BioE 135/235 Homework 1 Solutions

Due Date: Thursday, 2/11/2021, 5:00pm PST to Gradescope
Late work will be deducted 20% for each day it is late.

1. Brief Linear Algebra Review

(a) Perform the following matrix multiplication:

$$\vec{y} = A\vec{x} \tag{1}$$

$$A = \begin{bmatrix} 1 & 0 & 3 \\ 2 & -2 & 1 \end{bmatrix}, \vec{x} = \begin{bmatrix} 1 \\ 2 \\ 4 \end{bmatrix}$$

$$\vec{y} = \begin{bmatrix} 13 \\ 2 \end{bmatrix}$$

(b) For the following matrices, determine (1) the column space, (2) the null space, (3) the dimensions of the column and null spaces, and (4) the eigenvalues of the matrix.

(i) $\begin{bmatrix} 0 & 1 & 3 \\ 4 & 4 & 1 \\ 1 & 0 & 0 \end{bmatrix}$

Column Space: By inspection, the second two columns will allow one to reach any point $\begin{bmatrix} a \\ b \\ 0 \end{bmatrix}$. The addition of the first column allows one to reach every point in $\mathbb{R}^3$. Therefore the column space is all of $\mathbb{R}^3$ (dimension: 3), the null space is the empty set (dimension: 0), and from Wolfram Alpha, we have that the eigenvalues of this matrix are:
$\lambda_1 = 4.964, \lambda_2 = -2.047, \lambda_3 = 1.083.$

(ii) $\begin{bmatrix} 1 & 0 & 0 \\ 2 & 2 & 0 \\ 0 & 0 & 0 \end{bmatrix}$

Once again: by inspection, the third column does not contribute to the column space of the matrix, meaning that the first two columns constitute the column space $\Rightarrow$Col(A) = $\begin{bmatrix} a \\ b \\ 0 \end{bmatrix}$, $a, b \in \mathbb{R}$. Thus $\dim(Col(A)) = 2, \dim(Nul(A)) = 1$. For a triangular matrix, the eigenvalues are the diagonals $\Rightarrow \lambda_1 = 1, \lambda_2 = 2, \lambda_3 = 0$.

(iii) $\begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix}$

This is the zero matrix; its column space is the empty set (dimension 0), its nullspace is the entirety of $\mathbb{R}^3$ (dimension 3), and its eigenvalues are all 0.

2. Modeling The Central Dogma

Over the course of this question, you will develop a model for the central dogma, and define/defend the assumptions and limitations thereof. The emphasis of this question is less on the rigorous correctness of your model, and more on your ability to clearly state and explain your ideas. Please feel free to look things up, but cite sources when necessary.

(a) List a set of reactions that adequately encapsulates transcription and translation in *E. coli*. Your reactions should be sufficiently detailed as to describe open-complex formation during transcription, RNA polymerase binding, and ribosome binding.

Many different answers would have been acceptable here; to give an example of one:

$$DNA \leftrightarrow DNA_{OC}$$
$$DNA_{OC} + RNAP \leftrightarrow DNA_{OC} : RNAP[0]$$
$$DNA_{OC} : RNAP[i] + Base \rightarrow DNA_{OC} : RNAP[i+1]$$
$$DNA_{OC} : RNAP[n] \rightarrow DNA + RNAP + mRNA$$
$$mRNA + ribosome \leftrightarrow mRNA : ribosome$$
$$mRNA : ribosome \leftarrow protein + mRNA + ribosome$$
$$mRNA \leftarrow \emptyset$$
$$protein \leftarrow \emptyset$$

(b) How accurate is your model? Are there any shortcomings? If so, what are they?

Of course, answers will vary from person to person; one assertion could be that the model above – and, foreseeably, most models – miss out on the possibility of mutation in the system.

(c) For each of the following biological events, estimate the timescale thereof and order them from fastest to slowest: protein phosphorylation, bacterial cell division, ATP production, and bacterial chromosome replication.

ATP production: 10^6 - 0.001 sec (https://doi.org/10.1016/j.cell.2005.10.001)

Protein phosphorylation: 10^6 - 0.01 sec (enzyme catalysis varies, for example: carbonic anhydrase kcat = 10^6/s, alkaline phosphatase kcat = 100/s)

Bacterial chromosome replication: Assuming an average of 4.7 million base pairs (average for E. coli), an average bacterial chromosome will take 20 - 30 minutes to replicate.

Bacterial cell division: for E. coli in exponential growth at 37∘C = 20 - 40 min

3. Jupyter/iPython Simulation

For this question, see the Jupyter notebook in the Homework folder on bCourses. If you do not have Jupyter installed, feel free to check out this video, or email Nick if you have any questions.

See the attached Jupyter Notebook.

4. A Sample Reaction

For this question, consider the following reaction scheme:

$$A \to B$$
$$B \to 2C$$
$$B \to D$$
$$C + D \to A$$

(a) There is something wrong with this mechanism; what is it?

This reaction scheme defies the law of conservation of mass; with 3 molecules of A, we can only make 2 molecules of A.

(b) Ignoring this error, express deterministic differential equations that describe this system in the form:

$$\frac{d}{dt}\begin{bmatrix} A \\ B \\ C \\ D \end{bmatrix} = \sigma\vec{\nu}$$

where σ is the matrix of constants, and $\vec{\nu}$ is a vector of rate laws. What are the sizes of σ and $\vec{\nu}$?

$$\sigma = \begin{bmatrix} -1 & 0 & 0 & 1 \\ 1 & -1 & -1 & 0 \\ 0 & 2 & 0 & -1 \\ 0 & 0 & 1 & -1 \end{bmatrix}, \vec{\nu} = \begin{bmatrix} k_1[A] \\ k_2[B] \\ k_3[B] \\ k_4[C][D] \end{bmatrix}$$

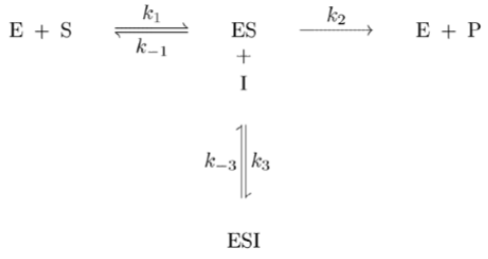
(c) Modify **one** reaction from above to fix the problem you identified in part (a).

A couple of things could work here; one viable one would be

$$2C + D \to 2A$$

5. Uncompetitive Enzyme Kinetics

Consider the following reaction scheme, demonstrating uncompetitive inhibition:



(a) Using either QEA or QSSA, show that the reaction rate $\frac{dP}{dt}$ can be written as $\frac{dP}{dt} = k_2[E_{tot}]\frac{[S]}{\frac{k_{-1}}{k_1} + C[S]}$ for some C. State all simplifying assumptions. What is the value of C?

We have the following 3 reactions:

$$E + S \leftrightarrow ES$$
$$ES + I \leftrightarrow ESI$$
$$ES \rightarrow E + P$$

The first two reactions are reversible, and the product-forming reaction is depicted as irreversible due to the large free-energy penalty of going in the reverse direction as well as the low concentration of the product. Thus, this final reaction is our rate-determining step. Note that the subscript 2 was dropped from the rate constant for clarity since this is the only rate constant we are tracking.

To derive the rate expression using a quasi-equilibrium assumption, we must first find the dissociation constants for the 2 non rate-determining steps in the reaction scheme. These are:

$$K_M = \frac{[E][S]}{[ES]}$$
$$K_I = \frac{[ES][I]}{[ESI]}$$

As stated above, we know that our reaction rate will be derived directly from the rate-determining step with rate k, and thus we have:

$$\begin{aligned}\frac{dP}{dt} &= k[ES] \\ &= k\frac{[E][S]}{K_M}\end{aligned}$$

Additionally, we know that the total enzyme concentration is constant, and thus:

$$[E_T] = [E] + [ES] + [ESI]$$

Substituting with the dissociation constants from above, we find that:

$$\begin{aligned}[E_T] &= [E] + \frac{[E][S]}{K_M} + \frac{[ES][I]}{K_I} \\ &= [E] + \frac{[E][S]}{K_M} + \frac{[E][S][I]}{K_M K_I} \\ &= [E]\left(1 + \frac{[S]}{K_M} + \frac{[S][I]}{K_M K_I}\right) \\ \Rightarrow [E] &= \frac{[E_T]}{\left(1 + \frac{[S]}{K_M} + \frac{[S][I]}{K_M K_I}\right)}\end{aligned}$$

Now, substituting this back into our previous expression, we get:

$$\begin{aligned}\frac{dP}{dt} &= k\frac{[E][S]}{K_M} \\ &= k\frac{[E_T][S]}{K_M\left(1 + \frac{[S]}{K_M} + \frac{[S][I]}{K_M K_I}\right)}\end{aligned}$$

Simplifying and rearranging this yields:

$$\frac{dP}{dt} = k_2[E_{tot}]\frac{[S]}{\frac{k_{-1}}{k_1} + \left(1 + \frac{[I]}{K_I}\right)[S]}$$

And thus $C = \left(1 + \frac{[I]}{K_I}\right)$.

(b) Putting this into the form of $\frac{dP}{dt} = V_{max}\frac{[S]}{K_M + [S]}$, what is the effect of changing the inhibitor concentration on K_M and V_{max}? How does this compare to competitive and noncompetitive inhibition?

Consolidating the C term into K_M and V_{max} yields that $K'_M = K_M/C$, $V'_{max} = V_{max}/C$. Given that $C > 1$, it follows that uncompetitive inhibition decreases both K_M and V_{max}.

This mechanism of decreasing is unique, and is further explained here. As inhibitor binds, the amount of ES complex is reduced. This reduction in the effective concentration of the ES complex can be explained by the fact that having the inhibitor bound to the ES complex essentially converts it to ESI complex, which is considered a separate complex altogether. This reduction in ES complex decreases the maximum enzyme activity (), as it takes longer for the substrate or product to leave the active site. The reduction in - the substrate concentration at which the enzyme can operate at half of its maximal velocity, often used to approximate an enzyme's affinity for a substrate - can also be linked back to the decrease in ES complex. Le Chatelier's principle opposes this decrease and attempts to make up for the loss of ES, so more free enzyme is converted to the ES form, and the amount of ES increases overall. An increase in ES generally indicates that the enzyme has a high degree of affinity for its substrate. decreases as affinity for a substrate increases, though it is not a perfect predictor of affinity since it accounts for other factors as well; regardless, this increase in affinity will be accompanied by a decrease in.

Competitive inhibitors can only bind to E and not to ES. They increase by interfering with the binding of the substrate, but they do not affect because the inhibitor does not change the catalysis in ES because it cannot bind to ES.

A noncompetitive inhibitor binds to a different site that is not the active site of the enzyme and changes the structure of the enzyme; therefore, it blocks the enzyme from binding to substrate, which stops enzyme activity. Thus, it decreases the rate of the chemical reaction of enzyme and substrate, which can not be changed by increasing concentration of substrate; the binding decreases and has no change on the of the chemical reaction.

(c) Describe how the mechanism of action of uncompetitive inhibition compares to that of competitive and noncompetitive inhibition. In what circumstances could this be useful to a cell?

See above for descriptions of uncompetitive inhibition compared to competitive inhibition and noncompetitive inhibition.

The unique traits of uncompetitive inhibition lead to a variety of implications for the inhibition's effects within biological and biochemical systems. Uncompetitive inhibition is present within biological systems in a number of ways. In fact, it often becomes clear that the traits of inhibition specific to uncompetitive inhibitors, such as their tendency to act at their best at high concentrations of substrate, are essential to some important bodily functions operating properly.

For example, it is part of the mechanism by which NMDA (N-methyl-D-aspartate) glutamate receptors are inhibited in the brain, for example. Specifically, this type of inhibition impacts the granule cells that make up a layer of the cerebellum. These cells have the aforementioned NMDA

receptors, and the activity of said receptors typically increases as ethanol is consumed. This often leads to withdrawal symptoms if said ethanol is removed. Various uncompetitive blockers act as antagonists at the receptors and modify the process, with one example being an inhibitor called memantine. In fact, in similar cases (involving over-expression of NMDA, though not necessarily via ethanol), it has been shown that uncompetitive inhibition helps in nullifying the over-expression due to its particular properties. Since uncompetitive inhibitors block high concentrations of substrates very efficiently, their traits alongside the innate characteristics of the receptors themselves lead to very effective blocking of NMDA channels when they are excessively open due to massive amounts of NMDA agonists.