HW 5: Synthetic transcriptional circuits.

(Dated: November 8, 2010)

1. PREVIEW OF FINAL PROJECT.

(a) (7.5 points). Find some synthetic biology paper that you find interesting and, in two paragraphs, explain (i) what the paper is about and (ii) why you picked it (why it is interesting). Use your own words rather than copying the abstract. You will be able to change topic for the final project but this is a good opportunity to start looking around. People whose work you might want to look at include: Adam Arkin, Jim Collins, Michael Elowitz, Andy Ellington, Drew Endy, Jeff Hasty, Farren Isaacs, Wendell Lim, Niles Pierce, Herbert Sauro, Christina Smolke, Jeff Tabor, Erik Winfree, Chris Voigt. Use pubmed or Google Scholar to look up papers.

(b) (7.5 points). Pick a second paper from the list of references in the paper you have chosen in (a) and, in one paragraph, explain how the two papers are related.

2. MODELING SIRNA DYNAMICS.

(a) (5 points). Simulation of mRNA and protein dynamics. Numerically solve the model

$$\dot{m} = \alpha_m - \gamma_m m, \quad (1)$$

$$\dot{P} = \alpha_P m - \gamma_P P. \quad (2)$$

Use the following parameter values:

$$\alpha_m = 200/cell/h; \quad (3)$$

$$\gamma_m = 2/h; \quad (4)$$

$$\alpha_P = 500/h; \quad (5)$$

$$\gamma_P = 0.35; \quad (6)$$

Most of these numbers are from D. W. Bartlett and M. E. Davis, Nucleic Acid Research **34**, 323 (2006). Choose a few different initial conditions and show that the concentrations approach equilibrium. Then, find the steady state values for m and P analytically and show the result in the same graph. Because of the differences in concentrations you may need to use different plots for mRNA and protein.

(b) (7.5 points). Now assume that an amount s_0 of siRNA is delivered to each cell at time $t = 0$. In an experiment, siRNA are generally designed to be fully complementary to their target mRNA. When an siRNA binds a complementary mRNA, the mRNA gets cleaved by an endonuclease that is part of the RISC complex. The siRNA itself is not cleaved and can act catalytically. As discussed in class, this is captured by the model

$$m + s \underset{k_-}{\overset{k_+}{\rightleftharpoons}} C \overset{k_2}{\rightarrow} s. \quad (7)$$

Here m is the mRNA, s the siRNA-RISC and C a complex between siRNA-RISC and mRNA. The catalytic constant k_{cat} and a Michaelis constant K_M have been measured in several *in vitro* experiments such as B. Haley and P. D. Zamore, Nature Structural and Molecular Biology **11**, 599 (2004) who report

$$k_2 = k_2 = 7.1 \times 10^{-3}/\text{s} = 25.6/\text{h}. \quad (8)$$

$$K_M = \frac{k_- + k_2}{k_+} = 8.4\,\text{nM} \approx 2 \times 10^4/\text{cell} \quad (9)$$

Here I assume that the cellular volume is 4×10^{-12} l. Use $k_- = 1/\text{h}$ to obtain k_+ form K_M. SiRNA are generally believed to be highly stable and not to be actively degraded. We here assume that they only get diluted by cell-division with a rate γ_s. To calculate γ_s assume that the time between cell divisions is 18h. Numerically solve the differential equation describing siRNA dynamics that we derived in class. Show plots of the siRNA, mRNA and protein concentration as a function of time. As initial conditions for your simulations use the steady state values of m and P before siRNA is added (see (a)). Compares $s0 = 100, 1000, 10000$.

(c) (7.5 points). In class, we solved the differential equations for siRNA dynamics in the steady state regime and obtained a simple expression for the mRNA concentration as a function of time and of the amount of siRNA. For one value of $S0$ Plot this approximation and the exact solution obtained in (b) in the same plot. In what regime is the approximation valid.

3. FITTING EXPERIMENTAL DATA.

Quantitative PCR methods can be used to determine how many copies of a specific mRNA molecule are expressed in a given cell type. The data below is for an experiment done in CHO cells where transcription of a red fluorescent protein (RFP) was switched one at time $t = 0$ using an inducible promoter system. Total RNA was taken from a subset of cells every few hours and the amount of the RFP mRNA was quantified. The experiment yielded the following time series:

Time (hrs)	0	0.2	1	2	3	4	5	6	7	8	20,
mRNA/cell	40	136	627	688	718	1616	1277	2085	1418	1906	1912.

This data can be fit to a simple model or mRNA production as introduced in class. However, because mRNA production is switched on at time $t = 0$, the system is not in steady state.

(a) (5 points). Analytically integrate Eq. (1) from Problem 2 to obtain an expression for mRNA as a function of time. Assume that the initial concentration is $m0$. Ideally, $m0 = 0$, but in practice there may be some leaky transcription even if the promoter is "off." For the integration use that $\frac{d}{dx}\ln(ax + b) = a/(ax + b)$.

(b) (10 points). Fit the data shown above to the model from (a). Use α_m, γ_m and m_0 as fitting parameters. For the fitting you can use Matlab (e.g. the "fmincon" function) or Excel. In Excel, us the "Solver" tool which can be found in the "Tool" menu (you may have to first activate it, which can be done under "Tools," then "Add-ins"). Use solver to minimize the mean squared error calculated from model and data. You will need to provide starting values for the parameters. You can guess a value for $m0$ from the first data point and can guess the other parameters from the last data points (with help from Problem 2).

4. SIRNA DESIGN.

(5 points). Use one of the many siRNA design engines available online to design an siRNA against gfp. You can find the sequence of gfp in the NCBI database. Provide the sequence of the siRNA sense strand and the position within the gfp mRNA or gene which matches the sense strand (position of first match).