ABE591 Principles of Systems & Synthetic Biology

HOMEWORK #2 **Due: September 18 at end of class (1:15 pm)**

1) Studies of the regulation of an unknown protein reveal that the presence of a ligand increases protein expression and subsequent activity. Experimental data is as follows:

Ligand (ng/ml)	Enzyme Activity (U/ml)
0	0.100
0.1	0.100
1	0.100
10	0.105
100	0.338
1000	0.400

a) Plot the experimental data and estimate parameter values for the Hill transfer function from this plot, justifying your reasoning. You may not be able to directly estimate all parameters using this approach (**4 points**)

b) The method of least squares may be used as a form of non-linear regression to empirically fit the data and calculate the transfer function parameters. Using a tool like SOLVER in Excel, calculate all parameter values for the Hill transfer function. How do these values compare to your estimates from part a? Include your initial values used for the optimization. (**5 points**)

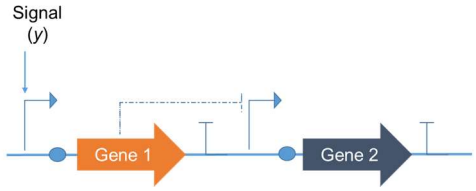
HINT: Recall that in regression, you are minimizing the sum of the square deviation between your predicted curve and the actual data (least squares). Youtube also has excellent tutorials

c) You then complete a ChIP assay around the putative promoter to reveal a monomeric protein that binds to this region. Could this protein be the transcription factor/activator protein responsible for the behavior? Why or Why not? [**1 points]**. If you are unable to solve part a, assume the Hill function is: $Activity = 0.1 + \frac{0.5x^{2.5}}{1700+x^{2.5}}$

2) Analyze the gene circuit below given by Hill functions with the following expression properties.

$$Gene1 = 2 + \frac{30y^2}{49 + y^2}$$

Gene 2: β = 30; K = 1; k' = 4; n = 5



Inputs (y) range in value from 0 to 100 AU.

a) Testing of this circuit reveals that the output of Gene 2 never changes. Why? Illustrate the problem with the range of outputs one might expect expect given the range of inputs. [**5 points**]

b) What parameters need to change? How might one change the underlying biology of **Gene 2** to fix the problem? [**5 points**]

3) A constitutive promoter (always on) is used to express a protein from cells grown in a bioreactor

a) Derive an expression that describes the steady state concentration of protein [**5 points**]

b) You need to increase protein concentration by 25% but know nothing about synthetic biology and **lack the tools to make any genetic intervention**. How might you change this system to increase protein concentration by the desired amount? [**2 points**]
c) Imagine that you now have an engineered strain where the protein half-life has been increased by 50%. How might you fine tune the fermentation process to increase protein concentration by only 25% relative to his initial construct? By how much? [**3 points**]

4) You've built a hyper biofuel producing strain and to prevent corporate espionage you have incorporated a kill switch using CRISPR that destroys your host genome when triggered (a real issue! – Caliendo & Voigt, *Nat Comm* doi:10.1038/ncomms7989). You create a system that represses death under lab conditions (a blend of magic nutrients) but will be triggered outside of the lab when these nutrients are absent.
a) How would you design your gene regulation **to definitively determine cell fate** and ensure accurate triggering of your kill switch? [**2 points**]
b) Your design initiates cell death when a key repressor protein is unable to bind DNA. This protein binds only in the presence of your magic nutrient, which, unfortunately, is present in the environment at low levels. How would you shift/transform the input-output relationship to ensure that the system is triggered when not present in lab conditions of high nutrient? It may help to draw the Hill functions [**3 points**]
c) What parameters would you actually control to effect this change? How would you physically implement this? [**5 points**]

5) The robustness of the steady state concentration of protein (P_{ss}) to fluctuations in the transcription rate (β) is quantified by a sensitivity coefficient defined as:

$$S(P_{ss},\beta) = \frac{\Delta P_{ss}/P_{ss}}{\Delta\beta/\beta} = \frac{\beta}{P_{ss}}\frac{dP_{ss}}{d\beta}$$

A sensitivity coefficient of 2 means that the steady state will change twice as much as the fluctuation in β.

a) For a simple repressor system where Y--| X, calculate the sensitivity coefficient for the steady state concentration of X. [**3 points**]
b) Calculate the sensitivity coefficient of the system if X is only subject to negative feedback from itself. How does sensitivity depend on Hill coefficient? [**5 points**]

6) **Design Project.** Outline the logic gates and major gene circuits (specific parts not necessary at this point, just positive/negative interactions etc) for your project. [**2 points**]