Harvey Mudd iGEM 2012: A Buffer Buffer

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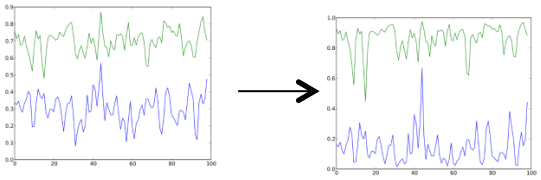
HARVEY MUDD COLLEGE

Goal:

Motivation:

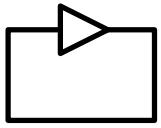


We want to be able to do this to our signals. We can't yet.

Approach 1: TALORs

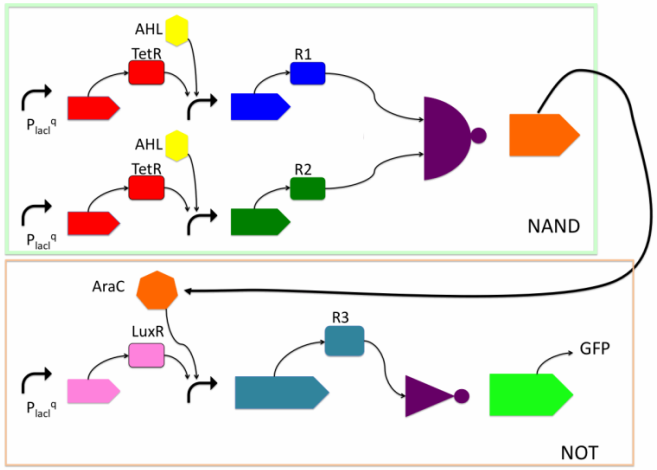
TALORs are customizable, sequence-specific transcription inhibition proteins.

We managed to mathematically prove that proteins with first-order binding (no cooperativity), including TALORs, cannot make a buffer amp. The key insight is that if you feed a buffer amp's output back into itself:



… then a good buffer will form a bistable circuit. However, first-order binding systems do not allow bistability, no matter how complex the system.

Approach 2: Hack a NAND gate.



Many groups have already developed NAND gates. We decided to hack one to make a buffer.

Our design uses the USTC 2007 iGEM team's NAND gate. The input, AHL, promotes two PoP signals, which are fed into the NAND gate. The two inputs are needed for second order behavior, so crosstalk between them does not matter. Since the NAND gate is inverting, the output is then fed through an inverting buffer (from the same group) through AraC.

Approach 3: Triplex Forming RNA (tfRNA)

Within the medical community, much research has been done into DNA oligos which bind specific DNA sequence to form triple-stranded helices. These oligos are then able to block transcription. Furthermore, these oligos can dimerize to increase bidning strength [cite]. Though less researched, it turns out that RNA can also form a third strand and block transcription [cite].

Our third approach attempts to use dimerizing, triplex-forming RNA to block a promoter. The work is still very much in progress, as we are just now beginning to test the first promoter/RNA pair.

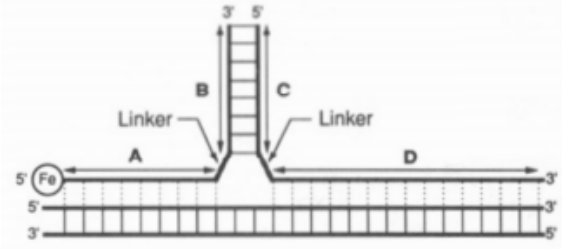


Diagram from Distefano, Shin and Dervan (1991). They found that DNA oligos can dimerize to strengthen triplex binding. We hope to do the same with RNA.