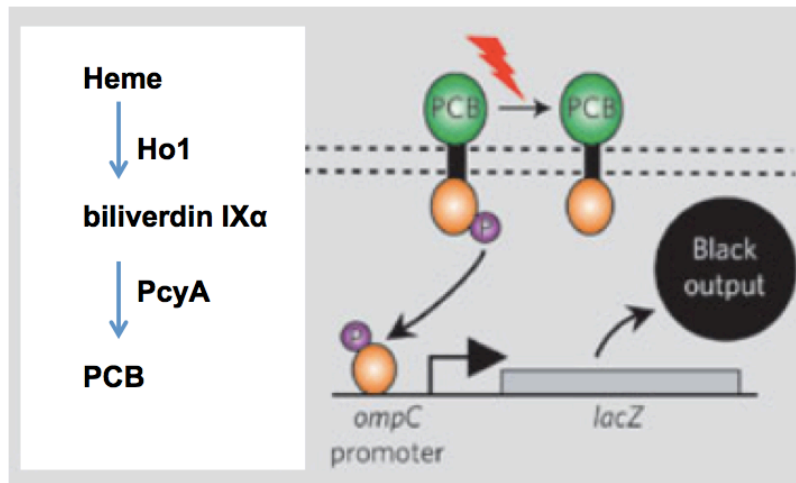
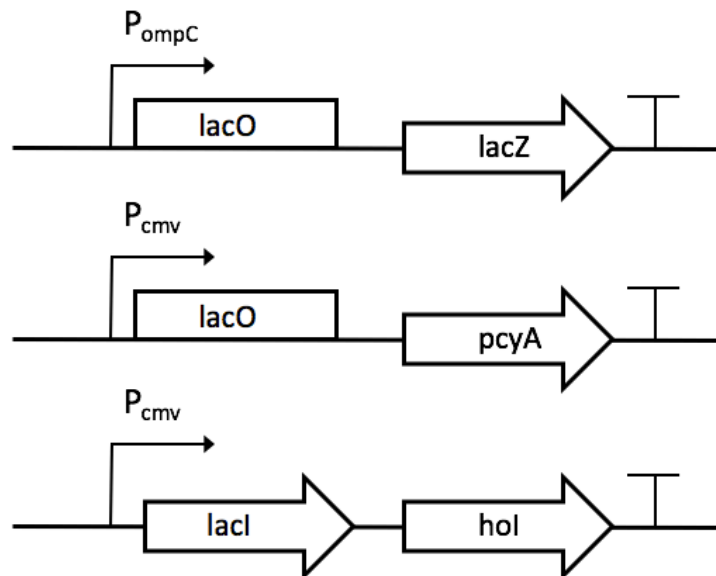


**Problem 1: Expression Regulation**

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
<b>Genes</b>	
<i>lacZ</i>	Codes for an enzyme that converts S-gal to a black pigment
<i>pcyA</i>	Codes for expression of an enzyme that converts biliverdin IX $\alpha$ to the Pcb receptor
<i>lacI</i>	Codes for Lac repressor which binds to the lac operator sequence ( <i>lacO</i> ), preventing transcription from any upstream promoter; IPTG binding causes lac repressor to dissociate from the lac operator
<i>ho1</i>	Codes for expression of an enzyme that converts heme to biliverdin IX $\alpha$
<b>Promoters, terminator, and RBS</b>	
P <sub>cmv</sub>	Constitutive promoter; always “on,” unless activity blocked by a repressor protein bound downstream
P <sub>ompC</sub>	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
<b>Operators</b>	
<i>lacO</i> : Lac operator	Bound by lac repressor
<b>Small molecules</b>	
IPTG: Isopropyl $\beta$ -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 RBSES where needed in the diagram above using asterisks.

(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
-	-			
+	-			
-	+			
+	+			

## Problem 2: Protein Expression and Folding

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  $1.5 \text{ hr}^{-1}$  and the second order rate constant for aggregation is  $2.0 \text{ hr}^{-1} \mu\text{M}^{-1}$  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  $0.003 \mu\text{M}$  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)
- (B) Under these conditions, what percent of the product will be properly folded?
- (C) Why might lowering the reaction temperature result in improved protein folding?