BIOE147 – Fall 2013 – PS2

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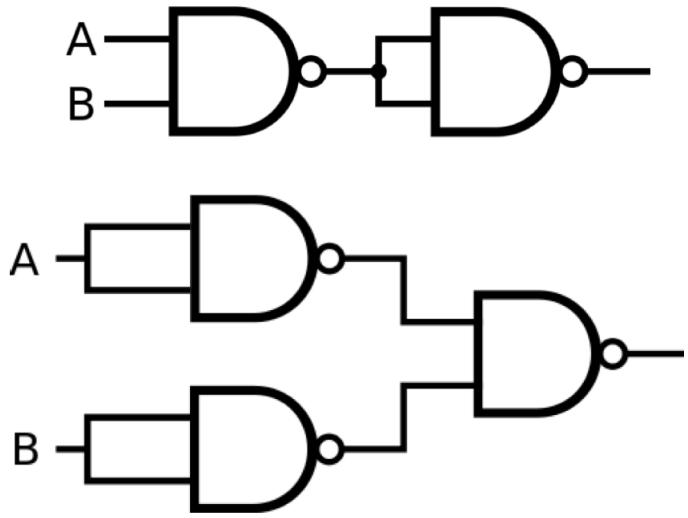
1. Logic Gates and Biology

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 logic gates can be represented by only NAND gates.

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



A	B	$\sim (A \wedge B)$	G1$(A \wedge B)$	G2$(A \vee B)$
1	1	0	1	1
1	0	1	0	1
0	1	1	0	1
0	0	1	0	0

(b.) Design a biological system 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 the course.

- **AND:** In order to implement the AND architecture we can using a two repressors. Let p_1, p_2 be two constitutive promoters and p_3, p_4 be two repressible promoters, r_3, r_4 be the repressor protein of p_3, p_4, and s_3, s_4 be the two small molecule repression inhibitors. p_1 promotes the transcription of r_3, and p_2 promotes the transcription of r_4. The reporter R is being repressed by both p_3 and p_4. It is assumed that the repression of either p_3 or

p_4 is enough to repress the transcription of R. So, only the presence of both s_3 **and** s_4 will activate transcription of the reporter. Options for repressible systems are LacO/LacI and cl-ts. The first gate in the figure is the equivalent of an AND gate.

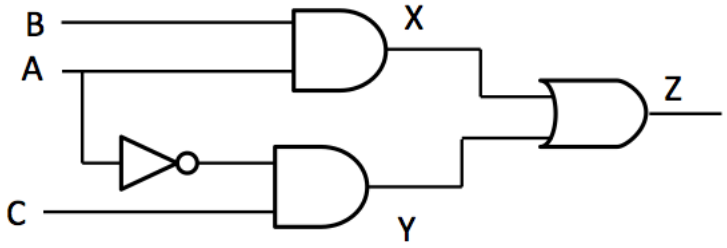
- **NAND:** The NAND gate can be created by implementing an AND gate and then simply flipping the output using a repressor. Starting with the AND gate above, make the output be another repressor protein r_5 (instead of the reporter R). This repressor protein will act on on p_5 and then repress the transcription of R. This will ensure that R is only repressed when both s_3 **and** s_4 are present. Since LacI/LacO and cl-ts were recommended for the AND gate, tetR can be used as the p_5/r_5 system.
- **OR:** The second gate in the figure is an OR gate. To construct this gate you can make two orthogonal circuits that both regulate the production of the same reporter R (GFP). Each of these circuits will act in the same way. Let p be the repressor, r be the repressor protein that modulates p, and s be the small molecule inhibitor that inactives r. The repressor proteins for each of the circuits will be transcribed constitutively. Therefore, the presence of either the s for one circuit **or** the other (or **both**) will activate the transcription of the reporter R.

(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?

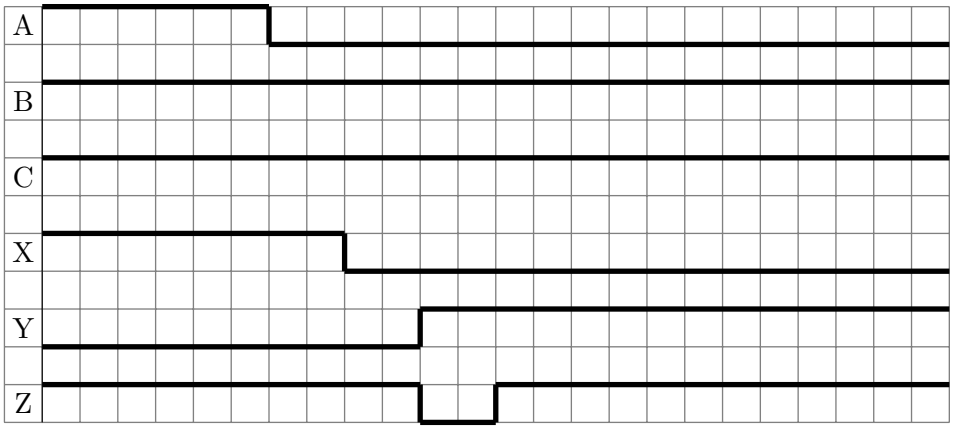
With the materials that we have been restricted to thusfar, no. If all parts known were available it may be feasible to construct the two example circuits, but beyond that it would not be feasible to construct larger systems with NAND only architecture. A key reason that NAND only architecture works in EE applications is that you can construct a many orthogonal NAND gates with ease, while in biology you do not have that luxury.

2. Time Delays in Circuits

(a.) Fill out the truth table and timing diagram. Assume all gates have a delay of one time unit.



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



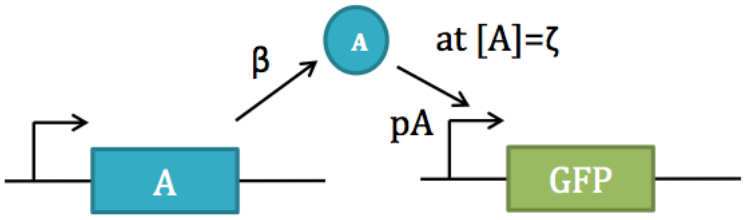
(b.) What happened to output Z when A switched signals?

There was a time delay of one time step when X turned to the 0-state, and a time step of two when Y turned to the 1-state. This meant that there was one time unit where both X and Y were in the 0-state, and therefore for the time step following, Z was also in the 0-state. If the system is designed for X and Y to fluctuate while Z maintains the 1-state invariant then the time delay observed that puts Z in the 0-state would cause issue with whatever behaved with the expectation that Z would alwasys be in the 1-state.

(c.) Redesign the circuit above to eliminate the problem caused by the delay.

An addition of a buffer gate between A and the X gate would stall the A signal for another time step as Y switched from the 0-state to the 1-state. This would eliminate the one time step gap where Z is in the 0-state.

(d.) 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.

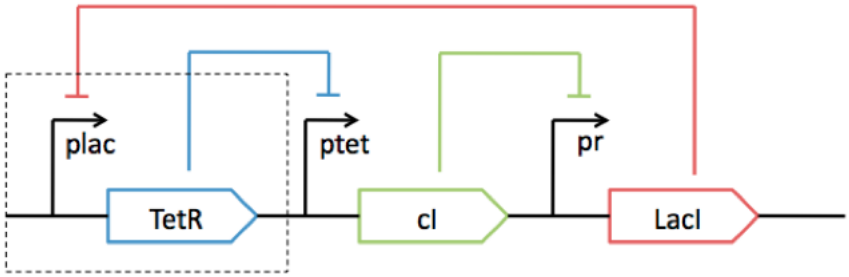


$$\frac{d[A]}{dt} = \beta - \alpha[A]$$

$$[A] = \frac{\beta}{\alpha} + e^{-\alpha t}$$

$$t = \frac{\ln\left(\zeta - \frac{\beta}{\alpha}\right)}{-\alpha}$$

3. Cooperative binding and part definitions



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

(a.) Find an expression for the concentration of promoter-repressor (dimerized) complex at equilibrium in terms of the constants and the concentration of LacI.

$$plac_0 + 2LacI \leftrightarrow plac_1 + LacI \leftrightarrow plac_2$$

$$\frac{d[plac_0]}{dt} = k_{off_{plac_1}}[plac_1] - k_{on_{plac_1}}[plac_0][LacI]$$

$$\frac{d[plac_1]}{dt} = k_{on_{plac_1}}[plac_0][LacI] - k_{off_{plac_1}}[plac_1]$$

$$\frac{d[plac_2]}{dt} = k_{on_{plac_1}}[plac_1][LacI] - k_{off_{plac_2}}[plac_2]$$

$$\log \frac{\frac{[plac_2]}{[plac_{total}]}}{1 - \frac{[plac_2]}{[plac_{total}]}} = 2 * \log [LacI] - \log K_d$$

Assumptions:

$n_H = 2$

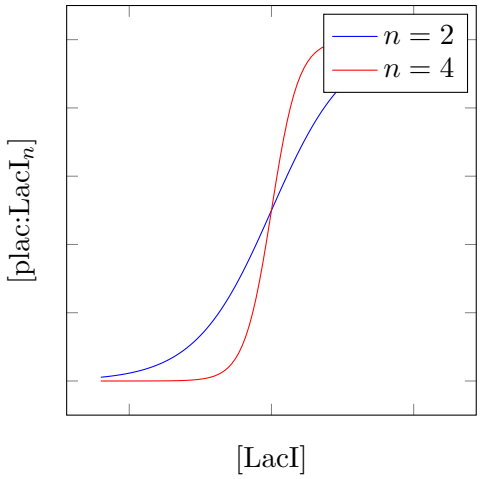
Cooperative binding

$plac_{total}$ is constant

(b.) Under what conditions does the above expression contain a Hill-like term?

The above expression will have a Hill-like term whenever there is n-merization of the repressor proteins when they interact with the promoter.

With these conditions, plot transfer 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 vice verse?

$n = 4$ would be more useful in any circuit where you need more digital response, and you have less concern on the effect of repressor protein production on cell fitness. On the other hand, $n = 2$ has its place in a system where you want to see a change in the system occur at very small concentration of repressor protein.

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 from above

(c.) Write differential equations for each species.

$$\frac{d[plac_0]}{dt} = k_{off_{plac_1}}[plac_1] - k_{on_{plac_1}}[plac_0][LacI]$$
$$\frac{d[plac_1]}{dt} = k_{on_{plac_1}}[plac_0][LacI] - k_{off_{plac_1}}[plac_1]$$
$$\frac{d[plac_2]}{dt} = k_{on_{plac_1}}[plac_1][LacI] - k_{off_{plac_2}}[plac_2]$$
$$\frac{d[mTetR]}{dt} = k_{ts_{mTetR}}([plac_0] + [plac_1]) - k_{deg_{mTetR}}[mTetR] - k_{ts_{plac:nLacI}}[plac_2]$$
$$\frac{d[TetR]}{dt} = k_{tl_{TetR}}[mTetR] - k_{deg_{TetR}}[TetR]$$

Then the same equations hold for the remaining two subsystems. For subsystem 2, replace plac $\rightarrow$ ptet and TetR $\rightarrow$ cl. Repeat for subsystem 3 replacing plac $\rightarrow$ pr and TetR $\rightarrow$ LacI. For a total of 15 equations.

(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.

One Part: For the one part system we have a fluctuating output, and no inputs(besides any inital input). This one part will alternate between 3 states: TetR expression, cl expression, and LacI expression. This alternating output, if fine-tuned, will happen cyclically.

3 Parts: For the three part system we have 3 repressible promoter / repressor protein pairs. Each of these parts takes as input the repressor protein output of another one of the three parts. In this way the output/input repressor proteins are coupled to make a 3 part system where each part cyclically increases/decreases its repressor protein output.

18 Parts: The 18 part system includes many of the often looked over transient parts of the central dogma. These parts include mRNA for each of the repressor proteins, the 1-(n-1) bound states of each of the repressible promoters, and the biological background that ensures that transcription, and translation occur. mRNA takes as input its respective gene as well as RNA polymerase, a(possibly a few) riobosome(s), and the promoter binding site. mRNA then outputs its respective protein.

(e.) Say you want to hook up a reporter to the circuit by introducting another plasmid with plac driving GFP. Qualitatively, how might the repressilator be affected? How might you minimize the interaction between the repressilator/reporter?

Any LacI molecules in the one plasmid circuit only had one plac site to bind to. Now there are two plac sites that will compete for LacI molecules. This could decrease the repression of TetR, and if the decrese in repression is large enough then the entire system will be in unstable equilibrium. In order to minimize the interaction between the repressilator and the reporter, you could introduce an siRNA at the 3' UTR of TetR that could regulate the expression of GFP orthogonally to the rest of the repressillator system.

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 high or low values of the following: n, k_{ts}, and k_{tl}?