rate (the rate if the reversibility of the enzyme-substrate complex formation is 0, \( k_{-1} = 0 \)) is \( V_{max} \).

Following, the domain of the flux vector is given in equation (4).

\[
Z = \begin{bmatrix}
[0, \infty) \\
V_{max} - V_{out} \\
[0, \infty) \\
V_{out}
\end{bmatrix}
\]  \( (4) \)
Before the linear program is fully developed, the final requirement is that an objective function 
be defined. However, for this network, this step is trivial, in that regardless of the choice of 
reaction flux to maximize, only a singular solution can exist to equation (1). However, for 
completeness, an objective vector, \( C \), will be defined, where the defined steady state removal of 
product is to be maximized.

\[
C = \begin{bmatrix}
0 \\
0 \\
0 \\
1
\end{bmatrix}
\]

(5)

With the above definitions, the following linear program is formulated.

\[
\max (v \cdot c) \text{ subject to } \\
v \in \mathbb{Z} \\
S \cdot v = 0
\]

(6)

Which, upon solving, reaction rates (fluxes) for each elementary step within the network are 
unambiguously defined. These flux values can be related to the rate constants that exist within 
the mass action model by solving equation (2) for a time \( t = \beta \), where \( \beta \) is some time at which the 
rate of formation of each species, \( i \), in the network is 0 or, equivalently, the system is at steady state.

\[
v_r = \begin{bmatrix}
v_1 \\
v_2 \\
v_3 \\
v_4 \\
v_5
\end{bmatrix} = \begin{bmatrix}
V_{max} \\
V_{max} - V_{out} \\
V_{out} \\
V_{out} \\
V_{out}
\end{bmatrix} = \begin{bmatrix}
k_1C_E(\beta)C_S(\beta) \\
k_{-1}C_{ES}(\beta) \\
k_2C_{ES}(\beta) \\
k_{-1}C_{ES}(\beta) \\
k_0C_P(\beta)
\end{bmatrix} = \begin{bmatrix}
k_1C_E(\beta)C_S(\beta) \\
k_{-1}C_{ES}(\beta) \\
k_2C_{ES}(\beta) \\
k_{-1}C_{ES}(\beta) \\
k_0C_P(\beta)
\end{bmatrix}
\]

(7)

However, this relationship is insufficient to determine the rate constant values, \( k_i \).

Additionally, the relations given in equation (2) do not determine a definitive relationship of \( k_i \) to
the flux vector, \( v_f \). Evaluating equation (2) when \( t = \beta \), yields the following relation for \( \frac{dC_E(t)}{dt} \).

\[
\frac{dC_E(\beta)}{dt} = 0 = k_1 C_E(\beta)C_S(\beta) + k_{-1} C_{ES}(\beta) + k_2 C_{ES}(\beta) = -v_1 + v_2 + v_3 \tag{8}
\]

Using equations (7) and (8), the system can be compacted into matrix form and reduced using Gaussian Elimination. Following reduction, the system returns to the relation given in equation (7) and is still underdetermined.

\[
\begin{bmatrix}
C_E(\beta)C_S(\beta) & 0 & 0 \\
0 & C_{ES}(\beta) & 0 \\
C_E(\beta)C_S(\beta) & -C_{ES}(\beta) & -C_{ES}(\beta)
\end{bmatrix}
\begin{bmatrix}
k_1 \\
k_{-1} \\
k_2
\end{bmatrix}
=
\begin{bmatrix}
v_1 \\
v_2 \\
v_3
\end{bmatrix}
\tag{9}
\]

\[
\Rightarrow
\begin{bmatrix}
C_E(\beta)C_S(\beta) & 0 & 0 \\
0 & C_{ES}(\beta) & 0 \\
0 & 0 & C_{ES}(\beta)
\end{bmatrix}
\begin{bmatrix}
k_1 \\
k_{-1} \\
k_2
\end{bmatrix}
=
\begin{bmatrix}
v_1 \\
v_2 \\
v_3
\end{bmatrix}
\tag{11}
\]

**Constraining the System**

To further constrain the system, two assumptions can be implemented. The first being that since the substrate concentration is much greater than the initial enzyme concentration, and substrate is being pumped into the system at exactly the same rate at which it is consumed at steady state, the concentration of substrate does not vary with time: \( \frac{dC_S(t)}{dt} = 0 \), and thus, \( C_S(t) = C_S(0) = S_0 \). Secondly, if enzyme cannot be transported across the system boundary, there is a conservation.

\[
C_E(0) = C_E(t) + C_{ES}(t) = E_0 \tag{10}
\]

These two assumptions can be implemented into equation (9), yielding equation (11).
\[
\begin{bmatrix}
C_E(\beta)C_S(\beta) & 0 & 0 \\
0 & C_{ES}(\beta) & 0 \\
0 & 0 & C_{ES}(\beta)
\end{bmatrix}
\begin{bmatrix}
k_1 \\
k_{-1} \\
k_2
\end{bmatrix}
= 
\begin{bmatrix}
v_1 \\
v_2 \\
v_3
\end{bmatrix}
= 
\begin{bmatrix}
(E_0 - C_{ES}(\beta) * S_0 & 0 & 0 \\
0 & C_{ES}(\beta) & 0 \\
0 & 0 & C_{ES}(\beta)
\end{bmatrix}
\begin{bmatrix}
k_1 \\
k_{-1} \\
k_2
\end{bmatrix}
\] (11)

**Computational Analysis**

This system, although still underdetermined, now exists with only one free variable. As such, the solution space and sensitivities can be reasonably explored computationally. The mass action model, equation (2), was implemented within a python script using the Numerical Python (van Der Walt et al., 2011) and Scientific Python (Jones, Oliphant, Peterson, & al., 2001) packages. The dynamics of this model were sampled by defining a value for each rate constant independently, following, the model was then parametrized by using equation (11). Following parameterization, the system was simulated using the odeint numerical differential equation solver for 700 time steps.

Each rate constant was systematically sampled with \( n = 1000 \) in a domain spanning four orders of magnitude, in correspondence with the domain of each rate constant. This domain was found from equation (11) and the conservation of enzyme which requires that \( C_{ES}(\beta) \in [0, E_0] \). These two relations form the below inequalities, equation (12), which serve as lower bounds for the rate constants values. The sampling interval was then designed with the lower bounds found from equation (12) and the upper bounds being four orders of magnitude greater.

\[
\frac{v_1}{S_0E_0} \leq k_1 \quad \frac{v_2}{E_0} \leq k_{-1} \quad \frac{v_3}{E_0} \leq k_2
\] (12)

Further, the stability of the network can be examined. From equation (11), the only variable that is not a rate constant is the steady state complex concentration, \( C_{ES}(\beta) \). As such, \( C_{ES}(\beta) \) can be solved for in terms of each rate constant independently, yielding equation (13).
\[ C_{ES}(\beta) = E_0 - \frac{v_1}{k_1S_0} = \frac{v_2}{k_{-1}} = \frac{v_3}{k_2} \]  

Then, the first derivative of \( C_{ES}(\beta) \) with respect to each rate constant can be found yielding equations (14-16).

\[
\frac{dC_{ES}(\beta)}{dk_1} = -\frac{v_1}{S_0k_1^2} \tag{14}
\]

\[
\frac{dC_{ES}(\beta)}{dk_{-1}} = -\frac{v_2}{k_{-1}^2} \tag{15}
\]

\[
\frac{dC_{ES}(\beta)}{dk_2} = -\frac{v_3}{k_2^2} \tag{16}
\]

This relation, along with the lower rate constant bound found from equation (12), allows for the maximal sensitivity with respect to each rate constant to be calculated, yielding equations (17-19).

\[
\max \left( \left| \frac{dC_{ES}(\beta)}{dk_1} \right| \right) = \frac{S_0E_0^2}{v_1} \tag{17}
\]

\[
\max \left( \left| \frac{dC_{ES}(\beta)}{dk_{-1}} \right| \right) = \frac{E_0^2}{v_2} \tag{18}
\]

\[
\max \left( \left| \frac{dC_{ES}(\beta)}{dk_2} \right| \right) = \frac{E_0^2}{v_3} \tag{19}
\]

Additionally, it can be shown that \( \lim_{k_1 \to \infty} \left| \frac{dC_{ES}(\beta)}{dk_1} \right| = 0 \) for all \( i \in \{1, -1, 2\} \), therefore \( C_{ES}(\beta) \) is asymptotically stable. To determine the dynamics of the near-stable regions of the system, \( k_1 \) and \( k_{-1} \) were chosen independently such that \( \left| \frac{dC_{ES}(\beta)}{dk_i} \right| \leq .0001 \). Using (11) and (14-15), the following inequalities are established for the rate constants in a near stable region, (20-21).

\[
k_1 \geq \frac{\sqrt{\frac{v_1}{(.0001)S_0}}} \tag{20}
\]
\[ k_{-1} \geq \sqrt{\frac{v_2}{0.0001}} \]  

(21)

The minimally stable systems were then parameterized by deducing all other rate constant values using (11) and computationally simulated using the numerical ODE solver mentioned above.
Results and Discussion

After rigorous mathematical analysis of the enzyme substrate binding model discussed above, it was shown that the deduced elementary reaction rates were not sufficient to define the rate constants embedded within the mass action kinetic model. However, after implementing many of the assumptions utilized in Michaelis Menten analysis, the system was reduced to a single free variable. To computationally examine the solution space of the model, an arbitrary product diffusion flux, $V_{\text{out}}$, was defined to be 13 and the maximal flux, $V_{\text{max}}$, was defined to be 15. Additionally, initial conditions were given with an initial substrate concentration, $S_0$, of 1000 and enzyme concentration, $E_0$, of 5, with the value of $k_0$ again being 1. For all other species, initial concentrations were set to 0. For this analysis, units are trivial. However, in practice flux values are often given in moles/second and concentration in moles. From (7), with $V_{\text{out}} = 13$ and $V_{\text{max}} = 15$, the following flux vector was produced.

$$
\mathbf{v}_f = \begin{bmatrix}
V_1 \\
V_2 \\
V_3 \\
V_4 \\
V_5
\end{bmatrix} = \begin{bmatrix}
V_{\text{max}} \\
V_{\text{max}} - V_{\text{out}} \\
V_{\text{out}} \\
V_{\text{out}} - V_{\text{in}} \\
V_{\text{out}} - V_{\text{in}}
\end{bmatrix} = \begin{bmatrix}
15 \\
13 \\
13 \\
2 \\
13
\end{bmatrix} = \begin{bmatrix}
k_1 C_E(\beta)C_S(\beta) \\
k_1(1 - C_E(\beta))(1000) \\
k_{-1} C_{ES}(\beta) \\
k_{-1} C_{ES}(\beta) \\
k_2 C_{ES}(\beta) \\
k_2 C_{ES}(\beta)
\end{bmatrix}
$$

(22)

To examine the solution space of this system, each rate constant was sampled individually, and the system was simulated for 1000 time steps. In the case of the first rate constant, $k_1$, near singular dynamics were observed from the network with only slight variations in substrate concentrations occurring. However, since substrate concentration was assumed constant throughout the simulation, the dynamics of the network can be considered identical. Additionally, final steady state concentrations of free enzyme were near zero for all sampled values of $k_1$. As such, nearly all the enzyme in the system was in the bound complex state. However, in examining the behavior of the system at values of $k_1$ near the lower bound, found
from (12), there was an increase in sensitivity with steady state enzyme concentrations varying rapidly as the value of $k_1$ changes.

![Graphs showing enzyme substrate binding kinetics under rate constant sampling. Each rate constant, $k_1$, $k_{-1}$, $k_2$, was independently sampled from a domain spanning nine orders of magnitude for $k_1$ and three for $k_{-1}$ and $k_2$. An increase in sensitivity was observed as rate constant values approach zero. Additionally, a greater dynamic range was produced over the sampling of $k_{-1}$ and $k_2$.](image)

In the case of $k_{-1}$, at large rate constant values free enzyme concentration approached zero, while the enzyme substrate complex concentrations approached five. This dynamic
signature is the opposite as what was seen when $k_1$ was sampled at large values. In keeping with the behavior seen in the previous exploration, there again was enhanced parameter sensitivity at $k_1$ values near the lower bound. At low values, steady state enzyme concentrations decreased rapidly, approaching the dynamic signature observed at high values of $k_1$. Similarly, in sampling $k_2$ at large values, an identical dynamic signature as that produced at large, $k_1$ was observed. However, the value at which the increased sensitivity began was greater than in the previous examination.

In all explorations, there existed sensitivity at rate constant values near the lower bound of their respective domain, and, further, the result of this sensitivity was instability in the steady state concentrations of the enzyme complex, as $Ces(\beta)$ varied smoothly in the interval $(0, E_0)$, shown in Figure 4. To examine this sensitivity in greater detail, equations (14-16) can be evaluated at rate constant values near their respective lower bounds. From this comparison, the maximal model sensitivity occurred with values of $k_1$ near its lower bound of 0.003, and the sensitivity asymptotically went zero with increasing rate constant values. Similarly, in $k_1$ and $k_2$, the maximal sensitivity occurred at the lower bound of the rate constant values, 0.4 and 2.6
respectively. However, the maximal rate of change in the steady state concentration of enzyme complex with respect to the rate constants, $k_1$ and $k_2$, was approximately three orders of magnitude less than that with respect to $k_1$, and the stability of $k_1$ increased as $k_1$ increased in value and did so more rapidly that the other two rate constants which underwent a more gradual transition into instability.

To fully describe the stable regions of this network, the dynamics were examined with rate constant values defined first for $k_1$ and then for $k_1$. This was sufficient as $k_1$ and $k_2$ differ only by a scaling factor, as shown above in equation (14) and in Figure 5. Following the analysis, the same stable dynamics were observed for a stable $k_1$ as for a stable $k_2$. To observe these stable dynamics, minimal rate constant values were chosen from the range given in equations (20-21), yielding the following two sets of rate constant parameters from equation (11):

$$\{k_1, k_1 - 1, k_2\} = \{12.24, 0.40009, 2.6006\},$$

$$\{0.003008, 141.4, 919.1\}.$$

These two parameter sets, describe the two, distinct, stable dynamical signatures, shown in Figure 6, which can be produced

Figure 5: Rate constants, $k_1, k_1 - 1, k_2$, as a function of $k_1$ and $k_1$. It is unnecessary to examine the rate constants as function of $k_2$ as they only differ from $k_1$ by a scale factor.
from this network. In the first, for large values of $k_1$, $k_{-1}$ and $k_2$, remain near their respective lower bound. As such, free enzyme concentrations were near zero at steady state, with bound complex concentrations near $E_0$. In the second parameter set, $k_{-1}$ and $k_2$ were large and $k_1$ was near its lower bound. Consequently, the opposite dynamics were observed: bound complex concentrations were near zero and free enzyme concentrations were near $E_0$. Therefore, it was concluded that when the steady state concentration of enzyme was on the order of the

Figure 6: Stable dynamics of the enzyme substrate binding network under investigation. The orange curves represent the solution of (2) with $\{k_1, k_{-1}, k_2\} = \{12.24, 0.40009, 2.6006\}$ and the blue curves with $\{k_1, k_{-1}, k_2\} = \{0.003008, 141.4, 919.1\}$.

Figure 7: Left: System dynamics when $k_1$ was sampled in a domain, [0.0045, 0.009], which generated steady state free and complexed enzyme concentrations intermediate to the dynamic signatures of the stable rate constant sets. Right: The relationship between $k_1$ and $k_{-1}$ is shown. In this domain, a more linear relationship existed between the rate constants then in the stable dynamics. The relationship between $k_1$ and $k_2$ was the same as that for $k_1$ and $k_{-1}$, with the only difference being a scale factor formed from the ratio of $v_3/v_2$.
steady state concentration of enzyme substrate complex, instability existed in the system where a small perturbation in rate constant values resulted in large variations in steady state chemical concentrations. Consequently, this scenario also resulted in a more linear relationship between $k_1$ and the other two rate constants.

In this context, biochemical network regulation factors into the interpretation and significance of the sensitivities and stable dynamics of the system. Rate constants can be considered to represent the activity of the enzyme, and although for most globular enzymes it is reasonable to assume their value will remain constant throughout a binding reaction (Érdi, 1989), they are dependent on external factors (temperature, pH, etc.) and structural considerations that alter their activity. The sensitivities that existed in the two stable dynamic states represent the aspects of the network with high potential for regulation. When steady state free enzyme concentration was near $E_0$, the system was very sensitive to $k_1$. Conversely, when enzyme complex concentration was near $E_0$, the system was highly sensitive to both $k_1$ and $k_2$. Making use of the dependence between $k_1$ and $k_2$, complete regulation of the system was possible.

Figure 8: Network dynamics at the steady dynamic signature produced when $\{k_1, k_2, k_3\} = \{0.003008, 141.4, 919.1\}$, but $k_1$ oscillates: $k_1(t) = (0.003008) + (0.003008)(0.05)\sin(3t) + \cos(5t))$, shown on the left. The perturbation translates through the network producing the more complex dynamics seen on the right.
through $k_1$ and $k_1$, which both belong to the preliminary binding reaction, $E + S \rightleftharpoons ES$. This reaction is then an ideal target to regulate the dynamics of this network.

The stability of the two opposite dynamical states were also suggestive of a biological switch. The network could easily transition between the two stable states, because at no dynamic state was the network insensitive to any rate constant. Through fine regulation of the enzyme activity, more complex time varying dynamics could be produced from this network simply through an oscillating temperature. To show this phenomenon, in the stable state defined by \( \{k_1, k_1, k_1, k_2\} = \{0.003008, 141.4, 919.1\} \), when $k_1$ oscillated within 5% of its value, after simulation the perturbed rate constant translated its value through the network due to the sensitivity.

Although complete parameterization of this network was not possible through definition of the lumped reaction rate and maximal rate, it did allow for rigorous mathematical analysis of the system at steady state. From this study, insight into the sensitivities of this network were obtained that allowed for determination of stable dynamic signatures that are unique to this network. In this section, a single set of reaction flux values were used in the analysis, but this approach generalizes nicely as shown in the Methodology.
Conclusion

This study, while limited in scope, demonstrates the complexity that exists in even the simplest of biological networks. Further, by elucidating the range of dynamics that can be obtained from such a system, the importance of rigorous modeling is amplified. Modern techniques developed for metabolic modeling, such as flux balance analysis, ignore these complexities, and although they allows for specific quantitative predictions, the large ambiguity that exists in these models must be recognized and treated appropriately. It may be that modeling frameworks that do not require kinetic information are incapable of predicting chemical concentrations and the mechanisms of regulation that exist in many such biological networks.

In addition to exposing the ambiguities in modern modeling frameworks, this study also provides a framework to experimentally derive the value of these rate constants. The mathematics developed in this study required that the rates of true and maximal (if no reversibility is assumed) product formation be known. An estimate of the latter can be easily achieved in a laboratory setting by placing a known quantity of substrate into a well-mixed solution, containing the enzyme, and measuring the quantity of product produced. If no reversibility existed in the network, obviously the amount of product formed should equal the amount of substrate. The ratio of substrate input, $S_{\text{in}}$, and product production, $v_{\text{actual}}$, then can be used as a scale factor to estimate the maximal reaction rate from the true overall reaction rate, $v_{\text{out}}$.

$$\zeta = \frac{S_{\text{in}}}{v_{\text{actual}}} \rightarrow S_{\text{in}} = v_{\text{max}} = \zeta v_{\text{out}}$$ (23)

Using a bioreactor like device, the system modeled in this study can be replicated by pumping substrate into the system that contains an initial enzyme concentration $E_0$, at a constant rate, such that at any time, $T$, $C_S(T) >> C_E(T)$. Then, measuring the rate of product produced,
\( v_{\text{out}} \), equation (23) can be used to calculate \( v_{\text{max}} \). To this point, the experimental procedure is very similar to that of Michaelis Menten analysis where the rate of reaction is calculated as function of substrate concentration. However, to deduce each individual reaction rate, instead of a lower resolution Michaelis Rate, either the steady state concentration of enzyme or complex must be measured. This crucial piece of information allows for unambiguous definition of rate constants due to the injectivity of equation (13).

In this study, the vast complexity and information that can be deduced from analysis of a simple network demonstrates the importance of quantitative approaches within biological systems. Through careful analysis, mechanisms of regulation can be found within mathematical models, and stable dynamics can be identified. Biological networks are too complex to infer using simplistic modeling, and there is a great need within the scientific community to develop frameworks to interpret and integrate high throughput biological data. However, before actual mechanisms can be discovered through analysis of this data, mechanistic models need to be developed such that the unknown parameters are identified and then approaches can be developed to either estimate these parameters from existing experimental data or protocols can be developed to glean the necessary biological insight. With computation, experimentation, and mathematical analysis operating in parallel, the biochemical networks that control life can be unraveled and modeled such that detailed quantitative predictions are made and emergent phenomena are identified.
References


