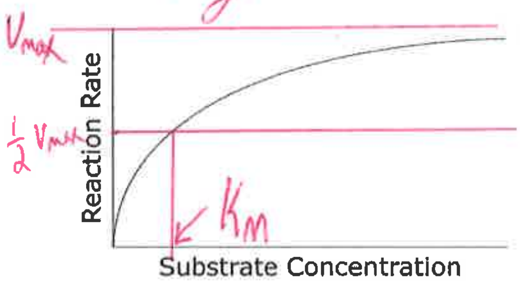
Quiz 17.3 – Reaction Mechanisms

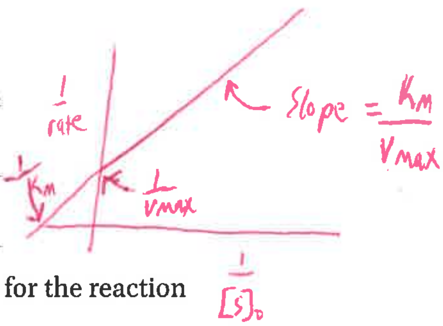
Name: Key



On the figure at left, mark the values of v_{max} and K_M

Reaction rates for an enzyme-catalyzed reaction were recorded at different substrate concentrations. The data are tabulated below:

Trial	Rate (M/s)	$[S]_o$ (M)
1	2.7×10^{-3}	0.0100
2	3.5×10^{-3}	0.0300



Use these data to give v_{max} and K_M for the reaction

trial	$\frac{1}{[S]}$ $(\frac{1}{M})$	$\frac{1}{rate}$ $(\frac{s}{M})$
1	100	370.4
2	33.33	285.7

$$\text{Slope} = \frac{(285.7 - 370.4)}{(33.33 - 100)} = 1.275$$

$$y = mx + b \rightarrow b = y - mx$$

$$b = -1.27 \cdot 100 + 370.4 = 243.4$$

$$V_{max} = \frac{1}{b} = 4.1 \cdot 10^{-3}\ M/s \qquad K_m = \text{Slope} \cdot V_{max} = 5.2 \cdot 10^{-3}\ M$$

For these trials, $[E]_o = 0.020M$. What is the catalytic efficiency, η, for the reaction?

$$V_{max} = k_{cat} \cdot [E]_o \rightarrow k_{cat} = \frac{V_{max}}{[E]_o} = \frac{4.1 \cdot 10^{-3}\ M/s}{0.020\ M} = 0.205\ s^{-1}$$

$$\eta = \frac{k_{cat}}{K_M} = \frac{0.205\ s^{-1}}{0.0052\ M} = 39\ \frac{1}{M \cdot s}$$

After an inhibitor is added, v_{max} remains the same but K_M is substantially greater. What type of inhibitor was added?

Competetive

A fluorophore is known to have $k_F = 3.0 \times 10^8 s^{-1}$, $k_{IC} = 1.0 \times 10^8 s^{-1}$, and $k_{ISC} = 6.0 \times 10^7 s^{-1}$

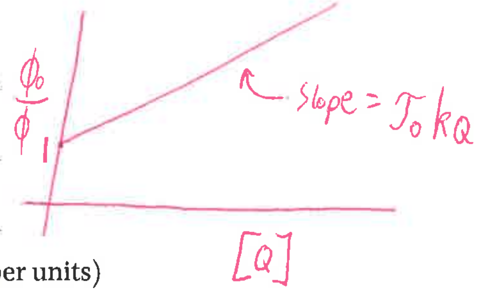
Give the observed fluorescence lifetime (τ) and the quantum efficiency (ϕ_F) for this fluorophore

$$\tau = \frac{1}{k_F + k_{IC} + k_{ISC}} = 2.17 \cdot 10^{-9} s = 2.17 ns$$

$$\phi_F = \frac{k_F}{k_F + k_{IC} + k_{ISC}} = k_F \cdot \tau = 0.652$$

A quencher is then added to the solution and the quantum efficiency is monitored. Data for the trials are shown in the table below

Trial	ϕ	$\frac{\phi_0}{\phi}$	[Q] (M)
1	0.513	1.27	0.0010
2	0.423	1.54	0.0020



Find the quenching rate constant k_Q (with proper units)

$$\text{Slope} = \frac{(1.54 - 1.27)}{0.0020 M - 0.0010 M} = 270 M^{-1}$$

$$k_Q = \frac{\text{Slope}}{\tau_0} = \frac{270 M^{-1}}{2.17 \cdot 10^{-9} s} = 1.24 \cdot 10^{11} M^{-1} s^{-1}$$

Assuming this quenching rate holds for auto-quenching, at what concentration of fluorophore will the quantum yield reach 10% of its value in the dilute limit?

$$\frac{\phi_0}{\phi} = 1 + \text{slope} \cdot [Q] \qquad \frac{\phi_0}{\phi} = 100 \qquad 100 = 1 + 270 M^{-1} [Q] \rightarrow [Q] = 0.37 M$$

A pair of fluorophores is capable of Förster resonant energy transfer with $R_0 = 3.9nm$. These fluorophores are placed on two sites of a protein, and the energy transfer is observed to have $\eta_T = 0.14$ (14% efficiency of energy transfer). What is the distance between the two sites on the protein?

$$\eta_T = \frac{R_0^6}{R_0^6 + R^6} \rightarrow 0.14 = \frac{3.9^6}{3.9^6 + x^6} \longrightarrow x = 5.3 nm$$