1. **Threshold response of an inducible auto-activator** A transcription factor (TF) A activates the transcription of a gene $g$, which codes for a protein $G$. As a result the rate with which $G$ is produced depends on the concentration $[A]$ of A as $\alpha G([A])$. Here $\alpha$ is the maximum protein synthesis rate, and $G([A])$ is the regulation function

$$G([A]) = \epsilon + [A]/K$$

where $K$ is the equilibrium dissociation constant for the TF-DNA binding and $\epsilon$ represents a basal leakage term. The protein $G$ is degraded at a rate $\beta$. [For simplicity assume the volume of cell is constant (=1 in suitable unit), so that you don’t need to distinguish between protein concentration and the number of proteins per cell.]

(a) Write down the deterministic rate equation describing the dynamics of the product $[G]$. What is the equilibrium value of $[G]$ given $[A]$?

(b) Now consider the case where $[G]$ actually activates its own gene (so that A and G are now one and the same protein). Write down the differential equation for the concentration $[G]$ and calculate its equilibrium value.

(c) Next we assume that G can activate the transcription of its own gene only if it is bound to some small molecule L (a ligand). We refer to the concentration of G not bound to L as $[G]$, to the concentration of G bound to a ligand as $[G_L]$, and call the total concentration $[G_{tot}] = [G] + [G_L]$.  
   • What is the equilibrium value of $[G_{tot}]$ if $[L]=0$? 
   • What is it if $[L]$ is very large? 
   • Explain in words why this circuit can be considered a cellular sensor for the concentration of the ligand.

(d) We define $r = [G_L]/[G_{tot}]$, i.e., $r$ is the fraction of G proteins bound to a ligand.  
   • Write down the differential equation for $[G_{tot}]$.  
   • Assume for simplicity that $\epsilon$ is negligible. What is the equilibrium concentration of $[G_{tot}]$ as a function of $r$? Sketch a plot of $[G_{tot}]$ as a function of $r$ (You can assume that $K < \alpha/\beta$). Some reasonable parameters would be $\alpha/\beta \approx 90$ nM and $K \approx 30$ nM.
• Explain in words why the behavior of the circuit as a function of the ligand concentration can be called a “threshold response”.

• Now assume that $\epsilon$ is not negligible, but nevertheless small: $\epsilon=0.05$. Sketch another plot of $[G_{tot}]$ as a function of $r$. If a strict threshold response is desired, what value should $\epsilon$ have?

2. Proteome allocation and Monod’s growth law: In this problem, we will work out key elements of the proteome allocation analysis for bacterial growth step-by-step using a concrete example, growth of *E. coli* cells on lactose. With a few assumptions, we will derive the growth rate and the expression of the lac operon in environment with different lactose concentration, hence derive Monod’s growth law and the phenomenon of catabolite repression.

In the following, all quantities correspond to amount derived from a 1-mL of culture at optical density (OD) $= 1$, referred to as “OD·mL” in short. 1 OD·mL of culture corresponds to $10^8 \sim 10^9$ bacterial cells in typical culturing conditions. We will refrain from using per cell quantity because the amount per cell can vary 10x due to change of cell size in different conditions. Instead, amount per OD·mL is more invariant. In particular, total dry mass contained in 1 OD·mL of culture is approx 0.5 mg and total cytoplasmic water contained in 1 OD·mL of culture is approximately 1 mg (or 1$\mu$L in volume) for almost all growth conditions characterized. The total protein content in OD·mL varies moderately, from 0.3 mg (at fast growth) to 0.4 mg (at slow growth). For simplicity, we will take total protein per OD·mL to be 0.35 mg.

Definition of symbols to be used below: $N_i$ and $M_i$ are, respectively, the number and mass of protein $i$ per OD·mL of culture. $M \approx 0.35 \text{ mg}$ is the total mass of cellular proteins per OD·mL of culture. $m_i$ is the molecular weight of protein $i$. $\phi_i \equiv M_i/M$ is the mass fraction of protein $i$ among all cellular proteins; it is also referred to as the “proteome fraction”.

(a) Conversion between proteome fraction and concentration: The average intracellular concentration of a protein $X$, $[X]$, can be taken as the number of proteins in OD·mL of culture, $N_X$, divided by the total cytoplasmic water volume in OD·mL of culture, $V$. For a protein with molecular weight $m_X$, derive a relation between its concentration $[X]$ and its proteome fraction $\phi_X$. If the protein is 300 aa in length (typical of many proteins), find its concentration in $\mu$M if its proteome fraction is 1‰ (part per thousand).

[Hint: It will be useful to convert the total protein abundance $M = 0.35 \text{ mg/(OD·mL)}$ into “mol of aa per OD·mL”, using the average mass of an amino acid, 110 Daltons.]

Note: In the problems below, we will only refer to protein concentrations as proteome fractions. It turns out that proteome fraction is more readily obtained experimentally (e.g., by proteomics and ribosome-profiling); it is also a natural quantity to work with in models. The purpose of this problem is to let you know that you can always convert proteome fraction to a more familiar concentration unit, e.g., $\mu$M.

(b) Protein synthesis flux by ribosomes: Let $J_R$ denote the flux of protein synthesis, in unit of no. of aa polymerized per OD·mL of culture. For a culture growing exponentially at
rate \( \lambda \), this is just \( \lambda \cdot M \) (with the total protein mass \( M \) in \#aa/OD\cdot mL). Molecularly, protein synthesis flux can be written as the product of the ribosome elongation rate (denoted as \( \varepsilon \)) and \( N_R \), the total number of ribosomes per OD\cdot mL of culture. [Here we have assumed that all ribosomes are engaged in translation at the same speed. This turns out to be a reasonably good approximation, breaking down only at very slow growth.]

- Show that the above leads to the growth law

\[
\lambda = \gamma \cdot \phi_R, \quad (1)
\]

where \( \phi_R \equiv M_R/M \) is the proteome fraction of ribosomal proteins, \( M_R \) being the total mass of r-proteins per OD\cdot mL of culture. Express \( \gamma \) in terms of \( \varepsilon \) and \( m_R \), the molecular weight of all r-proteins in a ribosome.

- Adding up the length of all r-proteins in a ribosomes gives 7336aa. However, for a ribosomes to do its job, many helper proteins such as elongation factors are also needed. These proteins add up to another 60% in mass. Thus, we can take the “molecular weight” of an effective ribosome as \( m_R = 1.6 \times 7336\text{aa} \). Further using \( \varepsilon = 16\text{aa/s} \), find the value of \( \gamma \) in unit of \( h^{-1} \).

- Note that this the maximum growth rate attainable if a cell contains only ribosomes. What is the corresponding doubling time? The fastest doubling time for \( E.\ coli \) is 20 min, when the culture is supplemented with many nutrient ingredients including all amino acids and nucleotides. What is the corresponding ribosomal fraction \( \phi_R \) at this fastest growth rate? The remaining fraction of the proteome, called \( \phi_Q \), are comprised of obligatory proteins needed for house-keeping functions, e.g., synthesis of the lipid membrane and cell wall.

(c) Carbon uptake flux: Consider growth of \( E.\ coli \) in minimal medium with a single substrate as a carbon source, without supplement of amino acid and other substances. Let \( J_C \) denote the flux of carbon uptake, in unit of no. of substrate molecules taken up per time per OD\cdot mL . Molecularly, this can be written as a product of \( k_E \), the turnover rate of the uptake enzyme, and \( N_E \), the number of enzymes per OD\cdot mL of culture.

- Express \( J_C \) in term of the proteome fraction of the uptake enzyme, \( \phi_E \equiv M_E/M \) and the molecular weight of enzyme \( E, m_E \).

- The condition of flux balance can be stated as \( J_R = c \cdot J_C \), where the coefficient \( c \) represents the conversion factor from the substrate molecule to aa. Using flux balance, and the expression you obtained above for \( J_R \) and \( J_C \), derive the relation \( \lambda = \nu_E \cdot \phi_E \) and find an expression for \( \nu_E \) in terms of the parameters for the enzyme \((k_E, m_E)\) and the coefficient \( c \).

- Consider the case where lactose is the sole carbon substrate. 1g of lactose is known to yield 1g of dry mass. Based on the protein:dry mass ratio given above, work out the conversion factor \( c \) for lactose.

- Given that the turnover rate for the lactose transporter (LacY, the lac permease), is \( k_Y = 3/s \), and the molecular weight of LacY is \( m_Y = 417\text{aa} \). Work out the value of \( \nu_E \) for lactose uptake (called \( \nu_Y \)) in unit of \( h^{-1} \).

(d) The lactose transporter is one of a suite of “carbon catabolic proteins” expressed when \( E.\ coli \) is short of carbon supply. The other proteins include beta-galactosidase (LacZ)
which degrades lactose into glucose and galactose, and other enzymes not specific to lactose degradation. Let the proteome fraction of all these carbon catabolic proteins be $\phi_C$. Since the expression of LacY is co-regulated with these proteins (by cAMP-CRP), we can write

$$\lambda = \nu_C \cdot \phi_C,$$

where $\nu_C = \alpha_Y \nu_Y$ if LacY is the bottleneck of lactose uptake.

For cells grown in minimal medium without the supplement of amino acids, etc, another significant fraction of the proteome is comprised of anabolic proteins (i.e., enzymes for biosynthesis of amino acids, etc.) Let the proteome fraction of all these enzymes be $\phi_A$. There is a linear relation between the growth rate $\lambda$ and $\phi_A$ similar to Eqs. (1) and (2):

$$\lambda = \nu_A \cdot \phi_A. \quad (3)$$

It turns out that $\nu_A \approx \gamma$.

Finally, there is the constraint that sum of all proteome fractions add up to 1, i.e.,

$$\phi_R + \phi_C + \phi_A = \phi_{\text{max}}, \quad (4)$$

where $\phi_{\text{max}} = 1 - \phi_Q$, $\phi_Q$ being is the same fraction of obligatory proteins encountered in part (b).

- Combine Eqs. (1)-(4) to show that the growth rate depends on the parameter $\nu_C$ (a measure of “nutrient quality”) as

$$\lambda = \frac{\nu_C}{\nu_C + K_C}. \quad (5)$$

Express $\lambda_C$ and $K_C$ in terms of $\gamma$ and $\phi_{\text{max}}$.

- For an “infinitely good” carbon source for which $\nu_C \to \infty$, what is the growth rate?

- For E. coli growing on lactose, the growth rate is found to be approximately 1/h. Find the corresponding value of $\nu_C$. Find the proteome fraction $\phi_R$, $\phi_C$, and $\phi_A$ devoted to ribosomal, catabolic, and anabolic enzymes during growth on lactose.

- Based on the value of $\nu_Y$ you calculated in part (c), what share of catabolic proteins is LacY? What fraction of the entire proteome is LacY?

(e) Next, let us consider the equilibration of lactose within the cytoplasm. As stated above, the influx of lactose is given by $k_Y \cdot N_Y$. The lactose brought into the cell is degraded by LacZ. The lactose degradation flux is $k_Z \cdot N_Z [L]_{\text{in}} / ([L]_{\text{in}} + K_Z)$, where $[L]_{\text{in}}$ is the intracellular lactose concentration, $k_Z$ and $K_Z$ are the turnover rate and binding constant of beta-galactosidase for lactose, and $N_Z$ is the number of enzymes. To keep the intracellular lactose concentration at the level of $K_Z$, how much LacZ must be expressed? i.e., what is the proteome fraction $\phi_Z$ for LacZ? What is the share of catabolic proteins do LacY and LacZ together comprise of?

[Properties of beta-galactosidase you need to know: $k_Z = 60/s$, molecular weight $m_Z = 4100$ aa (since LacZ has the length of 1024 aa, and functional enzyme is comprised of a LacZ tetramer).]
Finally, we derive the Monod law relating the growth rate at different substrate concentrations in the medium. Let the lactose concentration in the medium be \([L]\). Then, the lactose turnover rate per LacY becomes

\[
k_Y([L]) = k_Y^\infty \frac{[L]}{[L] + K_Y}
\]  

(6)

where \(k_Y^\infty = 3/s\) is the saturated turnover rate used above, and \(K_Y\) is the binding constant of lactose to LacY. Express \(\nu_C\) in terms of \(k_Y([L])\) and use it in Eq. (6) in Eq. (5) to derive Monod’s growth law:

\[
\lambda([L]) = \lambda^\infty \frac{[L]}{[L] + K_M}.
\]

(7)

Express the Monod constant \(K_M\) in terms of \(K_Y\) and the basic parameters of the growth laws in Eqs. (1)-(3). Find the value of the Monod constant if \(K_Y = 0.3\) mM.

Can you explain why the Monod constant, which describes the lactose concentration at which the growth rate is half of the maximum value, is much smaller than the binding constant \(K_Y\)?

(g) Solve for \(\phi_C([L])\), the fraction of catabolic proteins at different lactose concentration \([L]\). Show that

\[
\phi_C([L]) = \phi_C^{\max} \cdot (1 - \lambda([L]) / \lambda_C),
\]

(8)

and give the value of \(\phi_C^{\max}\). Eq. (8) describes a linear decline in the abundance of catabolic proteins with growth rate (the “C-line”). It is a quantitative statement of the phenomenon of “catabolite repression” ubiquitous phenomenon in microbiology, wherein the expression of catabolic enzymes is inhibited in medium with improved carbon availability. Try to explain in your own words why should cells reduce the expression catabolic proteins when carbon is more available.

3. Growth on two co-utilized carbon substrates. 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 \(\lambda_1\), and is \(\lambda_2\) with \(S_2\) as the sole carbon source. In this problem, we will derive the growth rate \(\lambda_{12}\) for medium containing both substrates \(S_1\) and \(S_2\). [In this problem, we will take the concentrations of the substrates to be always at saturating values.]

In Problem #2, we learned a lot about the growth of cells on a single substrate. Let the proteome fraction of the uptake proteins be \(\phi_{E,i}\) for substrate \(S_i\) in the absence of the other substrate. Then from Eq. (8) above (with \(\phi_{E,i} = \alpha_{E,i} \phi_C\)), we see that \(\phi_{E,i}\) and the growth rate \(\lambda_i\) are simply related by the C-line,

\[
\phi_{E,i} = \phi_{E,i}^{\max} \cdot (1 - \lambda_i / \lambda_C),
\]

(9)

where \(\phi_{E,i}^{\max}\) is an enzyme-specific constant, and \(\lambda_C\) is independent of the substrate. Note that \(\lambda_C\) is a ‘speed limit’, as cells cannot grow faster than \(\lambda_C\) no matter how carbon sources may be improved in the medium.

From Problem #2, we also learned that the carbon uptake flux \(J_{C,i}\) for substrate \(S_i\) is proportional to \(\phi_{E,i}\). The condition of flux balance applied to growth on substrate \(S_i\),
\(J_R = c_i \cdot J_{C,i}\) (where \(c_i\) is the conversion factor between substrate \(S_i\) and amino acid), leads to the relation

\[
\lambda_i = \nu_{E,i} \phi_{E,i}\]

(10)

where the parameter \(\nu_{E,i}\) can be expressed in terms of \(k_{E,i}, m_{E,i}\), and the conversion factor \(c_i\) as done in part (c) of the previous problem.

(a) With substrate \(S_i\) alone, combine Eqs. (9) and (10) to express the combination \(\nu_{E,i} \phi_{E,i}^{\text{max}}\) as a function \(g(\lambda_i, \lambda_C)\).

In medium with both substrates \(S_1\) and \(S_2\) present, we want to find the growth rate \(\lambda_{12}\). We expect \(\lambda_{12}\) to be different from \(\lambda_1 + \lambda_2\) since the latter can in principle exceed the ‘speed limit’ \(\lambda_C\). Let the proteome fraction of the uptake enzymes for the two substrates be \(\phi_{E,1}^*\) and \(\phi_{E,2}^*\). [The asterisks serve as a reminder that enzyme levels are different from the case in part (a) when only a single substrate is present.] The corresponding fluxes are \(J_{C,1}^*\) and \(J_{C,2}^*\), respectively, with each flux still proportional to the respective enzyme’s proteome fraction, \(\phi_{E,i}^*\).

(b) Write down the relation between the uptake flux \(J_{C,i}^*\) and its corresponding enzyme level \(\phi_{E,i}^*\) for the case where both substrates are present. If the proteome fractions \(\phi_{E,1}^*\) and \(\phi_{E,2}^*\) still follow the C-line (since they are both regulated by cAMP-Crp), write down their dependences on the growth rate \(\lambda_{12}\).

(c) The flux balance condition can now be generalized to \(J_R^* = c_1J_{C,1}^* + c_2J_{C,2}^*\). From \(J_R^* = \lambda_{12} \cdot M\), write down a simple relation among \(\lambda_{12}, \phi_{E,1}^*, \) and \(\phi_{E,2}^*\).

Use the result in part (a) and (b) to eliminate \(\phi_{E,1}^*\) and \(\phi_{E,2}^*\), and express \(\lambda_{12}\) in terms of only \(\lambda_C\) and \(\nu_{E,i} \phi_{E,i}^{\text{max}}\). Use the function \(g\) obtained in part (a) to express \(\lambda_{12}\) in terms of \(\lambda_C, \lambda_1\) and \(\lambda_2\) only. This is the “growth rate addition” formula which predicts the growth rate in the medium with both substrates in terms of the growth rates in single substrates and the speed limit \(\lambda_C\).

(d) Simplify the expression for \(\lambda_{12}\) if \(\lambda_i/\lambda_C \ll 1\). Also find \(\lambda_{12}\) if one of the single-substrate growth rates is close to \(\lambda_C\). E.g., find \(\lambda_{12}\) as a function of \(\lambda_1\) if \(\lambda_2 = 0.9 \cdot \lambda_C\). Plot \(\lambda_{12}\) vs \(\lambda_1\) for \(\lambda_2 = 0.3/h, 0.6/h, 0.9/h, 1.2/h\) and \(\lambda_C = 1.25/h\). Comment on your results.

4. **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 growth laws.

Let the fraction of proteome devoted to translational, catabolic, and anabolic processes be \(\phi_R, \phi_C, \) and \(\phi_A\), respectively. As we learned in Problem #2, flux balance conditions lead to simple linear relations between the growth rate \(\lambda\) and each of these 3 proteome fractions; see Eqs. (1) – (3), with the macroscopic parameters \(\gamma, \nu_C, \nu_A\) related to molecular parameters. The 3 proteome fractions are also constrained by (4).

(a) By eliminating \(\phi_R, \phi_C, \) and \(\phi_A\) in each the of the relations (1) – (3), find the growth rate \(\lambda\) as a function of \(\gamma, \nu_C, \nu_A,\) and \(\phi_{\text{max}}\).
A large class of antibiotics target the ribosome, and the effect of a good number of them can be described as slowing down the translational process. We can model the effect of a sublethal dose of such translation-retarding drugs by a reduction in the ribosome’s elongation rate, which would result in a reduction in the parameter $\gamma$; see Problem #2a. Let the value of $\gamma$ in the absence of drugs be $\gamma_0$ (whose value was obtained in Problem #1a), and let the corresponding growth rate be $\lambda_0$. For different carbon sources (different values of $\nu_C$), the growth rate varies as $\lambda_0(\nu_C)$.

(b) Suppose the antibiotics binds to the ribosome with a dissociation constant $K_D$ and reduces $\gamma$ as described below:

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

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

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

Find the IC50 value, $K_I$, in terms of the dissociation constant $K_D$ and the ratio of the drug-free growth rate $\lambda_0(\nu_C)$ and the maximum drug-free growth rate $\lambda_0(\nu_C \rightarrow \infty) \equiv \lambda_C$.

(c) Rewrite your result in part (b) 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 $K_D = 5 \mu M$, plot the doubling time $T$ vs drug concentration $[D]$ in 3 different growth medium, with carbon sources that support doubling time of $T_0 = 60\text{ min}, 90\text{ min}, 120\text{ min}$, respectively in the absence of the 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 ($\nu_C$), as manifested by its dependence on the drug-free growth rate $\lambda_0(\nu_C)$.