1.2.3   Solution to Homework #4
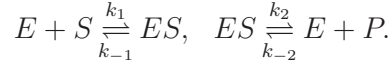
1. Let us consider a reversible enzyme reaction accoding to Michaelis-Menten kinetics:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES, \quad ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P.$$

(a) Following the law of mass action to write the system of ODEs for $e(t)$, $s(t)$, $x(t)$, and $p(t)$, the concentrations of E, S, ES, and P at time t:

$$\frac{de}{dt} = -k_1 es + k_{-1}x + k_2 x - k_{-2}ep$$

$$\frac{ds}{dt} = -k_1 es + k_{-1}x$$

$$\frac{dx}{dt} = +k_1 es - k_{-1}x - k_2 x + k_{-2}ep$$

$$\frac{dp}{dt} = +k_2 x - k_{-2}ep$$

(b) If we assume that the concentration of ES reaches a steady state rapidly, i.e., we can write $\dfrac{d[ES]}{dt} = 0$. while $[S]$ and $[P]$ are still changing with time, show that the rate of product formation

$$\frac{d[P]}{dt} = \frac{\dfrac{V_{max}^+([S])}{K_{MS}} - \dfrac{V_{max}^-([P])}{K_{MP}}}{1 + \dfrac{[S]}{K_{MS}} + \dfrac{[P]}{K_{MP}}}.$$

Give the parameters K_{MS}, K_{MP}, V_{max}^+ and V_{max}^- in terms of the k's.

$d[ES]/dt = 0$ gives

$$\frac{dx}{dt} = +k_1 es - k_{-1}x - k_2 x + k_{-2}ep = 0$$

If we introduce $e = e_0 - x$, then we can solve for x

$$x = \frac{e_0 \left(k_1\, s + k_{-2}\, p\right)}{k_1\, s + k_{-2}\, p + k_{-1} + k_2}$$

which gives us

$$\frac{dp}{dt} = \frac{\dfrac{e_0 k_1 k_2 s}{k_{-1} + k_2} - \dfrac{e_0\, k_{-1}\, k_{-2}\, p}{k_{-1} + k_2}}{\dfrac{k_1\, s}{k_{-1} + k_2} + \dfrac{k_{-2}\, p}{k_{-1} + k_2} + 1}$$

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Comparing with the form given, we get

$$K_{MS} = \frac{k_1}{k_{-1} + k_2}$$

$$K_{MP} = \frac{k_{-2}}{k_{-1} + k_2}$$

$$V_{max}^+ = e_0 k_2$$

$$V_{max}^- = e_0 k_{-1}$$

(c) To justify the quasi-steady state approximation, we carry out nondimension-alization. Let $e(t) + x(t) = e_0$ and $s(t) + x(t) + p(t) = s_0$, then we have

$$\frac{ds}{dt} = -k_1(e_0 - x)s + k_{-1}x$$

$$\frac{dx}{dt} = k_1(e_0 - x)s - (k_{-1} + k_2)x + k_{-2}(e_0 - x)p$$

$$\frac{dp}{dt} = k_2 x - k_{-2}(e_0 - x)p$$

$$\hat{e} = \frac{e}{e_0}, \quad \hat{s} = \frac{s}{s_0}, \quad \hat{x} = \frac{x}{e_0}, \quad \hat{p} = \frac{p}{s_0}, \quad \tau = k_1 e_0 t.$$

Then we have

$$\frac{d\hat{s}}{d\tau} = -\hat{e}\hat{s} + \left(\frac{k_{-1}}{k_1 s_0}\right)\hat{x}$$

$$\frac{d\hat{x}}{d\tau} = \frac{1}{\epsilon}\left\{(1 - \hat{x})\left[\hat{s} + \left(\frac{k_{-2}}{k_1}\right)\hat{p}\right] - \left(\frac{k_{-1} + k_2}{k_1 s_0}\right)\hat{x}\right\}$$

$$\frac{d\hat{p}}{d\tau} = \left(\frac{k_2}{k_1 s_0}\right)\hat{x} - \left(\frac{k_{-2}}{k_1}\right)\hat{e}\hat{p}$$

in which $\epsilon = e_0/s_0 \ll 1$. This implies that $d\hat{x}/d\tau$ is very large, $\hat{x}(\tau)$ change rapidly until the term inside $\{\cdots\}$ becomes zero. Hence, strictly speaking, the quasi-steady-state approximation is <u>not</u> $dx/dt = 0$, but the term inside $\{\cdots\} = 0$.

2. The chemical master equation for a single enzyme is

$$\frac{dp_E(t)}{dt} = -k_1 s p_E + (k_{-1} + k_2)p_{ES}$$

$$\frac{dp_{ES}(t)}{dt} = k_1 s p_E - (k_{-1} + k_2)p_{ES}$$

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in which we assume that the concentration for the substrate S, s, is constant. With a single enzyme, the change in the concentration of s is negligible.

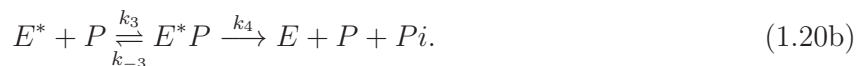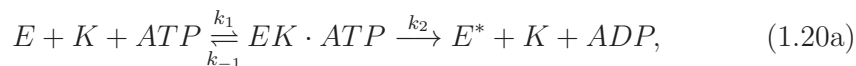(b) The steady state probabilities, with normalization, are

$$p_E^{ss} = \frac{k_{-1} + k_2}{k_1 s + k_{-1} + k_2}, \quad p_{ES}^{ss} = \frac{k_1 s}{k_1 s + k_{-1} + k_2}.$$

(c) The steady state rate for the formation of product is

$$J^{ss} = k_2 p_{ES}^{ss} = \frac{k_2 k_1 s}{k_1 s + k_{-1} + k_2} = \frac{k_2 s}{s + k_M}$$

in which $K_M = \dfrac{k_{-1} + k_2}{k_1}$. Comparing this with the Michaelis-Menten formula, we see that $V_{max} = k_2$. This is expected since there is only a single enzyme.

3. Let us consider the phosphorylation and dephosphorylation reactions catalyzed by corresponding enzymes called kinase (K) and phosphatase (P),

$$E + K + ATP \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EK \cdot ATP \overset{k_2}{\longrightarrow} E^* + K + ADP, \qquad (1.20a)$$

$$E^* + P \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E^*P \overset{k_4}{\longrightarrow} E + P + Pi. \qquad (1.20b)$$

ATP, ADP, and Pi are called adenosine triphosphate, adenosine diphosphate, and in-organic phosphate respectively. A complete phosphorylation-dephosphorylation cycle yield one ATP hydrolysis $ATP \rightleftharpoons ADP + Pi$. We shall again assume that the concentrations of ATP, ADP, and Pi are all sustained and constant. That means their concentrations are treated as constants.

(a) Following the theory of Michaelis-Menten kinetics, the two enzymatic reactions in (1.17a) and (1.17b) each has its own K_M and V_{max}. Let them be K_1 and V_1 and K_2 and V_1. Show that

$$\frac{d[E^*]}{dt} = \frac{V_1[E]}{K_1 + [E]} - \frac{V_2[E^*]}{K_2 + [E^*]}, \qquad (1.21)$$

in which $[E] + [E^*] = E_T$ is the total amount of substrate protein.

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Firstly we write down the ODEs governing the process

$$\frac{d[E]}{dt} = -k_1[E][K][ATP] + k_{-1}[EK \cdot ATP] + k_4[E^*P]$$

$$\frac{d[K]}{dt} = +(k_2 + k_{-1})[EK \cdot ATP] - k_1[E][K][ATP]$$

$$\frac{d[EK \cdot ATP]}{dt} = -(k_2 + k_{-1})[EK \cdot ATP] + k_1[E][K][ATP]$$

$$\frac{d[E^*]}{dt} = +k_2[EK \cdot ATP] - k_3[E^*][P] + k_{-3}[E^*P]$$

$$\frac{d[E^*P]}{dt} = +k_3[E^*][P] - (k_{-3} + k_4)[E^*P]$$

$$\frac{d[P]}{dt} = -k_3[E^*][P] + (k_{-3} + k_4)[E^*P]$$

Introducing the usual assumption $\dfrac{d[EK \cdot ATP]}{dt} = 0$ and $\dfrac{d[E^*P]}{dt} = 0$ and conservations $[K] = [K]_0 - [EK \cdot ATP]$, $[P] = [P]_0 - [E^*P]$, we get

$$[EK \cdot ATP] = \frac{k_1[K]_0[ATP][E]}{k_1[ATP][E] + k_{-1} + k_2}$$

$$[E^*P] = \frac{k_3[P]_0[E^*]}{k_3[E^*] + k_{-3} + k_4}$$

Plugging these quantities in to the equation of $[E^*]$, we get

$$
\begin{aligned}
\frac{d[E^*]}{dt} &= +k_2 \left(\frac{k_1[K]_0[ATP][E]}{k_1[ATP][E] + k_{-1} + k_2} \right) \\
&\quad - k_3[E^*] \left([P]_0 - \frac{k_3[P]_0[E^*]}{k_3[E^*] + k_{-3} + k_4} \right) + k_{-3} \left(\frac{k_3[P]_0[E^*]}{k_3[E^*] + k_{-3} + k_4} \right) \\
&= \frac{k_1 k_2 [K]_0[ATP][E]}{k_1[ATP][E] + k_{-1} + k_2} - \frac{k_4 k_3 [E^*][P]_0}{k_3[E^*] + k_{-3} + k_4} \\
&= \frac{k_2[K]_0[E]}{[E] + \frac{k_{-1} + k_2}{k_1[ATP]}} - \frac{k_4[P]_0[E^*]}{[E^*] + \frac{k_{-3} + k_4}{k_3}}
\end{aligned}
$$

As a result,

$$V_1 = k_2[K]_0, \quad V_2 = k_4[P]_0, \quad K_1 = \frac{k_{-1} + k_2}{k_1[ATP]}, \quad K_2 = \frac{k_{-3} + k_4}{k_3}.$$

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(b) Find the steady state fraction of phosphorylated protein $f = [E^*]/E_T$.

Setting Equation 1.21 equal to 0, we get the steady state solution of the fraction

$$
\begin{aligned}
f &= \frac{[E^*]}{E_T} \\
&= \frac{V_1 - V_2 - \frac{K_1}{E_T}V_2 - V_1\frac{K_2}{E_T}}{2(V_1 - V_2)} \\
&\pm \frac{\sqrt{\begin{array}{c}(V_1 - V_2)^2 - 2\frac{K_1}{E_T}V_1V_2 + 2\frac{K_1}{E_T}V_2^2 + 2\frac{K_2}{E_T}V_1^2 \\ -2\frac{K_2}{E_T}V_1V_2 + \frac{K_1}{E_T}^2V_2^2 + 2V_1\frac{K_2}{E_T}\frac{K_1}{E_T}V_2 + \frac{K_2}{E_T}^2V_1^2\end{array}}}{2(V_1 - V_2)}
\end{aligned}
$$

(c) $f = [E^*]/E_T$ is a function of $\hat{K}_1 = K_1/E_T$, $\hat{K}_2 = K_2/E_T$ and $\theta = V_1/V_2$. What is

$$
\lim_{\hat{K}_1, \hat{K}_2 \to 0} f\left(\theta, \hat{K}_1, \hat{K}_2\right) = ?
$$

Rewrite f

$$
f\left(\theta, \hat{K}_1, \hat{K}_2\right) = \frac{\theta - 1 - \hat{K}_1 - \theta\hat{K}_2 \pm \sqrt{\begin{array}{c}\left(\hat{K}_2\theta + \hat{K}_1\right)^2 + 2\hat{K}_2\theta^2 - 2\hat{K}_1\theta \\ -2\theta\hat{K}_2 + 2\hat{K}_1 + (\theta - 1)^2\end{array}}}{2(\theta - 1)}
$$

So

$$
\lim_{\hat{K}_1, \hat{K}_2 \to 0} f\left(\theta, \hat{K}_1, \hat{K}_2\right) = 1 \text{ or } 0.
$$

Whether the solution is $0, 1$ depend on the sign of $\theta - 1$, they switch when the sign changes. When $\theta = 1$,

$$
\hat{f} = \frac{\hat{K}_2}{\hat{K}_1 + \hat{K}_2}
$$

Then the limit is depending on how $\hat{K}_1, \hat{K}_2$ go to zero.

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(c)

$$\frac{V_1(E_T - [E^*])}{K_1 + E_T - [E^*]} - \frac{V_2[E^*]}{K_2 + [E^*]} = 0,$$

$$\theta(1-f)(\hat{K}_2 + f) - f(\hat{K}_1 + 1 - f) = 0,$$

$$\boxed{\left(1-\theta\right)f^2 - \left(\hat{K}_1 + \theta\hat{K}_2 - \theta + 1\right)f + \theta\hat{K}_2 = 0}\,,$$

$$f = \frac{\left(\hat{K}_1 + \theta\hat{K}_2 - \theta + 1\right) \pm \sqrt{\left(\hat{K}_1 + \theta\hat{K}_2 - \theta + 1\right)^2 - 4(1-\theta)\theta\hat{K}_2}}{2(1-\theta)}$$

$$= \frac{\left(\hat{K}_1 + \theta\hat{K}_2 - \theta + 1\right) \pm \sqrt{(1 - \theta + \hat{K}_1 - \theta\hat{K}_2)^2 + 4\theta\hat{K}_1\hat{K}_2}}{2(1-\theta)}$$

$$\simeq \begin{cases} 1 - \dfrac{\hat{K}_1}{(\theta-1)} & \theta > 1 \\[2ex] \dfrac{\hat{K}_2}{\hat{K}_1 + \hat{K}_2} & \theta = 1 \\[2ex] \dfrac{\theta\hat{K}_2}{(1-\theta)} & \theta < 1 \end{cases}$$

Therefore,

$$\lim_{\hat{K}_1, \hat{K}_2 \to 0} f = \begin{cases} 1 & \theta > 1 \\ 0 & \theta < 1 \end{cases}$$