BE 25 Winter 2025
Homework #2
Due at 9 AM PST, January 21, 2025

Problem 2.1 (Protein misfolding, 35 pts)**.**
This problem is based on Problem 15.17 of KKW. Imagine a test tube with buffer conditions such that it is full of denatured protein (D). The buffer conditions are suddenly changed such that the denatured protein can fold into one of two configurations, a natively folded configuration (N) or a misfolded configuration (M). That is, the following two reactions may happen.

$$\mathrm{D} \xrightarrow{k_1} \mathrm{N}, \tag{2.1}$$

$$\mathrm{D} \xrightarrow{k_2} \mathrm{M}. \tag{2.2}$$

In the experimental setup, we can only measure the concentration of folded (natively or otherwise) protein over time. That is, we can only monitor the reaction

$$\mathrm{D} \xrightarrow{k_{\mathrm{eff}}} \text{folded}. \tag{2.3}$$

It is determined that $k_{\mathrm{eff}} = 15\ \mathrm{s}^{-1}$. Though it is not fast enough to measure kinetics, another experimental technique can measure the ratio of natively folded to misfolded proteins. After a long time (presumably at steady state), it is determined that the ratio of the concentration of natively folded proteins to the concentration of misfolded proteins is 9. From these measurements, deduce the values of k_1 and k_2.

Problem 2.2 (Rate limiting steps, 30 pts)**.**
Section 15.10 of KKW provides a nice discussion on rate limiting steps in which they demonstrate through a simple cascade-style reaction $\mathrm{A} \longrightarrow \mathrm{B} \longrightarrow \mathrm{C}$ that the slowest step of a reaction scheme establishes its overall rate. While that example is illuminating, we can develop a more general result for cascading reactions without the need to explicitly integrate the resulting first-order linear differential equations using integrating factors, as KKW do in Box 15.2. Note that cascading reactions are commonly encountered in cellular dynamics, for example in signaling networks involving multiple phosphorylations.

Consider a transformation that requires n steps,

$$\mathrm{X}_1 \xrightarrow{k_1} \mathrm{X}_2, \tag{2.4}$$

$$\mathrm{X}_2 \xrightarrow{k_2} \mathrm{X}_3, \tag{2.5}$$

$$\vdots \tag{2.6}$$

$$\mathrm{X}_n \xrightarrow{k_n} \mathrm{X}_{n+1}, \tag{2.7}$$

where conversion from X_1 to X_{n+1} is the process we are interested in. For notational ease, let the concentration of species X_i be x_i. Say we initially start with $x_1(0) = x_1^0$ and $x_i = 0$ for all $i > 1$.

a) Write down a system of ODEs for this reaction system. To do so, you can write an expression for $\mathrm{d}x_1/\mathrm{d}t$, $\mathrm{d}x_{n+1}/\mathrm{d}t$, and $\mathrm{d}x_i/\mathrm{d}t$ for $2 \leq i \leq n$.

b) Show that there is a conservation law such that we can eliminate one of the ODEs. Specifically, use the conservation law to write an expression for x_{n+1} in terms of all of the other concentrations so that we no longer need to consider $\mathrm{d}x_{n+1}/\mathrm{d}t$ explicitly in the dynamical equations.

c) Write the dynamical equations as a matrix equation,

$$\frac{\mathrm{d}\mathbf{x}}{\mathrm{d}t} = \mathsf{A} \cdot \mathbf{x}, \tag{2.8}$$

where

$$\mathbf{x} = \begin{pmatrix} x_1 \\ x_2 \\ \vdots \\ x_n \end{pmatrix}. \tag{2.9}$$

That is, write down the matrix A.

d) The solution to a linear system of ordinary differential equations, as we have here, is

$$\mathbf{x}(t) = \sum_{i=1}^{n} a_i \, \mathbf{v}_i \, \mathrm{e}^{\lambda_i t}, \tag{2.10}$$

where λ_i is the ith eigenvalue of A and $\mathbf{v}_i$ is the corresponding eigenvector. The constants a_i are determined by the initial condition. Recall that the eigenvalues of A are found by solving $\det(\mathsf{A} - \lambda \mathsf{I}) = 0$, where I is the identity matrix. Recall also that the determinant of a lower triangular matrix is the product of its diagonal elements. Show that the eigenvalues of A are $\lambda_1 = -k_1$, $\lambda_2 = -k_2$, $\ldots$, $\lambda_n = -k_n$.

e) You have just shown that the dynamics are given by the sum of decaying exponentials with decay constants given by the rate constants. Imagine there is a spectrum of rate constants, with m of them being of a similar order of magnitude, and $n - m$ of them being much, much larger. Explain why the rate of production of X_{n+1} is determined only by the m small rate constants, meaning that only the slow reactions contribute appreciably to the dynamics of product formation.

Problem 2.3 (Switching time of bacteria, 35 pts)**.**
This problem is based on a thought experiment proposed by Robijn Bruinsma in Bruinsma, Physica A, ***313, 211–237, 2002.***

Say we are interested in assessing how fast a bacterial cell can respond to a change in environment. Specifically, imagine a cell is in a sea of delicious lactose and suddenly the lactose is washed away. The cell should then repress expression of β-galactosidase by having a repressor bind to the appropriate operator. Of course, many other mechanisms will be at play in the cellular response, but the fastest the response could possibly be is given by how fast the repressor could bind to its operator.

To establish this speed limit, imagine the following experiment. Many short oligonucleotides are in a buffered solution with concentration c_{D}^0 (where the subscript D means "DNA"). Suddenly, at $t = 0$, repressors are added to give a concentration c_{R}^0 with $c_{\mathrm{R}}^0 \ll c_{\mathrm{D}}^0$. The repressors are added in such a way that the volume of the reaction mixture does not change appreciably. The repressors bind reversibly according to

$$\mathrm{D} + \mathrm{R} \underset{k_{\mathrm{d}}}{\overset{k_{\mathrm{a}}}{\rightleftharpoons}} \mathrm{DR}. \tag{2.11}$$

In this experiment, the concentration of repressor, c_{R}, is monitored over time.

a) Show that at short times, $c_{\mathrm{R}}(t) \propto \mathrm{e}^{-t/\tau}$. Write an expression for τ.

b) In similar in vitro experiments, it was determined that $k_{\mathrm{a}} \approx 10^{10}\ \mathrm{M}^{-1}\mathrm{s}^{-1}$ and $k_{\mathrm{d}} \approx 10^{-2}\ \mathrm{s}^{-1}$. Given than an *E. coli* cell has a volume of about one femtoliter, estimate the characteristic time it takes for the repressor to bind the operator. That is, plug numbers into your expression for τ. (Note that in a cell, the condition that $c_{\mathrm{R}}^0 \ll c_{\mathrm{D}}^0$ does not hold, but we can still take the response time to be approximately τ.)

c) We already reasoned that τ is a lower bound on the switching time of a bacterium. We can say further that this is a lower bound on the time it takes repressor to bind. Why?