Building a Synthetic Biology "Circuit" Using Network Motif Models

(from William and Mary iGEM; contact: igem@wm.edu or mssaha@wm.edu)

Summary

One of the most phenomenal aspects of nature is the extreme diversity and sheer number of proteins that exist in nature. Thanks to the countless hours of work done by researchers across the world, and thanks to large, publicly available databases, these unique genes and their functions can be observed by all. Furthermore, by combining molecular biology understanding, along with the power of big data, and applying engineering principles, synthetic biology can harness these parts to accomplish novel and varied tasks. In order to accomplish these tasks, synthetic biology borrows a concept from electrical engineering, that of a circuit.

Circuits are engineered cell parts designed to perform a practical function. With our understanding of how biological entities regulate the central dogma of biology, we can apply mathematical and engineering concepts used in traditional electrical circuits to biological circuits. One of the most interesting examples of this are implementing network motifs such as feed forward loops, and autoregulatory components. While the ability of these motifs to create complex and varied circuits is fascinating, these motifs in it of themselves are also awe inspiring. To a biology student who has only thought of genes being able to be turned on and off, these motifs reveal to them that biology is not simply concerned with producing a protein end product, rather, life is concerned with regulating every aspect of the central dogma to produce the right protein product in the right amount at the correct time and place.

After learning about these motifs, students can be tasked with designing a circuit on paper, that will utilize these network motifs to accomplish a practical goal. Furthermore, after students draw out a blueprint or "pseudo-code" for their circuit, students can be directed to the iGEM Registry, a large, and searchable source for a variety of genetic parts. With access to the parts registry, students can actually pick out the parts that they would want to use in their circuit.

With all of these components, students will gain an appreciation of big data and bioinformatics, along with a deeper understanding of molecular biology, and synthetic biology.

Learning Goals

Students will:

- Gain a deeper understanding of molecular biology. Through knowledge of how gene regulation plays a fundamental role in the proper functioning of all organisms, students will be able to better conceptualize how regulatory functions they learn about in the classroom are actually applied and used in living organisms
- Attain increased familiarity with the presence of, and increased confidence in using large databases of genetic parts to search for sequences of DNA that allow for the creation and proper functioning of a biological circuit.
- Establish a deeper understanding of synthetic biology. By brainstorming, and then actually designing a biological circuit, students will become more familiar with how synthetic biology can take parts found in nature can be used to rationally accomplish desired tasks.
- Learn how molecular and cellular biology can be synthesized with big data and bioinformatics. Utilizing a large database of parts to search for DNA sequence with a specific function will demonstrate to students how bioinformatics and big data will play a crucial role in the future of biology. In the database, students will see how undergraduate scientists, like themselves, were able to use bioinformatics techniques to characterize, and document how the parts they deposited in the database actually function. Furthermore, the database in it of itself will show students the importance of big data and large data sources to allow researchers to choose the correct part for their task.

Procedure

First, introduce students to the concept of network motifs as utilized within living organisms, and within synthetic biology. As the idea of network motifs will be new to most students, they would most likely be able to best understand these concepts if given a few examples. An example of positive autoregulation would be quorum sensing systems used in bacteria, as when a bacteria detects a quorum sensing signal molecule (usually a Homoserine Lactone in gram negative bacteria, and a small signaling peptide in gram positive bacteria), that signal will cause the bacteria to produce more of the quorum sensing signaling molecule. An example of negative autoregulation would be the tryptophan synthetase operon. The product of the tryptophan synthesis operon is tryptophan itself, so when the operon is activated, it will eventually repress itself. Finally, an example of the incoherent feedforward loop in *E. coli* is the galactose utilization system. The IFFL motif allows bacteria to quickly translate proteins that allow for catabolism in the absence of glucose. When IFFL behaviour is removed in experimental studies, the bacteria lose the ultra fast response that bacteria with the IFFL network motif exhibit.

After students are introduced to the concept of network motifs, students should be tasked to utilize any one of the network motifs discussed in class, or any other network motif found in a peer reviewed journal, to create their own circuit. Students should first design their circuits on paper, or in a typed document. This “pseudo-code” should include what the students want this circuit to accomplish, what network motif they are utilizing and why, and should include a list of parts that they would need in order to get their circuit to function. Collaboration in this activity should be encouraged. Depending on the level of molecular biology understanding, students can either be assigned to design a circuit individually, or within groups.

Given enough time to design an outline for their circuits, students should be shown the iGEM parts registry (located at http://parts.igem.org). The iGEM parts registry contains a large catalog of every genetic regulatory element that the students would need in order to design their circuit. Students should be shown that the required elements for their circuit are the promoter, ribosome binding site, coding sequence, and terminator. With the knowledge of what parts are required, and where to find the required parts, students will be assigned to actually find parts on the registry that will allow them to construct their circuit. Parts should be listed with both the name of the gene (e.g. sfGFP) and the iGEM parts registry number (e.g. BBa_#########).

Now that they have a list of parts, students will then be instructed to draw a final diagram of how their circuit works. This, along with the previous documents, should be turned in to the instructor in whatever means is most convenient for the instructor. If there is sufficient time, or if the instructor wishes for the students to work on this activity outside of class, students can be asked to quickly present their circuit, what they wish it to accomplish, and how they designed the circuit to accomplish it. In larger lectures, the instructor can present circuits that they designed themselves ahead of time, or they can present their choice of circuits designed by students.

As mentioned briefly, this activity can, and should be scaled appropriate to the level of molecular biology that the students can understand. For students at a high school level of biology, they can be asked to work in groups, and can have this activity be assigned as a project that they can work on in class and at home. For students in an undergraduate introductory biology level, they can be assigned to work in small groups during a particular lecture, and then work on a presentation or a well laid out diagram at home. Finally, upper level biology students can be asked to work individually if the instructor wishes this activity to be a single day affair, or in groups for a take home or multiple day project. As they have a deeper understanding of biology, students can be asked to present exactly how their circuit will work at every level of the central dogma. Additionally, students can be asked to draw a complete circuit diagram, with all of the regulatory elements and interactions shown and annotated.