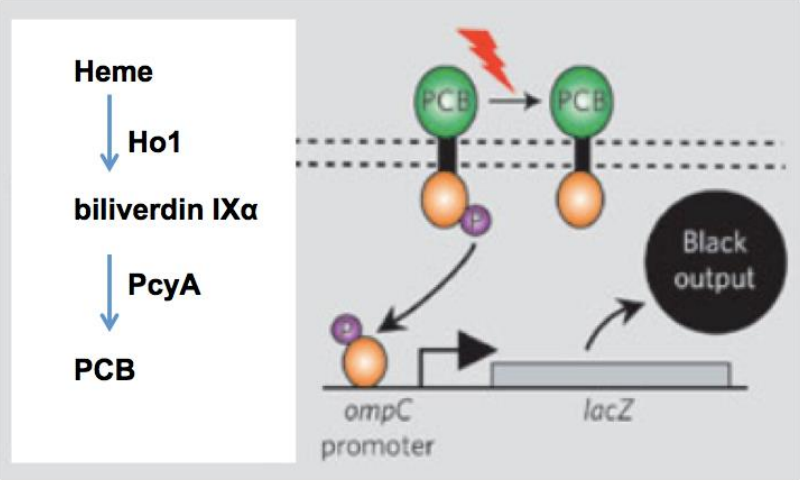
CHEMENG 355 Spring 2018

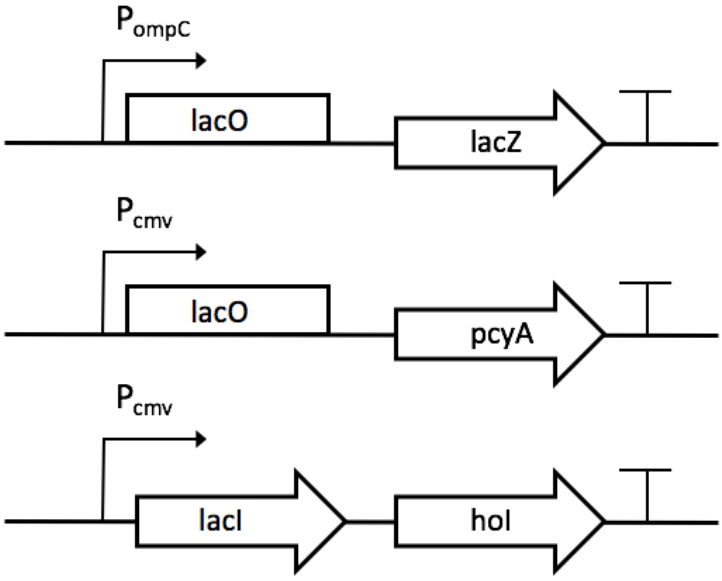
Problem Set 3 Due May 24, 2018

Problem 1: Expression Regulation (16 points)

Voigt and colleagues have engineered a system to control gene expression in *E. coli* through light exposure. They introduced enzymes, Ho1 and PcyA, to convert heme (already present in the bacteria) to a photosensitive receptor, Pcb. The Pcb receptor activates promoter P_{ompC} only if light is not present. They then linked the promoter to expression of the enzyme LacZ, which catalyzes the conversion of a small molecule present in the media (S-gal) into a dark compound for detection.



Here, we've altered the system to include additional forms of regulation, namely the addition of the lacI repressor to give another degree of control. (The table below summarizes the key parts of the engineered system):



Element	Function
Genes	
lacZ	Codes for an enzyme that converts S-gal to a black pigment
pcyA	Codes for expression of an enzyme that converts biliverdin Ixα to the Pcb receptor
lacI	Codes for Lac repressor which binds to the lac operator sequence (*lacO*), preventing transcription from any upstream promoter; IPTG binding causes lac repressor to dissociate from the lac operator
ho1	Codes for expression of an enzyme that converts heme to biliverdin IXα
Promoters, terminator, and RBS	
P_{cmv}	Constitutive promoter; always "on," unless activity blocked by a repressor protein bound downstream
P_{ompC}	Promoter that is only "on" if protein Pcb has not been activated by light
T: Terminator	Generic terminator that terminates transcription for any gene or operon
RBS: Ribosome binding site	Required to initiate translation
Operators	
lacO: Lac operator	Bound by lac repressor
Small molecules	
IPTG: Isopropyl β-D-1-thiogalactopyranoside	Binds to Lac repressor, causing it to dissociate from the lac operator; diffuses freely into the cell, but is not degraded

(A) Add RBSs where needed in the diagram above using asterisks. **(4 pts total: in front of LacZ, LacI, hoI and pcyA)**

(B) Fill in the following table with the outputs given the indicated inputs:

Use (+) to indicate an output is present and (-) to indicate the output is absent.

IPTG	light	pcyA	ho1	lacZ
-	-	-	+	-
+	-	+	+	+
-	+	-	+	-
+	+	+	+	-

(1pt per box, 12 total)

- ho1 will always be active due to the constitutive cmv promoter, the lacI gene only codes for the repressor but is itself not bound by the repressor
- Adding IPTG will inactivate the lac repressor, allowing for expression of pcyA
- lacZ expression requires pcyA, ho1, and IPTG are present; however, it also requires light not be present, since light will inactivate Pcb

Problem 2: Protein Expression and Folding (14 points)

We are seeking to make a 35,000 MW protein for a new Hepatitis B vaccine using the cell-free method, which is essentially *in vitro* protein synthesis. Our desired protein seems to have folding problems, and, at the moment, most of the protein accumulates as aggregates. We are going to attempt to improve folding by lowering the reaction temperature. After an analysis of *in vitro* protein folding, we estimate that the folding rate constant is 1.5 hr^{-1} and the second order rate constant for aggregation is 2.0 hr^{-1} μM^{-1} based on the estimated concentration of the nascent (unfolded) protein.

(A) In our cell-free system, the rate of translation is 4 amino acids per ribosome per second, and we have a steady-state mRNA concentration of 0.003 µM and 5 ribosomes per message. What will be the rate of production of *properly folded* protein? (Assume average amino acid MW of 110 g/mol) **(6 points)**

We need to first find the total rate of protein production, dP_T/dt, under these conditions.

$$\frac{dP_T}{dt} = [mRNA] \cdot \left(\frac{\#\ ribosome}{mRNA}\right) \cdot (translation\ rate)$$
$$= \left(\frac{0.003\ \mu mol\ mRNA}{L}\right) \cdot \left(\frac{5\ mol\ ribosome}{mol\ mRNA}\right) \cdot \left(\frac{4\ mol\ AA}{mol\ ribosome \cdot s}\right)\left(\frac{3{,}600\ s}{1\ hr}\right)$$
$$= 216\ {}^{\mu M\ AA}/_{hr}$$

Using the protein MW, convert this to µM protein / hr.

$$\frac{dP_T}{dt} = \left(\frac{216\ \mu M\ AA}{hr}\right) \cdot \left(\frac{110\ g}{mol\ AA}\right) \cdot \left(\frac{1\ mol\ protein}{35{,}000\ g}\right)$$
$$= 0.679\ {}^{\mu M\ protein}/_{hr}$$

Total rate of protein production = rate of protein properly folded + rate of protein aggregated

$$\frac{dP_T}{dt} = \frac{dP}{dt} + \frac{dA}{dt} = k_P N + k_A N^2$$
$$0.679\ {}^{\mu M\ protein}/_{hr} = (1.5\ hr^{-1}) \cdot N + (2.0\ hr^{-1}\mu M^{-1}) \cdot N^2$$

Solve for N using the quadratic equation or a polynomial solver.

$$N = 0.318\ \mu M$$

Now we can find dP/dt = $k_P N$.

$$\frac{dP}{dt} = k_P N = (1.5\ hr^{-1}) \cdot (0.318\ \mu M) = \mathbf{0.477\ {}^{\mu M}/_{hr}}$$

(B) Under these conditions, what percent of the product will be properly folded? **(4 points)**

Let *x* be the percent of product that is properly folded.

$$\frac{dP/dt}{dA/dt} = \frac{x}{100 - x}$$

In part (A), we already calculated dP/dt to be 0.477 μM/hr. For dA/dt:

$$\frac{dA}{dt} = k_A N^2 = (2.0\ hr^{-1}\mu M^{-1}) \cdot (0.318\ \mu M)^2 = 0.202\ {}^{\mu M}/_{hr}$$

$$\frac{0.477\ {}^{\mu M}/_{hr}}{0.202\ {}^{\mu M}/_{hr}} = \frac{x}{100 - x} = 2.36$$

$$x = \mathbf{70.2\%}$$

Alternatively, we could also simply calculate:

$$x = \frac{dP/dt}{dP_T/dt} = \frac{0.477\ {}^{\mu M}/_{hr}}{0.679\ {}^{\mu M}/_{hr}} = \mathbf{70.3\%}$$

(C) Why might lowering the reaction temperature result in improved protein folding? **(4 pts)**

Lowering the reaction temperature is likely slowing the kinetics of protein translation. It should be recognized that the given translation rate of 4 AA/ribosome-s is much slower than the typical prokaryotic rate discussed in class (16 AA/ribosome-s). If the mRNA is being translated at a lower rate, this will give the folding machinery (e.g. chaperones, foldases) more time to fold the nascent protein properly. Additionally, if the translation rate is lower, the nascent protein concentration (N) will also be lower. A lower N will have a larger negative effect on dA/dt (which varies as N^2) than on dP/dt (varies as N). Partial or full credit was also given for the following reasons:

- Lowering the reaction temperature can help us avoid the potential for protein denaturation caused by higher temperatures. The activity of proteases, enzymes which are responsible for breaking down proteins, may also be decreased at lower temperatures.
- Thermodynamically, the Gibbs free energy for the folding process is given by $\Delta G = \Delta H - T\Delta S$. Since $\Delta S < 0$ for folding, decreasing T will lessen the contribution from the entropy term towards ΔG, making the folding process more thermodynamically favorable.