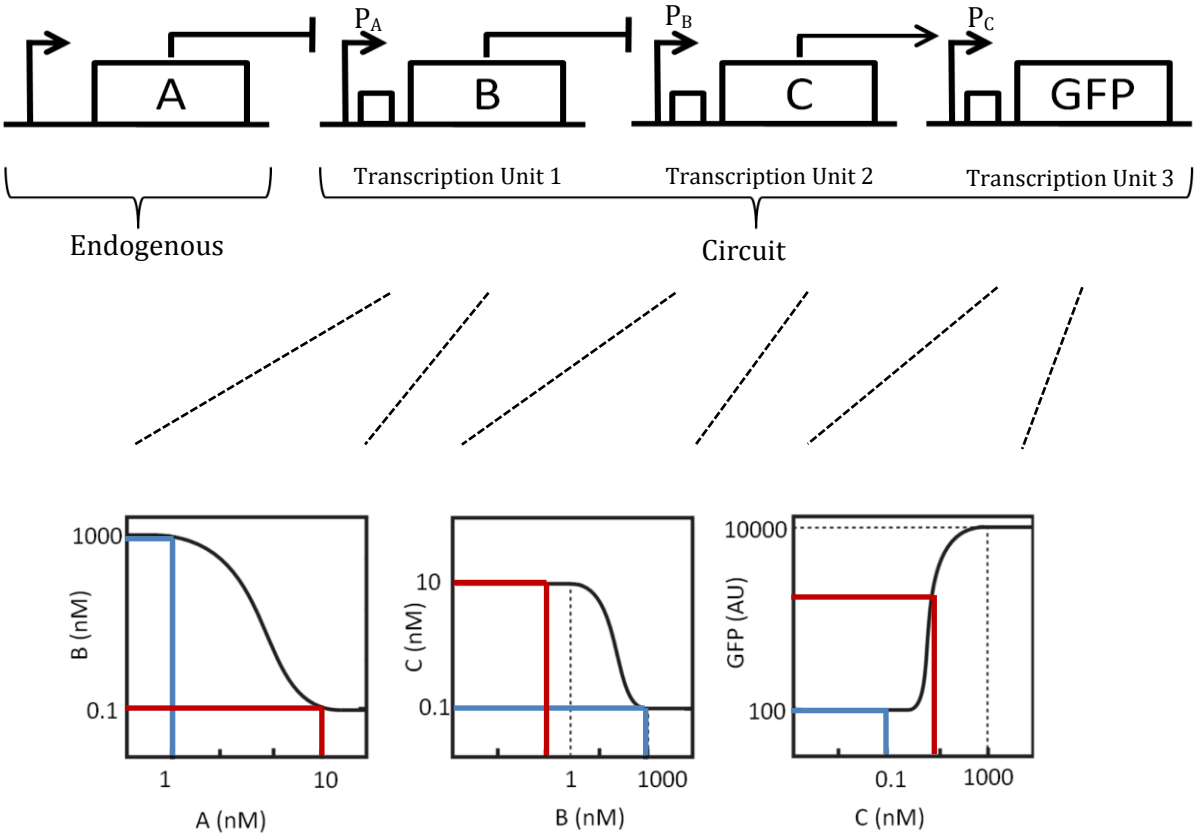
1. Cascades, Input matching, Circuit Tuning, and DNA Assembly

You have designed the following cascade to report concentrations of endogenous repressor A in a cell. You have characterized the individual transfer curves for A:B, B:C and C:GFP and are trying to obtain 100-fold change in GFP fluorescence when A changes from 1 to 10 nM.



a. Approximately what fold change would you expect in GFP output when A varies from 1 nM to 10 nM? [3pt]

You might expect about 10-fold change as shown above, though variation could be large since at [A]=10nM, GFP will be in the transition region.

b. What advantages/disadvantages are there for having a cascade rather than having A directly repress GFP? [3pt]

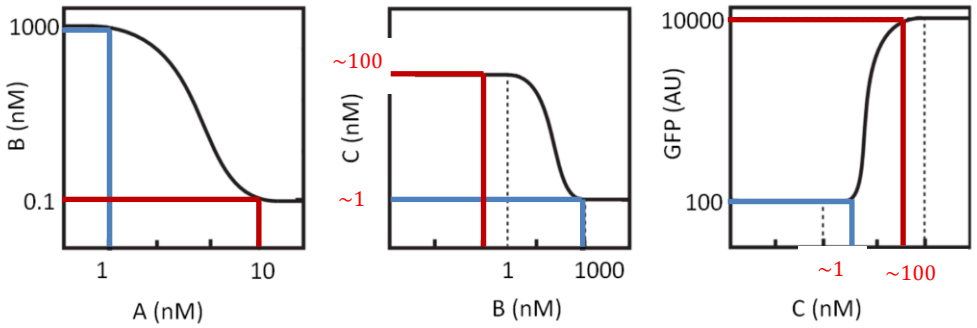
Some advantages: time delay, more DNA elements to tune, possible amplification, noise reduction
Some disadvantages: time delay, more DNA elements to assemble and tune, higher metabolic load (more answers are possible)

c. What biological parameters determine how fast GFP turns on and what parameters determine how fast GFP turns off? [3pt]

Transcription/translation for C and GFP along with transcript/protein degradation rates for B will likely be most important for GFP on rate. Degradation of GFP protein should be most important for GFP off rate.

d. Describe two approaches for redesigning the circuit to give binary GFP output (either 100 or 10000 AU) in response to concentrations of A that are outside the transition region.[6pt]

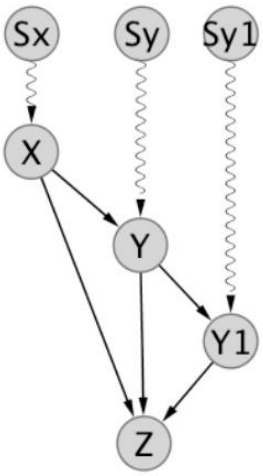
The RBS on transcription unit 2 can be replaced with a stronger RBS to try to shift up the concentrations of C for a given concentration of B. This would shift relevant part of the C:GFP transfer curve to the right to take up most of the transition region.



Alternatively we could have B directly repressing GFP output by removing transcription unit 2 and replacing P_C with P_B. Or we might have A directly repress GFP by a similar modification.

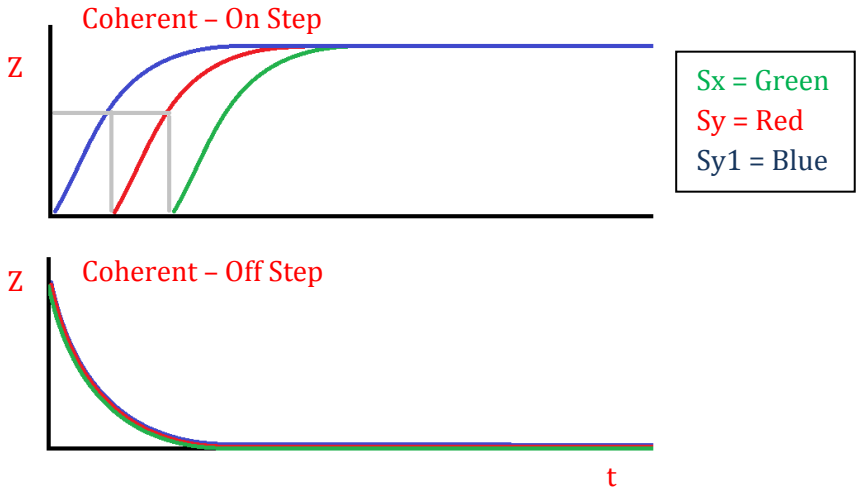
2. Feedforward

Consider a coherent type-1 feedforward loop with nodes X, Y, and Z, which is linked to another coherent type-1 FFL in which Y activates Y1, which activates Z. Expression of X requires signal Sx, expression of Y requires X and Sy, while expression of Y1 requires Y and Sy1.



a. Sketch the dynamics of Z expression in response to steps of the signals Sx, Sy, and Sy1 (steps in which one of the signals goes ON or OFF in the presence of the other signals). There should be six traces in total [10pt]

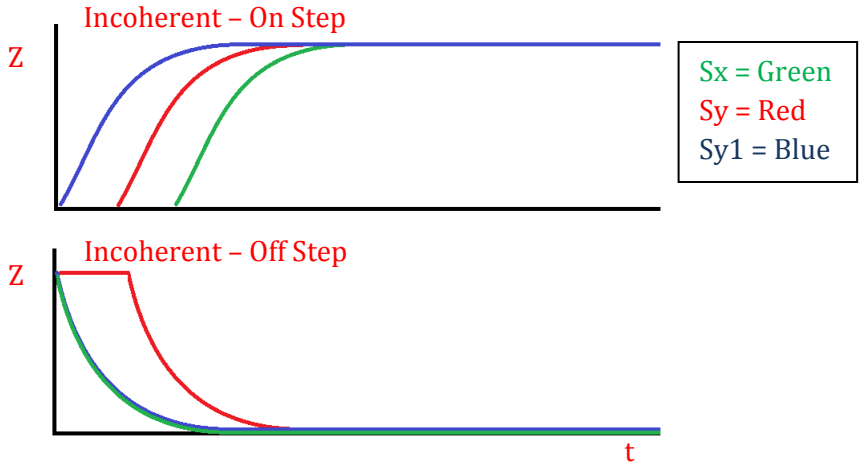
We will use a simplified model where activator concentration above a threshold activates transcription of the associated promoter. For the on step, we get a time delay for each feedforward loop the signal has to go through before reaching Y1. For the off step, removal of any signal means that the final AND gate will decrease Z concentration as soon as any signal is removed.



b. Repeat part a for the case where Y represses Z, so that the X, Y, Z FFL is an incoherent type-1 FFL. Assume that Y1 binding to the Z promoter can alleviate the repressing effect of Y. [10pt]

For the on step in the incoherent case, we again need Y1 to for Z to be output so we will get a time delay for each feedforward loop until we reach Y1. This is identical to the coherent case. For the off step, removal of Sx or Sy1 will result in decay of Z without a time delay because those are needed to activate the final AND gate. Removal of Sy derepresses the AND

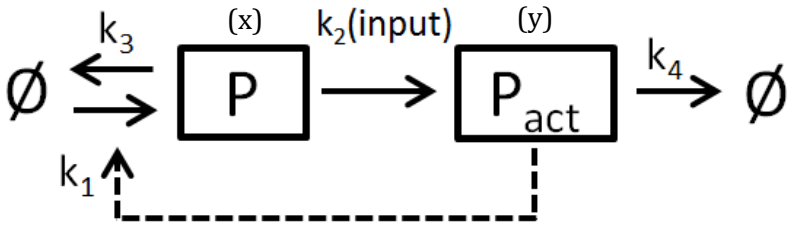
gate but since Y1 already overrides Y, the signal remains for a time until Y1 is no longer activated.



c. Can the dynamics of the interconnected circuit be understood based on the qualitative behavior of each FFL in isolation? [5pt]

Generally the dynamics of interconnected FFL circuits cannot be understood based only on qualitative behavior of isolated FFLs. Unless we know how the components of the FFL interact, the predicted behavior can be incorrect. For instance, with the 3 input AND gates considered here, there will be no output in any of the isolated FFLs as not all of the required nodes will be present (eg. in incoherent case, isolated X-Y FFL is missing Y1 and Y-Y1 FFL is missing X). However, in the interconnected circuit high output is possible as shown in part b.

3. Stability



You are trying to simulate a simplified circuit which consists of a protein (x) that becomes activated by the input with 1 st order rate constant k_2 (input) which is a function of some input signal. Activated protein (y) produces more x with rate of the form $k\ 1 * y^n / (y^n + K_d)$. x is degraded with first order rate constant k_3 and y with rate constant k_4. There is also basal production of x with rate constant k_5. Write differential equations for x and y. [3pt]

$$\frac{dx}{dt} = k_1 \frac{y^n}{K_d + y^n} - k_2 x - k_3 x + k_5$$

$$\frac{dy}{dt} = k_2 x - k_4 y$$

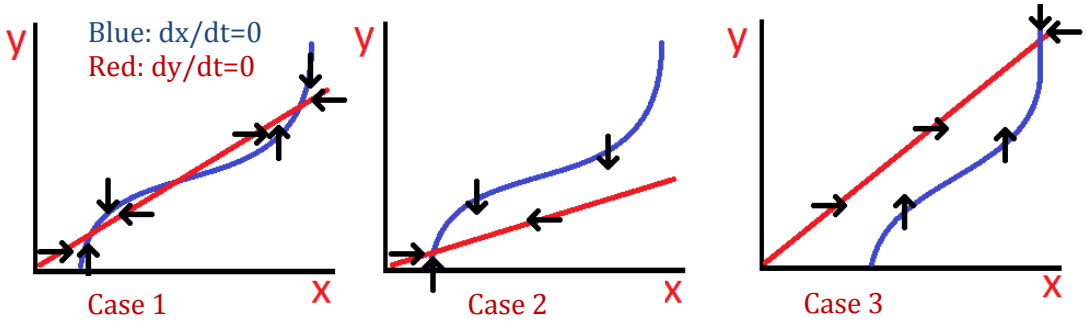
a. Sketch nullclines and the vector field for x and y if n=2. [3pt]

For $\frac{dx}{dt} = 0$: $x = \frac{k_1}{k_2+k_3} \frac{y^n}{K_d+y^n} + \frac{k_1 k_5}{k_2+k_3}$

For $\frac{dy}{dt} = 0$: $y = \frac{k_2}{k_4} x$

There are three potential steady states with two stable.

b. Sketch nullclines and the vector field for x and y if n=2. Be sure to sketch all possibilities for how the nullclines may intersect. How many potential steady states are there and which ones are stable? How many failure modes are there? [15pt]



Three potential steady states. Two are stable (low x/low y, high x/high y). Two failure modes where there is only one steady state

c. For the failure mode(s) you described in b, describe how you might modify one of the system parameters (eg. k_1 , k_2 , k_3 , k_4 , k_5 , n, K_d) to alter the nullclines in order to make multiple steady states more favorable. [7pt]

One of the following answers is required (except k2):

k1 alters the amplitude of the dx/dt=0 nullcline. Increasing k1 in the case 2 failure mode will scale the dx/dt=0 nullcline to the right, perhaps allowing for multiple intersections between the nullclines. Likewise, decreasing k1 in the case 3 failure mode will scale the dx/dt=0 nullcline to the left, again making multiple intersections between the nullclines more favorable.

It is difficult to predict the effect of k2 since k2 appears in both nullcline equations.

k3 alters the amplitude of the dx/dt=0 nullcline (but this effect depends on the value of k2). Increasing k3 in the case 2 failure mode will scale the dx/dt=0 nullcline to the left, perhaps allowing for multiple intersections between the nullclines. Likewise, decreasing k3 in the case 3 failure mode will scale the dx/dt=0 nullcline to the right, again making multiple intersections between the nullclines more favorable.

k4 alters the slope of the dy/dt=0 nullcline. Decreasing k4 in the case 2 failure mode will increase the slope of the dy/dt=0 nullcline, perhaps allowing for multiple intersections between the nullclines. Likewise, increasing k4 in the case 3 failure mode will decrease the slope of the dy/dt=0, again making multiple intersections between the nullclines more favorable.

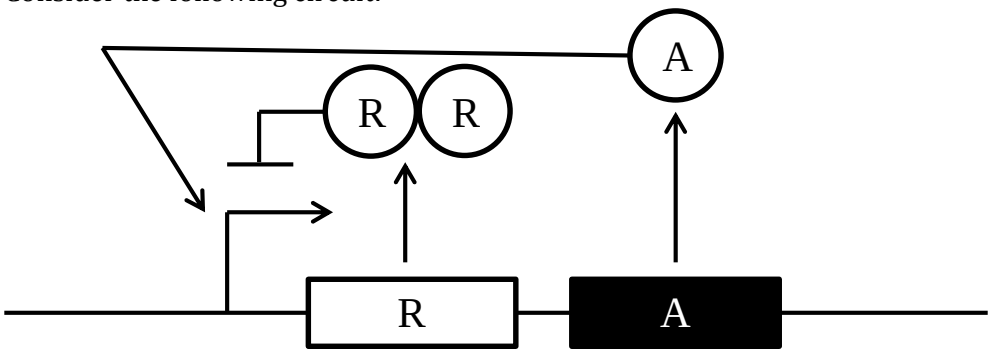
n alters the sharpness of the sigmoidal dx/dt=0 nullcline. Increasing n in either failure mode case may make it more likely for the two nullclines to intersect.

Kd alters the [y] where the sigmoidal transition region occurs for the dx/dt=0 nullcline. Decreasing Kd for case 2 will shift up the dx/dt=0 nullcline to make it more likely to get multiple steady states. Likewise increasing Kd for case 3 will shift down the dx/dt=0 nullcline to make multiple steady states more likely.

k5 shifts the dx/dt=0 nullcline left or right. Increasing k5 can help make 3 steady states in case 2 and 4 by moving the dx/dt=0 nullcline to the left, while decreasing k5 can help in cases 3 and 5 by moving the nullcline to the right.

4. Positive-negative feedback

Consider the following circuit:



The promoter P is creating its own repressor and activator. The repressor must dimerize before being able to bind the activator. The following reactions are taking place:

$P + A \overset{K_{Da}}{\longleftrightarrow} P_A$

$P_A \overset{k_{tr}}{\longrightarrow} P_A + A + R$

$R + R \overset{K_{Dr}}{\longleftrightarrow} R_2$

$R_2 + P \overset{K_{Dr_2}}{\longleftrightarrow} P_R$

$A \overset{k_{degA}}{\longrightarrow} \Phi$

$R \overset{k_{degR}}{\longrightarrow} \Phi$

a. Write down the expression for the rate of production of A in terms of reaction constants, total promoter concentration, [A] and [R]. Assume rapid equilibrium in binding reactions. [10pt]

(1) $\frac{dA}{dt} = k_{tr}[P_A] - k_{degA}[A]$

(2) $[R]^2 = k_{Dr}[R_2]$

(3) $[P][R_2] = k_{Dr_2}[P_R]$

(4) $[P][A] = k_{Da}[P_A]$

From (4): $[P] = k_{Da}\frac{[P_A]}{[A]}$

From (2) and(3): $[P_R] = \frac{1}{k_{Dr}k_{Dr_2}}[R]^2[P] = \frac{1}{k_{Dr}k_{Dr_2}}[R]^2(k_{Da}\frac{[P_A]}{[A]})$

$[P_A] = P_{tot} - [P] - [P_R]$

$[P_A] = P_{tot} - k_{Da}\frac{[P_A]}{[A]} - \frac{1}{k_{Dr}k_{Dr_2}}[R]^2(k_{Da}\frac{[P_A]}{[A]})$

$$[P_A] = P_{tot} - k_{Da}\frac{[P_A]}{[A]}(1 + \frac{[R]^2}{k_{Dr}k_{Dr_2}})$$

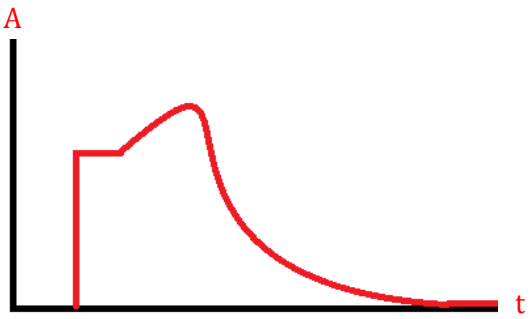
$$[P_A] = \frac{P_{tot}}{1 + \frac{k_{Da}}{[A]}(1 + \frac{[R]^2}{k_{Dr}k_{Dr_2}})}$$

$$[P_A] = \frac{P_{tot}[A]}{[A] + k_{Da} + \frac{k_{Da}[R]^2}{k_{Dr}k_{Dr_2}}}$$

From (1): $\frac{dA}{dt} = \frac{k_{tr}P_{tot}[A]}{[A]+k_{Da}+\frac{k_{Da}[R]^2}{k_{Dr}k_{Dr_2}}} - k_{degA}[A]$

b. Assuming no initial concentration of R or A, a pulse of A is added to the system. What happens to the system if K_{Dr} and K_{Dr2} are much lower than K_{Da}? [5pt]

When K_{Dr} and K_{Dr2} are low, $\frac{dA}{dt} \propto \frac{[A]}{[R]^2}$, and with the initial [R] at low values, [A] initially increases, but as [R] accumulates with A, the $[R]^2$ dependency in the denominator dominates and [A] decreases to a low value.



c. You would like a short pulse of A to create oscillations of A in the system. How would you need to alter the degradation rates of A and R to get to this behavior? [5pt]

There is a quick increase of A and R with the initial pulse of A, followed by repression by R, resulting in decreased formation of A and R. Following repression, if R is degraded much faster than A, A would stick around longer, allowing again for an increase in A and R. This process may repeat to cause oscillations. The degradation rate has to be slower than the production rate and there has to be a large difference between the degradation rate of A and R where the degradation rate for R is higher.

d. The circuit is used by some bacteria in avoiding antibiotic where A is a gene that also activates the production of general antibiotic resistance mechanisms. When antibiotic is present, it binds R and stops it from repressing the promoter. From your answer above, why do you think cells utilize this strategy? [5pt]

Cells can use this oscillatory network to periodically check for antibiotics. A system that turns on autonomously is more likely to be able to instantaneously respond to an antibiotic that may otherwise shut down all protein translation. It may also be more energy efficient to have antibiotic resistance on for only a short period of time depending on whether the cost of having to make lots of R to counteract increased degradation is less than the cost of expressing and maintaining the resistance mechanism.