BE 25 Winter 2025
Homework #4
Due at 9 AM PST, February 4, 2025

Problem 4.1 (Britton Chance and enzyme kinetics, 50 pts)**.**
The stopped-flow method for studying chemical kinetics was pioneered by Britton Chance. He developed the technology during his Ph.D. thesis in the late 1930s and used it to study enzyme-catalyzed reactions. By 1940, he had achieved key results, but was drafted to work in the secret radar lab at MIT as part of the war effort. Nonetheless, in 1943 he managed to publish is results in a landmark paper (Chance, *J. Biol. Chem.*, **151**, 553–577, 1943).

In this paper, he used his newly developed stopped-flow technology to measure the kinetics of the breakdown of hydrogen peroxide (the substrate) by peroxidase (the enzyme) with a colorimetric readout. Though Michaelis and Menten had written down their famous reaction mechanism

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{\mathrm{k_2}} \mathrm{E} + \mathrm{P}, \tag{4.1}$$

in 1913, and Briggs and Haldane had expanded on their ideas in 1925, prior to Chance's stopped-flow experiments, no one had ever seen the proposed enzyme-substrate complex. Chance sought to directly observe the complex. He also sought to examine if the rapid steady-state approximation of Michaelis and Menten or the quasi-steady state approximation of Briggs and Haldane was more appropriate.

a) The Michaelis-Menten equation gives the rate of production of product P as a function of the total enzyme concentration c_{E}^0 and the substrate concentration c_{S}. In lecture, we used the QSSA to derive the Michaelis-Menten equation, as done by Briggs and Haldane in 1925. Michaelis and Menten, however, did not use the QSSA. Rather, they applied a fast steady state approximation in which they assumed that the forward and reverse rates of the enzyme-substrate binding/unbinding reactions were equal. Derive the Michaelis-Menten equation (that is, the expression for $\mathrm{d}c_{\mathrm{P}}/\mathrm{d}t$) using this approximation. Be sure to show explicitly what the expressions are for $v_{\max}$ and K_{M}.

b) In his stopped-flow experiment, Chance measured both the concentration of the product (P, degraded hydrogen peroxide) and the enzyme-substrate complex (ES, peroxidase bound to hydrogen peroxide). Chance wanted to see how his measurements compared to the theory given by the Michaelis-Menten reaction scheme. Note that he was explicitly observing the enzyme-substrate complex, so he needed to solve the full mass action kinetics system of ordinary differential equations without making any approximations such as the rapid steady-state approximation or the quasi-steady state approximation. He had to resort to numerical calculation in the late 1930s to do so. In Chance's words,

"This was the first study of enzyme kinetics by a machine computer. The mechanical equation solver, approximately 75 feet long, consisted of geared multipliers and ball-and-disc integrators that set up each parameter and turned out mechanical solutions of the differential equations for exhaustion of substrate and formation and decomposition of the enzyme-substrate compounds." The computer is shown in the picture below.

Figure 1: The computer Chance used to numerically solve the system of ODEs arising from the Michaelis-Menten chemical reaction scheme. Taken from Chance, *Photosynthesis Research*, **80**, 387–400, 2004.

Fortunately, we now know how to numerically solve the ODEs with an *electronic* computer (and maybe even someday with a quantum computer)! We will use this capability to perform a curve fit to estimate the parameters k_1, k_{-1} and k_2. We will fit the enzyme-substrate complex data to the theoretical curve found from solving the ODEs. Toward that end, complete the code below to obtain the parameter estimates. Report your estimates.

```
import numpy as np
import scipy.optimize

# Chance's concentrations in his experiment
cs0 = 4.0  # Initial substrate concentration in micromolar
ce0 = 1.0  # Total enzyme concentration in micromolar

# Time points for product in units of seconds
t_p = np.array([0.032, 0.061, 0.102, 0.175, 0.274, 0.344,
                0.451,  0.624, 0.700, 0.874, 0.967, 1.043,
                1.219, 1.525])

# Concentration of product in units of micromolar
c_p = np.array([0.131, 0.172, 0.344, 0.569, 0.918, 1.237,
                1.854, 2.554, 3.103, 3.656, 3.901, 4.010,
                4.033, 4.007])

# Time points for enzyme-substrate complex in units of seconds
```

```
t_es =  np.array([0.007, 0.040, 0.073, 0.134, 0.245, 0.306,
                  0.411, 0.583, 0.657, 0.832, 0.935, 0.997,
                  1.181, 1.351, 1.712])

# Conc. of enzyme-substrate complex in units of micromolar
c_es = np.array([0.353, 0.688, 0.811, 0.844, 0.855, 0.844,
                  0.802, 0.711, 0.655, 0.472, 0.340, 0.282,
                  0.137, 0.074, 0.019])


# YOU MAY NEED TO WRITE OTHER FUNCTIONS HERE


# Initial concentrations
c0 = np.array([cs0, ce0, 0, 0])


def ces_theor(t, log_k1, log_km1, log_k2):
    """Theoretical concentration of ES as a function of time.
    Rate constants are inputted as logarithms to ease curve
    fitting.

    Parameters
    ----------
    t : Numpy array
        Time points for which the solution of desired
    log_k1 : float
        Logarithm of the rate constant k1.
    log_km1 : float
        Logarithm of the rate constant k_minus_1.
    log_k2 : float
        Logarithm of the rate constant k2.

    Returns:
    output : Numpy array, same length as `t`
        Concentration of enzyme-substrate complex over time.
    """
    # COMPLETE THIS FUNCTION
    # It can and should use `c0`, even though it is not
    # passed in.


# Perform curve fit
popt, _ = scipy.optimize.curve_fit(ces_theor, t_es, c_es)

# Convert parameters from logs
k1, km1, k2 = np.exp(popt)
```

c) Plot Chance's data for both the enzyme-substrate complex concentration and the product concentration versus time. Overlay the respective curves given by solving the kinetic equations. Comment on what you see. (Note that there may

be some systematic variation of the measured product concentrations from the theoretical curve, and Chance commented on these data in particular in his paper: "The scatter of points is thought to represent an instrumental rather than intrinsic irregularity.")

d) Given the parameter estimates you obtained, which approximation is more appropriate in this case, the rapid steady-state approximation of Michaelis and Menten or the quasi-steadty state approximation of Briggs and Haldane? Could we have figured this out without directly observing the enzyme-substrate complex (that is, by only observing substrate depletion and/or product production)?

Problem 4.2 (HIV protease inhibitors and pH dependence, 50 pts)**.**
This problem is based on problem 4.10 of WTHS. Some enzymes, such as HIV protease, exhibit pH-dependence on their catalytic activity. As a simple example, imagine an enzyme that can bind substrate in its protonated state, but not in its unprotonated state. That is, it has the following reaction scheme.

$$\mathrm{E}^- + \mathrm{H}^+ \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} \mathrm{EH}, \tag{4.2}$$

$$\mathrm{EH} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{SEH} \xrightarrow{k_2} \mathrm{EH} + \mathrm{P}. \tag{4.3}$$

a) Derive an expression for the reaction velocity,

$$v_0 = \frac{\mathrm{d}c_\mathrm{P}}{\mathrm{d}t}. \tag{4.4}$$

This should be an analytical expression, and you will need to make approximations to derive it. Be sure to clearly state which approximations you use. It should be written in terms of c_E^0, c_S, and c_{H^+}. Does the resulting expression match a Michaelis-Menten form? If so, what are the effective k_cat and K_M?

b) In the presence of an inhibitor, such as HIV protease inhibitors used in some treatments, the situation gets more interesting. In an inhibitor could also bind the enzyme in either the protonated or unprotonated form, giving additional reactions

$$\mathrm{E}^- + \mathrm{I} \underset{k_{-i}^-}{\overset{k_i^-}{\rightleftharpoons}} \mathrm{IE}^-, \tag{4.5}$$

$$\mathrm{EH} + \mathrm{I} \underset{k_{-i}}{\overset{k_i}{\rightleftharpoons}} \mathrm{IEH}. \tag{4.6}$$

The inhibitor-bound unprotonated enzyme may also be protonated.

$$\mathrm{IE}^- + \mathrm{H}^+ \underset{k_{-a}^i}{\overset{k_a^i}{\rightleftharpoons}} \mathrm{IEH}, \tag{4.7}$$

though, for reasons we will learn about later in the course, this last reaction is dispensible.

For this inhibited scheme, derive an expression for the reaction velocity, again making appropriate approximations. It should be written in terms of c_{E}^{0}, c_{S}, $c_{\mathrm{H^+}}$, and now also c_{I}. Does the resulting expression still match a Michaelis-Menten form?

c) How do the effective k_{cat} and K_{M} you found in part (b) depend on pH, if at all?

d) Does it matter whether the inhibitor binds more readily to the unprotonated or protonated state of the enzyme? Explain.