5440 Prelim 1 — Jonathan Albo — 4/13/21

1. a.

$$G_j + R^0_x \rightleftharpoons (G_j : R_x)_C$$
$$(G_j : R_x)_C \rightarrow (G_j : R_x)_O$$
$$(G_j : R_x)_O \rightarrow R_x + G_j$$
$$(G_j : R_x)_O \rightarrow m_j + R_x + G_j$$

G_j : gene j
R^0_x : free RNAP conc
$(G_j : R_x)_O$: open complex concentrations
$(G_j : R_x)_C$: closed complex concentrations

$$r_{x_j} = k_{E_j} (G_j : R_x)_O$$

r_{x_j} : kinetic limit of transcription
k_{E_j} : elongation rate constant for gene j

$$R_x T = R_x^0 + (G_j : R_x)_C + (G_j : R_x)_O + \sum_{i=1,j}^{N} \{(G_i : R_x)_C + (G_i : R_x)_O\}$$

material balance around closed and open loop complex

From Notes:

$$\frac{d}{dt}(G_j : R_x)_C = k_+ (G_j)(R_x) - k_- (G_j : R_x)_C - k_I (G_j : R_x)_C$$

$$\frac{d}{dt}(G_j : R_x)_O = k_I (G_j : R_x)_C - k_A (G_j : R_x)_O - k^x_{E_j} (G_j : R_x)_O$$

Assume steady state, rearrange

$$\frac{d(G_j : R_x)_C}{dt} \to 0 \qquad k_- (G_j : R_x)_C + k_I (G_j : R_x)_C = k_+ (G_j)(R_x)$$

$$(G_j : R_x)_C = \left(\frac{k_+}{k_- + k_I}\right)(G_j)(R_x)$$

$$\frac{d(G_j : R_x)_O}{dt} \to 0 \qquad k_A (G_j : R_x)_O + k^x_{E_j} (G_j : R_x)_O = k_I (G_j : R_x)_C$$

$$(G_j : R_x)_O = \left(\frac{k_I}{k_A + k^x_{E_j}}\right)(G_j : R_x)_C$$

$$(G_j : R_x)_O = \left(\frac{k_I}{k_A + k^x_{E_j}}\right)\left(\frac{k_+}{k_- + k_I}\right)(G_j)(R_x)$$

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$$K_{x,j}^{-1} \equiv \frac{K_{Ij}}{K_{-j}+K_{Ij}}$$

$$\tau_{x,j}^{-1} \equiv \frac{K_{Ij}}{K_{Aj}+K_{Ej}^{x}}$$

$$(G_j : R_x)_1 : K_{x,j}^{-1}(G_j)(R_x) \quad ; (G_i : R_x)_1 : K_{x,i}^{-1} G_i R_x$$

$$(G_j : R_x)_0 : \tau_{x,j}^{-1}(K_{x,j}^{-1})(G_j)(R_x) \; ; (G_i : R_x)_b : \tau_{xi}^{-1} K_{xi}^{-1} G_i R_x$$

$$\sum_{i=1,j}^{N}\{(G_i:R_x)_1 + (G_i:R_x)_0\} : \sum_{i=1,j}^{N}\{(K_{x,i}^{-1})(G_i)(R_x) + \tau_{x,i}^{-1}(K_{x,i}^{-1})(G_i)R_x\}$$

From proposed elementary steps and RNAP balance:

$$R_{xT} = R_x + K_{x,j}^{-1}(G_j)(R_x) + \tau_{x,j}^{-1}(K_{xj}^{-1})(G_j)(R_x) + \sum_{i=1,j}^{N}\{K_{x,i}^{-1}(G_i)(R_x) + \tau_{x,i}^{-1}(K_{x,i}^{-1})(G_i)\}^{(R_x)}$$

Assume R_x not dependent on i, so can pull out front

$$R_{xT} = R_x\left(1 + K_{x,j}^{-1}(G_j) + \tau_{x,j}^{-1}(K_{x,j}^{-1})(G_j) + \sum_{i=1,j}^{N}\{K_{x,i}^{-1}(G_i) + \tau_{x,i}^{-1}(K_{x,i}^{-1})(G_i)\}\right)$$

$$R_x = \frac{R_{xT}}{1 + K_{x,j}^{-1}G_j + K_{xj}^{-1}\tau_{xj}^{-1}G_j + \sum_{i=1,j}^{N}\{K_{x,i}^{-1}G_i + K_{xi}^{-1}\tau_{x,i}^{-1}G_i\}}$$

rearrange: $\sum_{i=1,N}^{N} G_i \cdot \left(\frac{1}{K_{xi}} + \frac{1}{K_{xi}\tau_{xi}}\right)$

verify this works: $\left(\frac{1}{5} + \frac{1}{5\cdot 10}\right) = 0.22$

$$\sum_{i=1,j}^{N} G_i \cdot \left(\frac{\tau_{xi}}{K_{xi}\tau_{xi}} + \frac{1}{K_{xi}\tau_{xi}}\right) \qquad \left(\frac{10}{5\cdot 10} + \frac{1}{5\cdot 10}\right) = 0.22 \checkmark$$

$$\sum_{i=1,j}^{N} \frac{G_i}{K_{xi}\tau_{xi}}\left(1+\tau_{xi}\right)$$

Plug in to kinetic limit of transcription $r_{xj} = K_{Ej}(G_j : R_x)_0$

$$r_{xj} = K_{Ej} R_{xT} \cdot \left(\frac{G_j}{\tau_{xj}K_{xj} + \tau_{xj}G_j + G_j + \sum_{i=1,j}^{N}\frac{\tau_{xj}K_{xj}}{K_{xi}\tau_{xi}}(1+\tau_{xi})G_i}\right)$$

$(1+\tau_{xj})G_j$; $\sum_{i=1,j}^{N}\frac{\tau_{xj}K_{xj}}{K_{xi}\tau_{xi}}(1+\tau_{xi})G_i$ $= \xi_j$

$$\therefore \boxed{r_{xj} = K_{Ej} R_{xT} \cdot \left(\frac{G_j}{\tau_{xj}K_{xj} + (1+\tau_{xj})G_j + \xi_j}\right)}$$

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1. b. In class for 1-gene system, we derived

$$r_{x,j} = K_{E,j}^{x} R_{x,T} \left(\frac{G_j}{\tau_{xj} K_{xj} + (\tau_{xj}+1) G_j} \right)$$

For N-gene system ($N>>1$):

$$r_{xj} = K_{Ej} R_{xT} \left(\frac{G_j}{\tau_{xj} K_{xj} + (1+\tau_{xj}) G_j + \varepsilon_j} \right)$$

where $\varepsilon_j = \sum_{i=1,i\neq j}^{N} \frac{K_{xj}\tau_{xj}}{K_{xi}-\tau_{xi}} (1+\tau_{xi}) G_i$

So essentially, we want $\varepsilon_j \approx 0$ in order to be equivalent to the 1-gene system
if τ_{xj} or K_{xj} were $>>1$ or $<<1$, this would modify parts of the equation outside of ε_j and $\neq$ 1-gene system
if $\tau_{xi} << 1$, $\varepsilon_j > 0$ $\left(\text{ex } \frac{1}{0.001} \cdot (1+0.001) = 1001\right)$
if $\tau_{xi} >> 1$, $\varepsilon_j \neq 0$ $\left(\text{ex } \frac{1}{100} \cdot 100 = 1\right)$, could be minimized by low G_i or K_{xi}
if $K_{xi} << 1$, $\varepsilon_j > 0$ $\left(\text{ex } \frac{1}{0.001} = 1000\right)$
if $K_{xi} >> 1$, ε_j may ≈ 0 $\left(\text{ex } \frac{1}{1000} = 0.001\right)$
if $G_i << 1$, ε_j may ≈ 0 $\left(\text{ex } \frac{5}{2}(0.001) = 0.0025 \text{ also } \frac{2}{5}\cdot 0.001 = 0.0004\right)$ <- if dissimilar magnitudes, number could be higher
if $G_i >> 1$, $\varepsilon_j > 0$ $\left(\text{ex } \frac{5}{2}\cdot 100 = 250,\ \frac{2}{5}\cdot 100 = 40\right)$

So an N-gene system could be approximately equivalent to the 1-gene system if $\sum_{i=1,i\neq j}^{N} G_i << 1$. If the concentration of N genes was very small, the system could be approximated just for G_j.

or

If $\sum_{i=1,i\neq j}^{N} K_{xi} >> 1$, the two systems could be equivalent. A large saturation constant would mean that the system would take a long time to be saturated. Looking at the formulas for $K_{x,i}$ and $\tau_{x,i}$,

$$K_{x,i} = \frac{K_{Ii}}{K_{-i} + K_{Ii}} \qquad \tau_{x,i} = \frac{K_{Ii}}{K_{Ai} + K_{Ei}}$$

Assuming that $K_{Ii} \neq 0$, a small K_{-i} would not make $K_{x,i}$ large
$\therefore$ $K_{+i} >> 1$ to make $K_{x,i}$ large enough to minimize ε_j such that an N-gene system could be approximated to a 1-gene system

4/6

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k_{+i} is the on rate constant for RNAP at the promoter for gene i (rate constant from first equation) and would have to be $>> k_{-i}$ which is the off rate constant for RNAP at the promoter for gene j, and $>> k_{I_i}$ which denotes the rate constant governing open complex formation. Having a large k_+ would encourage a higher abundance of $(G_i:R_x)_i$ from the first elementary reaction to be available/formed.

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2. $\hat{r}_j = r_j v(..)$ where r_j is the given Pfk kinetic limit reaction, $v(..)_j$ is control variable (i.e. lecture 6, pg 4)

$$v(...)_j = \frac{N}{O}$$

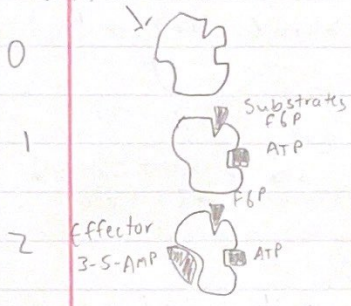
N: sum of micro states leading to catalysis/activation

O: sum of all possible microstates

Substrates: f6P, ATP

Enzyme: Pfk

W_1 - substrate, W_2 - effector



microstate	weight	Catalysis? or Activation?	
0	1	—	
1	W_1	+	N
2	$W_2 f_I$	+	N

regulated

So, $$v(...)_j = \frac{1 + W_1 + W_2 f_I}{1 + W_1 + W_2 f_I}$$

Unregulated

Microstate 0 represents only having the enzyme. Since there is no substrate or effector, the microstate weight is 1 and there is no catalysis/activation. For microstate 1, there are substrates present and looking at the data, there is activity when 3-5-AMP is 0. So W_1 is the activity correction with no 3-5-AMP.

For microstate 2, there are substrates and effector present so this will have microstate weight W_2 (which is with 3-5-AMP), multiplied by f_I which is a binding function that is dependent on the fraction of bound activator. The kinetic limit is constant because F6P and ATP (the substrates) are constant

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2. cont b.

Estimate W_1 and W_2:

$$\hat{r}_j = r_1 \left(\frac{W_1 + W_2 f_I}{1 + W_1 + W_2 f_I} \right)$$

$\hat{r}_j$: overall rate of PFK (know); r_1: kinetic limit (know); f_I: don't know

W_1: No 3-5-AMP

So $f_I = 0$ and when 3-5-AMP = 0 so first data point

$$\frac{\hat{r}_1}{r_1} = \frac{W_1}{1 + W_1}$$

$$\frac{\hat{r}_1}{r_1} + \frac{\hat{r}_1}{r_1} W_1 = W_1 \rightarrow \frac{\hat{r}_1}{r_1} = W_1 \left(1 - \frac{\hat{r}_1}{r_1} \right)$$

$$W_1 = \frac{\frac{\hat{r}_1}{r_1}}{1 - \frac{\hat{r}_1}{r_1}}$$

At 3-5-AMP = 0, $\hat{r}_1 = 3.003$

$r_1 = 69.5798$ (see code)

$$W_1 = \frac{\frac{3.003}{69.5798}}{1 - \frac{3.003}{69.5798}} \rightarrow W_1 = 0.045$$

W_2: with 3-5-AMP

Assume at last data point (6), binding funct is it's saturated so $f_I = 1$

$$\hat{r}_6 = r_1 \left(\frac{W_1 + W_2}{1 + W_1 + W_2} \right)$$

r_1: only kinetic limit

$$\frac{\hat{r}_6}{r_1} (1 + W_1 + W_2) = W_1 + W_2 \rightarrow \frac{\hat{r}_6}{r_1} + W_1 \frac{\hat{r}_6}{r_1} + W_2 \frac{\hat{r}_6}{r_1} = W_1 + W_2$$

$$\frac{\hat{r}_6}{r_1} + W_1 \frac{\hat{r}_6}{r_1} - W_1 = W_2 \left(1 - \frac{\hat{r}_6}{r_1} \right)$$

$$\frac{\frac{\hat{r}_6}{r_1} + W_1 \left(\frac{\hat{r}_6}{r_1} - 1 \right)}{\left(1 - \frac{\hat{r}_6}{r_1} \right)} = W_2 \rightarrow W_2 = 74.028$$

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f_I: Hill-type binding function

$$f_i = \frac{(x/K_i)^{n_i}}{(1 + (x/K_i)^{n_i})}$$

x: fraction of bound activator. Assumed for H_2 that data point 6 was fully saturated so at 3-5-AMP conc of 0.990, $f_i = 1$

K_i: binding constant, don't have more info so estimate to get $f \approx 1$

n_i: order parameter, do same as K_i

Estimate parameters at saturation ($f_i = 1$), fully bound activator at 0.990mM

$$f_i = \frac{(x/K_i)^{n_i}}{(1 + (x/K_i)^{n_i})} \qquad x = 1 \text{ because } \frac{0.990}{0.990} = 1$$

So to get f_i close to 1, $(x/K_i)^{n_i} >> 1$

since $x = 1$, make $K_i < 1$, try $K_i = 0.3$

To make the value larger, try $n = 6$:

$(x/K_i)^{n_i} = 1,371.74$

$$f_i = \frac{1371}{1372} \approx 0.999$$, seems to be close to 1

Not unique but try C and see how well it matches

C. see code and attached graph.

Based on how close the measured data points are to the model, it appears that this model for allosteric regulation works for PFK.