Problem Set #1
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1 Part A

Starting with the mental model of transcription:

$$G_j + R_X \rightleftharpoons (G_P : R_X)_C$$

$$(G_j : R_X)_C \rightarrow (G_j : R_X)_O$$

$$(G_j : R_X)_O \rightarrow G_j + R_X$$

$$(G_j : R_X)_O \rightarrow m_j + G_j + R_X$$

Where G_j represents some gene concentration and R_X represents free RNAP concentration and $(G_j : R_X)_C$,$(G_j : R_X)_O$ represent the closed and open complexes respectively. We can define the kinetic rate of transcription as being proportional to the open complex by the elongation rate rate constant k_{Ej}:

$$r_{Xj} = k_{Ej}(G_j : R_X)_O$$

Now differential equations can be written on the closed and open complexes:

$$\frac{d}{dt}(G_P : R_X)_C = k_+(G_j)(R_X) - k_-(G_j : R_X)_C - k_I(G_P : R_X)_C$$

$$\frac{d}{dt}(G_P : R_X)_O = k_I(G_j : R_X)_C - k_A(G_j : R_X)_O - k_{Ej}(G_P : R_X)_O$$

Each rate term refers to the rate of formation on the closed complex (second order k_+), the dissociation of the closed complex (k_-), the formation of the open complex from the closed (k_I), and the rate of abortive initiation (k_A).
Now, a mass balance on total RNAP can be made:

$$R_{XT} = R_X + (G_P : R_X)_C + (G_P : R_X)_O$$

From here, the quasi steady state assumption must be made to solve for the open and closed complexes. This means that the concentrations of the complexes after an initial change, will not change much and the rate of mRNA production will dominate. There is a separation of timescales between mRNA production and the complexes' rate of change. We can set the complexes' rate of change to 0. The solutions are thus:

$$(G_P : R_X)_C = \frac{k_+}{k_- + k_I}(G_j)(R_X)$$

$$(G_P : R_X)_O = \frac{k_I}{k_A + k_E}(G_j : R_X)_C$$

We can define the saturation constant of the gene and RNAP binding as:

$$K_{Xj}^{-1} = \frac{k_+}{k_- + k_I}$$

The time constant comparing various paths the complex of the gene and RNAP can take is defined as:

$$\tau_{Xj}^{-1} = \frac{k_I}{k_A + k_E}$$

Taking these new parameters, the open complex can be related to the free gene and RNAP by:

$$(G_P : R_X)_O = K_{Xj}^{-1}\tau_{Xj}^{-1}G_jR_X$$

Substituting into the mass balance:

$$R_{XT} = R_X + K_{Xj}^{-1} G_j R_X + K_{Xj}^{-1} \tau_{Xj}^{-1} G_j R_X$$

Free RNAP can be isolated to:

$$R_X = \frac{R_{XT} K_{Xj} \tau_{Xj}}{K_{Xj} \tau_{Xj} + (\tau_{Xj} + 1) G_j}$$

Finally, the open conformation can be rewritten and plugged into our equation for rate:

$$(G_j : R_X)_O = \frac{R_{XT} G_j}{K_{Xj} \tau_{Xj} + (\tau_{Xj} + 1) G_j}$$

$$r_{Xj} = k_{Ej} R_{XT} \frac{G_j}{K_{Xj} \tau_{Xj} + (\tau_{Xj} + 1) G_j}$$

This kinetic term can be transformed into the overall specific rate of transcription with a control term $u(I)$ defined in Part C:

$$\hat{r}_{Xj} = r_{Xj} u(I)$$

$$\hat{r}_{Xj} = k_{Ej} R_{XT} \frac{G_j}{K_{Xj} \tau_{Xj} + (\tau_{Xj} + 1) G_j} u(I)$$

Now, we have to define all these parameters which are in Table One:

Table One: Relevant Parameters

Parameter Name	Symbol	Value	Units	Source
Gene concentration	G_j	2500	plasmids/cell	Given
Gene concentration	G_j	6.196	μM	See Note 2 Below
Gene length	L	3075	nucleotides	Given
E.coli Elongation Rate	e_X	42	nt/s	Gotta[1]
Rate of Elongation	k_{Ej}	0.0137	s^-1	See Note 1 Below
RNAP Concentration	R_{XT}	8000	molecules/cell	Bremer[2]
RNAP Concentration	R_{XT}	19.8	μM	See Note 2 Below
Rate of Open Complex Formation	k_I	1/42	s^-1	McClure[3]
Rate of Closed Complex Formation	k_+	5	$\mu M^{-1} s^{-1}$	See Note 3 Below
Rate of Close Complex Dissociation	k_-	0.1	s^{-1}	See Note 3 Below
Rate of Abortive Initiation	k_A	0	s^{-1}	Assume $k_A << k_I or k_E$

Note 1:

$$k_{Ej} =< k_E > \frac{L}{L_j}$$

Where $< k_E >$ is the average elongation rate and L,L_j refer to the characteristic gene length (average here) and the current gene length (3075 nt). This simplifies to the elongation rate e_X (42 nt/s) divided by the gene length:

$$k_{Ej} = e_X / L_j = 42/3075 = 0.0137 s^{-1}$$

Note 2:

Some numbers were converted from molecules per cell to μM via Avogadro's number and the volume of an E. Coli cell (6.7e-10μL from Wang[4]).

Note 3:

The values of k_+ and k_- were derived from McClure's[3] formula and value for the slope of a tau plot in *E.Coli* D promoters:

$$McClureSlope = 1.04 \mu M * s = \frac{k_- + k_I}{k_+ k_I}$$

$$1.04 k_+ (1/42) = k_- + 1/42$$

We can arbitrarily assign $k_+ = 5\mu M^{-1}s^{-1}$ which leads to $k_- = 0.1s^{-1}$. This is valid because when these values are used later in calculating K_{Xj}, the ratio of k_-/k_+ or some dissociation constant k_D is restored. This is best described below:

$$McClureSlope = \frac{k_- + k_I}{k_+ k_I}$$

$$K_{Xj} = \frac{k_- + k_I}{k_+}$$

Thus:

$$K_{Xj} = McClureSlope * k_I$$

Plugging into either equation for K_{Xj} returns the same value. It also happens that K_D or k_-/k_+ for *E.Coli* with the lac promoter is $550nM$ (Bintu[5]) which is only one order of magnitude off our arbitrary assignment ($20nM$).

2 Part B

We can write the equation for τ_{Xj} and plug in the values from Table One:

$$\tau_{Xj}^{-1} = \frac{k_I}{k_A + k_E}$$

$$\tau_{Xj} = \frac{k_A + k_E}{k_I}$$

$$\tau_{Xj} = \frac{0.0137s^{-1}}{1/42s^{-1}}$$

$$\tau_{Xj} = 0.57$$

Since τ_{Xj} is less than 1, the rate of open complex formation is faster than the rate of elongation making **transcription elongation limited.**

3 Part C

Performing a mass balance on mRNA yields:

$$\frac{dm_j}{dt} = r_{Xj}u_j - k_{Xj}^d m_j - m_j B^{-1}B$$

Where m_j is the concentration of mRNA, r_{Xj} is the rate of transcription, u_j is some sort of operator deciding the fraction of transcription occurring [0,1], k_{Xj}^d is the rate of degradation of mRNA, and $B^{-1}B$ relates to the dilution of mRNA. These dilution terms can be simplified into the specific growth rate μ. The degradation rate can be extracted from half-life and both these numbers are in Table Two:

$$ln(2) = k_{Xj}^d t_{0.5}$$

$$k_{Xj}^d = 0.0058s^{-1}$$

Table Two: Relevant Parameters

Parameter Name	Symbol	Value	Units	Source
mRNA half-life	$t_{0.5}$	120	s	Li[6]
Specific growth rate of *E.coli*	μ	1.14	hr^{-1}	Selvarasu[7]

Now setting the rate of change of mRNA equal to zero in steady state yields:

$$0 = r_{Xj}u_j - k^d_{Xj}m_j - m_j\mu$$

$$m_j = \frac{r_{Xj}u_j}{k^d_{Xj} + \mu}$$

u_j can be defined with a Voigt type model (Moon[8]) as a function of one inducer as ($u(I)$):

$$u_j = \frac{W_1 + W_2 f_I}{1 + W_1 + W_2 f_I}$$

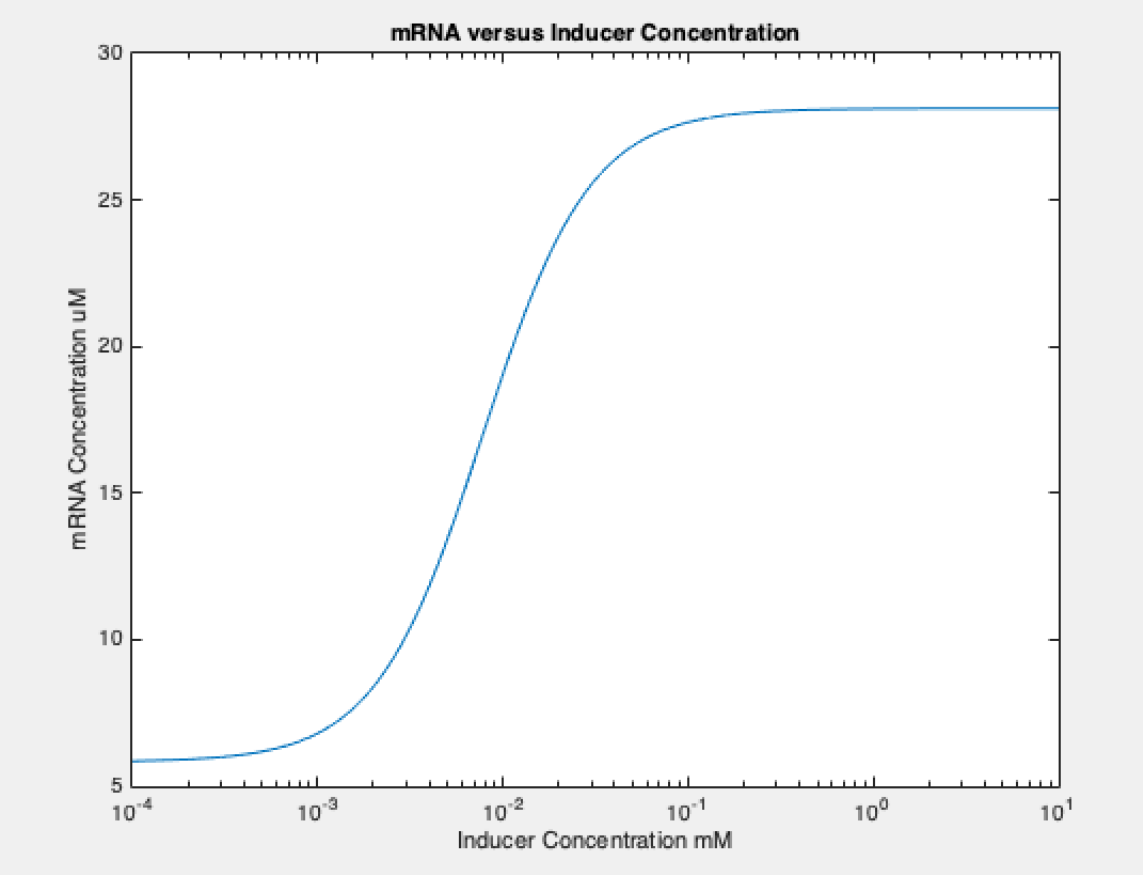
Where:

$$f_I = \frac{I^n}{K^n + I^n}$$

And $I = [0.0001, 10.0mM]$, $W_1 = 0.26$, $W_2 = 300.0$,$K = 0.30mM$, and $n = 1.5$. Plugging everything in yields the following equation of $m_j\mu M$ as a function of inducer ImM:

$$m_j = \frac{k_{Ej}R_{XT}\frac{G_j}{K_{Xj}\tau_{Xj}+(\tau_{Xj}+1)G_j} * \frac{W_1+W_2f_I}{1+W_1+W_2f_I}}{k^d_{Xj} + \mu}$$

Plotted on a semi-log scale with attached code README_CHEME5440HW1.m yields a familiar step-like function. Inducer is required for any significant transcription to occur:



References

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