CHEME 7770 HW1

tm545

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1 1a

Derivation of the rate of transcription for a protein P, given a single inducer I given by the following equation.

$$\hat{r}_{x,p} = r_{x,p} * u(I) \tag{1}$$

First let us derive the kinetically limited rate. The following equations show the kinetics behind transcription. Let G_p be the gene of protein p, R_x be the amount of free RNA polymerase. $(G_p : R_x)_c$ and $(G_p : R_x)_o$ give the closed and open complexes, respectively, and m_p is the mRNA transcript of the gene. All rate constants are shown below.

$G_p + R_x \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (G_p – R_x)_c$

$(G_p – R_x)_c \xrightarrow{k_I} (G_p – R_x)_o$

$(G_p – R_x)_o \xrightarrow{k_A} G_p + R_x$

$(G_p – R_x)_o \xrightarrow{k_E} m_p + G_p + R_x$

We assume that k_A is much smaller and negligible when compared to k_E. The following equation gives us the rate of transcription.

$$\frac{d[m_p]}{dt} = r_{x,p} = k_E(G_p : R_x)_o \tag{2}$$

We can begin solving for the rate by finding concentration of the open complex and closed complex from a steady state assumption and solving the following ordinary differential equations.

$$\frac{d}{dt}(G_p : R_x)_c = k_1(G_p)(R_x) - k_{-1}(G_p : R_x)_c - k_I(G_p : R_x)_c \tag{3}$$

$$\frac{d}{dt}(G_p : R_x)_o = k_I(G_p : R_x)_c - k_E(G_p : R_x)_o - k_A(G_p : R_x)_o \tag{4}$$

Since we assumed k_A is neglibile we can rewrite equation 4 as:

$$\frac{d}{dt}(G_p : R_x)_o = k_I(G_p : R_x)_c - k_E(G_p : R_x)_o \tag{5}$$

Our steady state assumptions leads to the following two equations:

$$(G_p : R_x)_o = \frac{k_I}{k_E}(G_p : R_x)_c \tag{6}$$

$$(G_p : R_x)_c = \frac{k_1}{k_{-1} + k_I}(G_p)(R_x) \tag{7}$$

We can plug equation 7 into equation 6 as well as rename our fractions into the following:

$$\tau_x^{-1} = \frac{k_I}{k_e} \tag{8}$$

$$\kappa_x^{-1} = \frac{k_1}{k_{-1} + k_I} \tag{9}$$

All this simplification results in:

$$(G_p : R_x)_o = \tau_x^{-1} * \kappa_x^{-1} * G_p * R_x \tag{10}$$

R_x is difficult to measure within a cell and harder to use in a model, however we can rewrite equation 10 in terms of total RNA polymerase within a cell as shown by $R_{x,T}$ and the following equation.

$$R_{x,T} = R_x + \frac{G_p * R_x}{\kappa_x} + \frac{G_p * R_x}{\kappa_x * \tau_x} \tag{11}$$

Equation 11 represents the total amount of RNA polymerase as the sum of the free RNA polymerase, polymerase within the closed complex, and polymerase within the open complex. This results in the following equation for R_x:

$$R_x = \frac{R_{x,T} * G_p}{\kappa_x * \tau_x + (\tau_x + 1)(G_p)} \tag{12}$$

Plugging in equation 12 into equation 10 results in the following:

$$(G_p : R_x)_o = \frac{R_{x,T} * G_p}{\kappa_x * \tau_x + (\tau_x + 1)(G_p)} \tag{13}$$

Therefore the kinetically limited rate of transcription is given as follows:

$$r_{x,p} = \frac{k_E * R_{x,T} * G_p}{\kappa_x * \tau_x + (\tau_x + 1)(G_p)} \tag{14}$$

We still need to solve for u(I) which represents a Voigt type model for how activated the gene is, where u(I) is a number between 0 and 1 given by the following equation:

$$u_j = \frac{W_{R,j} + \Sigma W_{n,j} f_{n,j}}{1 + W_{R,j} + \Sigma W_{n,j} f_{n,j}} \tag{15}$$

In our case we have the following:

$$u(I) = \frac{W_1 + W_2 f_I}{1 + W_1 + W_2 f_I} \tag{16}$$

Where W_j are weight fractions and f_I is given as follows for the binding fraction:

$$f_I = \frac{I^n}{k + I^n} \tag{17}$$

So the final equation for the rate of transcription is as follows:

$$\hat{r}_{x,p} = r_{x,p} * u(I) = \frac{k_E * R_{x,T} * G_p}{\kappa_x * \tau_x + (\tau_x + 1)(G_p)} * \frac{W_1 + W_2 f_I}{1 + W_1 + W_2 f_I} \tag{18}$$

The following parameters are given: $W_1 = 0.26$, $W_2 = 300.0$, k = 0.30 mM, L = 3075 nt $G_p = 2500$ and n = 1.5. And the following table shows other parameters as found from literature.

Parameter	Value	Citation
k_I	$.33sec^{-1}$	[1]
e_x	$62\frac{nt}{sec}$	[2]
K_{obs}	$1.04 * 10^{-3} mM * sec$	[3]
$R_{x,T}$	$30 * 10^{-6} mM$	[4]
$V_{cytoplasm}$	$.67 * 10^{-18} m^3$	[5]

From these parameters we can solve for everything else.

$$k_E = e_x * L^{-1} = .020\frac{1}{sec} \tag{19}$$

$$\kappa_x = k_obs * k_I = 1.04 * 10^{-3} mM * sec * .33\frac{1}{sec} = 3.432 * 10^{-4} mM \tag{20}$$

$$\tau_x = \frac{k_E}{K_I} = \frac{.020sec^{-1}}{.33sec^{-1}} = .061 \tag{21}$$

Let $G_{p,c}$ be the concentration of the plasmid within a cell.

$$G_{p,c} = \frac{\frac{2500}{6.02*10^23}moles}{.67 * 10^{-18} m^3} = .0062M = 6.2mM \tag{22}$$

So $r_{x,p}$ can be given as the following after having plugged in all the parameters shown above (note this isn't $\hat{r}_{x,p}$)

$$r_{x,p} = 5.655 * 10^{-7} mM/s \tag{23}$$

2 1b

Transcription for this gene is elongation limited as shown by both our Tau value being so small as well as when comparing the size of k_E and k_I we see that k_I which denotes that initiation happens on a much smaller time scale than elongation. The rate constants that intitiation happens in about three seconds while elongation takes about 50 seconds showing that elongation is the rate limiting step.

3 1c

To plot the steady state concentration of m_p we must solve the following differential equation.

$$\frac{d[m_p]}{dt} = r_{x,p} * u(I) - k_d * m_p - m_p * B^{-1} * B \tag{24}$$

We assume steady state and so the steady state m_p concentration would be given by:

$$m_p = \frac{r_{x,p} * u(I)}{k_d + B^{-1} * B} \tag{25}$$

We use bionumbers to find half life of mRNA and the doubling rate of E. coli to then calculate k_d and dilution rate.

Parameter	Value	Citation
$k_{half-life}$	900 sec	[6]
doubling time	1.14 hr^{-1}	[7]

$$k_d = \frac{ln(2)}{k_{half-life}} = 7.701 * 10^{-4} sec^{-1} \tag{26}$$

$$B^{-1} * B = \mu = 1.14 \frac{1}{hr} * \frac{1hr}{3600sec} = 3.17 * 10^{-4} sec^{-1} \tag{27}$$

Plotting this equation in Excel yields the following graph which is similar to others for Hill function kinetics with step growth. Appreciable amounts of protein expression requires significant amounts of the inducer.

References

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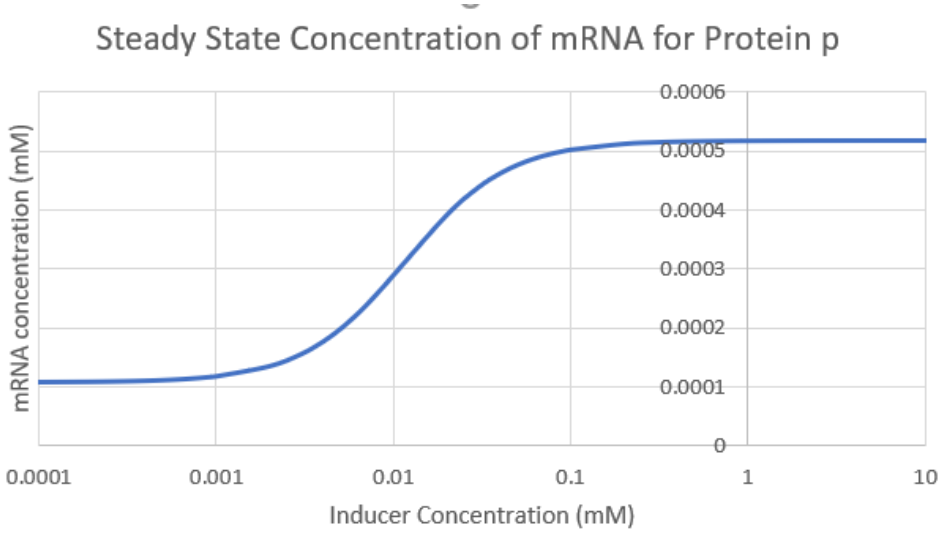

Figure 1: Graph of the steady state mRNA concentration

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