Worksheet: Biochemical reactions

Read: Chapters 2.3-2.7 from Bistability in the lactose operon on *Escherichia coli*: A comparison of differential equation and Boolean network models., by R. Robeva, and N. Yildirim. Pages 40–73.

1. Consider the following biochemical reaction:

\[ A + 2B \xrightarrow{k_1} C \xrightarrow{k_3}{k_2} D. \]

Assuming mass-action kinetics (the reaction rate is proportional to the number of molecular collisions), write down a formula for \( d[C]/dt \).

2. Consider the reactions where two substrates \( S \) and \( T \) compete for binding to an enzyme \( E \) to produce two different products \( P \) and \( Q \):

\[
E + S \xrightarrow{p_1}{p_2} ES \xrightarrow{p_3} P + E \\
E + T \xrightarrow{q_1}{q_2} ET \xrightarrow{q_3} Q + E
\]

Assume that each reaction follows the Michaelis-Menten kinetics. Also, assume that the initial enzyme concentration is \( E_0 = [E] + [ES] + [ET] \).

(a) Derive rate equations for \( P \) and \( Q \) in this system in terms of \([ES]\) and \([ET]\). That is, determine \( d[P]/dt \) and \( d[Q]/dt \).

(b) Derive rate equations for \( ES \) and \( ET \).

(c) Assume that the enzyme-substrate complexes reach equilibrium quickly: \( d[ES]/dt \approx 0 \) and \( d[ET]/dt \approx 0 \). Solve for \( [E] \) in each of these equations.

(d) Equate the two expressions for \( [E] \) from Part (c) and solve for \( [ET] \).

(e) Solve for \( [ES] \) by plugging your answers to Parts (c) and (d) into \( E_0 = [E] + [ES] + [ET] \). You should not have \( [E] \) or \( [ET] \) in your final answer.

(f) Plug this into the original ODE for \( d[P]/dt \).

(g) Repeat the previous three steps but solve for \( ES \) instead of \( ET \).

(h) Explain the effects of the competition occurring.

3. The Hill equation is an approximation for multi-molecule binding and it assumes simultaneous binding of \( n \)-molecules of a substrate \( S \) to the enzyme \( E \). Suppose that two molecules of the substrate \( S \) are undergoing a reaction with an enzyme in an ordered manner as follows:

\[
E + S \xrightarrow{k_1}{k_2} ES + S \xrightarrow{k_3}{k_4} ES_2 \xrightarrow{k_5} P + E.
\]

Assume that the reaction follows the Michaelis-Menten kinetics and that the initial enzyme concentration is \( E_0 = [E] + [ES] + [ES_2] \).
(a) Derive rate equations for $P$, $ES$, and $ES_2$. Be very careful with the $ES \xrightarrow{k_2} E + S$ reaction when deriving the equation for $[ES]$.

(b) Assume that $d[ES_2]/dt \approx 0$ and solve for $[ES]$.

(c) Assume that $d[ES]/dt \approx 0$. Plug your answer to Part (b) into this and solve for $[E]$.

(d) Plug your expressions for $[E]$ and $[ES]$ back into $E_0 = [E] + [ES] + [ES_2]$ and solve for $[ES_2]$.

(e) Derive an ODE for $[P]$ of the form $d[P]/dt = f([S])$.

(f) Compare your answer to Part (d) to the Hill equation with Hill coefficient $n = 2$:

$$\frac{d[P]}{dt} = \frac{V_{\text{max}}[S]^2}{K_m + [S]^2}.$$  

When do these two equations become roughly the same?

4. Recall another model of the lac operon that we saw last time:

$$f_M = A,$$

$$f_B = M,$$

$$f_A = (B \land L_m) \lor L \lor (A \land \overline{B}).$$

Does this model exhibit bistability? Why or why not?

5. Recall our original 3-variable Boolean model of the lac operon:

$$f_M = \overline{G_e} \land (L \lor L_e),$$

$$f_E = M,$$

$$f_L = \overline{g_e} \land ((E \land L_e) \lor (L \land \overline{E})).$$

Since this model cannot distinguish between basal, medium, and high levels of lactose, it cannot exhibit bistability. Add a new parameter $L_{em}$ that stands for “at least medium levels of extracellular lactose”. Modify the Boolean functions so the new model exhibits bistable behavior for medium lactose concentrations.