BE 25 Winter 2026
Homework #4
Due at 9 AM PST, February 3, 2025

Problem 4.1 (The Stern-Volmer relation, 20 pts)**.**
The kinetics of emission of a sample of excited fluorophores have been shown to follow first-order kinetics. That is, the fluorescence intensity I follows

$$I(t) \propto -\frac{\mathrm{d}c_*}{\mathrm{d}t} = k\, c_*, \tag{4.1}$$

where c_* is the concentration of excited fluorophores in a sample that was illuminated and then had the excitation light suddenly turned off. The characteristic fluorescence decay time is $\tau = 1/k$, usually between 3 and 30 nanoseconds.

Multiple processes contribute to this decay. They are shown below, where M_* is the excited fluorophore and M is the fluorophore in its ground state.

$$\text{fluorescence emission: } \mathrm{M}_* \xrightarrow{k_\mathrm{f}} \mathrm{M} \tag{4.2}$$

$$\text{thermal deactivation: } \mathrm{M}_* \xrightarrow{k_\mathrm{t}} \mathrm{M} \tag{4.3}$$

$$\text{photochemical dactivation: } \mathrm{M}_* \xrightarrow{k_\mathrm{p}} \mathrm{M} \tag{4.4}$$

$$\text{quenching: } \mathrm{M}_* + \mathrm{Q} \xrightarrow{k_\mathrm{Q}} \mathrm{M} + \mathrm{Q}_*. \tag{4.5}$$

a) Write down an expression $I(t)$ over time. Use α as the constant of proportionality between $I(t)$ and $\mathrm{d}c_*/\mathrm{d}t$. Assume that the quencher concentration c_Q is constant.

b) Write an expression for the **quantum yield**, ϕ_f, which is the ratio of the number of photons fluoresced to the total number of photons that excited the fluorophores.

c) The **Stern-Volmer relation** relates the ratio of quantum yield in the absence of quenchers, ϕ_f^0, to that in the presence of quenchers, ϕ_f.

$$\frac{\phi_\mathrm{f}^0}{\phi_\mathrm{f}} = 1 + K\, c_\mathrm{Q}. \tag{4.6}$$

Derive this result making sure to specify how K relates to the rate constants.

Problem 4.2 (HIV protease inhibitors and pH dependence, 45 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.

$$\mathrm{E}^- + \mathrm{H}^+ \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} \mathrm{EH}, \tag{4.7}$$

$$\mathrm{EH} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{SEH} \xrightarrow{k_2} \mathrm{EH} + \mathrm{P}. \tag{4.8}$$

a) Derive an expression for the reaction velocity,

$$v_0 = \frac{\mathrm{d}c_\mathrm{P}}{\mathrm{d}t}. \tag{4.9}$$

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

$$\mathrm{E}^- + \mathrm{I} \underset{k_{-i}^-}{\overset{k_i^-}{\rightleftharpoons}} \mathrm{IE}^-, \tag{4.10}$$

$$\mathrm{EH} + \mathrm{I} \underset{k_{-i}}{\overset{k_i}{\rightleftharpoons}} \mathrm{IEH}. \tag{4.11}$$

The inhibitor-bound unprotonated enzyme may also be protonated.

$$\mathrm{IE}^- + \mathrm{H}^+ \underset{k_{-a}^i}{\overset{k_a^i}{\rightleftharpoons}} \mathrm{IEH}, \tag{4.12}$$

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_{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.

Problem 4.3 (Time scale of relaxation experiment, 35 pts)**.**
You wish to study the kinetics of a reaction

$$2\,\mathrm{A} + \mathrm{B} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{C}. \tag{4.13}$$

a) Derive an expression for the relaxation time τ of the relaxation experiment after a T-jump perturbation. Write your expression in terms of the rate constants k_1 and k_{-1} and the steady state concentrations of A, B, and C.

b) What set of relaxation experiments could you do to obtain good estimates for k_1 and k_{-1}?