CHEME 5440, Spring 2019	**Problem Set 2**
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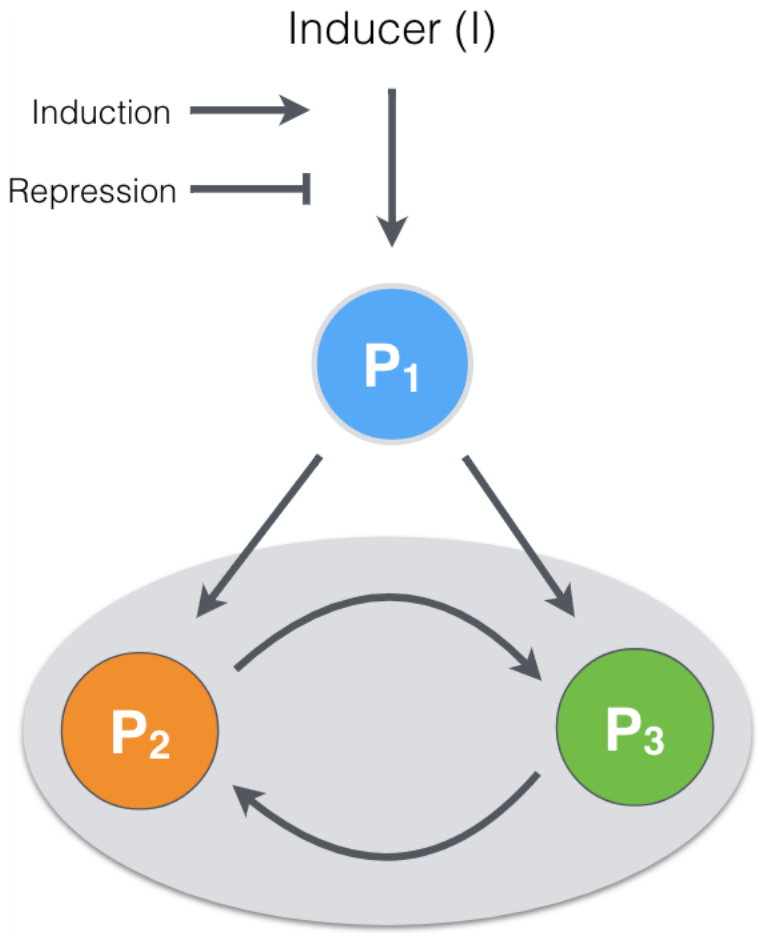


1.

2. Assumptions:

   - Circuit encoded on plasmid present at a constant 200 copies per cell
   - Characteristic lengths $\mathcal{L}_X = 1000$ nt, $\mathcal{L}_T = 333$ AA
   - Promoter controls follow Moon and Voigt formulation
   - Translation operates at kinetic limit
   - RNAP and ribosome levels constant
   - $\mathcal{L}_{X,1} = 1200$ nt, $\mathcal{L}_{X,2} = 2400$ nt, $\mathcal{L}_{X,3} = 600$ nt

(a) Looking first at the mRNA...

$$\frac{dm_1}{dt} = r_{X,1}u_1 - k^d_{X,1}m_1 - m_1\mathcal{B}^{-1}\dot{\mathcal{B}}$$
$$\frac{dm_2}{dt} = r_{X,2}u_2 - k^d_{X,2}m_2 - m_2\mathcal{B}^{-1}\dot{\mathcal{B}}$$
$$\frac{dm_3}{dt} = r_{X,3}u_3 - k^d_{X,3}m_3 - m_3\mathcal{B}^{-1}\dot{\mathcal{B}}$$

We can use the substitution $\mu = \mathcal{B}^{-1}\dot{\mathcal{B}}$

$$r_{X,j} = k^X_{E,j}R_{X,T}\left(\frac{G_j}{\tau_{X,j}K_{X,j} + (\tau_{X,j}+1)G_j}\right)$$

$$u_1 = \frac{W_{R_T,1} + W_I f_I}{1 + W_{R_T,1} + W_I f_I}$$
$$u_2 = \frac{W_{R_T,2} + W_{12}f_{12} + W_{32}f_{32}}{1 + W_{R_T,2} + W_{12}f_{12} + W_{32}f_{32}}$$
$$u_3 = \frac{W_{R_T,3} + W_{13}f_{13} + W_{23}f_{23}}{1 + W_{R_T,3} + W_{13}f_{13} + W_{23}f_{23}}$$

And now at the protein...

$$\frac{dp_1}{dt} = r_{L,1}w_1 - k^d_{L,1}p_1 - p_1\mathcal{B}^{-1}\dot{\mathcal{B}}$$
$$\frac{dp_2}{dt} = r_{L,2}w_2 - k^d_{L,2}p_2 - p_2\mathcal{B}^{-1}\dot{\mathcal{B}}$$
$$\frac{dp_3}{dt} = r_{L,3}w_3 - k^d_{L,3}p_3 - p_3\mathcal{B}^{-1}\dot{\mathcal{B}}$$

$$r_{L,j} = k^L_{E,j}R_{L,T}\left(\frac{m_j}{\tau_{L,j}K_{L,j} + (\tau_{L,j}+1)m_j}\right)$$

$w_j = 1$ (since operating at kinetic level)

$$\frac{d\mathbf{x}}{dt} = \mathbf{A}\mathbf{x} + \mathbf{S}\mathbf{r}$$

$$\frac{d}{dt}\begin{bmatrix} m_1 \\ m_2 \\ m_3 \\ p_1 \\ p_2 \\ p_3 \end{bmatrix} = -\begin{bmatrix} k^d_{X,ex} & 0 & 0 & 0 & 0 & 0 \\ 0 & k^d_X + \mu & 0 & 0 & 0 & 0 \\ 0 & 0 & k^d_X + \mu & 0 & 0 & 0 \\ 0 & 0 & 0 & k^d_{L,ex} & 0 & 0 \\ 0 & 0 & 0 & 0 & k^d_L + \mu & 0 \\ 0 & 0 & 0 & 0 & 0 & k^d_L + \mu \end{bmatrix}\begin{bmatrix} m_1 \\ m_2 \\ m_3 \\ p_1 \\ p_2 \\ p_3 \end{bmatrix}$$
$$+ \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix}\begin{bmatrix} r_{\hat{X},1} \\ r_{\hat{X},2} \\ r_{\hat{X},3} \\ r_{\hat{L},1} \\ r_{\hat{L},2} \\ r_{\hat{L},3} \end{bmatrix}$$

(b) Solving for parameters...

$$k_{E,j}^X = \frac{e_X}{\mathcal{L}_j} \text{ (from last week)}$$
$$k_{E,j}^X = \frac{42}{\mathcal{L}_j}$$

$$\mu = \frac{\ln 2}{t_d}$$
$$\mu = \frac{\ln 2}{30 \text{ min}}$$
$$\mu = 0.0231 \text{ min}^{-1} = 3.85 \times 10^{-4} \text{ s}^{-1}$$

$$\tau_{X,j}^{-1} \equiv (\frac{k_I}{k_A + k_E})$$
$$\tau_{X,j}^{-1} \equiv (\frac{0.04}{k_{E,j}^X})$$

This gives us time constants $\tau_{E,1}^X = 0.875$, $\tau_{E,2}^X = 0.4375$, $\tau_{E,3}^X = 1.75$

$$\kappa_{X,j}^{-1} \equiv (\frac{k_+}{k_- + k_I})$$
$$\kappa_{X,j}^{-1} = (\frac{10.42}{0.01 + 0.04})$$
$$\kappa_{X,j} = 0.0048$$

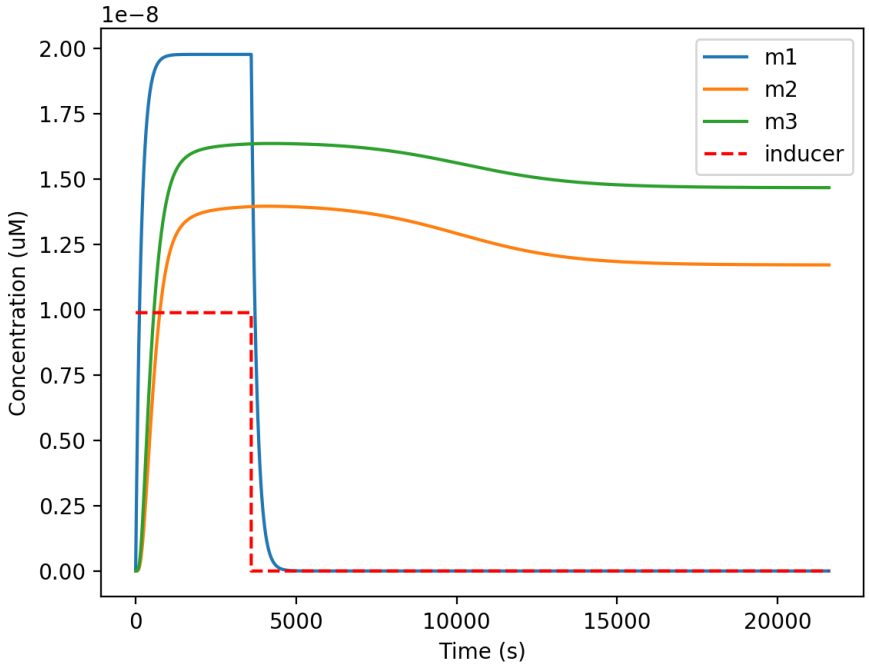
We need the copy number, RNA polymerase concentration, and ribosome concentrations in μM units:

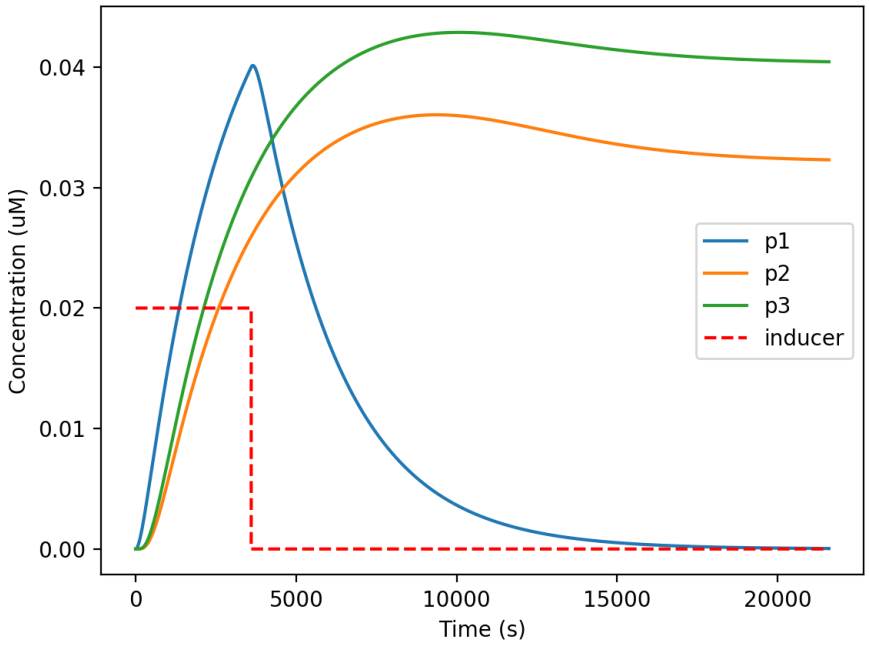
$$200\frac{\text{plasmids}}{\text{cell}}(\frac{1 \text{ mol}}{6.02 \times 10^{23} \text{ plasmids}})(\frac{1 \text{ cell}}{6.7 \times 10^{-10}\mu L})(\frac{10^6 \mu L}{1L})(\frac{10^6 M}{1\mu M}) = 0.496\mu M$$

We can derive the RNAP and ribosome concentrations similarly

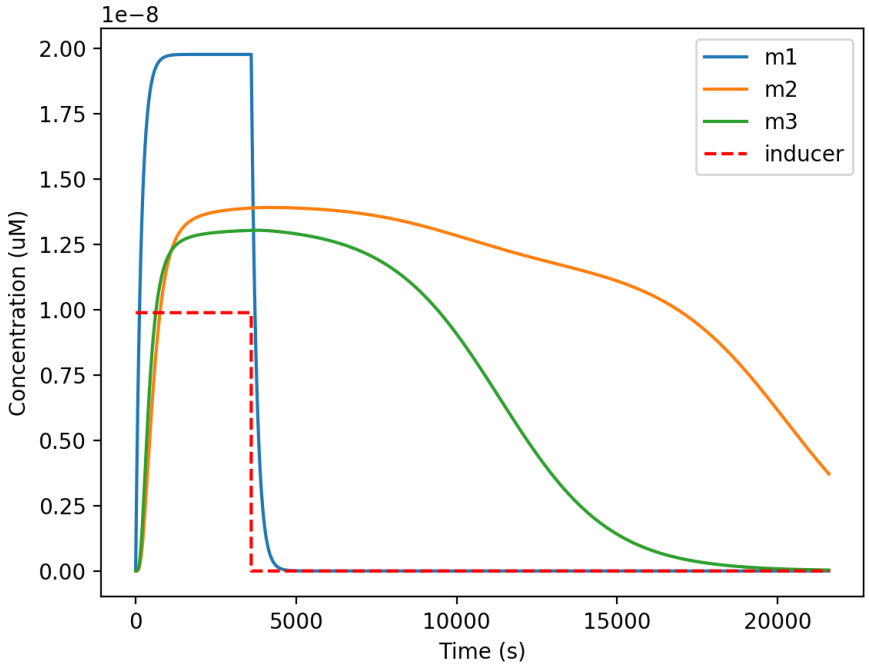
Parameter	Symbol	Value	Units	Source
Specific growth rate	μ	3.85×10^{-4}	s^{-1}	Given
mRNA elongation rate	e_X	42	nt/s	PS1
Gene 1 length	$\mathcal{L}_{X,1}$	1200	nt	Given
Gene 2 length	$\mathcal{L}_{X,2}$	2400	nt	Given
Gene 3 length	$\mathcal{L}_{X,3}$	600	nt	Given
TX elongation rate constant	$k^X_{E,1}$	0.035	s^{-1}	See above
TX elongation rate constant	$k^X_{E,2}$	0.0175	s^{-1}	See above
TX elongation rate constant	$k^X_{E,3}$	0.07	s^{-1}	See above
TX initiation rate constant	k^X_I	0.04	s^{-1}	PS1
TX abortive rate constant	k^X_A	0	s^{-1}	PS1
TX time constant	$\tau_{X,1}$	0.875	n/a	See above
TX time constant	$\tau_{X,2}$	0.4375	n/a	See above
TX time constant	$\tau_{X,3}$	1.75	n/a	See above
TX saturation constant	$\kappa_{X,123}$	0.0048	μM	See above
Gene concentration	G_{123}	200	plasmids/cell	Given
Gene concentration	G_{123}	0.496	μM	See above
RNAP Concentration	$R_{X,T}$	8,000	molecules/cell	Bremer [1]
RNAP Concentration	$R_{X,T}$	19.8	μM	See above
Ribosome Concentration	$R_{L,T}$	50,000	ribosomes/cell	Mackie [2]
Ribosome Concentration	$R_{L,T}$	123.96	μM	See above
Basal transcription rate	$W_{R_T,123}$	0.00001		
Translation elongation rate	e_L	14.5	aa/s	Dalbow [3]
Peptide 1 length	$\mathcal{L}_{L,1}$	400	aa	Given
Peptide 2 length	$\mathcal{L}_{L,2}$	800	aa	Given
Peptide 3 length	$\mathcal{L}_{L,3}$	200	aa	Given
TX elongation rate constant	$k^L_{E,1}$	0.03625	s^{-1}	See above
TX elongation rate constant	$k^L_{E,2}$	0.0181	s^{-1}	See above
TX elongation rate constant	$k^L_{E,3}$	0.0725	s^{-1}	See above
TL time constant	$\tau_{L,123}$			Defined same as TX
TL saturation constant	$\kappa_{L,123}$			Defined same as TX
mRNA degradation rate constant	k^X_d	5.5×10^{-3}	s^{-1}	Tanagachi [4]
Protein degradation rate constant	k^L_d	3.85×10^{-6}	s^{-1}	Maurizi [5]

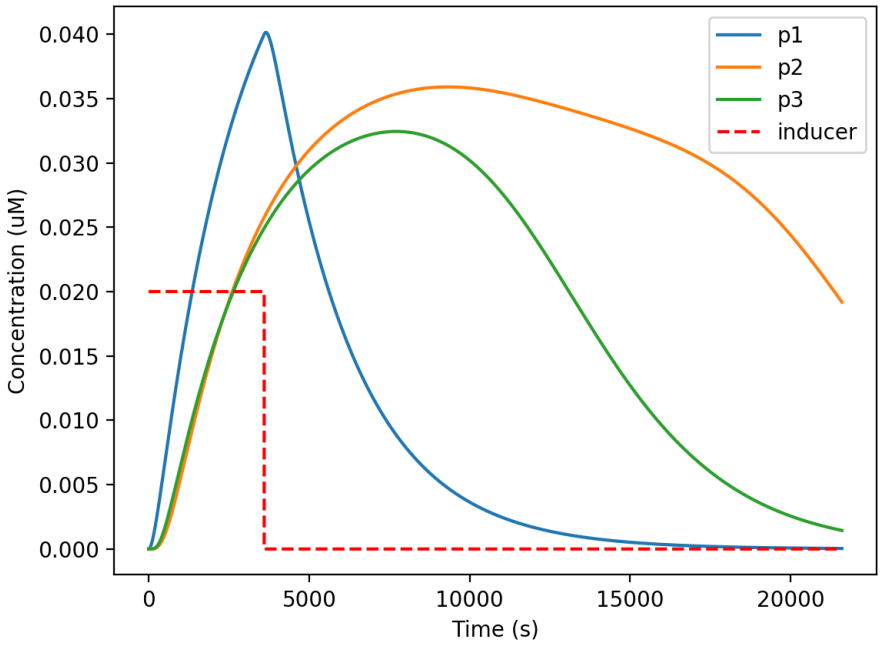
For the unbroken circuit, we found the expected result that this circuit functions as a memory circuit. Even after the inducer is removed from the surroundings, thus deactivating the transcription of P1, we still have P2 and P3 being transcribed since they are able to induce each other's transcription.





However, when we break the circuit, preventing P2 from inducing P3, we see that P3's expression drops shortly after the inducer is removed from the system. And without P3 present to induce P2, P2 also drops soon after.





References

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[4] Y. Taniguchi, P. J. Choi, G.-W. Li, H. Chen, M. Babu, J. Hearn, A. Emili, and X. S. Xie, "Quantifying e. coli proteome and transcriptome with single-molecule sensitivity in single cells," *Science*, vol. 329, no. 5991, pp. 533–538, 2010.

[5] M. Maurizi, "Proteases and protein degradation inescherichia coli," *Experientia*, vol. 48, no. 2, pp. 178–201, 1992.