Quantitative Molecular Biology
Problem Set #5
due: Thursday, March 18, 2021

1. **Uptake vs de novo synthesis of amino acid.** An amino acid a can be taken up by the transporter T if it is available in the environment or it can be synthesized *de novo* by a series of enzymes collectively referred to as E. It is advantageous for the cell to synthesize the transporter instead of the enzyme E when a is available from the environment because synthesizing E costs a lot more than synthesizing T. In this problem, you will investigate a simple regulatory strategy that allows the cell to synthesize the enzyme E only when needed, i.e., when the external concentration of amino acid $[a]_{\text{ext}}$ is low.

   Let the specific rate of uptake by the transporter be k_T, and specific rate of synthesis by the enzyme be k_E. Then changes in the cellular concentration of the amino acid, $[a]$, can be described by

$$\frac{d[a]}{dt} = k_T \cdot [T] + k_E \cdot [E] - j, \tag{1}$$

   where j is the flux of a demanded by cell growth and is proportional to the growth rate. The uptake rate k_T itself depends on the external concentration of a as will be specified below.

   (a) Both the transporter and enzyme concentrations are regulated by a common regulatory scheme called "end-product inhibition", with

$$[T] = T_0 \frac{1 + ([a]/K_T)^n}{1 + f \cdot ([a]/K_T)^n}, \tag{2}$$

   and

$$[E] = E_0 \frac{1 + ([a]/K_E)^n}{1 + f \cdot ([a]/K_E)^n}. \tag{3}$$

   For simplicity, we use here regulatory functions with the same capacity $f \gg 1$ and Hill coefficient $n \to \infty$. Sketch (by hand) the dependence of $[T]$ and $[E]$ on $[a]$ and explain the meanings of the parameters T_0, K_T, E_0, and K_E.

   (b) For $K_T < K_E$, sketch the dependence of the concentrations $[T]$, $[E]$, and $[a]$ on the flux demand, j. (You can take the steady state where $d[a]/dt = 0$.) Find an expression for the critical value of the flux at which the enzyme E is 'turned on'? How does this critical value depend on the values of K_T, K_E, and f? What happens for $K_E < K_T$?

   (c) In part (b) above, you will notice that there is no solution for very small and very large values of j. Write down the lower and upper limit in terms of the model parameters, and explain what "goes wrong" in each case. Show how the problem at small-j end disappears if n is taken to be finite.

   (d) The specific uptake rate depends on the external concentration of a via the standard Michaelis-Menton form,

$$k_T = k_{T,max} \frac{[a]_{\text{ext}}}{[a]_{\text{ext}} + K_a}, \tag{4}$$

   where $k_{T,max}$ is the maximal uptake rate for saturating concentration of a, and K_a is the half-saturation constant for the transporter. Sketch the dependence of the concentrations $[T]$, $[E]$, and $[a]$ on the external concentration $[a]_{\text{ext}}$ for a fixed value of

the demand flux j. What is the critical concentration of external a below which the enzyme E is 'turned on'? Describe or sketch qualitatively how the dependences of $[T]$, $[E]$, and $[a]$ on $[a]_{\text{ext}}$ would change if n has a finite value, e.g., $n = 2$?

2. **Effect of antibiotics on cell growth:** Bacteriostatic antibiotics slows down cell growth by interfering with a spectrum of bacteria-specific functions without killing cells. The drug efficacy can be quantified by the IC50 value, which is the concentration that slows down growth by 50%. In this problem, we will compute IC50 for antibiotics which targets protein synthesis using the approach of quantitative growth physiology.

   Let the fraction of proteins in the proteome devoted to translational processes be ϕ_R, and let the fraction of proteins devoted to metabolism be ϕ_P. An exponentially growing bacterial culture with specific growth rate λ is known to obey the growth laws:

$$\phi_R = \lambda/\gamma, \qquad \text{and} \qquad \phi_P = \lambda/\nu,$$

   where γ, the average translation rate, is only affected by the applied drug, and ν, the nutrient assimilation rate, is only affected by the type of nutrients supplied. [Take the concentrations of nutrient be saturating.] Furthermore, it is known that

$$\phi_R + \phi_P = \phi_{\max},$$

   where $\phi_{\max} \approx 50\%$ for all nutrient and drug combinations.

   (a) By eliminating ϕ_R and ϕ_P in the above 3 relations, find the growth rate λ as a function of γ, ν, and $\phi_{\max}$.

   (b) The translation rate in the absence of drugs, γ_0, is $\sim 5/hr$. Find the value of the largest possible growth rate, $\lambda_{\max}$, i.e., the growth rate corresponding to the best possible nutrient ($\nu \to \infty$). What is the corresponding doubling time? (remember the factor of $\ln 2$.) Now for nutrients of limited quality (i.e., for finite ν), write down an expression describing how the growth rate in the absence of drug, λ_0, depends on $\lambda_{\max}$, γ_0, and ν.

   (c) Suppose the antibiotics binds to the ribosome with a dissociation constant K and reduces the translation rate from γ_0 to

$$\gamma = \frac{\gamma_0}{1 + [D]/K}$$

   where $[D]$ is the drug concentration. Show that the growth rate depends on drug concentration as

$$\lambda = \frac{\lambda_0}{1 + [D]/K_I}$$

   and find IC50, K_I, in terms of the dissociation constant K and the ratio of the drug-free growth rate λ_0 and the maximum drug-free growth rate $\lambda_{\max}$.

   (d) Rewrite your result in part (c) in terms of the doubling time T in the presence of drug, and the doubling time T_0 in the absence of drugs. For a drug with a dissociation constant $K = 5\mu M$, plot the doubling time T vs drug concentration $[D]$ in 3 different growth medium, which support doubling time of $T_0 = 20\text{min}, 60\text{min}, 100\text{min}$ respectively in the absence of drug. Indicate the value of K_I on the plot for each case. Explain qualitatively why the IC50 value should depend on the quality of the nutrient, as manifested by the dependence on the drug-free growth rate λ_0.

3. **Growth on two co-utilized carbon sources.** A culture of bacteria can simultaneously utilize two carbon substrates, S_1 and S_2. Suppose the growth rate in minimal medium with S_1 as the sole carbon source is λ_1, and is λ_2 with S_2 as the sole carbon source. In this problem, we will derive the growth rate λ_{12} for medium containing both substrates S_1 and S_2. [Take the concentration of the substrates to be always saturating.]

   A lot is known about the growth of cells on a single substrate S_i, where $i \in \{1, 2\}$. Let the concentration of the uptake proteins for substrate S_i be $[E_i]$ in the absence of the other substrate. We showed in class that for different carbon sources, the concentration of the enzyme E_i depends on the growth rate λ_i by the so-called C-line,

$$[E_i] = E_{i,\max} \cdot (1 - \lambda_i/\lambda_C)$$

   where $E_{i,\max}$ is a enzyme-specific constant (and hence may be different for different substrates), and λ_C is independent of the substrate. λ_C in fact presents a 'speed limit', as cells cannot grow faster than λ_C no matter how carbon sources may be improved in the medium.

   It is also known that the carbon influx J_i for substrate S_i is proportional to $[E_i]$, i.e.,

$$J_i = k_i \cdot [E_i]$$

   where k_i is an effective nutrient uptake rate. Finally, the carbon influx is related to the growth rate as $\lambda_i = c_i \cdot J_i$, where the constant c_i describes the conversion between the substrate mass and the cell's dry mass.

   (a) With substrate S_i alone, find the growth rate λ_i as a function of the combination $c_i k_i E_{i,\max}$ and λ_C. Simplify this expression for a very poor substrate ($k_i \to 0$) and a very good substrate ($k_i \to \infty$). Invert this function to express the combination $c_i k_i E_{i,\max}$ as a function $g(\lambda_i, \lambda_C)$ which depends on λ_i and λ_C.

   In medium with both substrates S_1 and S_2 present, we want to find the growth rate λ_{12}. Let the concentration of the uptake enzymes for the two substrates be $[E_1^*]$ and $[E_2^*]$. [The asterisk are reminders that enzyme concentrations are different from the case in part (a) when only a single substrate is present.] The corresponding fluxes are J_1^* and J_2^*, respectively, with each flux still given by $J_i^* = k_i[E_i^*]$.

   (b) If the enzyme concentrations still follow the C-line, write down the relation between $[E_i^*]$ and the growth rate λ_{12}.

   (c) The relation between growth rate and the carbon fluxes can now be generalized to $\lambda_{12} = c_1 J_1^* + c_2 J_2^*$. Use the result in part (b) to eliminate $[E_1^*]$ and $[E_2^*]$, and express λ_{12} in terms of only λ_C and $c_i k_i E_{i,\max}$. Use the function g obtained in part (a) to express λ_{12} in terms of λ_C, λ_1 and λ_2 only. This is the "growth rate addition" formula which gives the growth rate in the medium with both substrates in terms of the growth rates in single substrates and the speed limit λ_C.

   (d) Simplify the expression for λ_{12} if $\lambda_i/\lambda_C \ll 1$. Also find λ_{12} if one of the single-substrate growth rates is close to λ_C. Finally, find λ_{12} for $\lambda_1 = \lambda_2 = 0.5/h$, and for $\lambda_1 = 0.3/h$, $\lambda_2 = 0.7/h$, if $\lambda_C = 1/h$.