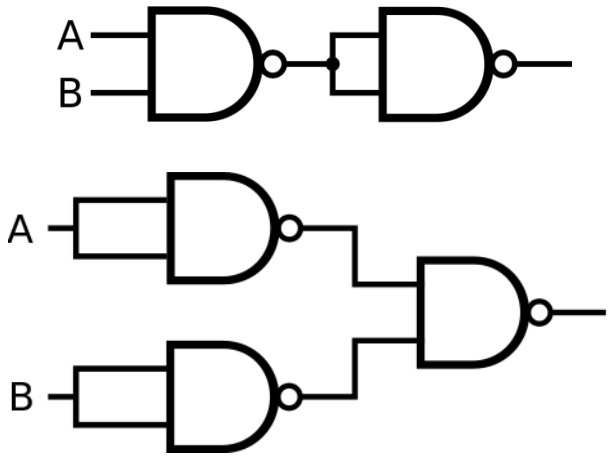
1. Logic gates and biology

The NAND gate is often the only one used in electronic circuits. One of the main reasons for this is that the NAND gate is functionally complete which means that all other logics can be represented by only NAND gates.

a. Write the truth table for the following NAND gate circuits and show what gate they represent:

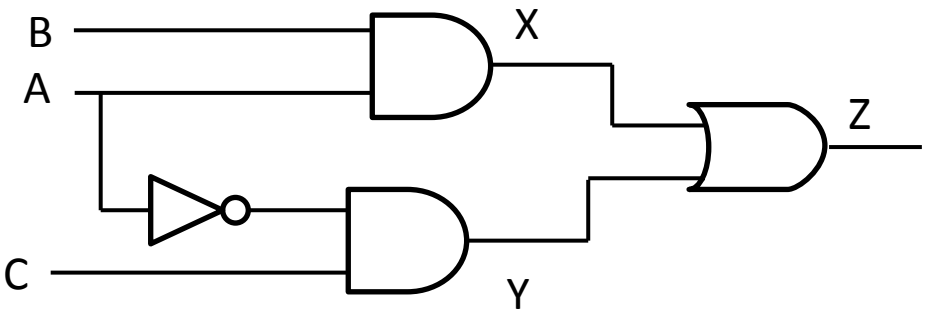


b. Design a biological circuit that performs the function of a NAND gate and the two gates above. Use only transcriptional regulation and use only the specific biological parts covered in class (tetR, lacI, cI, etc.)

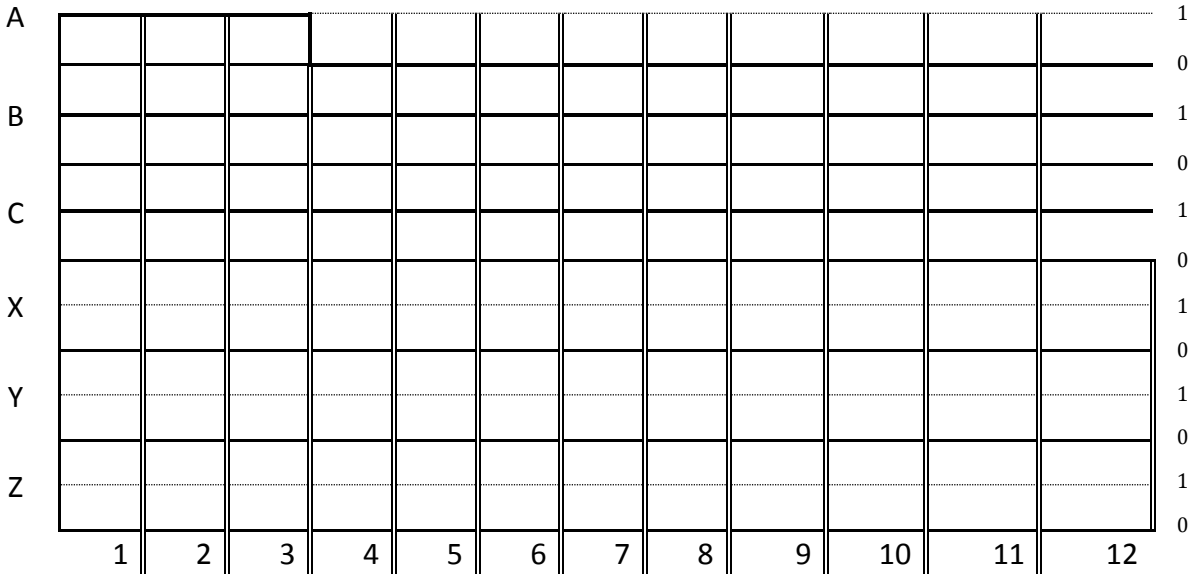
c. Could we construct the two gates above using only the NAND gate in biological systems? Is it feasible to utilize the NAND only architecture in biological systems?

2. Time delays in circuits

a. Logic devices are not perfect. They do not give instant output when inputs are presented, rather logic devices have delays. Fill out the truth table and the timing diagram. Assume all gates have a delay of 1 time unit. The timing diagram is a way to represent changes of a signal over time. For example, signal A was initially at "1". It switched to "0" at end of the third time interval, and then kept its status. Signal B and signal C were at "1" all the time



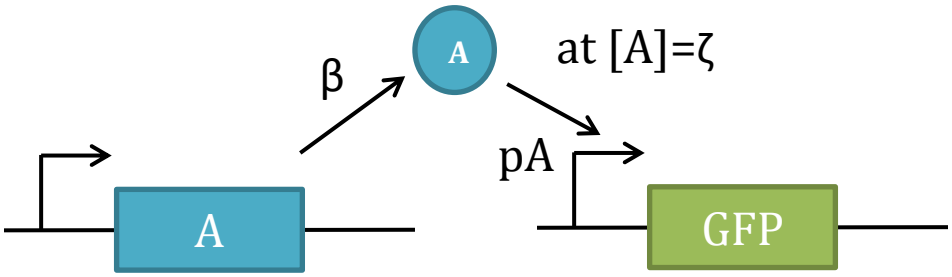
A	B	C	X	Y	Z
0	0	0			
0	0	1			
0	1	0			
0	1	1			
1	0	0			
1	0	1			
1	1	0			
1	1	1			



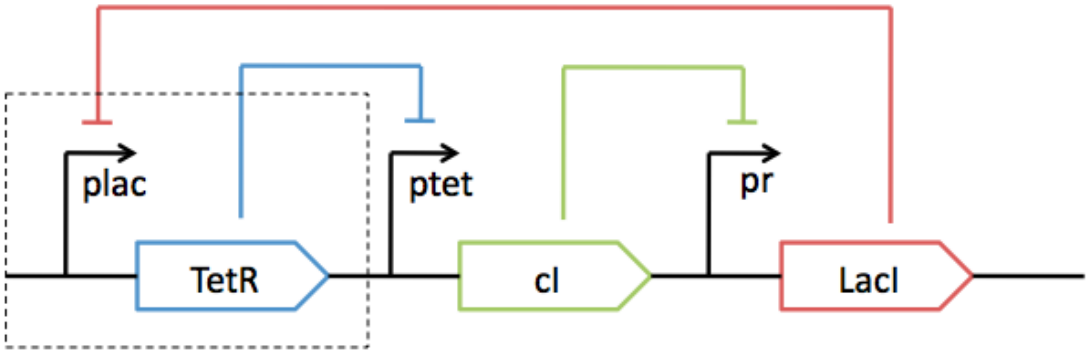
b. What happened to output Z when A switched signals. Examine the logic table and the timing diagram when B and C are held high and A is switched. What kind of problems could this system create?

c. Redesign the circuit above to eliminate the problem caused by delay. Try to make the fewest number of changes you can.

d. Let's look at how time delay can come through in biological systems. We have a simple circuit where protein A is expressed from an inducible promoter with strength β that turns on very quickly once activated. A, in turn, can activate promoter pA to induce expression of GFP. This activation occurs immediately upon A reaching a threshold concentration ζ. A is not destroyed over time, but does undergo dilution at a rate α. Schematic of the system is shown below. If there is no A in the system at time 0 and its expression is turned on, find how much time it would take for the cell to begin expressing GFP.



3. Cooperative binding and part definitions



Consider the repressilator design from Elowitz and Leibler (Nature, 2000) with the following approximations: Unbound or singly bound promoter transcribes mRNA in a first order reaction (example rate constant: k_{ts_plac}); mRNA transcript is translated in a first order reaction (k_{tl_mTetR}); degradation of mRNA and repressors is also first order (k_{deg_mTetR}, k_{deg_TetR}); promoter-repressor binding takes place in two steps, one repressor protein binds to promoter in a reversible bimolecular reaction with $k_{on\_plac\_1}$ and $k_{off\_plac\_1}$ then the singly bound promoter can reversibly bind in another bimolecular reaction to another repressor ($k_{on\_plac\_1}$ and $k_{off\_plac\_2}$), the doubly bound repressor has repressed transcription with rate $k_{ts\_plac:nLacI}$

First consider the plac-TetR transcription unit that is repressed by a source of LacI.

a. Find an expression for the concentration of the promoter- repressor (dimerized) complex at equilibrium in terms of constants and the concentration of LacI.
b. Under what conditions does the above expression contain a Hill-like term? With these conditions, plot transfer curves (output vs. input curves) for [plac:$LacI_n$] as a function of [LacI] when the Hill coefficient is n=2, and n=4. In what circuits might n=4 be more useful than n=2 and vise versa?

Now consider the whole circuit. Assume that repressor-promoter binding is much faster than the other reactions and use the same assumptions made in deriving the Hill equation form above.

c. Write differential equations for each species
d. Break the system into 1, 3 and 18 parts. For each view of the system, briefly summarize the function of each part and list the inputs and outputs (give expressions for the outputs).
e. Say you want to hook up a reporter to circuit by introducing another plasmid with plac driving GFP. Qualitatively how might the repressilator be affected? How might you minimize interactions between the repressilator/reporter?

4. Simulation using parts and compositors

a. Simulate the repressilator using the SimPartComposition scripts. Model the repressor promoter binding as you did in deriving the Hill form. It may be helpful to use the following parameters listed below.
b. Are oscillations more likely for low or high values of the following: n, k_{ts}, and k_{tl} ? Explain.

Repressilator parameters:

```
plac_copy = 50; %copy number for plac promoter (# of molec/cell)
pr_copy = 50; %copy number for lambda promoter
ptet_copy = 50; %copy number for ptet promoter

n_LacI = 2.2; %hill coeff for LacI
n_cI = 2.2; %hill coeff for cI
n_TetR = 2.2; %hill coeff for TetR

k_on_plac = 10; %on rate for repressor-promoter binding [1/(molec^n*min)]
k_off_plac = 1; %off rate for repressor-promoter binding [1/min]
k_on_pr = 10; %on rate for repressor-promoter binding
k_off_pr = 1; %off rate for repressor-promoter binding
k_on_ptet = 10; %on rate for repressor-promoter binding
k_off_ptet = 1; %off rate for repressor-promoter binding

k_ts_plac = 100; %transcription rate for unbound promoter [1/min]
k_ts_pr = 100; %transcription rate for unbound promoter
k_ts_ptet = 100; %transcription rate for unbound promoter
k_ts_low_plac = 0.0005; %transcription rate for bound promoter
k_ts_low_pr = 0.0005; %transcription rate for bound promoter
k_ts_low_ptet = 0.0005; %transcription rate for bound promoter

k_deg_mLacI = 10; %degradation rate for transcript [1/min]
k_deg_mcI = 10; %degradation rate for transcript
k_deg_mTetR = 10; %degradation rate for transcript

k_tl_mLacI = 5; %translation rate [1/min]
k_tl_mcI = 5; %translation rate
k_tl_mTetR = 5; %translation rate

k_deg_LacI = 0.1; %degradation rate for repressor [1/min]
k_deg_cI = 0.1; %degradation rate for repressor
k_deg_TetR = 0.1; %degradation rate for repressor
```

(Also: start with all promoters initially unbound except plac which is 1% bound)