The Development of Genetic Circuitry

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– Anonymous student author

Instructor
Julia Khodor

Parallels can be seen in the historical analysis of the development of modern electrical computational devices verses current attempts at computation using organic devices. Although these approaches have drastically different origins, they both started from a basic scientific discovery; electricity and conductive metals verses DNA and Central Dogma. Electrical computational devices have come rapidly from very basic understanding at the beginning of the last century to the plethora of devices that we interact with everyday. Currently organic devices such as combinations of DNA or even whole cells are only recently being explored as computational devices. There are a few parallel steps that can be seen in the development of each that I would like to cover.

First, the basic science is necessary for the development of the device. Electronic computational devices in the 1930's relied on the first electric switches. Biological computational devices require a switch as well. Engebrecht et al. (1983) describe the Lux operon in *Vibrio fischeri* which acts as a switch. This switch uses quorum sensing to act as a method for activating a genetic circuit. The quorum sensing relies on a low level production a dimer-auto inducer. Once it forms a dimmer, the auto inducer returns to the operon to up regulate the production of the promoter and the protein product.

binds promoter
produces protein.

In the case of electronic devices, basic science allowed later accomplishments by incorporation of these switches into complicated devices. Between 1937 and 1941 the electric switches were used to build a simple computational device at Iowa State that acted like a simple calculator. During World War II Alan Turing built Colossus for the British Military to help break the German codes.

Simple biological computational devices have taken much longer to put into practice, even for such simple calculations. In 2000 Weiss and Knight used the Lux operon in conjunction with other genetic information to produce a very simple circuit. Their design split the Lux operon in to the inducer protein with a promoter of its own and the indicator protein with the normal operon inducer that responds normally to the dimer ~~promoter~~ inducer protein. This circuit allowed Weiss and Knight to control the production of the Green Florescent Protein through the promoter of the Lux I protein. This type of control allows a limited design of genetic circuitry.

The limits of genetic circuitry have to do with the kinetic balances of the transcription as well as translation. Quoting Yokobayashi et al. "biological circuit engineers will have to confront their inability to predict the precise behavior of even the most simple synthetic networks, a serious shortcoming and challenge for the design and construction of more sophisticated genetic circuitry in the future." In their 2002 paper Directed evolution of a genetic circuit they describe one method attempting to balance the kinetics of the components of one such genetic switch.

Using the genes lac I (the lac operon inhibitor), cI (a repressor), ECFP (a florescent protein) and EYFP (another florescent protein) in two plasmids, they designed a simple circuit that they succeeded balancing via directed mutations. By changing either the protein

or the ribosomal binding site the overall circuit kinetics could be changed to benefit the overall design.

The possibility of using this for computational devices that compete with modern electronic computers is not likely anytime soon. But, just like those computers the biological computational devices will change and improve.

Engineering Benefits

While there are many uses for a genetic circuit, from WMD detectors to nano-scale engineering; the computational uses for a single logic gate are minimal. For a genetic circuit to be of any computational value, a wide array of protein interactions would need to understood and balanced. For example, if one logic gate used a particular inducer or inhibitor then that protein could not be used again inside that circuit. For gates to be reused in the same broader computational system a multi-cell communication system would need to be used similar to the experiment by Weiss and Knight which used the Lux operon. This segregation would allow separation of circuit models that use the same protein.

Oscillator Circuit Balance Method

In regards to future methods of circuit development, I propose the use of a two-operon system with reciprocal inhibitors balance via targeted mutation. Each operon would have different inhibitor genes, antibiotic resistance genes and a florescent protein genes. The reciprocal inhibitors system would force a balance in between the strengths of the inhibitors association to the inhibitor-binding site and the RBS strength of each operon. This would ensure that each inhibitor and each reporter is produced in approximately equal amounts. Despite the homeostatic balance of the oscillator it could be easily tipped by the addition of more inhibitor protein to the cell would tip the chemical balance of the oscillator

to produce more of the same inhibitor applied. This tipping action would allow two further non-exclusive responses, either the heightened production of a reporter protein on the same operon or the inhibitor blocking the opposing signals propagation.

Two issues arise with the system illustrated above. The first is in the construction where a double knockout of the inhibitors would create positive response. Focusing the directed mutation to only one gene a time could minimize this concern. The other issue would be a reset of the oscillator. Should one inhibitor be overwhelmingly over produced the circuit would not be able to reset until a balance was renewed. One method of oscillator reset would be placing a timed inhibitor response into the circuit. The timing could be based on transcription-translation cycles. For example the operon could hold a dimer or higher order auto-inhibitor that would down-regulate the transcription after one two or more translation runs. Another possible oscillator reset method would be to include into the circuit a multi gene pathway that would produce a strong inhibitor at the end of the path.

Path of Genetic Circuits

Clear limits exist in the science of genetic circuitry today; the number of genes that can be used, the overlap of with the cellular machinery and the difficulties balancing the responses are just a few. But these few hurdles can be overcome with more research. The uses of genetic circuits are limited at the moment but like their forebears, the electronic computer, no one can guess where it will go.

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