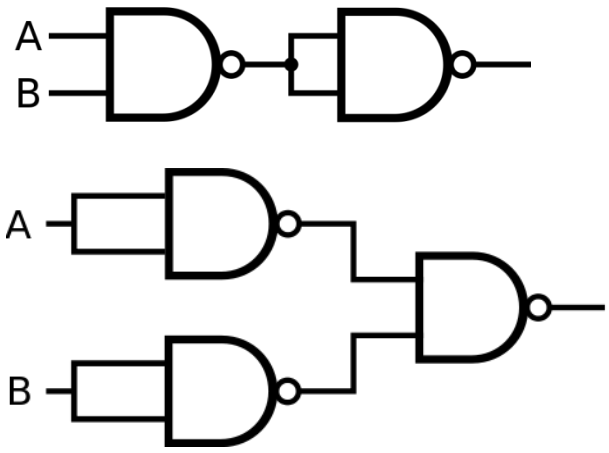
1. **Logic gates and biology**

   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:



A	B	Out
0	0	0
0	1	0
1	0	0
1	1	1

AND gate

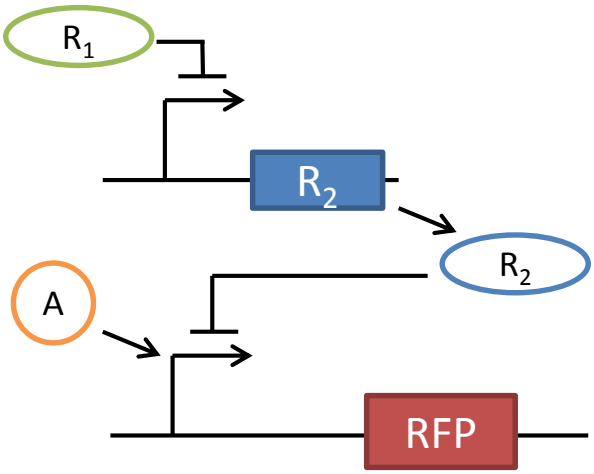
A	B	Out
0	0	0
0	1	1
1	0	1
1	1	1

OR gate

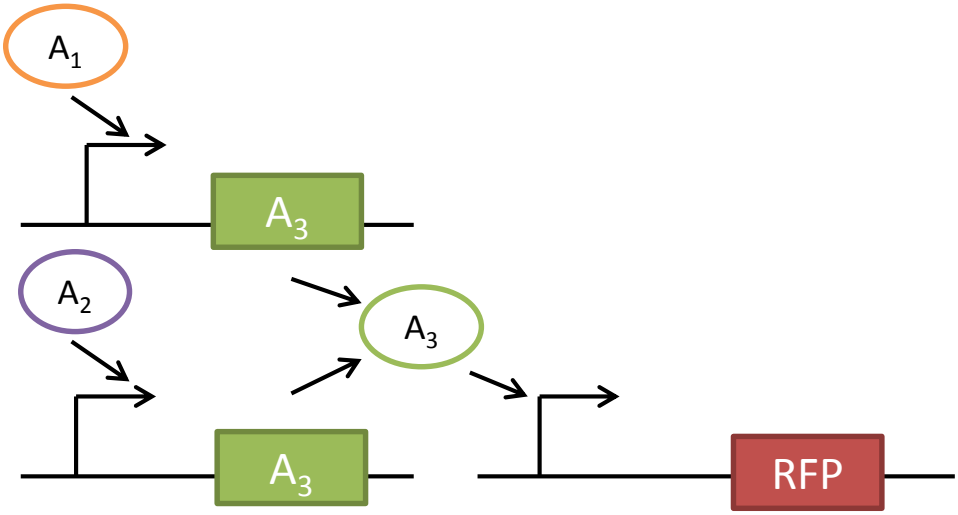
   b. Design a biological circuit that performs the function of a NAND gate and the two gates above. Use only transcriptional regulation and try to only use parts encountered so far in class.

Multiple answers possible. Examples:

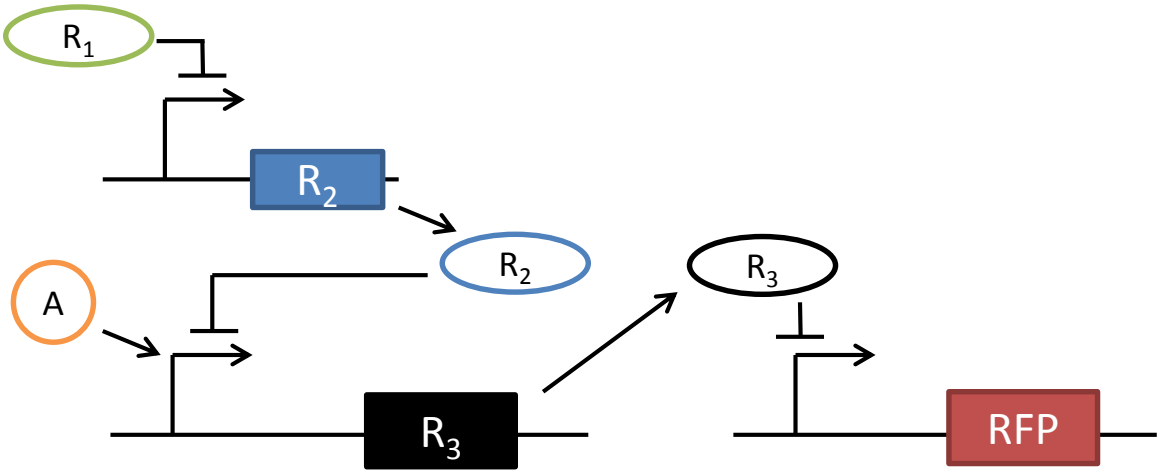
AND gate: repressor R1 AND activator A = RFP



OR gate: activator A1 OR activator A2 make A3 = RFP



NAND gate: AND gate + NOT gate



c. Could we construct the two gates above using only the NAND gate in biological systems? What would be the limitations?

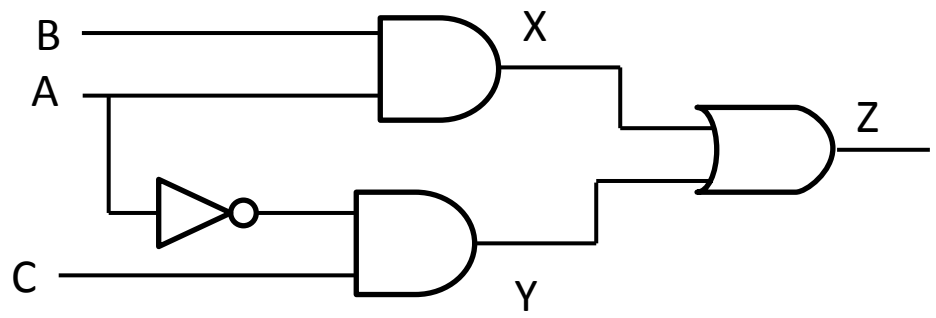
It's possible to construct the gates that way, but since each NAND gate requires 2-4 control elements, construction of a single OR gate would require at least 6 orthogonal elements that do not affect each other. Large logic systems cannot support so many orthogonal repressors/activators.

d. Is it feasible to utilize the NAND only architecture in biological systems?

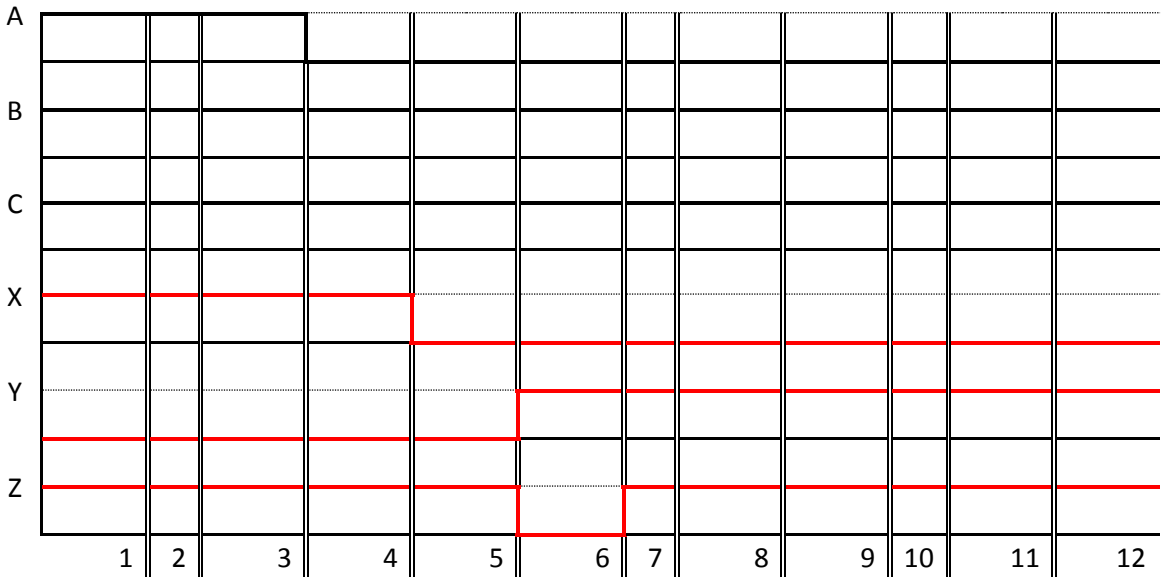
Not really. Since the whole circuit exists in the same space, each effector has to be separate from others, and parts cannot be reused. It is much easier to change base level architecture for each gate rather than chain modular gates together.

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. Timing diagram is 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	0
0	0	1	0	1	1
0	1	0	0	0	0
0	1	1	0	1	1
1	0	0	0	0	0
1	0	1	0	0	0
1	1	0	1	0	1
1	1	1	1	0	1



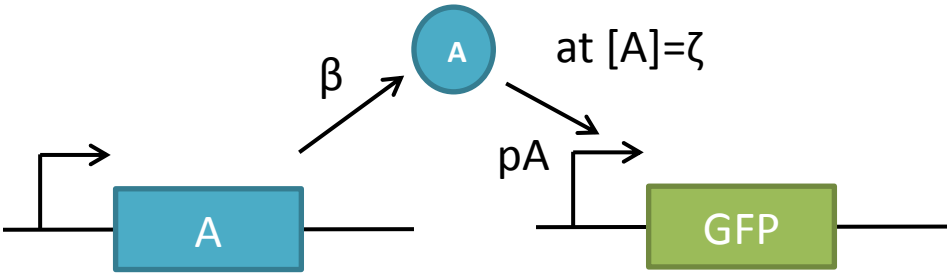
b. What happened to output Z when A switched signals. Examine the logic table and the timing diagram when B and C are held high and A is switched. What kind of problems could this system create?

Z had a brief moment where it switched from 1 to 0 and the back to 1. In the logic table at C=1 and B=1, A=1 or 0 have the same value => Z=1. However, if a change in A occurs, in the timing diagram we can see that Z has a small time fluctuation. This could cause a lot of problems downstream as the system exhibits behavior that is not predicted by the logic functions of its components.

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

The easiest way to make the circuit work is to include a buffer gate on the A input into gate X. This would cause the same time delay coming from any fluctuation to go into X and Y gates and should not interfere with the function of the Z gate.

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 it's expression is turned on, find how much time it would take for the cell to begin expressing GFP.



$\frac{d[A]}{dt} = \beta - \alpha[A]$

$[A] = \frac{\beta}{\alpha} - Ce^{-\alpha t}$ since at t=0, [A]=0;

$[A] = \frac{\beta}{\alpha}(1 - e^{-\alpha t})$.

At $t=t_{expression}$; [A] = ζ;

$\zeta = \frac{\beta}{\alpha}(1 - e^{-\alpha t_{expression}})$

$t_{expression} = \frac{1}{\alpha}\ln\left(\frac{\beta}{\beta - \zeta\alpha}\right)$

3. a.) We have the following system

$$plac \underset{k_{off1}}{\overset{k_{on1}}{\rightleftharpoons}} plac\cdot LacI \underset{k_{off2}}{\overset{k_{on2}}{\rightleftharpoons}} plac\cdot LacI_2$$

$\downarrow k_{ts\_plac}$

TetR mRNA

$\downarrow k_{tl\_mTetR}$

TetR

At equilibrium, $\frac{d\,plac}{dt} = 0$ $\quad$ $\frac{d\,plac\cdot LacI_2}{dt} = 0$

① $\frac{d\,plac}{dt} = 0 = -k_{on1}[plac]_{ss}[LacI]_{ss} + k_{off1}[plac\cdot LacI]_{ss}$

② $\frac{d\,plac\cdot LacI_2}{dt} = 0 = k_{on2}[plac\cdot LacI]_{ss}[LacI]_{ss} + -k_{off2}[plac\cdot LacI_2]$

~~Substitute ① into ②~~

↳ $\frac{k_{on1}}{k_{off1}}[plac]_{ss}[LacI]_{ss} = [plac\cdot LacI]_{ss}$

↳ ~~plug into~~

~~$[plac\cdot LacI_2] = \frac{k_{on1}\,k_{on2}}{k_{off1}\,k_{off2}}[plac]_{ss}[LacI]_{ss}^2$~~

b.) ③ $[plac] = plac_{tot} - [plac\cdot LacI] - [plac\cdot LacI_2]$ (plac total conc is constant)

plug ③ into ①

↳ $[plac][LacI] = K_{d1}[plac\cdot LacI]$

$(plac_{tot} - [plac\cdot LacI] - [plac\cdot LacI_2])[LacI] = K_{d1}[plac\cdot LacI]$

$[plac\cdot LacI]\cdot\left(1 + \frac{[LacI]}{K_{d1}}\right) = \frac{[LacI]}{K_{d1}}(plac_{tot} - [plac\cdot LacI_2])$

Ⓐ $[plac\cdot LacI] = \frac{[LacI]}{[LacI]+K_{d1}}(plac_{tot} - [plac\cdot LacI_2])$

plug Ⓐ into ②

$$[plac \cdot LacI] = \frac{Kd_2}{[LacI]}[plac \cdot LacI_2]$$

$$\frac{[LacI]}{[LacI] + Kd_1}(plactot - [plac \cdot LacI_2]) = \frac{Kd_2}{[LacI]}[plac \cdot LacI_2]$$

$$\frac{[LacI]^2}{Kd_2([LacI] + Kd_1)}(plactot - [plac \cdot LacI_2]) = [plac \cdot LacI_2]$$

$$\frac{[LacI]^2}{Kd_2([LacI] + Kd_1)}\, plactot = \left(1 + \frac{[LacI]^2}{Kd_2([LacI] + Kd_1)}\right)[plac \cdot LacI_2]$$

$$\frac{[LacI]^2}{Kd_2([LacI] + Kd_1)}\, plactot = \frac{Kd_2([LacI] + Kd_1) + [LacI]^2}{Kd_2([LacI] + Kd_1)}[plac \cdot LacI_2]$$

$$[plac \cdot LacI_2] = \frac{[LacI]^2\, plac\,tot}{[LacI]^2 + Kd_2[LacI] + Kd_1Kd_2}$$

$$= \frac{[LacI]^2\, plactot}{\left([LacI] + \frac{Kd_2}{2}\right)^2 + Kd_1Kd_2 - \frac{Kd_2}{4}}$$

Reminder: Hill term looks like (for n=2) $\frac{v_{max}[S]^2}{(K_A)^2 + [S]^2}$

So we need Kd2 ->0 while Kd1>>Kd2, which allows Kd1Kd2 to converge to a constant, while the remaining Kd2 terms go toward zero, leaving an expression in the form of the Hill equation. This shows that once the first repressor binds, we consider all remaining n-1 repressors to bind instantaneously. Note that this allows us to consider the binding as a single step nth order reaction.

3c 6 parts (or subparts) per repressor: Hill-style binding/unbinding, transcription from free promoter, transcription from n·repressor bound promoter, degradation of transcript, translation, degradation of repressor

v [promoter]: (from repressor binding)

$v_1[p_{lac}] = -k_{on\text{-}plac}[p_{lac}][LacI]^{n\_lacI} + k_{off\text{-}plac}[p_{lac} \cdot nLacI]$

$v_2[p_{tet}] = -k_{on\text{-}ptet}[p_{tet}][TetR]^{n\_TetR} + k_{off\text{-}ptet}[p_{tet} \cdot nTetR]$

$v_3[p_r] = -k_{on\text{-}pr}[p_r][cI]^{n\text{-}cI} + k_{off\text{-}pr}[p_r \cdot ncI]$

v [mRNA]: (from free promoter)

$v_4[mTetR] = k_{ts\text{-}plac} \cdot [p_{lac}]$

$v_5[mcI] = k_{ts\text{-}ptet} \cdot [p_{tet}]$

$v_6[mLacI] = k_{ts\text{-}pr} \cdot [p_r]$

v [mRNA]: (from bound promoter)

$v_7[mTetR] = k_{ts\text{-}plac\text{-}low} \cdot [p_{lac} \cdot nLacI]$

$v_8[mcI] = k_{ts\text{-}ptet\text{-}low} \cdot [p_{tet} \cdot nTetR]$

$v_9[mLacI] = k_{ts\text{-}pr\text{-}low} \cdot [p_r \cdot ncI]$

v [mRNA]: (from degradation)

$v_{10}[mTetR] = -k_{mTetR,deg}[mTetR]$

$v_{11}[mcI] = -k_{mcI,deg}[mcI]$

$v_{12}[mLacI] = -k_{mLacI\text{-}deg}[mLacI]$

v [repressor]: (from translation)

$v_{13}[TetR] = k_{tl\text{-}mtetR}[mTetR]$

$v_{14}[cI] = k_{tl\text{-}mcI}[mcI]$

$v_{15}[LacI] = k_{tl\text{-}mLacI}[mLacI]$

v [repressor]: (from degradation)

$v_{16}[TetR] = -k_{deg\text{-}TetR}[TetR]$

$v_{17}[cI] = -k_{deg\text{-}cI}[cI]$

$v_{18}[LacI] = -k_{deg\text{-}LacI}[LacI]$

$$\frac{d[plac]}{dt} = v_1 [plac]$$

$$\frac{d[plac \cdot nLacI]}{dt} = -v_1 [plac]$$

$$\frac{d[mTetR]}{dt} = v_4 [mTetR] + v_7 [mTetR] + v_{10} [mTetR]$$

$$\frac{d[TetR]}{dt} = v_{13} [TetR] + v_{16} [TetR]$$

$$\frac{d[ptet]}{dt} = v_2 [ptet]$$

$$\frac{d[ptet \cdot nTetR]}{dt} = -v_2 [ptet]$$

$$\frac{d[mcI]}{dt} = v_5 [mcI] + v_8 [mcI] + v_{11} [mcI]$$

$$d[cI]/dt = v_{14} [cI] + v_{17} [cI]$$

$$\frac{d[pr]}{dt} = v_3 [pr]$$

$$\frac{d[pr \cdot ncI]}{dt} = -v_3 [pr]$$

$$\frac{d[mLacI]}{dt} = v_6 [mLacI] + v_9 [mLacI] + v_{12} [mLacI]$$

$$\frac{d[LacI]}{dt} = v_{15} [LacI] + v_{18} [LacI]$$

18 Parts: Since these are all basic reactions, inputs are simply all concentrations that appear in the appropriate v equations; eg. for v_1 inputs are [plac], [LacI], [plac·nLacI]
output is v_1

plac binding – inputs: [plac] and [LacI], outputs: v1
ptet binding – inputs: [ptet] and [TetR], outputs: v2
lambda pr binding – inputs: [pr] and [cI], outputs: v3
transcription from plac – inputs: [plac], outputs: v4
transcription from ptet – inputs: [ptet], outputs: v5
transcription from lambda pr – inputs: [pr], outputs: v6
leakage transcription from bound plac– inputs: [plac*nLacI], outputs: v7
leakage transcription from bound ptet – inputs: [ptet*nTetR], outputs: v8
leakage transcription from bound pr – inputs: [pr*ncI], outputs: v9
mTetR degradation– inputs: [mTetR], outputs: v10
mcI degradation – inputs: [mcI], outputs: v11
mLacI degradation – inputs: [mLacI], outputs: v12
translation from mTetR – inputs: [mTetR], outputs: v13
translation from mcI – inputs: [mcI], outputs: v14
translation from mLacI – inputs: [mLacI], outputs: v15
TetR degradation – inputs: [TetR], outputs: v16
cI degradation – inputs: [cI], outputs: v17
LacI degradation – inputs: [LacI], outputs: v18

3 Parts: Similarly, inputs are all concentrations appearing in relevant v equations:
eg. for placTetRpart
outputs ⇒ v_1, v_4, v_7, v_{10}, v_{13}, v_{16}
inputs ⇒ [plac], [LacI], [plac·nLacI], [mTetR], [TetR]

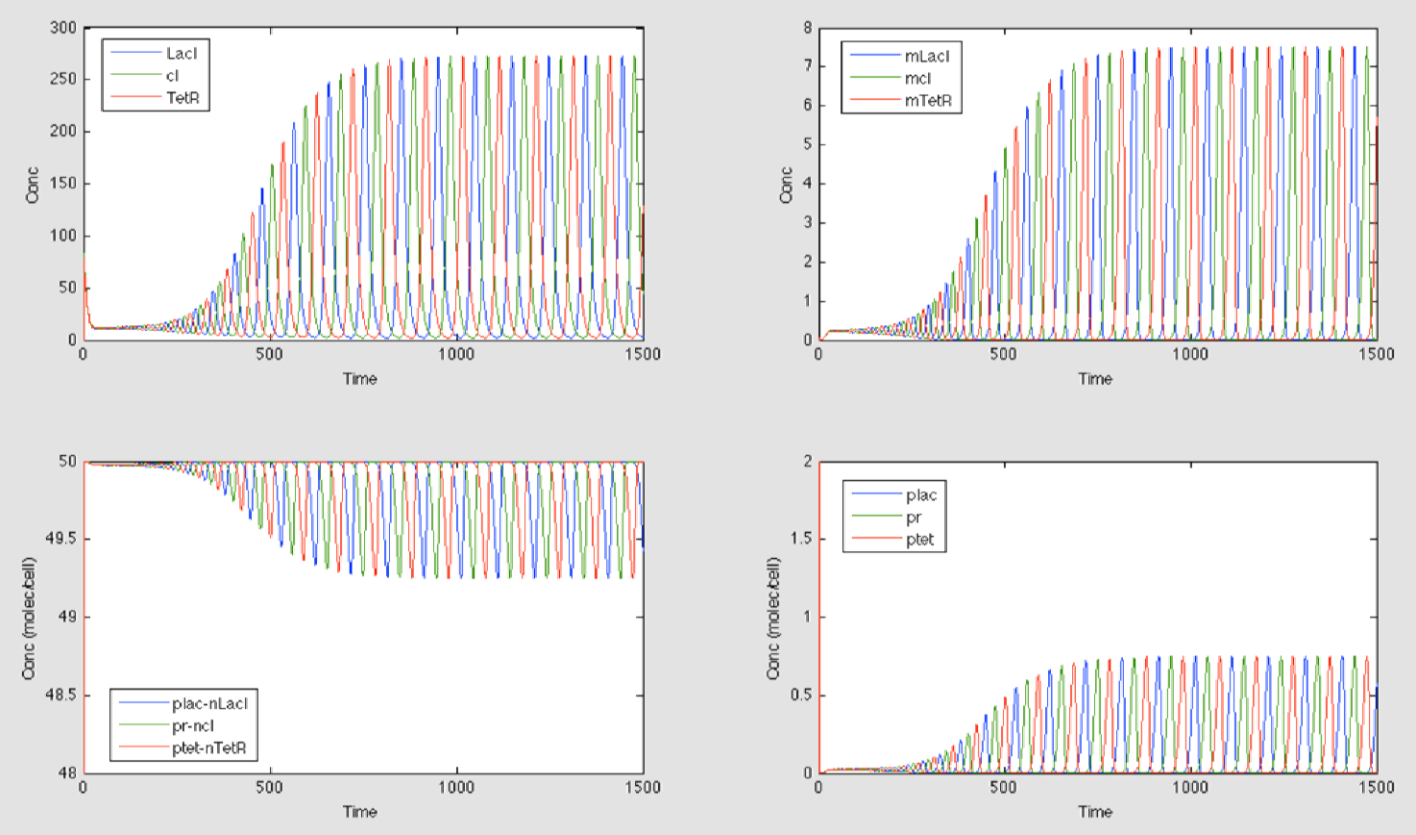
Plac -TetR – inputs: [plac], [LacI],[plac*nLacI], [mTetR],[TetR]; outputs: v1, v4, v7, v10, v13, v16
Ptet - cI – inputs: [ptet], [cI],[ptet*ncI], [mcI],[cI]; outputs: v2, v5, v8, v11, v14, v17
Pr - LacI – inputs: [pr], [LacI],[pr*nLacI], [mLacI],[LacI]; outputs: v3, v6, v9, v12, v15, v18

1 Part: All relevant concentrations are inputs
v_1 through v_{18} are outputs

→ Different views are useful for different things; 18-part view useful for seeing how parameters change individual rates of change. 3-part view useful for getting [TetR] concentration

3c. There might be less available LacI to bind the repressilator's plac depending on system parameters. This could lead to less robust oscillation behavior as strong repression is needed for oscillations. One might build a sort of insulator that takes LacI as input and amplifies it - then connect that output to the reporter. The requirement for the insulator is that it not titrate away significant amounts of LacI

4a. Final output should look something like:



4b. The repressilator relies on good repression for each repressor-promoter pair. Thus high cooperativity (n), and high repressor expression (kts, ktl) will make it more favorable for the system to generate oscillations