PRINCIPLES OF SYNTHETIC BIOLOGY: MIDTERM EXAM

November 1st, 2011

NAME (PRINT):

STUDENT ID:

EMAIL ADDRESS:

POLICY:

There are six problems in total. We understand they may be too many for 90 minutes. You can choose N (N=4-6) problems out of these 6 problems to answer for your midterm, and leave the rest as your homework problem set 3. The score will be calculated following the equation:

$$Score = Midterm + 50\% \cdot Homework\,3$$

1. When you are done, please hand in both the Problem set together with your exam booklets.

2. This midterm problem set will be posted to the web later.

3. Please first decide which problems you want to take as your midterm, and circle them below.

4. The problems that you don't circle will be your homework 3, and have a reduced weight.

PROBLEMS CHOSEN FOR MIDTERM (PLEASE CIRCLE):

1	2	3	4	5	6

PRINCIPLES OF SYNTHETIC BIOLOGY: MIDTERM EXAM

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1. SYNTHETIC TRANSCRIPTIONAL CASCADES (30PT)

Cascades are ubiquitous regulatory motifs in cells. To study the functions of cascading motifs and their functions, you are about to construct synthetic cascades in bacteria cells.

First, you construct a cascade that is composed of two activators as shown in Figure 1. Protein A_1 is initially present in the cell in its inactive form. The input signal X_1 is added at time t = 0. Depending on the concentration of X_1, certain amount of A_1 ***rapidly*** becomes active and binds to the promoter of A_2, so that protein A_2 starts to be produced at rate β. When A2 levels exceed a threshold K_1, GFP begins to be produced at γ.

All assume all proteins don't degrade, and they only have a dilution rate of α when the cell divides.

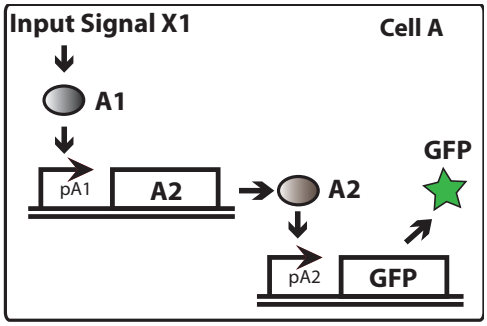

Figure 1

(A) After you constructed the circuit, you added 1uM of input signal molecule X1. However, you didn't observe any GFP signal after a long time. So you characterized the transfer curves for promoters pA1 and pA2, and the induction relationship between X1 and A1 (all axes are in logarithm10 scale). (5PT)

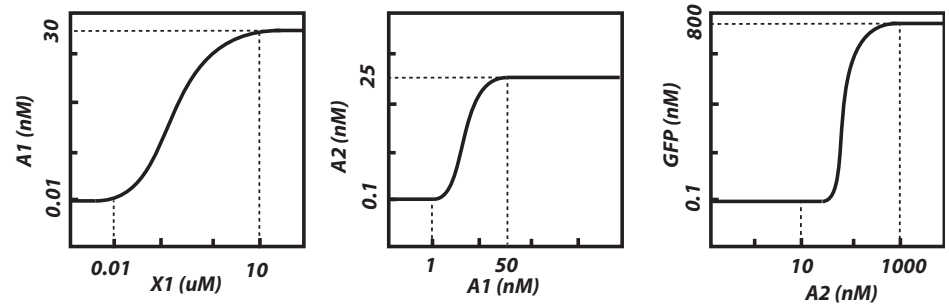

Figure 2

Explain why you didn't observe GFP signal.

(B) Your advisor points to you that these transfer curves are potentially limiting the GFP signal you might observe. Identify the problem, and propose how you might improve the circuit. (5PT)

(C) After improving the circuit, you are finally able to observe GFP signal after you add the input signal X1. The time delay of the circuit τ, is defined as the duration between the addition of

the input signal (t = 0) and initial expression of GFP (t = τ). Explain briefly the source of the time delay in this circuit, and calculate how much is the delay using the given parameters (5PT).

Then you set to construct a second cascade that is composed of two repressors as shown in Figure 3. Protein R_1 is initially present in the cell in its inactive form. The input signal Y_1 is added at time t = 0. As a result, R_1 ***rapidly*** becomes active and binds to the promoter of R_2, so that expression of R_2 is ***completely*** shut off. Assume the initial concentration of R_2 is η. When R_2 drops below a threshold of K_2, GFP begins to be produced at rate γ.

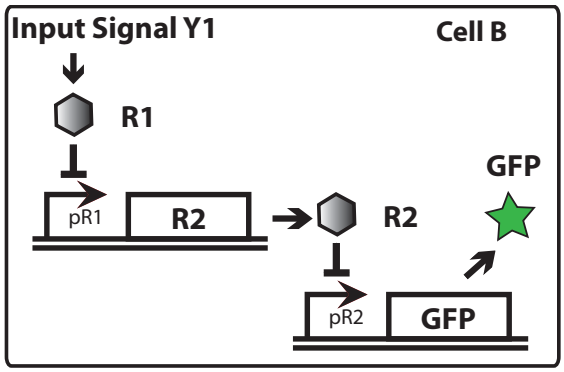

Figure 3

(D) Calculate the time delay using the given parameters, and explain the source of the time delay in this circuit. (5PT)

(E) A good approximation for repressible promoter activity is

$$f(X) = \frac{\varphi}{1 + (\frac{X}{\kappa})^n}$$

Where X is the concentration of repressor protein, and $f(X)$ is the promoter activity. Briefly explain the physical meanings of the parameters φ, κ and n, and the assumption(s) making this expression valid. (5PT)

(F) Both R1 and R2 bind to their promoters in the monomer form. Do you expect the steady-state transfer curve of the cascade circuit in Cell B will exhibit a higher sensitivity than that of the single repressor-promoter circuit? Write equations to explain. (5PT)

2. NEGATIVE FEEDBACK (20PT)

Feedbacks are important network motifs in biological systems. To understand their functions deeper, you will build and model a synthetic feedback circuit. You plan to use one transcription repressor and its promoter to build a simple circuit, so expression of the repressor protein will repress itself by repressing the promoter as shown in Figure 4.

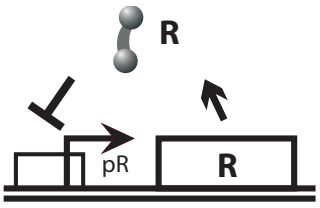

Figure 4

(A) Write down the chemical reactions for the circuit. (5PT)

(B) Comparing the negative feedback circuit to the simple circuits where there is no repression on the promoter, choose the correct answers. (5PT)

- The negative feedback has ( ) speed to reach its steady state;

a. faster

b. slower

c. same

- The negative feedback is ( ) to the fluctuations in gene expression;

a. more robust

b. more susceptible

c. similar

(C) Assume mRNA is always at its steady state. Also assume the promoter activity follows the step function:

$$f(R) = \varphi, \text{ if } R < \kappa$$

$$f(R) = 0, \text{ if } R \geq \kappa$$

φ and κ are all constants, and φ is very large. Write down the ODE equations to describe the expression of R (5PT).

(D) You test the feedback circuit, and find that after a few cycles of oscillation, the circuit reaches its steady state concentration that equals to κ. Explain why there are oscillations and why the steady state concentration is κ. (5PT)

3. POSITIVE FEEDBACK (20PT)

Now consider a positive feedback circuit as shown in Figure 5, where a promoter drives the production of one activator protein A to activate the promoter. Activator A binds to the promoter in the monomer form.

(A) Assume mRNA is always at steady state, and follow a similar promoter expression from Problem 1(E), write one equation to describe the system. (5PT) (*hint: you need an activated promoter expression*)

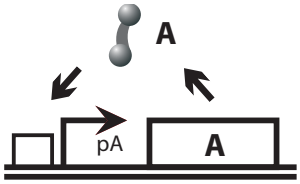

Figure 5

(B) Your expression should look like

$$\frac{dA}{dt} = f(A) - g(A)$$

On a 2D plot where the x-axis is the amount of A, y-axis is the $\frac{dA}{dt}$, draw a curve of f(A) and a curve of g(A) as a function of A, and label the critical points. (5PT)

(C) How many steady states does this circuit have? Circle the stable steady states, and explain why it is stable. (5PT)

(D) Switches that possess two stable critical points, also called bistable switches, are especially interesting and useful in biology. Positive feedback loops are important for building bistable switches. To engineer the above circuit to be a bistable switch, you think of the following strategies:

1) Engineer the activator A so it only activates its promoter when it forms a dimer;

2) Change the protein degradation rate of activator A;

3) Tune the RBS to increase or decrease the translation rate of activator A;

4) Constitutively express another protein B that can sequester A from activating the promoter;

Identify which strategy(s) might allow you to engineer bistable switches, and explain why. (5PT)

4. TOGGLE SWITCH (25PT)

A toggle switch is a mutually inhibitory circuit. Repressor R2, produced from constitutive promoter pR1, blocks expression of promoter pR2. Promoter pR2 constitutively expresses repressor R1 that blocks promoter pR1.

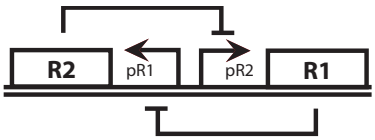

Figure 6

This may also be considered as another implementation of positive feedback where the repressor blocks the expression of the protein that would shut down its expression; this effectively equals activation. Assume full expression strengths are $\alpha 1, \alpha 2$ for P1 and P2 respectively, the protein degradation rate $\gamma = 1$ for both repressors.

(A) Write equations using the promoter function from Problem 1.B to describe the system. (5PT)

(B) Intuitively, how many steady states does the system have, and how many of them are stable steady states? (5PT)

(C) Draw the nullclines for both R1 and R2 on a 2D plot qualitatively, with R1 as the x axis, and R2 as the y axis. Circle the stable steady states. Give brief explanation. (5PT)

(D) A toggle switch is useful for storing information and for making decisions, when both steady states can be reached, however an imbalanced toggle switch has only one steady state. Applications with imbalanced toggle switches include a biological timer. Assume that binding of repressor R2 to promoter pR2 can be inhibited by a chemical as shown in Figure 5. Assume the inhibition on R2 from the chemical is very fast. How would you use this information to set up a circuit that makes a biological timer? Describe the operation. Remember we need to have all cells behave similarly, not to have some of them in one state, and the rest in another state. (5PT)

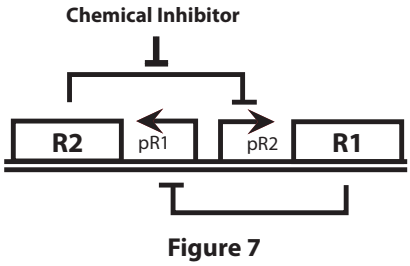

Figure 7

(E) Draw the nullclines for the imbalanced toggle switch, and explain if the critical points are stable. (5PT)

5. DISCRETE STOCHASTIC SIMULATION (30PT)

Biological systems are noisy and noise has been shown to be a critical driving force of various biological events. ODE simulations cannot adequately capture this noise.

(A) Briefly explain why this is true and give an example (5PT).

Gillespie's stochastic systems analysis (SSA) simulates the biological system by treating reactions as probabilistic events. Let's consider a closed system with the reaction dynamics:

$$2S_1 \xrightarrow{0.1/\text{sec}} 2S_2$$

$$S_1 + S_2 \xrightarrow{0.4/\text{sec}} 2S_1$$

$$S_2 \xrightarrow{0.6/\text{sec}} S_3$$

(B) Using SSA, if the initial state is defined as $[S_1] = 1$, $[S_2] = 2$, $[S_3] = 0$, what reaction is most likely to occur for the first reaction step? (5PT)

(C) Keeping the same initial state, what is the probability that after two reaction steps, $[S_3] = 0$? (5PT)

(D) Now, let us redefine the initial state to be $[S_1] = 1$, $[S_2] = 1$, $[S_3] = 0$; again, what is the probability that after two reaction steps, $[S_3] = 0$? (5PT)

(E) What is the expected time for the system with the same initial conditions as defined in (D) to reach stability? (5PT)

(F) If you are designing a circuit and you would like to take advantage of biological noise, would you choose positive feedback or negative feedback? Intuitively explain your choice. (5PT)

6. LOGICAL DEVICES (25PT)

You are tasked with designing a biological reporter that monitors two separate threshold concentration sensors (S1 and S2) for two different biological species. Each sensor is comprised of two concentration level detectors (a and b), one set to activate at a lower threshold, and one set to activate at a higher threshold concentration, respectively. This enables concentration segmentation into 3 separate regions:

- Low – both a and b are inactive;
- Middle – only a is active;
- High – both a and b are active.

Your biological reporter should take in the output signals S1a, S1b, S2a, and S2b and produce GFP if neither sensor reports concentrations in the high region, but at least one sensor reports in the middle region.

(A) Derive a simplified logic expression for the system f(S1a, S1b, S2a, S2b) = GFP (10PT).

(B) Sketch a corresponding logic circuit using the least number of total gates. Multi-input gates are allowed (5PT).

(C) Reduce this to an optimized number of AND, OR, and NOT gates such that if each gate has a delay of 1, the time from change in input to change in output is minimized (5PT).

(D) You are asked to additionally incorporate the feature of "blinking" fluorescence if either S1 or S2 reports concentration in the low region. Describe what design features you would need to implement this (5PT).