BIOE 147 - Detailed Project Description

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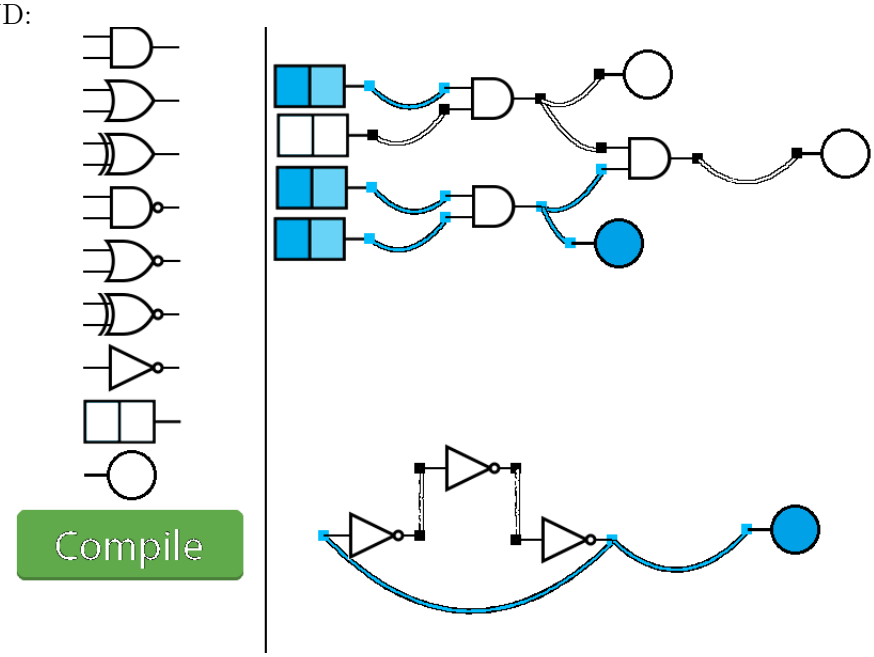
Overview

The goal of this project is create a tool for the rational design of biological logic circuits. The interface is a graphical logic circuit tool with drag and drop functionality of individual gates, switches, and output bulbs. Pictured here with a repressilator analog and a 4-input AND:



Following the graphical design of a circuit, there is a compilation option that takes as input the circuit as designed and outputs a set of parts and compositors that are a good starting point for realizing the circut in vivo. The details of part and compositor generation are described in the next section. This type of design flow pulls from design flows that are used in the design of microelectronic circuits. Analogies can be drawn between the consituents of semiconductors and biological gates, and these analogies open the door for the application of analogous design processes [7].

Compilation

Since the goal is to generate gates based on user-input, there has to be some type of rational, mechanism for generating these gates. Of the many types of regulation that occurs in the cell, riboregulation proves to be the most easily modeled because of the canonical base pairing interactions that dominate its structure [2]. Because of this, it seemed natural to select riboregulation as the form of regulation that will be rationally generated by the circuit compiler. Many models have been created that rely, to varying degrees, on the quantification of riboregulator libraries [6], and on thermodynamic free energy calculations [5]. This project does not attempt to devise a new scheme for predicting RNA-RNA, and RNA-ligand interactions. The chosen scheme for rational design will be the use of ligand actuated self-cleaving ribozymes as discussed in Smolke and Win's article [8].

The initial inputs to the given circuit will be exogenously added small molecule ligands. These ligands will bind and modulate the ON/OFF state of a generated ribozyme. The ON state is defined as having cleaving ability activated, and conversely, the OFF state is defined as lacking cleaving ability. The cleaving activity always acts to repress the signal that is downstream of it, whether that be an siRNA effector or protein coding sequence. The final output signal(s) of the circuit is/are depicted as bulb(s) in the graphical depiction, and they are fluorescent protein(s) (xFP, $x \in \{G, R, Y, EB, EC\}$). Now that the initial input signal and final output signal are defined, the only remaining piece of the circuit is internal signal transduction. A design choice for this tool is to simply use ribozyme-siRNA pairs. Each of these pairs consists of a ribozyme that is modulated (ON/OFF) by an effector oligonucleotide, followed by an siRNA. This siRNA acts as the effector oligonucleotide for the next ribozyme in the circuit cascade. [9] There are many available programs that assist in secondary structure prediction, but for the purposes of this project RNAstructure v.5.3 will be used. This tool has been sucessfully applied by Smolke in the rational design of ribozymes.

The tunable parameters to be considered are: binding affinities of siRNA/ligand to a specific ribozyme, and to other non-specific ribozymes, rate of transcription of each of the ribozyme-siRNA compositors, degradation rate of each of the ribozyme-siRNA compositors, activiating/respressing ability of ribozyme when bound/not bound.

Some conceptual constructions of ribozymes upstream of a signal siRNA show how fundamental gates can be constructed. The construction of a NOT gate would involve a ribozyme that is ON in the presence of an effector oligonucleotide or ligand. An AND gate can be represented by two ribozymes, adjacent to one another, that are each in the OFF state in the presence of two different effector/ligand inputs. Natrually, the two of these gates strung one after another will form a functionally complete NAND gate.

Additional features

There are many opportunities to expand on this core design functionality. Given the time constraint, many of these features will not be realized, but they play a key role in describing the power of this type of design tool.

- Logic optimization of graphical logic with *espresso*
- Graphical parameter optimization that displays transfer curves updated as parameters are changed.
- Optional use of compositors other than ribozymes and siRNAs.
- Modeling options during the compilation process (e.g. continuous/stochastic model, varying structure prediction algorithms)
- Automated construction of plasmids, and/or the exporting of compositors to a tool like SimVector.
- Importing logic circuits based on the *espresso* input format
- Optional library import for regulators that have been quantified

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