CHEME 5440/7770: Take Home Prelim 1 S2021

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1. Take Home Prelim 1 has two questions which are collectively worth 50 points.
2. Take Home Prelim 1 is due on T April 13, 2021 by 11:59 PM Ithaca time
3. You may use your course notes, literature, the internet, or other course materials to formulate your solutions.
4. You *cannot* consult with any other person regarding the prelim (except ZM, MP or JV). You *cannot* use any form of electronic communication to discuss the prelim questions with any other person (except ZM, JV or MP via a direct message in Slack). Violation of this policy will result in a ZERO for the prelim, and an honor code violation.
5. Mistakes/corrections/clarifications to the prelim document will be made on the #general Slack channel by ZM, JV or MP.
6. In all problems, show your work and state all assumptions or simplifications and **clearly state your answers**.
7. **Submission**: Submit your solution to the teaching team email by the deadline. Your solution should include all written material, links to source code if posted on GitHub or the source code itself, and instructions to reproduce your calculations/figures.

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1. (25 points). In class we considered the expression of a single gene. Let's now derive the *kinetic limit* of transcription for gene j ($r_{X,j}$) in a set of $\mathcal{N}$ genes. Assume the same four elementary steps proposed in class:

$$\begin{aligned} \mathcal{G}_j + R_X^\circ &\rightleftharpoons (\mathcal{G}_j : \mathrm{R_X})_C \\ (\mathcal{G}_j : \mathrm{R_X})_C &\longrightarrow (\mathcal{G}_j : \mathrm{R_X})_O \\ (\mathcal{G}_j : \mathrm{R_X})_O &\longrightarrow R_X + \mathcal{G}_j \\ (\mathcal{G}_j : \mathrm{R_X})_O &\longrightarrow m_j + R_X + \mathcal{G}_j \end{aligned}$$

where $\mathcal{G}_j$, R_X° denote the gene and *free* RNAP concentration, and $(\mathcal{G}_j : \mathrm{R_X})_O$, $(\mathcal{G}_j : \mathrm{R_X})_C$ denote the open and closed complex concentrations, respectively. Let the kinetic limit of transcription be directly proportional to the concentration of the open complex:

$$r_{X,j} = k_{E,j} \left(\mathcal{G}_j : \mathrm{R_X}\right)_O$$

where $k_{E,j}$ is the elongation rate constant for gene j.

a) Starting from the proposed elementary steps and the RNAP balance:

$$R_{X,T} = R_X^\circ + (\mathcal{G}_j : \mathrm{R_X})_C + (\mathcal{G}_j : \mathrm{R_X})_O + \sum_{i=1,j}^{\mathcal{N}} \Big\{ (\mathcal{G}_i : \mathrm{R_X})_C + (\mathcal{G}_i : \mathrm{R_X})_O \Big\}$$

show that:

$$r_{X,j} = k_{E,j} R_{X,T} \left(\frac{\mathcal{G}_j}{\tau_{X,j} K_{X,j} + (1 + \tau_{X,j}) \mathcal{G}_j + \mathcal{E}_j} \right)$$

where:

$$\mathcal{E}_j = \sum_{i=1,j}^{\mathcal{N}} \frac{K_{X,j} \tau_{X,j}}{K_{X,i} \tau_{X,i}} \left(1 + \tau_{X,i}\right) \mathcal{G}_i$$

The saturation and time constants are defined as $K_{X,j}^{-1} \equiv k_{+,j} / \left(k_{-,j} + k_{I,j}\right)$ and $\tau_{X,j}^{-1} \equiv k_{I,j} / \left(k_{A,j} + k_{E,j}\right)$, respectively.

b) Under what circumstances would an $\mathcal{N}$-gene system ($\mathcal{N} \gg 1$) be approximately equivalent to the 1-gene system we derived in class?

2. (25 points). Derive and test an expression for the allosteric regulation of enzyme activity. Allosteric regulation is a fast mechanism that regulates the catalytic activity of metabolic enzymes. In this type of regulation, the ability of an enzyme to catalyze a reaction depends upon the concentration of effector molecules which do not directly participate in the chemical reaction. For example, Phosphofructokinase (PFK), a key glycolytic enzyme which catalyzes the conversion of D-fructose 6-phosphate (F6P):

$$\mathrm{ATP} + \mathrm{D-fructose\ 6-phosphate} \longrightarrow \mathrm{ADP} + \mathrm{D-fructose\ 1,6-bisphosphate} \quad (1)$$

is strongly activated in the presence of 3′-5′-AMP, a signalling molecule produced when glucose is transported into cells.

In lecture we suggested that we could use a statistical mechanical approach, similar to our promoter modelling strategy, to describe allosteric regulation. Let's explore this idea by building and a testing a model of the allosteric regulation of PFK using the experimental dataset for PFK activity (3′-5′-AMP versus reaction rate) posted in the *#prelims* channel in Slack.

Proposal: Let the model take the form $\hat{r}_j = r_j v\left(\ldots\right)_j$ where $\hat{r}_j$, the overall rate of the PFK reaction (μM h^{-1}), is the product of a kinetic limit r_j (μM h^{-1}), i.e., the maximum rate of conversion, and a control variable $0 \leq v\left(\ldots\right)_j \leq 1$ (dimensionless) that describes the influence of effector molecules. Let $v\left(\ldots\right)_j$ take the form:

$$v\left(\ldots\right)_j = \frac{\sum\limits_{i \in \{\mathcal{X}_j\}} W_i f_i\left(\ldots\right)}{\sum\limits_{j \in \mathcal{C}_j} W_j f_j\left(\ldots\right)} \quad (2)$$

where W_i (dimensionless) denotes the weight of configuration i, while $f_i\left(\cdots\right)$ (dimensionless) is a hill-type binding function $f_i = (x/K_i)^{n_i}/(1 + (x/K_i)^{n_i})$ which describes the fraction of bound activator/inhibitor (x) for configuration i; K_i denotes a binding constant (mM), and n_j denotes an order parameter (dimensionless). The summation in the numerator of $v\left(\ldots\right)_j$ is over those configurations that lead to activity (denoted by set $\mathcal{X}_j$), while the summation in the denominator is over all possible configurations for enzyme j (denoted as $\mathcal{C}_j$). Let the kinetic limit for PFK (E_1, μM)

be given by:

$$r_1 = k_{cat} E_1 \left(\frac{F6P}{K_{F6P} + F6P} \right) \left(\frac{ATP}{K_{ATP} + ATP} \right) \tag{3}$$

Assume: (i) the concentration of F6P in the assay equals 0.1 mM and is constant; (ii) the concentration of ATP in the assay equals 2.3 mM and is constant; (iii) the concentration of PFK in the assay equals 0.12 μM and is constant; (iv) K_{F6P} = 0.11 mM, K_{ATP} = 0.42 mM, and k_{cat} = 0.4 s^{-1}.

a) Propose and describe the terms in a three-state model for PFK activity; State 0: no effector+no substrate, State 1: no effector+substrate and State 2: effector+substrate.

b) Estimate the parameter(s) W_1 (no 3′-5′-AMP), W_2 (with 3′-5′-AMP), the binding constants and order parameters from the dataset. **Note**: this can be done analytically, but need not be.

c) Plot your estimated overall rate $\hat{r}_1$ (y-axis), along with the measured rate (with errorbars), versus the 3′-5′-AMP concentration (x-axis) on the same axes. How well does the proposed model describe the data?