CHEME 5440/7770: Take Home Prelim 1 S2020

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1. Take Home Prelim 1 has two questions which are collectively worth 100 points.
2. Take Home Prelim 1 is due at the beginning of class on T May 12, 2020.
3. You may use your course notes, literature, the internet, or any other course materials to formulate your solutions.
4. You *cannot* consult with any other person regarding the prelim (except the TA, JV or MP). You *cannot* use any form of electronic communication to discuss the prelim questions with any other person (except the TA, JV or MP via a direct message in Slack). Violation of this policy will result in a ZERO for the prelim, and an honor code violation.
5. Mistakes/corrections/clarifications to the prelim will be made on the #general Slack channel by the TA, JV or MP.
6. In all problems, show your work and state all assumptions or simplifications. Start from the general, and work your way to the specific.
7. **Submission**: Submit a link to the #prelim-1 channel on Slack that points to your solutions stored on GitHub, Box, Google Drive etc. Your solutions should include all written material, source code/spreadsheets, and instructions to reproduce your calculations/figures.

Table 1: Average lacZ messenger RNA copy number $< n >$ per cell for the P_{lac} promoter as a function of extracellular IPTG (inducer). Data reproduced from Golding and coworkers (1).

IPTG (mM)	$< n >$ (mRNA/cell)	low (mRNA/cell)	high (mRNA/cell)
0.0	19	18	20
5e-4	21	17	26
0.005	41	37	44
0.012	67	65	69
0.053	86	84	88
0.216	93	91	95
1.0	93	92	94

1. (50 points) Golding and coworkers measured the average mRNA copy number per cell for several promoters using single-molecule fluorescence in situ hybridization (smFISH) in single dividing *E. coli* cells (1). Let's use the data from the P_{lac} promoter expressing the *lacZ* gene, and parameters from literature, `BioNumbers` etc to test the effective biophysical gene expression model we developed in class.

   Assume: (i) all experiments were conducted in an exponentially growing population of *E. coli* cells with a doubling time of $\tau_d \simeq$ 40 min, (ii) OD600 = 0.1 is equivalent to 1×10^8 cells ml^{-1}, (iii) write the promoter function in terms of extracellular inducer (ignore inducer transport) (iv) assume the lacZ gene is present at two copies/cell, (v) assume the lacZ mRNA half-life is 5 minutes, (vi) use a characteristic transcript length of 1000 nt.

   Parameters: Parameters for this problem can be found at `BioNumbers`, and in the study of McClure (2). Hint: there is also a lot of information stored in the shape, and extremes of the inducer range.

   a) Convert the $< n >$ values in Table 1 to the specific volume basis $\mathcal{B} = < m_c > \hat{N}_c V$ if a sample size of 1 ml of culture at OD600 = 0.1 was used. Let $< m_c >$

denote the average mass/cell (gDW/cell), $\hat{N}_c$ denotes the cell count in the sample (number of cells/ml) and V denotes the sample volume (ml).

b) Derive the gain function $\mathcal{K}_X$. Starting from the mRNA balance developed in class:

$$\dot{m}_i = r_{X,i}\bar{u}_i - (\mu + \theta_{m,i})m_i \tag{1}$$

where the `dot` notation denotes the derivative with respect to time. The term $r_{X,i}\bar{u}_i$ (units: nmol/gDW-hr) denotes the specific rate of transcription of gene i (production rate of mRNA i), and $\bar{u}_i$ denotes the promoter activity function describing both regulated and unregulated gene expression activity ($0 \leq \bar{u}_i \leq 1$). Show the pseudo steady state abundance of the lacZ mRNA (m^*; nmol gDW^{-1}) can be written as:

$$m^* = \mathcal{K}_X\left(\mathcal{G}, \theta\right)\bar{u}(I, \kappa) \tag{2}$$

where $\mathcal{K}_X$ denotes a gain function, $\mathcal{G}$ denotes the lacZ gene abundance, I denotes the inducer abundance, $\bar{u}(\cdot)$ denotes the promoter function, and κ, θ denotes parameter vectors (various kinetic and promoter model constants).

c) Use the data in Table 1 (converted to the correct units), the promoter modeling approach of Moon et al. (3), and your best parameter guesses (presented in a table with value, units and source listed), to estimate $\mathcal{K}_X\left(\mathcal{G}, \kappa\right)$ and $u(I, \theta)$ such that m^* is consistent with the measured copy number as a function of IPTG concentration. To make the problem easier, assume P_{lac} is a positively inducible promoter that responds to IPTG.

d) Plot (on a semilogx axis) the converted data, and the estimated lacZ concentration from your model as a function of IPTG. Does the model fit and have the correct shape? (if not: what parameters control the fit and shape?)

References

1. So LH, Ghosh A, Zong C, Sepúlveda LA, Segev R, Golding I. General properties of transcriptional time series in Escherichia coli. Nat Genet. 2011;43(6):554–60. doi:10.1038/ng.821.
2. McClure WR. Rate-limiting steps in RNA chain initiation. Proc Natl Acad Sci U S A. 1980;77(10):5634–8.
3. Moon TS, Lou C, Tamsir A, Stanton BC, Voigt CA. Genetic programs constructed from layered logic gates in single cells. Nature. 2012;491(7423):249–53.

2. (50 points) Perez-Carrasco and colleagues conducted an analysis of an alternate current (AC)-direct current (DC) circuit that combines the repressilator and toggle switch circuits: "Combining a Toggle Switch and a Repressilator within the AC-DC Circuit Generates Distinct Dynamical Behaviors," *Cell Systems* 6, 521-530, 2018. Note that *Cell Systems* is a sub-journal of Cell Press and has a high impact factor. In this problem you will test and analyze some of the key results of Perez-Carrasco. Begin by looking over results presented in Figures 1-3.

a) The model corresponding to the network diagram that was used to construct the bifurcation diagram in Figure 1B is not explicitly shown. Note that the network diagram shows a toggle switch where the activity of X can be induced by a signal, S. Following the approach presented in the STAR METHODS for the AC-DC circuit (See Equation 2), construct a dynamic system of equations for the network diagram shown in Figure 1B that includes the action of signal, S. Write your ODE system in dimensional form.

b) Non-dimensionalize your system using the relevant non-dimensional quantities given by Equations 3-6 of the STAR METHODS. Note that one of the expressions presented in Equations 3-6 may have a small error in it. If so, state what this error is. Provide your non-dimensional system equations.

c) Create a plot of stable steady-state values of X (non-dimensional) versus S similarly to that shown in Figure 1B. Use the following non-dimensional parameters:
   - $\alpha_x = 1.5$
   - $\beta_x = 5.0$
   - $z_x = 0.4$
   - $n_{zx} = 2.7$
   - $x_z = 1.5$
   - $n_{xz} = 2.7$

   Are the solid black lines of the bifurcation diagram in Figure 1B qualitatively reproducible?

   <u>BONUS</u>: Also find the unstable steady-states for varying S and graph both stable and unstable steady-states to construct the full bifurcation diagram of 1B.

d) Now consider the AC-DC circuit whose dynamic behavior is described by the non-dimensional system of ODEs presented in Equation 1 and analyzed in Figure 2. Confirm that the results of Figure 2 are reproducible. Solve for time varying values of X, Y, and Z (non-dimensional) for S = 0.02, 10, and 10^5. You do not need to solve

over the whole range of S as the authors did in Figure 2B, just the three values of S specified. Initial conditions are as in Figure 2, $X_o = Y_o = Z_o = 0$. Use the mode parameter values presented in Table S.1 of the paper (you have to download the supplemental information containing Table S.1 separately from the main paper). Provide plots of X vs. time for the three values of S.

e) The results presented in Figure 3 lead to one of the most important conclusions of the paper. The authors conclude that oscillations arising by passing through the Hopf bifurcation are incoherent whereas those arising by passing through the saddle node bifurcation are coherent. This result gives us valuable insight into how we might be able to engineer a multicellular system to oscillate in synchrony using an AC-DC circuit. Review Figure 1 and accompanying legend to familiarize yourself with what is meant by the Hopf and saddle node bifurcations.

   Find a stable steady-state for a value of signal, S, near but below the Hopf bifurcation point. Report your selected value of S and the corresponding steady-state values of X, Y, and Z. Consider the dynamics of three cells that have been stimulated with your value of signal, S, for enough time to achieve states near the predicted steady-state. Cell 1 has values of X, Y, Z exactly at the steady state values. In cell 2, X, Y, and Z are 25% higher than the expected steady-state values at the signal level. In cell three, they are 25% lower than expected steady-state values. Plot Z vs time when signal, S, is suddenly changed to a value of 100 in the three cells. Are the oscillations incoherent or coherent? Note that for this problem, you do not have to simulate noise in your system as done by Perez-Carrasco.

   Find another value of S near but above the saddle node bifurcation, where there is only one stable steady state. Report your selected value of S and the corresponding steady-state values of X, Y, and Z. Conduct a similar analysis as above for three cells initially near this steady-state (Cell 1 has expected steady-state values of X, Y, Z; Cell 2 has values 25% higher; Cell 3 has values 25% lower). Plot Z vs time when S is suddenly changed to a value of 100 in the three cells. Are the oscillations incoherent or coherent? Why? Explain using your results and the discussion in section “Coherent or Incoherent Oscillations” of the paper.

f) In the legend of Figure 3E, the authors state that they achieved coherent oscillations by decreasing the signal form S=105 to S=100. Is this possible if they used the parameters values of Table S.1. as we believe they did? Explain your answer.

Reference

Perez-Carrasco R, Barnes CP, Schaerli Y, Isalan M, Briscoe J, Page KM. Combining a toggle switch and a repressilator within the AC-DC circuit generates distinct dynamical behaviors. Cell Systems. 2018; 6: 521-30. Doi: 10.1016/j.cels.2018.02.008.