

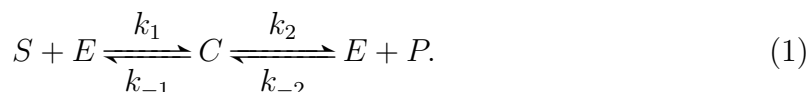
Systems Biology, Homework # 3, Part 2:

Biochemical Kinetics

Due Wednesday Feb 16th 11:59 pm

1. Reversible Michaelis-Menten kinetics

Recall the derivation of the Michaelis-Menten rate law for an enzyme-catalysed reaction, which began with the scheme:



(a) Write a differential equation model for the system dynamics (**not** assuming $k_{-2} = 0$).

(b) Applying a quasi-steady state assumption to the complex C , verify that

$$c^{qss} = \frac{k_1 e_T s + k_{-2} e_T p}{k_1 s + k_{-2} p + k_{-1} + k_2}, \quad (2)$$

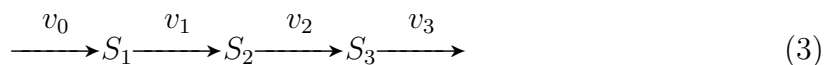
where e_T is the total concentration of the enzyme.

(c) Determine the overall reaction rate (i.e. the net rate of production of product) as a function of the concentrations of substrate and product.

(d) Confirm that when k_{-2} is taken to be zero, the irreversible Michaelis-Menten rate law is recovered.

2. First-order approximation of Michaelis-Menten kinetics

Consider the four-step reaction chain shown below. Here the label v_i is the **reaction rate**; it is *not* a mass action rate constant. So, for example $\frac{d}{dt}s_2(t) = v_1 - v_2$. (This is the conventional notation when labelling the rates of enzyme-catalysed reactions. It is unfortunately the same convention that's used for mass-action rate constants. We use the context and the variable name (v vs. k) to distinguish which case we're considering.)



Suppose the rate v_0 is fixed and presume the other rates are given by Michaelis-Menten kinetics, with

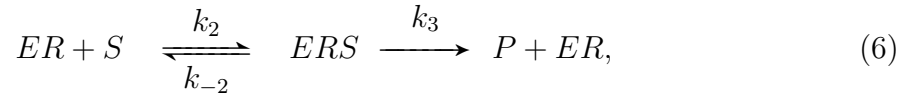
$$v_i = \frac{V_{\max}^i s_i}{K_{Mi} + s_i}. \quad (4)$$

Take parameter values $v_0 = 2$ mM/min, $V_{\max}^1 = 9$ mM/min, $V_{\max}^2 = 12$ mM/min, $V_{\max}^3 = 15$ mM/min, $K_{M1} = 1$ mM, $K_{M2} = 0.4$ mM, $K_{M3} = 3$ mM.

- (a) Simulate the system from initial conditions (in mM) $(s_1, s_2, s_3) = (0.3, 0.2, 0.1)$ and $(6, 4, 4)$.
- (b) Construct an approximate model in which the rates of reactions 1, 2, and 3 are replaced by mass action rate laws: $v_i = k_i s_i$. For $i = 1, 2, 3$, choose rate constants k_i so that the mass action rate is the linearization of the corresponding Michaelis-Menten rate law, centered at $s_i = 0$. This is called the *first-order approximation* for the Michaelis-Menten rate.
- (c) Simulate your simpler (mass-action based) model from the two sets of initial conditions in part (a). Explain why the approximation is better in one case than the other.

3. Allosteric activation

Consider an allosteric *activation* scheme in which an allosteric activator must be bound before an enzyme can bind substrate. This is called *compulsory activation*. The reaction scheme resembles a two-substrate reaction, but the enzyme-activator complex stays intact after the product dissociates:



where R is the allosteric activator (regulator).

- (a) Apply a quasi-steady-state assumption to the two complexes ER and ERS (and use enzyme conservation) to verify that the rate law takes the form

$$v = \frac{srk_3e_T}{r\frac{k_{-2}+k_3}{k_2} + \frac{k_{-1}(k_{-2}+k_3)}{k_1k_2} + sr} = \frac{V_{\max}sr}{K_1r + K_2 + rs} \quad (7)$$

where r is the regulator concentration and s is the substrate concentration.

- (b) Next, consider the case in which catalysis can only occur after n regulator molecules have bound. Assuming the binding involves strong cooperativity, we can approximate the regulator-binding events by a single meta-reaction:



Verify that in this case the rate law takes the form

$$v = \frac{V_{\max}sr^n}{K_1r^n + K_2 + r^n s} \quad (9)$$

- (c) Confirm that when regulator and substrate are at very low concentration, the rate law in part (b) can be approximated as

$$v = \frac{V_{\max}}{K_2} sr^n. \quad (10)$$

4. **Negative cooperativity.**

In our discussion of cooperative binding, we focused on *positive cooperativity*, in which the substrate binding affinity increases as substrates bind. Some proteins, such as the enzyme glyceraldehyde-3-phosphate dehydrogenase, exhibit *negative cooperativity*—substrate affinity drops as substrates bind.

- (a) Consider a version of the Adair equation [(3.18) in the Ingalls text] for a protein with two binding sites (as in Exercise 3.3.1; there's no need to derive this equation). Plot the curve for a negative cooperative case (e.g. for $K_2 > K_1$.) Is the curve sigmoidal?
- (b) An extreme case of negative cooperativity is known as *half-of-the-sites reactivity*, in which the affinity drops to zero once half of the binding sites are occupied. Referring again to the Adair equation for two sites, what is the form of the binding curve in this extreme case?