**BE 25 Winter 2024**
**Homework #3**
Due at 9 AM PST, January 25, 2024

**Problem 3.1** (Evaluating the QSSA in Michaelis-Menten mechanisms, 50 pts)**.**
In this problem, you will explore the regimes in which the quasi-steady state approximation (QSSA) is a good approximation for Michaelis-Menten reaction scheme,

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}. \tag{3.1}$$

As we have learned, the Michaelis-Menten equation,

$$\frac{\mathrm{d}c_\mathrm{P}}{\mathrm{d}t} \equiv v_0 = k_\mathrm{cat}\, c_\mathrm{E}^0 \, \frac{c_\mathrm{S}/K_\mathrm{M}}{1 + c_\mathrm{S}/K_\mathrm{M}}, \tag{3.2}$$

can be derived from the mass action ODEs for the reaction scheme using the QSSA, which gives $k_\mathrm{cat} = k_2$ and $K_\mathrm{M} = (k_{-1} + k_2)/k_1$. Here, you will directly solve the Michaelis-Menten ODEs numerically without approximation and compare your result to the numerical results to assess the validity of the QSSA.

a) The ODEs corresponding to the Michaelis-Menten chemical reaction scheme can be written as

$$\frac{\mathrm{d}c_\mathrm{S}}{\mathrm{d}t} = -k_1(c_\mathrm{E}^0 - c_\mathrm{ES})c_\mathrm{S} + k_{-1}\, c_\mathrm{ES}, \tag{3.3}$$

$$\frac{\mathrm{d}c_\mathrm{ES}}{\mathrm{d}t} = k_1(c_\mathrm{E}^0 - c_\mathrm{ES})c_\mathrm{S} - (k_{-1} + k_2)c_\mathrm{ES}, \tag{3.4}$$

$$\frac{\mathrm{d}c_\mathrm{P}}{\mathrm{d}t} = k_2\, c_\mathrm{ES}, \tag{3.5}$$

where I have used conservation of enzyme to eliminate $c_\mathrm{E}$ from the dynamical equations. There are four parameters ($k_1$, $k_{-1}$, $k_2$, and $c_\mathrm{E}^0$) in the above equations, which makes exploration of parameter space in our numerical calculations difficult. **Nondimensionalization** is a common procedure to reduce the number of parameters we need to consider. To nomdimensionalize a set of equations, we divide all variables (in this case, $c_\mathrm{S}$, $c_\mathrm{P}$, $c_\mathrm{ES}$, and $t$) by a constant defined by some combination of the parameters. Show that by defining dimensionless variables

$$\tilde{t} = \frac{k_2\, c_\mathrm{E}^0}{K_\mathrm{M}}\, t \tag{3.6}$$

$$\tilde{c}_\mathrm{S} = c_\mathrm{S}/K_\mathrm{M}, \tag{3.7}$$

$$\tilde{c}_\mathrm{P} = c_\mathrm{P}/K_\mathrm{M}, \tag{3.8}$$

$$\tilde{c}_{ES} = c_{ES}/c_E^0, \tag{3.9}$$

the dynamical equations may be written as

$$\kappa \frac{d\tilde{c}_S}{d\tilde{t}} = -(1-\tilde{c}_{ES})\tilde{c}_S + (1-\kappa)\tilde{c}_{ES}, \tag{3.10}$$

$$\kappa\zeta \frac{d\tilde{c}_{ES}}{d\tilde{t}} = (1-\tilde{c}_{ES})\tilde{c}_S - \tilde{c}_{ES}, \tag{3.11}$$

$$\frac{d\tilde{c}_P}{d\tilde{t}} = \tilde{c}_{ES}, \tag{3.12}$$

allowing us to reduce the number of parameters from four to two. Be sure to clearly write $\zeta$ and $\kappa$ in terms of the respective parameters that comprise them. Henceforth for notational convenience, you can drop the tildes from the dimensionless variables, operating with the understanding that all variables are dimensionless.

b) Provide a physical interpretation of the two parameters $\zeta$ and $\kappa$.

c) Using a QSSA, write down the dimensionless Michaelis-Menten equation using the nondimensionalization scheme from part (a). In so doing, show that the dimensionless Michaelis-Menten equation is parameterless.

d) Write a code to numerically solve the dimensionless system of ODEs given by equations (3.10), (3.11), and (3.12). Use initial conditions $\tilde{c}_S = 1$ and $\tilde{c}_P = \tilde{c}_{ES} = 0$. Plot the result. Do this for various values of the parameters $\kappa$ and $\zeta$. Overlay a plot of the solution to the appropriately nondimesionalized Michaelis-Menten equation (3.2). You can either use the analytical solution of the Michaelis-Menten equation or a numerical solution. If you choose to plot the analytical solution, the `scipy.special.lambertw()` function may be useful. Comment on what you see. Specifically, for what parameter regimes is the QSSA a good approximation?

**Problem 3.2** (The Polach-Widom experiment and excess enzyme, 50 pts)**.**
*This problem is inspired by Chapter 8 of Helmut Schiessel's book* Biophysics for Beginners, 2nd Ed.

In eukaryotic cells, DNA is wrapped around histones and packaged into chromosomes. The transcription machinery is sterically occluded from accessing the DNA when it is wrapped around the histone. The DNA "breathes" on the histone, becoming unwound on occasion. The more time the DNA is unwrapped, the easier it is for the transcription machinery to engage and for the gene associated with the segment of DNA to be expressed. So, a quantitative understanding of the dynamics of DNA-histone interactions is valuable to learn about regulation of gene expression.

To address this question, Polach and Widom (*J. Mol. Biol.*, **254**, 130, 1995) devised a now-classic experiment, depicted in Fig. 1. They purified histone-DNA complexes where the DNA sequence contains a recognition sequence for a restriction enzyme. The restriction enzyme cuts the DNA at the restriction site. They can then measure the number of cut fragments over time to learn about the dynamics of unwinding. In this problem, we will work out the chemical kinetics to see how we can interpret the experiment. The ultimate goal is to figure out the probability that the DNA is unwound from the histone.

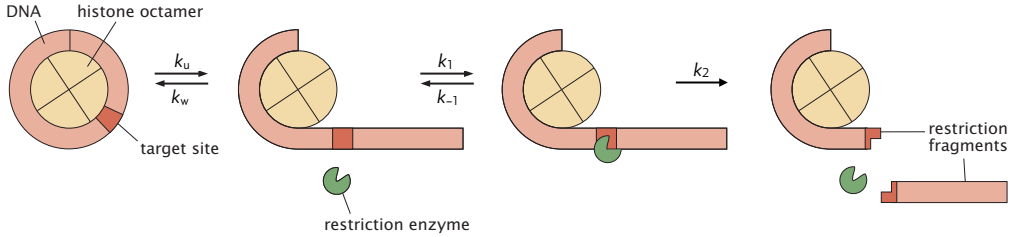


Figure 1: Schematic of the chemical reactions of the Polach-Widom experiment. DNA wrapped around a nucleosome reversibly unwinds exposing a target site whose sequence is the recognition sequence for a restriction enzyme. The restriction enzyme then binds reversibly to the target site. Bound restriction enzyme can then irreversibly cleave the DNA. Figure adapted from Fig. 10.22 of Phillips, Kondev, Theriot, and Garcia, *Physical Biology of the Cell, 2nd Ed.*, 2012, which was itself adapted from Polach and Widom, *J. Mol. Biol.*, **254**, 130, 1995.

a) As will become clear as we work out this problem, Polach and Widom needed a measurement of the rate of cleavage for bare DNA in the absence of DNA. The reaction scheme for this scenario is the same as in Fig. 1, except without the first step. We can write it in text as

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}. \tag{3.13}$$

Here, E denotes the restriction enzyme, S the restriction site, and P is the cut fragment. Not surprisingly, this is the reaction scheme for Michaelis-Menten kinetics. In Problem 3.1, we worked out the conditions under which the quasi-steady state approximation (QSSA) holds and we can use the Michaelis-Menten equation to describe the dynamics. In Polach and Widom's experimental setup, the QSSA does *not* hold. But not to worry! Polach and Widom set up their experiment such that the total concentration of restriction enzyme was much greater than the total concentration of cleavage sites on the DNA, such that $c_\mathrm{E}^0 \gg c_\mathrm{S}^0$. As a result, we can make another simplification, which is that $c_\mathrm{E} \approx c_\mathrm{E}^0$, a constant. So that with this approximation, the dynamics may be written as a linear system of equations, which can be written in matrix form as

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix} c_\mathrm{S} \\ c_\mathrm{ES} \end{pmatrix} = \mathsf{A} \cdot \begin{pmatrix} c_\mathrm{S} \\ c_\mathrm{ES} \end{pmatrix}. \tag{3.14}$$

Write down the matrix A. Why do we not need to include $c_P$ in the equation (and in fact should not include it)?

b) Show that if

$$r_{\text{bare}} \equiv \frac{k_1 k_2 c_E^0}{(k_1 c_E^0 + k_{-1} + k_2)^2} \ll 1, \tag{3.15}$$

the slowest time scale of the dynamics is $1/k_{\text{bare}}$, such that $c_P(t) \approx c_S^0(1 - e^{-k_{\text{bare}}t})$, assuming we start with no cleaved product. Be sure to write an expression for $k_{\text{bare}}$.

c) Now consider the full reaction scheme in Fig. 1. Denote by $c_W$ the concentration of wound cut sites, represented by the leftmost image in the figure. We will make a rapid steady state approximation for the winding/unwinding reaction such that the dynamics of that reaction are much faster that those of the others. Let $f$ be the fraction of sites that are available for cleavage. Note that $f$ is the key quantity of interest. We want to know how much of the time a segment of DNA is free of the histone. Show that $f = k_u/(k_u + k_w)$.

d) Show that the dynamics may be written as

$$\frac{d}{dt}\begin{pmatrix} c_W + c_S \\ c_{ES} \end{pmatrix} = \mathsf{B} \cdot \begin{pmatrix} c_W + c_S \\ c_{ES} \end{pmatrix}. \tag{3.16}$$

Be sure to write down an expression for **B**. You should include $f$ in your expressions; that is, do not write it out at $k_u/(k_u + k_w)$.

e) Show that if

$$r_{\text{hist}} \equiv\ll \frac{k_1 k_2 c_E^0 f}{(k_1 c_E^0 f + k_{-1} + k_2)^2} \ll 1, \tag{3.17}$$

that, analogously to part (b), $c_P(t) \approx (c_W^0 + c_S^0)(1 - e^{-k_{\text{hist}}t})$. Be sure to write an expression for $k_{\text{hist}}$. You can use previously derived results if they are useful.

f) Show that if $k_1 c_E^0/(k_{-1} + k_2) \ll 1$, then $f = k_{\text{hist}}/k_{\text{bare}}$. This means that Polach and Widom could measure the production of cleaved product modeled as a simple exponential for bare DNA and also in the presence of histones, and from those measurements they could work out the fraction of time the DNA is detached from a histone.

g) *Not graded.* There were a lot of assumptions that led to the handy, experimentally very useful result that $f = k_{\text{hist}}/k_{\text{bare}}$. These do, in fact, hold! You can read the analysis in Prinsen and Schiessel, *Biochimie*, **92**, 1722, 2010, where they investigate measured parameter values and verify that the assumptions hold.