# CHEME 7770 Prelim1

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## 1 Question 1

### 1.1 1a

Given the following elementary steps a kinetic rate limit, given by $r_{Xj} = k_{Ej}(G_j : R_x)_o$, can be derived for transcription of genes without the open complex term that is hard to experimentally find.

$$\mathrm{G}_j + \mathrm{R}_x^o \underset{\mathrm{k_-}}{\overset{\mathrm{k_+}}{\rightleftharpoons}} (\mathrm{G}_j\text{–}\mathrm{R}_x)_c$$
$$(\mathrm{G}_j\text{–}\mathrm{R}_x)_c \xrightarrow{\mathrm{k_I}} (\mathrm{G}_j\text{–}\mathrm{R}_x)_o$$
$$(\mathrm{G}_j\text{–}\mathrm{R}_x)_o \xrightarrow{\mathrm{k_A}} \mathrm{G}_j + \mathrm{R}_x$$
$$(\mathrm{G}_j\text{–}\mathrm{R}_x)_o \xrightarrow{\mathrm{k_{E,j}}} \mathrm{m}_p + \mathrm{G}_j + \mathrm{R}_x$$

These equations may be used to derive the following differential equations for the concentration of the open and closed complexes of the RNA polymerase and the gene j.

$$\frac{d}{dt}(G_j : R_x)_c = k_+(G_j)(R_x^o) - k_-(G_j : R_x)_c - k_I(G_j : R_x)_c \tag{1}$$

$$\frac{d}{dt}(G_j : R_x)_o = k_I(G_j : R_x)_c - k_{E,j}(G_j : R_x)_o - k_A(G_j : R_x)_o \tag{2}$$

The change in the concentration of both complexes can be assumed to be at psuedo-steady state and therefore equal to 0, resulting in the following equations.

$$0 = k_+(G_j)(R_x^o) - k_-(G_j : R_x)_c - k_I(G_j : R_x)_c \tag{3}$$

$$0 = k_I(G_j : R_x)_c - k_{E,j}(G_j : R_x)_o - k_A(G_j : R_x)_o \tag{4}$$

Solving equation 3 for the closed complex results in the following equation.

$$(G_j : R_x)_c = \frac{K_+}{k_- + k_I}(G_j)(R_x^o) \tag{5}$$

The following saturation parameter is defined in order to reduce the clutter of the equation 5.

$$K_{X,j}^{-1} = \frac{k_{+,j}}{k_{-,j} + k_{I,j}} \tag{6}$$

Similarly the open complex can be defined as the following.

$$(G_j : R_x)_o = \frac{k_I}{k_A + k_{E,j}}(G_j : R_x)_c \tag{7}$$

Similarly to equation 6, we can define the following time constant to further simplify equation 7.

$$\tau_{X,j}^{-1} = \frac{k_{I,j}}{k_{A,j} + k_{E,j}} \tag{8}$$

Substituting equation 7 and 5 into equation 8 allows us to express the open complex concentration in terms of more measurable parameters.

$$(G_j : R_x)_o = \tau_{X,j}^{-1} K_{X,j}^{-1}(G_j)(R_x^o) \tag{9}$$

The final parameter that we can alter to make it easier to measure experimentally is the concentration of the RNA polymerase. We have no way of knowing if the RNA polymerase is an in open complex, closed complex, free or bound to another gene, but instead we can rewrite equation 9 in terms of the total RNA polymerase concentration.

$$R_{X,T} = R_X^o + (G_j : R_x)_c + (G_j : R_x)_o + \sum_{i=1,j}^{N} \{(G_i : R_x)_c + (G_i : R_x)_0\} \tag{10}$$

We can rewrite some of the terms within this equation as with the values we have found already for the open and closed complexes. The summation term for other genes follows the same equation as for gene j but only uses different subscripts to denote the other genes.

$$R_{X,T} = R_X^o + K_{X,j}^{-1}(G_j)(R_x^o) + K_{X,j}^{-1}\tau_{X,j}^{-1}(G_j)(R_x^o) + \sum_{i=1,j}^{N}\{K_{X,i}^{-1}(G_i)(R_x^o) + K_{X,i}^{-1}\tau_{X,i}^{-1}(G_i)(R_x^o)\} \tag{11}$$

We need an expression for the free RNA polymerase which we can find by just rearranging terms from equation 11 and by multiplying everything by $\frac{K_{X,j}\tau_{X,j}}{K_{X,j}\tau_{X,j}}$ results in the following expression.

$$R_X^o = \frac{R_{X,T}K_{X,j}\tau_{X,j}}{K_{X,j}\tau_{X,j} + (1+\tau_{X,j})G_j + \sum_{i=1,j}^{N}\frac{K_{X,j}\tau_{X,j}}{K_{X,i}\tau_{X,i}}(1+\tau_{X,i})G_j} \tag{12}$$

Lets rewrite the summation part of that expression to save some space as the following parameter.

$$\mathcal{E}_j = \sum_{i=1,j}^{N}\frac{K_{X,j}\tau_{X,j}}{K_{X,i}\tau_{X,i}}(1+\tau_{X,i})G_j \tag{13}$$

After all this work we can now rewrite free RNA polymerase term in equation 9 in terms of measurable coefficients.

$$(G_j : R_x)_o = \tau_{X,j}^{-1}K_{X,j}^{-1}(G_j) * \frac{R_{X,T}K_{X,j}\tau_{X,j}}{K_{X,j}\tau_{X,j} + (1+\tau_{X,j})G_j + \mathcal{E}_j} \tag{14}$$

The kinetic rate limit of transcription is given as:

$$r_{X,j} = k_{E,j}(G_j : R_x)_o \tag{15}$$

We can now use equation 14 and clean it up a little to end up with following expression for the kinetic rate limit of transcription.

$$r_{X,j} = \frac{R_{X,T}G_j k_{E,j}}{K_{X,j}\tau_{X,j} + (1+\tau_{X,j})G_j + \mathcal{E}_j} \tag{16}$$

### 1.2 1b

In class we derived the 1-gene system as compared to this derivation which also includes a system of N genes. Comparison of the two kinetic limits show that the only change is the $\mathcal{E}_j$ term. This term is given by equation 13 and by examining this equation we can see that this term approaches zero if the $K_{X,i}\tau_{X,i}$ denominator of the equation is much larger than the numerator: $K_{X,j}\tau_{X,j}$ suggesting that initiation and elongation of other genes is much longer than the gene of interest. Another way this term could approach zero is if $G_i$ approaches zero which could describe the case if a plasmid was introduced into the cell which has a much higher concentration than the other genes so this term can be neglected.

## 2 Question 2

### 2.1 2a

The GRNSimKit package was utilized from the Varner Lab github repository. The provided .json file was editted in terms of the binding constants for proteins 1 and 2. The provided values were given no units, therefore the assumption was made that they were in petamoles per gram dry weight of cell. This assumption converted the binding constants to values that were close to the nanomoles produced of protein 1 and allowed protein 1 to begin to bind and activate or repress proteins 2 and 3, respectively. The altered .json file is included in this github repository. The GRNSimKit was utilized to build a data dictionary given this .json file and run a discrete solution of this network motif as shown by the protein concentrations expressed over time below.

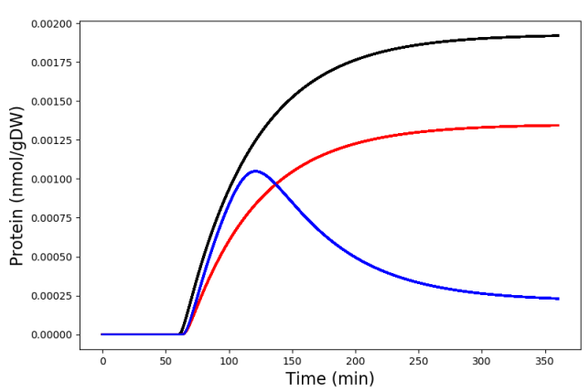


Figure 1: The protein concentrations of Protein 1 (black), Protein 2 (red), and Protein 3 (blue).

## 2.2 2b

Calculation of the scaled sensitivity parameters in each state for all parameters is walked through below.

$$s_{ij}(t) = (\frac{p_j}{x_i}) * \frac{\partial x_i}{\partial p_j}|_t \tag{17}$$

The scalar term within equation 17 was set as 1 when the magnitudes of the states were small (before the inducer was added - window 1). The partial term was calculated by using the finite difference method as shown below.

$$\frac{\partial x_i}{\partial p_j}|_t = \frac{f(p_j + h) - f(p_j)}{h} \tag{18}$$

H for this problem was set to .05 of the parameters magnitude in all cases. By scaling h to the magnitude of the parameter, the sensitivity coefficients could be rewritten as follows for cases where the small state assumption could not be used (windows 2 and 3).

$$s_{ij}(t) = \frac{p_j}{x_i} * \frac{x_i(p_j + h) - x(p_j)}{p_j * .05} = \frac{x_i(p_j + h) - x(p_j)}{.05 * x_i} \tag{19}$$

All states equations were written by pulling from a vector of all parameters that is shown below.

$$P = \begin{bmatrix} kE_{avgx} \\ L_x^o \\ L_{x1} \\ L_{x2} \\ L_{x3} \\ RNAP_{gDW} \\ gene_{gDW} \\ \kappa_x \\ kI_X \\ W_{11} \\ W_{I1} \\ I_{on} \\ k_I \\ n \\ W_{22} \\ W_{12} \\ K_{12} \\ W_{33} \\ W_{13} \\ K_{13} \\ W_{23} \\ K_{23} \\ n_{23} \\ kE_{avgl} \\ L_p^o \\ L_{p1} \\ L_{p2} \\ L_{p3} \\ Ribosome_{gDW} \\ \kappa_l \\ kI_l \\ kd_m \\ kd_p \\ \mu \end{bmatrix} \tag{20}$$

All 34 parameters were used to calculate a sensitivity coefficients within a window of 20 consecutive time points before the inducer was added, window 1, early after the inducer was added, window 2, and late after the inducer was added and protein levels begin to grow to steady state, window 3.

## 2.3 2c

Calculations of the sensitivity coefficients over 20 time points was then utilized to calculate a time averaged sensitivity coefficient by adapting the function created by the Varner Lab and shown in the Lac Operon example repository. The time averaged sensitivity coefficients for each state were grouped up to create networks based on the window the data was taken from. The magnitude of the calculated coefficients was found by dividing these network matrices by the magnitude of the first column of the U matrix from Singular Value Decomposition. The final matrices are shown below for each window.

Comparison of the mRNA1 within each window showed that at the first window most sensitivity coefficients were barely correlated with the level of mRNA1 possibly due to the inducer being completely absent from the cell and not even present in minute conditions which might have been a problem with this model. Looking at windows 2 and 3 showed no large changes with the most important parameters remaining the RNA polymerase concentration and the rate of initiation for this gene. Additionally looking at the ratio between the rate of initiation and elongation showed that this gene was limited by initiation.

| Window 1 | mRNA1 | mRNA2 | mRNA3 | Protein 1 | Protein 2 | Protein 3 |
|---|---|---|---|---|---|---|
| kE avg x | 1.89E-09 | 0.206738 | 0.078988 | 1.53E-08 | 2.20E-08 | 1.32E-08 |
| $L^0x$ | 9.69E-10 | 0.206738 | 0.078988 | 1.53E-08 | 2.20E-08 | 1.32E-08 |
| Lx1 | -9.69E-10 | -1.09E-15 | -5.15E-15 | -1.56E-08 | -2.77E-09 | -2.15E-09 |
| Lx2 | 0 | -0.207281 | 0 | 0 | -1.91E-08 | 0 |
| Lx3 | 0 | 0 | -0.081466 | 0 | 0 | -1.13E-08 |
| RNAP (gDW) | 1.61E-08 | 0.448497 | 0.44846 | 5.11E-08 | 5.18E-08 | 7.04E-08 |
| gene (gDW) | 8.15E-10 | 0.012894 | 0.02 | 1.92E-09 | 1.54E-09 | 3.05E-09 |
| kappa_x | -8.15E-10 | -0.013499 | -0.020904 | -2.01E-09 | -1.61E-09 | -3.18E-09 |
| kI_x | 2.96E-08 | 0.230852 | 0.362921 | 3.47E-08 | 2.81E-08 | 5.57E-08 |
| W_11 | 9.16E-08 | 0 | 0 | 5.11E-08 | 1.00E-08 | 7.75E-09 |
| W_I1 | 0 | 0 | 0 | 0 | 0 | 0 |
| I on | 0 | 0 | 0 | 0 | 0 | 0 |
| k_I | 0 | 0 | 0 | 0 | 0 | 0 |
| n | 0.00E+00 | 3.27E-15 | 6.70E-14 | 0 | -2.86E-08 | -2.21E-08 |
| W_22 | 0.00E+00 | 1.63E-09 | 0 | 0 | 3.90E-08 | 0 |
| W_12 | 0 | 0.015541 | 0 | 0 | 2.33E-09 | 0 |
| K_12 | 0.00E+00 | -1.09E-15 | 0 | 0 | -8.24E-09 | 0 |
| W_33 | 0 | 0.00E+00 | 7.49E-08 | 0 | 0 | 6.05E-08 |
| W_13 | 0 | 0 | 0.371586 | 0 | 0 | 1.81E-09 |
| K_13 | 0 | 0.00E+00 | -1.86E-14 | 0 | 0 | -6.38E-09 |
| W_23 | 0 | 0 | -0.354493 | 0 | 0 | 0 |
| K_23 | 0 | 0 | 0 | 0 | 0 | 0 |
| n_23 | 0 | 0 | 0 | 0 | 0 | 0 |
| kE avg I | 0 | 0 | 0.00E+00 | 4.29E-16 | 6.40E-16 | 3.79E-16 |
| $L^0_p$ | 0 | 0 | 0.00E+00 | 4.29E-16 | 6.40E-16 | 3.79E-16 |
| Lp1 | 0 | 0 | 0.00E+00 | -4.50E-16 | -8.19E-17 | -6.34E-17 |
| Lp2 | 0 | 0 | 0 | 0.00E+00 | -5.90E-16 | 0 |
| Lp3 | 0 | 0 | 0 | 0 | 0.00E+00 | -3.35E-16 |
| Ribosome (gDW) | 0 | 0 | 1.44E-14 | 5.11E-08 | 5.18E-08 | 7.04E-08 |
| kappa_I | 0 | -1.09E-15 | -1.65E-14 | -4.87E-08 | -4.72E-08 | -6.54E-08 |
| $kI_l$ | 0 | 0 | 1.44E-14 | 5.11E-08 | 5.18E-08 | 7.04E-08 |
| kd_m | -3.27E-12 | -5.30E-05 | -1.81E-05 | -1.83E-12 | -3.42E-12 | -5.71E-13 |
| kd_p | 0 | 0 | 0.00E+00 | -1.09E-14 | -2.92E-15 | -3.11E-15 |
| mu | -1.72E-13 | -2.78E-06 | -9.52E-07 | -4.89E-13 | -2.85E-13 | -1.42E-13 |

Figure 2: Sensitivity Coefficients for all states and parameters in window 1

| Window 2 | mRNA1 | mRNA2 | mRNA3 | Protein 1 | Protein 2 | Protein 3 |
|---|---|---|---|---|---|---|
| kE avg x | 6.38E-02 | 0.098169 | 0.037506 | 6.34E-02 | 9.75E-02 | 3.75E-02 |
| $L^0x$ | 0.063784 | 0.098169 | 3.75E-02 | 6.34E-02 | 9.75E-02 | 0.037478 |
| Lx1 | -6.50E-02 | -5.18E-16 | -2.45E-15 | -6.46E-02 | -4.56E-16 | -2.35E-15 |
| Lx2 | 0 | -0.098427 | 0 | 0.00E+00 | -0.097815 | 0 |
| Lx3 | 0 | 0 | -0.038683 | 0 | 0.00E+00 | -0.038655 |
| RNAP (gDW) | 0.212961 | 0.212967 | 2.13E-01 | 2.12E-01 | 2.12E-01 | 0.212781 |
| gene (gDW) | 0.008023 | 0.006123 | 9.50E-03 | 7.98E-03 | 6.08E-03 | 0.00949 |
| kappa_x | -0.008392 | -0.00641 | -9.93E-03 | -8.35E-03 | -6.37E-03 | -0.009919 |
| kI_x | 0.144734 | 0.109619 | 1.72E-01 | 1.44E-01 | 1.09E-01 | 0.172196 |
| W_11 | 8.00E-12 | 0 | 0.00E+00 | 7.95E-12 | 0.00E+00 | 0 |
| W_I1 | 0.000762 | 0 | 0 | 0.000758 | 0 | 0 |
| I on | 2.45E-09 | 0 | 0 | 2.44E-09 | 0 | 0 |
| k_I | -2.98E-09 | 0 | 0 | -2.96E-09 | 0 | 0 |
| n | 6.98E-09 | 1.55E-15 | 3.18E-14 | 6.94E-09 | 1.59E-15 | 3.20E-14 |
| W_22 | 0.00E+00 | 7.73E-10 | 0 | 0.00E+00 | 7.69E-10 | 0 |
| W_12 | 0 | 0.007379 | 0 | 0.00E+00 | 0.007332 | 0 |
| K_12 | 0.00E+00 | -5.18E-16 | 0 | 0.00E+00 | -4.56E-16 | 0 |
| W_33 | 0.00E+00 | 0.00E+00 | 3.56E-08 | 0 | 0.00E+00 | 3.56E-08 |
| W_13 | 0 | 0 | 0.17644 | 0 | 0.00E+00 | 0.176307 |
| K_13 | 0.00E+00 | 0.00E+00 | -8.81E-15 | 0 | 0.00E+00 | -8.82E-15 |
| W_23 | 0 | 0 | -0.168324 | 0 | 0 | -0.168207 |
| K_23 | 0 | 0 | 0 | 0 | 0 | 0 |
| n_23 | 0 | 0 | 0 | 0 | 0 | 0 |
| kE avg I | 0 | 0.00E+00 | 0.00E+00 | 6.68E-04 | 9.85E-04 | 6.56E-05 |
| $L^0_p$ | 0 | 0.00E+00 | 0.00E+00 | 6.68E-04 | 9.85E-04 | 6.56E-05 |
| Lp1 | 0 | 0.00E+00 | 0.00E+00 | -7.01E-04 | 0.00E+00 | 0 |
| Lp2 | 0 | 0 | 0.00E+00 | 0.00E+00 | -0.001034 | 0 |
| Lp3 | 0 | 0 | 0 | 0.00E+00 | 0.00E+00 | -6.89E-05 |
| Ribosome (gDW) | 0 | 0.00E+00 | 6.85E-15 | 2.13E-01 | 2.13E-01 | 0.212941 |
| kappa_I | 0.00E+00 | -5.18E-16 | -7.83E-15 | -2.02E-01 | -2.02E-01 | -0.202661 |
| $kI_I$ | 0 | 0.00E+00 | 6.85E-15 | 2.12E-01 | 2.12E-01 | 0.212868 |
| kd_m | -1.99E-05 | -2.52E-05 | -1.47E-06 | -1.98E-05 | -2.50E-05 | -1.47E-06 |
| kd_p | 0 | 0.00E+00 | 0.00E+00 | -5.66E-08 | -3.08E-09 | -8.31E-09 |
| mu | -1.05E-06 | -1.32E-06 | -7.72E-08 | -3.08E-06 | -1.42E-06 | -3.76E-07 |

Figure 3: Sensitivity Coefficients for all states and parameters in window 2

| Window 3 | mRNA1 | mRNA2 | mRNA3 | Protein 1 | Protein 2 | Protein 3 |
|---|---|---|---|---|---|---|
| kE avg x | 0.063784 | 0.098159 | 3.75E-02 | 6.34E-02 | 9.75E-02 | 0.03748 |
| $L^0x$ | 0.063784 | 9.82E-02 | 3.75E-02 | 6.34E-02 | 0.09752 | 0.03748 |
| Lx1 | -6.50E-02 | -5.18E-16 | -2.45E-15 | -6.46E-02 | -5.95E-16 | -2.46E-15 |
| Lx2 | 0 | -0.098417 | 0.00E+00 | 0 | -0.097805 | 0 |
| Lx3 | 0 | 0 | -0.038684 | 0.00E+00 | 0 | -0.038657 |
| RNAP (gDW) | 0.212963 | 2.13E-01 | 2.13E-01 | 2.12E-01 | 0.211524 | 0.21279 |
| gene (gDW) | 0.008023 | 6.12E-03 | 9.50E-03 | 7.98E-03 | 0.006083 | 0.00949 |
| kappa_x | -0.008392 | -6.41E-03 | -9.93E-03 | -8.35E-03 | -0.006369 | -0.009919 |
| kI_x | 0.144736 | 1.10E-01 | 1.72E-01 | 1.44E-01 | 0.108893 | 0.172203 |
| W_11 | 8.00E-12 | 0.00E+00 | 0.00E+00 | 7.95E-12 | 0 | 0 |
| W_I1 | 0.000762 | 0 | 0 | 0.000758 | 0 | 0 |
| I on | 2.45E-09 | 0 | 0.00E+00 | 2.44E-09 | 0 | 0 |
| k_I | -2.98E-09 | 0 | 0.00E+00 | -2.96E-09 | 0 | 0 |
| n | 6.98E-09 | 1.55E-15 | 3.18E-14 | 6.94E-09 | 1.55E-15 | 3.17E-14 |
| W_22 | 0.00E+00 | 7.73E-10 | 0.00E+00 | 0.00E+00 | 7.69E-10 | 0 |
| W_12 | 0 | 0.007379 | 0.00E+00 | 0 | 0.007332 | 0 |
| K_12 | 0.00E+00 | -5.18E-16 | 0.00E+00 | 0.00E+00 | -5.95E-16 | 0 |
| W_33 | 0.00E+00 | 0.00E+00 | 3.56E-08 | 0.00E+00 | 0.00E+00 | 3.56E-08 |
| W_13 | 0 | 0 | 0.176448 | 0.00E+00 | 0 | 0.176315 |
| K_13 | 0.00E+00 | 0.00E+00 | -8.81E-15 | 0.00E+00 | 0.00E+00 | -8.80E-15 |
| W_23 | 0 | 0 | -0.168331 | 0 | 0 | -0.168214 |
| K_23 | 0 | 0 | 0 | 0 | 0 | 0 |
| n_23 | 0 | 0 | 0 | 0 | 0 | 0 |
| kE avg I | 0.00E+00 | 0.00E+00 | 0.00E+00 | 6.68E-04 | 9.86E-04 | 6.56E-05 |
| $L^0_p$ | 0.00E+00 | 0.00E+00 | 0.00E+00 | 6.68E-04 | 9.86E-04 | 6.56E-05 |
| Lp1 | 0.00E+00 | 0.00E+00 | 0.00E+00 | -7.01E-04 | 0 | 0 |
| Lp2 | 0 | 0.00E+00 | 0.00E+00 | 0 | -0.001034 | 0 |
| Lp3 | 0 | 0 | 0.00E+00 | 0.00E+00 | 0.00E+00 | -6.89E-05 |
| Ribosome (gDW) | 0.00E+00 | 0.00E+00 | 6.85E-15 | 2.13E-01 | 0.212943 | 0.212943 |
| kappa_I | 0.00E+00 | -5.18E-16 | -7.83E-15 | -2.02E-01 | -0.201574 | -0.202663 |
| $kI_I$ | 0.00E+00 | 0.00E+00 | 6.85E-15 | 2.12E-01 | 0.211857 | 0.21287 |
| kd_m | -2.01E-05 | -3.01E-06 | -8.61E-06 | -2.00E-05 | -2.99E-06 | -8.60E-06 |
| kd_p | 0.00E+00 | 0.00E+00 | 0.00E+00 | -5.95E-08 | -1.38E-08 | -6.61E-09 |
| mu | -1.05E-06 | -1.58E-07 | -4.52E-07 | -3.19E-06 | -6.54E-07 | -6.90E-07 |

Figure 4: Sensitivity Coefficients for all states and parameters in window 3

TABLE 1

*Simplified, fundamental reaction set for protein production*

| | |
|---|---|
| Transcription initiation: | $G + \text{RNAP} \xrightarrow{\varepsilon_1} G^*$ |
| Transcription: | $G^* + n\text{NTP} \xrightarrow{\varepsilon_2} \text{mRNA} + G + \text{RNAP} + 2n\text{P}_i$ |
| mRNA decay: | $\text{mRNA} \xrightarrow{\varepsilon_3} n\text{NMP}$ |
| Translation initiation: | $\text{mRNA} + \text{rib} \xrightarrow{\varepsilon_4} \text{rib}^*$ |
| Translation: | $\text{rib}^* + a\text{AAtRNA} + 2a\text{GTP} \xrightarrow{\varepsilon_5} a\text{tRNA} + 2a\text{GDP} + 2a\text{P}_i + \text{rib} + \text{mRNA} + \text{protein}$ |
| tRNA charging: | $\text{AA} + \text{tRNA} + \text{ATP} \xrightarrow{\varepsilon_6} \text{AMP} + 2\text{P}_i + \text{AAtRNA}$ |
| Exchange fluxes: | $\text{AA}_{ext} \xrightarrow{b_1} \text{AA}$ |
| | $\text{NTP}_{ext} \xrightarrow{b_2} \text{NTP}$ |
| | $\text{protein} \xrightarrow{b_3} \text{protein}_{ext}$ |
| | $\text{NMP} \xrightarrow{b_4} \text{NMP}_{ext}$ |
| | $\text{ATP}_{ext} \xrightarrow{b_5} \text{ATP}$ |
| | $\text{AMP} \xrightarrow{b_6} \text{AMP}_{ext}$ |
| | $\text{GTP}_{ext} \xrightarrow{b_7} \text{GTP}$ |
| | $\text{GDP} \xrightarrow{b_8} \text{GDP}_{ext}$ |
| | $\text{P}_i \xrightarrow{b_9} \text{P}_{i\,ext}$ |

Figure 5: Reaction Mechanism as taken from Palsson paper Table 1

mRNA2 and mRNA3 show similar trends to mRNA1 in that the RNA polymerase concentration was the most sensitive parameter within this model. mRNA2 shows an equal limitation between initiation and elongation while mRNA3 was more limited by initiation. Larger weights were placed for both mRNA on their Moon-Voigt parameters for the activation of their genes by protein 1.

Protein sensitivity coefficients also showed the high sensitivty to RNA polymerase concentration. Window 1 showed diminshed coefficients across the board as very little protein was expressed during this window. The rankings of the coefficients did not change across windows although the magnitude of how much proteins depended on the saturation coefficient of genes did change between windows 2 and 3 as the levels of proteins began to activate and cause more mRNA to be produced led to the diminshed importance of this saturation coefficient.

Both mRNA and protein states were tied to each other as the mRNA levels were directly correlated with protein levels as shown by the sensitivity coefficients, however even though protein levels were tied with activation or repression of genes the sensitivity coefficients barely showed this relation.

# 3 3

## 3.1 3a

Beginning from the Palsson paper detailing a reaction mechanism, as shown below, a Stoichiometric matrix was constructed.

The Stoichiometric Matrix constructed from this table of reactions is shown below.

| | v1 | v2 | v3 | v4 | v5 | v6 | b1 | b2 | b3 | b4 | b5 | b6 | b7 | b8 | b9 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Gene | -1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RNAP | -1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gene* | 1 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NTP | 0 | -924 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| mRNA | 0 | 1 | -1 | -1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pi | 0 | 1848 | 0 | 0 | 616 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 |
| NMP | 0 | 0 | 924 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 |
| Ribosome | 0 | 0 | 0 | -1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ribosome* | 0 | 0 | 0 | 1 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| AAtRNA | 0 | 0 | 0 | 0 | -308 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GTP | 0 | 0 | 0 | 0 | -616 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| tRNA | 0 | 0 | 0 | 0 | 308 | -1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GDP | 0 | 0 | 0 | 0 | 616 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 |
| Protein | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Amino Acid | 0 | 0 | 0 | 0 | 0 | -1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ATP | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| AMP | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 |

Figure 6: Stoichiometric matrix generated from Palsson paper

In addition to creation of the stoichiometric matrix, a bounds matrix was also created by constraining the reactions present within the network. The second reaction was constrained by the kinetically limited rate of transcription and modulation by an inducer as modelled by Moon-Voigt. The third reaction was given an upper bound of the degradation rate constant of mRNA. The fourth reaction was given an upper bound of the average rate of initiation as derived from the rate of elongation divided by tau. The fifth reaction rate was given an upper bound of the kinetically limited rate of translation. Both rates of transcription and translation are shown below.

$$r_x = kE_x * RNAP * \frac{G_j}{\kappa_x * \tau_x + (\tau_x + 1)G_j} * u(I) \tag{21}$$

$$u(I) = .26 + 300 * \frac{I^{1.5}}{.30^{1.5} + I^{1.5}} \tag{22}$$

$$r_l = kE_l * ribosome * \frac{M_j}{\kappa_l * \tau_l + (\tau_l + 1)M_j} \tag{23}$$

$$M_j = r_x / k_{d,mRNA} \tag{24}$$

I represents the inducer concentration in this case with all parameters given. The mRNA concentration was found by assuming steady state for the change in mRNA concentration and solving for the mRNA balance with $k_{d,mRNA}$ representing the degradation constant for mRNA.

### 3.1.1 3b

By altering the Inducer concentration and performing Flux Balance Analysis at the varying concentrations of inducer we can generate the following plot of the optimized rate of translation as a log concentration of the inducer.

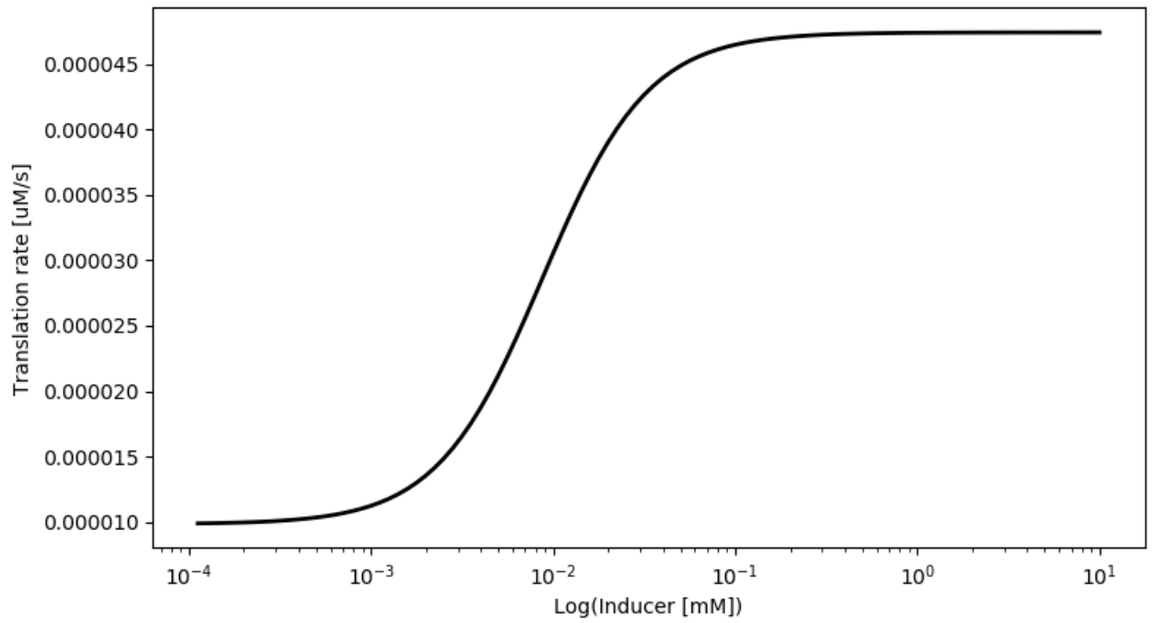


Figure 7: Varied rates of translation as compared to the log of inducer concentration

### 3.2 3c

We can find the exchange flux that the rate of translation is most sensitive to by altering the rates of exchange individually and monitoring the change in the rate of translation as compared to the original rate of translation with all exchange rates at normal levels.

This analysis reveals that the exchange fluxes must first be reduced drastically to have any affect on the rate of translation and that the most influential exchange flux was that of the phosphate being shuttled out from the system. This seems rather surprising in that energy is not the binding constraint in terms of GTP or ATP availability but is rather the buildup of waste.