A Detailed Balance Approach to the Analysis of Enzymatic Reactions

Introduction

In 1913, German biochemist Leonor Michaelis and Canadian chemist Maud Menten published their seminal work describing the catalysis of a simple reaction by an enzyme. In brief, they investigated the following reaction scheme:

$$\mathrm{E} + \mathrm{S} \underset{\mathrm{k}_{-1}}{\overset{\mathrm{k}_1}{\rightleftharpoons}} \mathrm{E} \cdot \mathrm{S} \xrightarrow{\mathrm{k}_f} \mathrm{E} + \mathrm{P} \qquad \text{(Eq. 1)}$$

Michaelis and Menten asked what the rate of product formation, or $\frac{d[\mathrm{P}]}{dt}$, was. In this scheme, [E] represents free enzyme concentration, while [E ·S] represents enzyme substrate complex concentration—the sum of these, or total enzyme concentration, is given by $[\mathrm{E}]_0$. [S] and [P] refer to free substrate and free product concentrations, respectively.

Michaelis and Menten posited that in the early-time kinetic regime, where substrate concentration is far larger than the total concentration of enzyme, the system will be in a quasi-steady state (QSS)—that is, the concentrations of any chemical intermediates are constant. In this case, the intermediate in question is the enzyme-substrate complex.

In doing so, they derive the famous **Michaelis-Menten** equation:

$$\frac{d[\mathrm{P}]}{dt} = \frac{V_{max}}{1 + \frac{K_M}{[\mathrm{S}]}} \qquad \text{(Eq. 2)}$$

where V_{max} is the maximum reaction velocity for the enzyme in a solution of substrate, given by $k_f[\mathrm{E}]_0$, while K_M has earned the title of the Michaelis constant, and is given by $\frac{k_{-1}+k_f}{k_1}$. The typical derivation for this equation is ubiquitous in textbooks detailing enzymatic kinetics, and is described in full in the next section.

This work aims not to question the validity of Michaelis and Menten's work, but to instead bring to the surface pedagogically useful insights that may shed light on the rather uninterpretable parameters that come out of the derivation.

Review of Steady-State Theory

Before beginning with the central thrust, it is prudent to review the original Michaelis-Menten work. Consider again the scheme given in Equation 1 and note that the below rate equations follow when substrate concentration is far larger than total enzyme concentration (this implies that free substrate concentration is only marginally smaller, and for our purposes, equal to, total substrate concentration; i.e. [S] is equal to the total substrate concentration):

$$\frac{d[\mathrm{E} \cdot \mathrm{S}]}{dt} = k_1[\mathrm{E}][\mathrm{S}] - k_{-1}[\mathrm{E} \cdot \mathrm{S}] - k_f[\mathrm{E} \cdot \mathrm{S}]$$

$$\frac{d[\mathrm{P}]}{dt} = k_f[\mathrm{E} \cdot \mathrm{S}]$$

The principle we invoke now is that at QSS, the concentration of enzyme substrate complex must be constant. Thus the first equation above becomes

$$\frac{d[\mathrm{E} \cdot \mathrm{S}]}{dt} = 0 = k_1[\mathrm{E}][\mathrm{S}] - k_{-1}[\mathrm{E} \cdot \mathrm{S}] - k_f[\mathrm{E} \cdot \mathrm{S}]$$

From here it follows that

$$[\mathrm{E} \cdot \mathrm{S}] = \frac{k_1[\mathrm{S}]}{k_{-1} + k_f}[\mathrm{E}]$$

Note also that [E] represents only the free enzyme concentration, so we may write that

$$[\mathrm{E}]_0 = [\mathrm{E}] + [\mathrm{E} \cdot \mathrm{S}] = [\mathrm{E} \cdot \mathrm{S}] \left(\frac{k_{-1} + k_f}{k_1[\mathrm{S}]} + 1 \right)$$

Solving for [E · S] gives:

$$[\mathrm{E} \cdot \mathrm{S}] = \frac{[\mathrm{E}]_0}{\frac{k_{-1}+k_f}{k_1[\mathrm{S}]} + 1}$$

From here, the rate of product formation follows quickly as:

$$\frac{d[\mathrm{P}]}{dt} = k_f[\mathrm{E}\cdot\mathrm{S}] = \frac{k_f[\mathrm{E}]_0}{\frac{k_{-1}+k_f}{k_1[\mathrm{S}]}+1}$$

Defining $K_M = \frac{k_{-1}+k_f}{k_1}$ and $V_{max} = k_f[\mathrm{E}]_0$ yields exactly Eq. 2 as desired.

There is pedagogical utility in exploring the meaning of V_{max} and K_M. In particular, V_{max} is the rate of reaction when substrate concentration grows far larger than K_M. Mathematically, we can think of this is a horizontal asymptote for the Michaelis-Menten equation. The physical (and biochemical) interpretation is more interesting. The reaction is limited to being catalyzed by enzyme, so the reaction proceeds maximally when all the enzyme is saturated by product. Of course, this must mean that $[\mathrm{E}\cdot\mathrm{S}] = [\mathrm{E}]_0$ whence follows the definition of V_{max} from the rate equation for product formation.

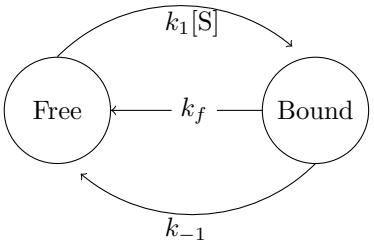
Perhaps the more interesting quantity is the Michaelis constant, K_M, which is an odd combination of rate constants–in fact, as we will later show, perhaps not so odd at all. Biochemistry students are often taught Michaelis-Menten kinetics soon after learning about protein-ligand dissociation and have thus seen an equation representing the equilibrium bound fraction of a protein in a ligand solution:

$$f = \frac{1}{\frac{K_D}{[\mathrm{L}]}+1}$$

Why does the structure of this equation bear so much resemblance to that of Michaelis and Menten? Many students aim to understand K_M as a dissociation constant, but the consensus varies—it is not technically true to call K_M a dissociation constant because it is a fundamentally different entity from the binding dissociation constant we conventionally discuss. As we show here, the students intuition is correct on a deep level—K_M does have a useful interpretation as a dissociation constant, such an interpretation that small problems in enxzymatic biochemistry can be addressed through its understanding.

An Equilibrium Detailed Balance Approach to Kinetics

In the provided kinetic picture, enzyme is initially free; it then binds a substrate, catalyzes a reaction, and then releases the product, returning to the free state. While in the kinetic picture, there are 3 sets of reactant/product "states" (either free enzyme and free substrate or free product, and the complex), we can think of this scheme as the enzyme shuttling between a free and bound state. Importantly, the steady state condition implies that since bound enzyme is an intermediate in the overall reaction scheme, its concentration is constant—which means the below Markov chain is in equilibrium, and thus obeys detailed balance.



2 State Model of Enzyme Kinetics

Note that there is only one transition from the free state to the bound state, whose rate is given by the forward effective rate constant for substrate loading onto the enzyme, while there are two transitions heading in the opposite direction, one of which has the rate of substrate dissociation while the other has the rate of product catalysis and release.

Our goal here should be to phenomenologically develop the Michaelis-Menten equation. We can begin by asserting that the rate of product formation should be given as

$$\frac{d[\mathrm{P}]}{dt} = \eta V_{max} \qquad \text{(Eq. 3)}$$

where V_{max} is the maximum reaction velocity possible, while η is a fraction that is at most 1, thus leading the rate to be a fraction of the maximum velocity. It is evident that the maximum reaction velocity occurs, as before, when all enzymes are totally saturated—that is, the free enzyme concentration goes to 0. In this limit, we will have that $[\mathrm{E}\cdot\mathrm{S}] = [\mathrm{E}]_0$, and necessarily the reaction velocity is $k_f[\mathrm{E}]_0$. This is no different than the V_{max} from Eq 2.

What is η? Intuitively, we can think of it as the fraction of total enzyme which is "active"—that is, primed to create product. In this case, that fraction is just the fraction of total enzyme which is in the complexed state—in other words, $\eta = p_{bound}$

Since our transition diagram is in equilibrium in our current perspective, we can make use of the following detailed balance principle.

Result 1 (2-State Probabilities in Detailed Balance)**.** *Given a transition scheme* $\mathrm{A} \underset{\mathrm{k_{-1}}}{\overset{\mathrm{k_1}}{\rightleftharpoons}} \mathrm{B}$*, in detailed balance the following holds.*

$$\begin{cases} k_+ p_A = k_- p_B \\ p_A + p_B = 1 \end{cases} \implies p_B = \frac{k_+}{k_+ + k_-} = \frac{1}{1+\frac{k_-}{k_+}}$$

The 2 state model proposed is now immediately useful. Note the analogy that $\eta = p_{bound} = p_B$, $k_+ = k_1[\mathrm{S}]$ and $k_- = k_{-1} + k_f$. From this we must have

$$\eta = \frac{1}{1+\frac{k_-}{k_+}} = \frac{1}{1+\frac{k_{-1}+k_f}{k_1[\mathrm{S}]}}$$

But this of course, if we define K_M as we did before, is just the result that

$$\eta = \frac{1}{1 + \frac{K_M}{[\mathrm{S}]}}$$

Thus if we maintain our definitions of V_{max} and K_M, we arrive at

$$\frac{d[\mathrm{P}]}{dt} = \eta V_{max} = \frac{V_{max}}{1 + \frac{K_M}{[\mathrm{S}]}}$$

which is the desired result of Eq. 2.

The approach described above is capturing the same mathematical details as the existing algebraic approach. Inherent to this formulation, though, is the deep intuition that quasi-steady state, though not explicitly equilibrium, does carry with it some signatures of the equilibrium of a 2 state system, an intricacy that persists up to the final result where it manifests as the similarity between the Michaelis-Menten equation and the equation describing the equilibrium bound fraction of a protein in a solution of its ligand.

Detailed Balance Provides a Rapid Approach to Difficult Extensions

What happens when we complexify? Consider the following reaction scheme:

$$\mathrm{E} + \mathrm{S} \underset{\mathrm{k_{-1}}}{\overset{\mathrm{k_1}}{\rightleftharpoons}} \mathrm{E}\cdot\mathrm{S} \underset{\mathrm{k_{-2}}}{\overset{\mathrm{k_2}}{\rightleftharpoons}} \mathrm{E}\cdot\mathrm{P} \xrightarrow{\mathrm{k_f}} \mathrm{E} + \mathrm{P} \qquad \text{(Eq. 4)}$$

Biochemically, the above reaction scheme describes the situation of slow product dissociation, slow enough that it merits consideration as its own reaction step. Importantly, this allows for product to back-convert to substrate while still in the active site of the enzyme, a feature not previously possible as product would have dissociated too quickly for back-conversion to occur. The classical algebraic QSS approach tackles this challenge head on, and arrives at a "Michaelis-Menten" equation of the same form as Eq. 2, but with

$$V_{max} = \frac{k_2 k_f [\mathrm{E}]_0}{k_2 + k_{-2} + k_f}$$

$$K_M = \frac{k_{-1}k_{-2} + k_{-1}k_f + k_2 k_f}{k_1(k_2 + k_{-2} + k_3)}$$

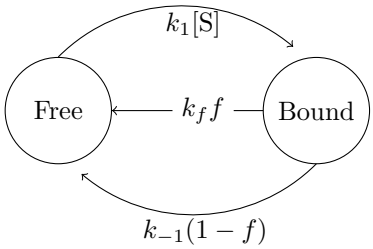
There is no easy reprieve from the algebra needed to arrive at these equations, and while not inherently difficult, the solution offers nothing instructive outside of practice in manipulating equations. Instead, we look to derive the above result via phenomenological arguments alongside the detailed balance method described previously.

We begin by positing that the velocity must take the form

$$\frac{d[\mathrm{P}]}{dt} = \eta V_{max}$$

for some η and V_{max}. Intuitively, the maximum velocity can no longer be $k_f[\mathrm{E}]_0$—while the rate of reaction we desire is still $k_f[\mathrm{E}\cdot\mathrm{P}]$, there is no way to fully load the enzyme with product. This is because there exists a reversible reaction between enzyme-substrate and enzyme-product complexes which is independent of all parameters except $k_{\pm 2}$; so long as these two rates are not orders of magnitude apart, there should be appreciable fraction of enzyme in each of the two states. It is true, however, that by maximally loading the enzyme with ligand in general, the probability that the enzyme is bound to product is also maximized. This indicates that $V_{max} = k_f[\mathrm{E}_0]f$ where f denotes the conditional probability that the enzyme is bound to product given it is bound to ligand. In other words, $f = p(\mathrm{E}\cdot\mathrm{P}|\mathrm{E}\cdot\mathrm{X})$ where X can represent either S or P.

Before solving for f, let us return to η. This must the probability that the enzyme is bound to anything at all, since the overall rate is given by $K_f[\mathrm{E}\cdot\mathrm{P}]$ and we have accounted for the conditional probability that given the enzyme is in complex with a ligand, it is in complex with the product. We can devise a 2 state transition model:



Weighted 2 State Model

Note here an incredibly important difference. Whereas before, the reverse rates where merely k_f and k_{-1}, now they are weighted. This is because the transition rate for exiting the bound state differs based on whether the enzyme is bound to substrate (in which case it is k_{-1}) or whether it is bound to product (in which case it is k_f). The solution here is to then weight the transition rates by the probabilities of their relative initial states: we know that a fraction f of the bound state is bound to product, so the transition rate attributable to product-bound complexes must be $k_f f$. The same logic applies to the substrate-bound complexes to yield both rates above.

We can then apply our previous work to arrive at

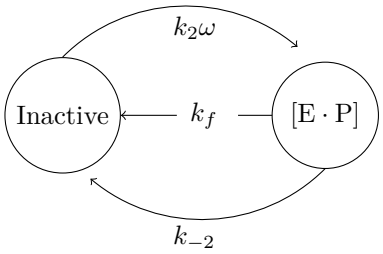
$$\eta = \frac{1}{1 + \frac{K_M}{[\mathrm{S}]}}$$

with

$$K_M = \frac{k_{-1}(1-f) + k_3 f}{k_1}$$

It is clear now that if we identify what f is, we will have solved the problem in its entirety. So what is the conditional probability that, given an enzyme is complexed to a molecule at all, it is bound to the product?

This question is addressable with another nifty 2 state transition model. Suppose we ask about the following scheme:



Active-Inactive Transition Model

Here, the off rates are easily interpretable; from the active state, you can either fall backwards or forwards through the reaction scheme at rates k_{-2} and k_f, respectively. However, the on rate is given by $k_2\omega$, where here ω represents the conditional probability that the enzyme is bound to substrate *given that it is not bound to product.* Explicitly, $\omega = p([\mathrm{E}\cdot\mathrm{S}]|\sim[\mathrm{E}\cdot\mathrm{P}]) = \frac{p([\mathrm{E}\cdot\mathrm{S}])}{p(\sim[\mathrm{E}\cdot\mathrm{P}])}$.

Detailed balance yields that

$$\frac{p([\mathrm{E}\cdot\mathrm{P}])}{p(\sim[\mathrm{E}\cdot\mathrm{P}])} = \frac{k_2\omega}{k_{-2}+k_f} = \frac{k_2}{k_{-2}+k_f}\cdot\frac{p([\mathrm{E}\cdot\mathrm{S}])}{p(\sim[\mathrm{E}\cdot\mathrm{P}])}$$

This readily yields that

$$\frac{p([\mathrm{E}\cdot\mathrm{P}])}{p([\mathrm{E}\cdot\mathrm{S}])} = \frac{k_2}{k_{-2}+k_f}$$

This is a useful result in the journey to obtaining f:

$$f = \frac{p([\mathrm{E}\cdot\mathrm{P}])}{p([\mathrm{E}\cdot\mathrm{S}])+p([\mathrm{E}\cdot\mathrm{P}])} = \frac{1}{\frac{p([\mathrm{E}\cdot\mathrm{S}])}{p([\mathrm{E}\cdot\mathrm{P}])}+1}$$

And so

$$f = \frac{1}{\frac{k_{-2}+k_f}{k_2}+1} = \frac{k_2}{k_2+k_{-2}+k_f}$$

While the algebra is not shown, it is easy to work out that, using this f in the previously derived V_{max} and K_M, one will obtain exactly the same result as was previously stated at the beginning of this section for V_{max} and K_M explicitly in terms of the rate constants.

Arguably, though, we see in the present method the mechanism by which each of these constants arrives in their final location. Instead of arriving at

$$V_{max} = \frac{k_2k_f[\mathrm{E}]_0}{k_2+k_{-2}+k_f}$$

$$K_M = \frac{k_{-1}k_{-2}+k_{-1}k_f+k_2k_f}{k_1(k_2+k_{-2}+k_f)}$$

we arrive at the equivalent

$$V_{max} = k_f[\mathrm{E}_0]f$$

$$K_M = \frac{k_{-1}(1-f)+k_ff}{k_1}$$

$$f = \frac{k_2}{k_2+k_{-2}+k_f}$$

The latter series of equations offers more interpretability, moving away from "rate constant soup" to a phenomenological description of the kinetic mechanism occurring underneath the final formulas.