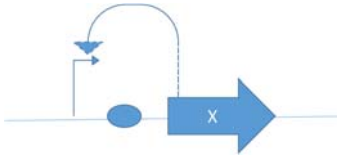
ABE591 Principles of Systems & Synthetic Biology

HOMEWORK #3 **Due: Tuesday, October 23, 2018**

1. Some gene circuits display what is known as bistability where multiple stable stationary points exist. An example of this is the genetic toggle switch where the precise combination of inputs determine what state the system goes towards. This is a consequence of the positive feedback in the system (in a toggle switch, A represses B, a repressor for A, thus increasing expression of A). Let's explore a simpler system that only has one protein. Suppose a transcriptional activator, X, is transcribed from a promoter that is activated by X.

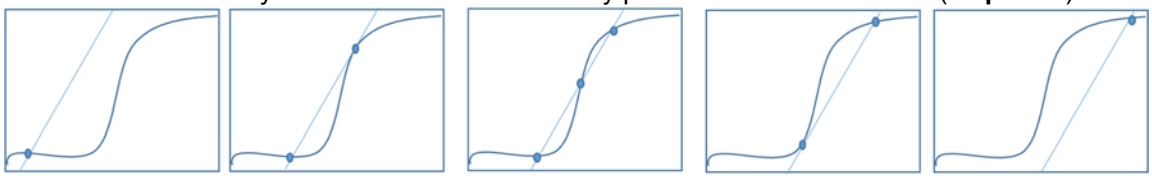


The promoter has two binding sites that display strong cooperative binding. Thus, each binding site displays distinct association constants, K_1 and K_2. Assuming background expression of k' and gain of β, and degradation of X is negligible due to growth, the concentration of x mRNA may be given by:

$$\frac{dx}{dt} = \frac{k' + \beta K_1 K_2 x^2}{1 + K_1 K_2 x^2} - k_d x$$

$$\rightarrow \frac{dx}{dt} = f(x) - g(x)$$

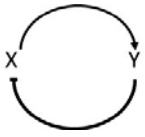
Stationary points or steady states occur at values of *x* at which the generation rate, *f(x)*, and degradation rate, *g(x)*, are equal. Set $k_d = 1$, $K_1K_2 = 1$ and explore the intersections of *f(x)* and *g(x)* numerically as k' and β are varied. Figures (i) through (v) below show schematically the types of behaviors that can occur. **Plot examples of types (i), (iii), and (v) indicating the parameter values that generated them**. Label those values of x for which *f(x) > g(x)* with a right pointing arrow, and those for which *f(x)<g(x)* with a leftward arrow. **Which solutions are stable?** Recall a solution is stable if any deviation from the stationary point returns to that value. (**10 points**)



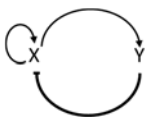
(i) (ii) (iii) (iv) (v)

2. In class, we discussed how 3 repressing proteins (the repressilator) may display sustained oscillatory behavior. However, may plants and animal cells will display a circadian rhythm (i.e. oscillatory cycle) using only 2 proteins via different network motifs. We will consider two possible system architectures to examine how they may display oscillation

A



B



The dynamics of these two systems may be described by the following normalized system of equations

$$A\begin{cases}\frac{dx}{dt} = \gamma\left(k_x + \beta_x \frac{1}{1+y} - x\right) = f(x,y)\\ \frac{dy}{dt} = k_y + \beta_y x - y = \ g(x,y)\end{cases} \qquad B\begin{cases}\frac{dx}{dt} = \gamma\left(k_x + \beta_x \frac{x^2}{1+x^2}\frac{1}{1+y} - x\right) = f(x,y)\\ \frac{dy}{dt} = k_y + \beta_y x - y = g(x,y)\end{cases}$$

Assume γ= 10, k_x = 0.1, β_x=4.0, k_y = 0.0, β_y = 2.0.
*Hint: it may be useful to use MATLAB or similar programs for this section

a) On a graph of y vs x, plot the nullclines (f(x,y)= 0 and g(x,y) =0) for each system. **(4 points each – 8 total)**
b) The intersections of your nullclines in part a will divide your plot into regions. Show the trajectory of an initial condition from each of these quadrants. Based on these figures, comment on the stability of the stationary point. (**2 points**)
c) For system A, prove that the system will always converge to a stable fixed point regardless of parameter value. What is the fixed point? (**5 points**)
d) Let γ be a variable parameter. If the fixed point of system B becomes unstable, sustained oscillations will arise. Numerically compute the eigenvalues of the Jacobian as a function of γ. Write down the conditions on γ under which sustained limit cycles may be possible. (**5 points**)
e) On a graph of y vs x, plot the nullclines (f(x,y)= 0 and g(x,y) =0) for system B for two values of γ: one which gives stable oscillations, and one which approaches the stationary point. Draw trajectories of initial points to confirm your prediction. **(5 points)**

3. You are creating a gene circuit in *E. coli* and decide to use the Biobrick standard to assemble the final product but you notice an issue in the region of nts 242 – 268 of gene 2 given below:

5’ TTCGGAGAATTCCGGT 3’

a) What is wrong with the sequence? (**3 points**)
b) Once you’ve overcome this hurdle, you assemble this gene in a pET vector with a kanamycin selection marker. The rest of your circuit is encoded on a pUC vector with ampicillin resistance. Will your complete gene circuit be stable? Why or why not? (**2 points**) Note:

Common Vectors	Copy Number+	ORI	Incompatibility Group	Control
pUC	~500-700	pMB1 (derivative)	A	Relaxed
pBR322	~15-20	pMB1	A	Relaxed
pET	~15-20	pBR322	A	Relaxed
pGEX	~15-20	pBR322	A	Relaxed
pColE1	~15-20	ColE1	A	Relaxed
pR6K	~15-20	R6K*	C	Stringent
pACYC	~10	p15A	B	Relaxed
pSC101	~5	pSC101	C	Stringent
pBluescript	~300-500	ColE1 (derivative) and F1**	A	Relaxed
pGEM	~300-500	pUC and F1**	A	Relaxed

Source: addgene.org

4. You are trying to create an N-terminal fusion of RFP with GFP via **Golden Gate**. The last five codons on the 3' end of the GFP gene you are using are given below: 5' CTA TAC AAA TAA TAA 3'. The first five codons on the 5' end of the RFP gene you are using are given below:5' ATG GCT TCC TCC GAA 3'.

Assuming you are only removing the STOP codons (TAA) and are omitting a linker sequence, **how might you design the 3’ GFP and 5’ RFP primers**? (**10 points**)