Design and Evolution of Engineered Biological Systems

Jason Kelly, Josh Michener, and Drew Endy
(http://openwetware.org/wiki/Jason_Kelly)

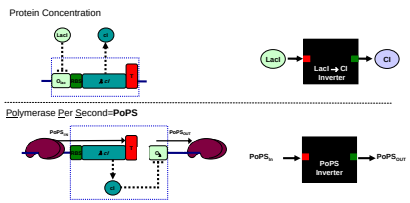
Abstract

To date, engineered biological systems have been constructed via a variety of *ad hoc* approaches. The resulting systems should be thought of as pieces of art. We are interested in exploring how existing forward engineering approaches might be combined with directed evolution to make routine the construction of engineered biological systems. We have specified a procedure for construction of biological systems via screening of subcomponent libraries and rational re-assembly. We have begun development of tools to enable this approach, including a FACS-based screening system to rapidly measure the input/output function of a genetic circuit. Additionally, we have designed a microfluidic system that enables more sophisticated screening and selection functions. Specifically, a microfluidic chemostat integrated with a cell sorter (i.e., a sortostat). This microscope-based system will enable us to evaluate whether or not more complicated screens and selections will be of practical use in service of evolving engineered biological systems.

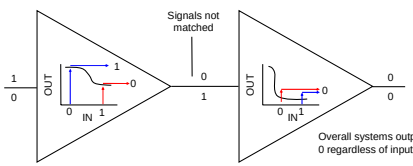
Motivation

In our framework, engineered biological systems are made from devices. Devices, in turn, are made from parts. Parts are units of basic biological function (e.g., a ribosome binding site). Devices are combinations of parts that perform some simple operation (e.g., a Boolean NOT function). We have to solve several problems to reliably combine devices into functional systems.

1. Common Signal Carrier
A carrier signal which is independent of the specifics of the device is essential to composing devices into systems.



2. Signal Level Matching
The levels of the common signal must match between devices. For example, in analogy to digital systems, both devices must have the same definition of a 1 or 0 if they are to communicate.



3. Resistance to internal noise
Our devices must work inside living cells. The intracellular environment is noisy, small, dense, and uncertain. Devices should be designed to function robustly in the presence of expected fluctuations in their local environment. (Or local environment should be made more receptive to hosting devices)

4. Stability of the information encoding the system
Engineered genetic systems are subject to mutation and natural election. As a result, we must develop methods to control the stability of the genetic information encoding our devices.

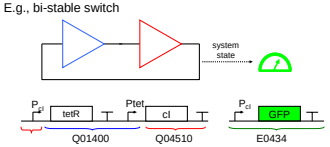
Library-Based Construction

A strategy of constructing engineered biological systems via directed evolution from standard parts libraries offers several advantages over a purely rational approach.

1. Some characterization of devices becomes implicit in their construction due to screening for I/O function of device.
2. Signal matching between devices is more easily accomplished[1]
3. Library diversity yields functional subcomponents with diverse characteristics not specifically selected for (latency, etc), improving likelihood of achieving a functional final system.
4. Complexity of biological substrate makes effective modeling of system performance in response to rational changes very challenging.

1. Rationally design system from standard devices

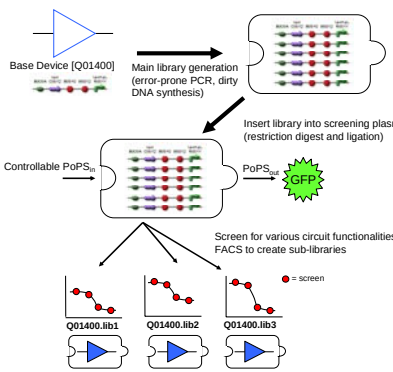
E.g., bi-stable switch



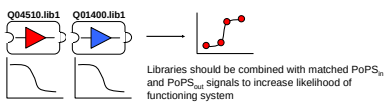
2. Identify devices with screenable I/O functions

For instance, oscillatory behavior would not be easily screenable – however, some of the subcomponents of an oscillating system might be screenable. In this simple system, subcomponents as well as the final system can be screened for function.

3. Generation of sub-libraries from a base device

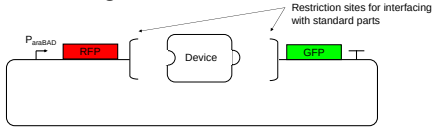


4. Combine libraries of devices to build system and screen for functionality

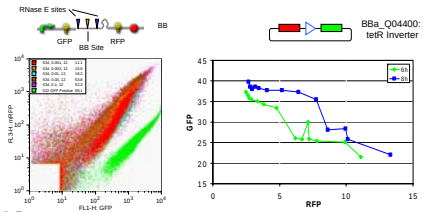


Since no screening was done for characteristics such as the switching speed in response to inducer, we expect that bistable switches with a variety of speeds will be isolated from the final system library.

Screening Plasmid

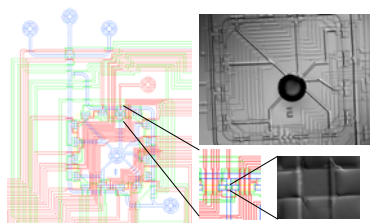


The plasmid will be based on pSB2K3 [Registry of Standard Biological Parts]. pSB2K3 is derived from the variable copy plasmid system, pSCANS[2]. The plasmid contains the F' replication origin (copy number <10) and also the P1 lytic origin (copy number >100). Replication at the lytic origin can be induced by IPTG. This will facilitate screening of devices at low copy number (expected operating conditions for our systems) while allowing for induction to high copy number to increase DNA preparation yield for subsequent construction steps.



The latest version of the screening plasmid contains RNase E sites to create independence between the mRNA stability of the device being screened and the mRNA stability of the fluorescent proteins. In particular, we suspect mRFP1 to contain internal RNaseE cut sites and have added a hairpin 5' of the coding region to slow degradation by RNase E. [4] An earlier version of the screening plasmid was tested with a tetR-based inverter. The curves look qualitatively correct, however expression levels of the fluorescent proteins were rather low.

Sortostat Design & Motivation

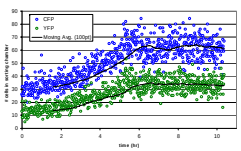


- Total Reactor Volume = 16nL
- Sorting chamber = 1/50th of total reactor volume
- Modification and extension of design by Balagadde et al. [5]

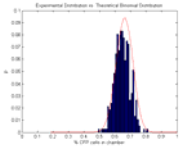
A microfluidic chemostat integrated with a cell sorter, which we call a "sort-o-stat", will enable more complicated selections to be applied to a population of cells in continuous culture. In particular, time varying selective pressures as well as very specific selection strengths can be applied. We will evaluate whether or not these more sophisticated selective pressures will be of practical use in service of evolving engineered biological systems. Selection can be based on any characteristic that can be reliably measured via microscopy. Lastly, since this is a physical selection (rather than a chemical one), it may be more difficult for cells to find unexpected methods to evade selection.

Sortostat Preliminary Results

Normal Chemostat Operation

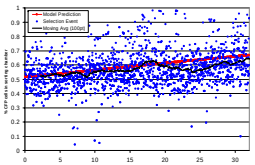


Sortostat was run with no selective pressure after being inoculated with cells growing in log phase from a batch culture.

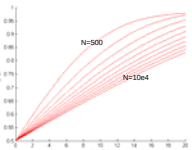


Analysis of steady state region (>6hrs) suggests that the %CFP cells found in the sorting chamber is binomially distributed (0.01 significance level)

Selective Pressure Turned ON



Sortostat was run with selective pressure, sorting against cells expressing YFP. Based on the rate of sorting events (1/3 min^{-1}) and initial cell counts, the mathematical model predicted the effect of sorting on the population relatively well.



Graph depicts the performance limits of the device based on a mathematical model at the maximum screening rate for populations 500-10e4 cells / reactor. Smaller populations have wider distribution and thus will face a greater selective pressure.

Future Work

- Further characterization and specification of device performance
- Tuning of oscillation frequency by selective pressure
- Selection for reduction in noise in gene expression across population
- Selection for a specific expression level of a fluorescent protein.
- Other ideas?

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References

[1] Yokobayashi et al., Directed evolution of a genetic circuit. Proc Natl Acad Sci U S A. 2002 Dec 24;99(26):16587-91. Epub 2002 Nov 25.

[2] pSCANS vector developed by John Dunn, Brookhaven (NCBI Accession #AY007424)

[3] Khlebnikov et al,Modulation of gene expression from the arabinose-inducible araBAD promoter. J Ind Microbiol Biotechnol. 2002 Jul;29(1):34-7.

[4] Effect of gene location, mRNA secondary structures, and RNase sites on expression of two genes in an engineered operon. Biotechnol Bioeng. 2002 Dec 30;80(7):762-76.

[5] Balagadde et al., Long Term Monitoring of Bacteria Undergoing Programmed Population Control in a Microchemostat. Science. 2005 Jul 1;309(5731):137-40.