Engineering the Interface Between Cellular Chassis and Integrated Biological Systems

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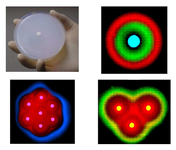
1 Abstract

The engineering of biological systems with predictable behavior is a challenging problem. One reason for this difficulty is that engineered biological systems are embedded within complex and variable host cells. To help enable the future engineering of biological systems, we are studying and optimizing the interface between an engineered biological system and its host cell or "chassis". Other engineering disciplines use modularity to make interacting systems interchangeable and to insulate one system from another. Engineered biological systems are more likely to work as predicted if system function is decoupled from the state of the host cell. Also, specifying and standardizing the interfaces between a system and the chassis will allow systems to be engineered independent of chassis and allow systems to be interchanged between different chassis. To this end, we are assembling orthogonal transcription and translation systems employing dedicated machinery, independent from the equivalent host cell machinery. By analogy to software programming, these orthogonal systems form the basis of a biological virtual machine. In parallel, we are developing test systems and metrics to measure the interactions between an engineered system and its cellular chassis. We hope these metrics will allow us to characterize cellular chassis and ensure they are well matched to the engineered systems that they power.

2 Engineering biological systems

It is becoming possible to engineer simple multi-component systems in living organisms based on transcriptional logic. An example of pattern forming bacteria is shown to the right [1]. However, the engineering of functional systems is still difficult and time consuming, more akin to art than engineering. Furthermore, current engineered systems are highly sensitive to host physiology and environmental conditions [2, 3].



The engineering of biological systems will be facilitated by adopting concepts that have proved useful in other engineering disciplines. Central among these concepts is standardization of components (http://parts.mit.edu) and abstraction, which lead to the concept of modularity. Currently, engineered biological systems are dependent on natural host cells. Constructing modular systems is made difficult by the complexity of the host cells and the numerous interactions between the host cell and the engineered system. The development of engineered systems would be accelerated if system engineers did not have to consider all the details of the host cell. Modularization can be achieved by making the interactions between the engineered system and the host cell simpler and standardized.

3 The chassis/system interface

Engineered biological systems typically rely on the host cell for the processes of replication, transcription, translation and degradation and the requisite energy and materials to power those processes. In this way, the cell acts as a power supply and chassis that insulates and drives the system [Knight, T.F. Jr., personal communication].

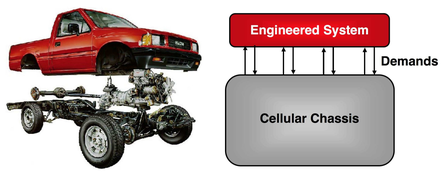


Figure 1 - Just as the power supply and chassis of an automobile support the driver and the accessory systems, so the cell supports an engineered biological system.

Desirable characteristics of a chassis/system interface:

- Perturbations in the environment or the chassis should not be transmitted to the system. Similarly, changes in the function of the system should not affect the function of the chassis.
- The system and the chassis should share different resource pools.
- A simple and standard chassis/system interface will allow interchangeability of systems and chassis.

4 References

[1] Basu, S., Gerchman, Y., Collins, C. H., Arnold, F. H., and Weiss, R. (2005) *Nature* **434(1476-4687)**, 1130–4.

[2] Elowitz, M. B., Levine, A. J., Siggia, E. D., and Swain, P. S. (2002) *Science* **297(5584)**, 1183–6.

[3] Rosenfeld, N., Young, J. W., Alon, U., Swain, P. S., and Elowitz, M. B. (2005) *Science* **307(5717)**, 1962–1965.

[4] Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) *Methods Enzymol* **185(0076-6879)**, 60–89.

[5] Brink, M. F., Verbeet, M. P., and deBoer, H. A. (1995) *Gene* **156(2)**, 215–22.

5 Dedicated systems

- By creating an orthogonal or dedicated protein production channel for an engineered system, we can decouple its function from that of the host.
- This dedicated channel can form a standard interface between the system and the cellular chassis.

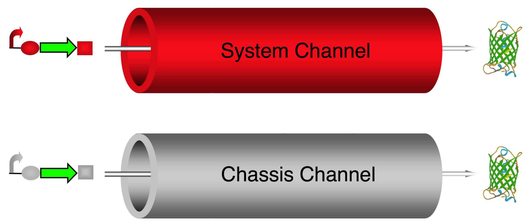


Figure 2 - Dedicated systems separate the gene expression of an engineered system from that of the cellular chassis. This means the behavior of the engineered system should become decoupled from the behavior of the chassis.

6 A biological virtual machine

Software virtual machine: Software that isolates an application from the computer. Any application written for the virtual machine can be operated on any platform for which a virtual machine exists. [http://en.wikipedia.org/wiki/Virtual_machine]

Biological virtual machine: A genetic network that isolates an engineered system from the cellular chassis. Any engineered system that uses the virtual machine can be operated in any cellular chassis for which a virtual machine exists.

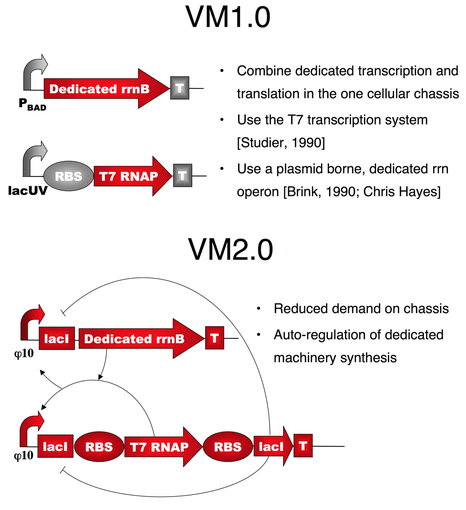


Figure 3 - Two early versions of a virtual machine. The components of VM1.0 have been assembled and shown to function as described later. The combined system is currently being tested. VM2.0 is an improved design with better regulation of the dedicated systems. The behavior of VM2.0 is currently being tested by a computational model.

7 Reporter Devices

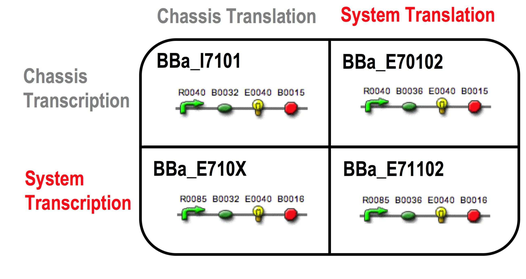


Figure 4 - BL21(VM1.0) contains two transcription systems and two translation systems. For both transcription and translation there is the native chassis system and the virtual machine's dedicated system. We have built reporter devices for each combination of transcription and translation system. These devices, built from standard biological parts, are described in more detail at http://parts.mit.edu

8 Dedicated Transcription

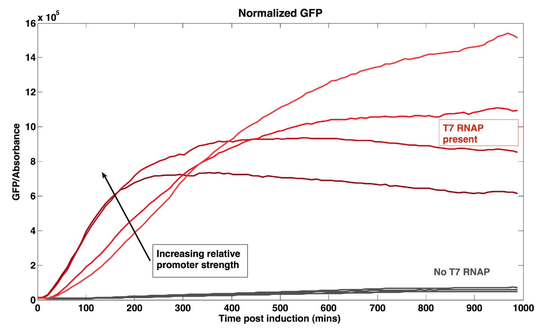


Figure 5 - Reporter devices using 4 variants to the consensus T7 promoter were tested. A very high rate of GFP accumulation in the presence of T7 RNAP and no accumulation in the absence of T7 RNAP showed that dedicated transcription was working effectively. Furthermore it showed that a range of transcription rates could be achieved.

9 Dedicated Translation

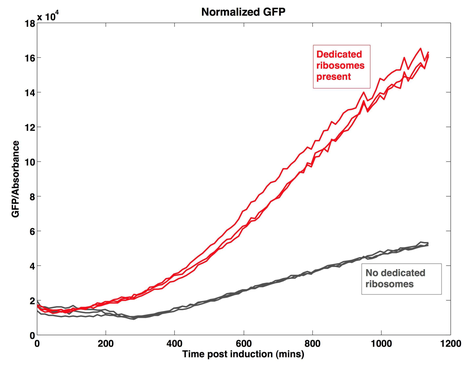


Figure 5 - A reporter device with a ribosome binding site (RBS) that is recognized by dedicated ribosomes. Three replicates of the cultures with and without dedicated ribosomes induced are shown. Induction of the dedicated ribosomes is necessary to see accumulation of GFP. The fluorescence of the induced cultures is similar to cells without a GFP reporter.

10 Measuring chassis response to an applied demand

- Can we quantitatively measure and predict the demand a system places on a chassis?
- How does a cellular chassis respond to an applied demand for machinery, materials or energy?

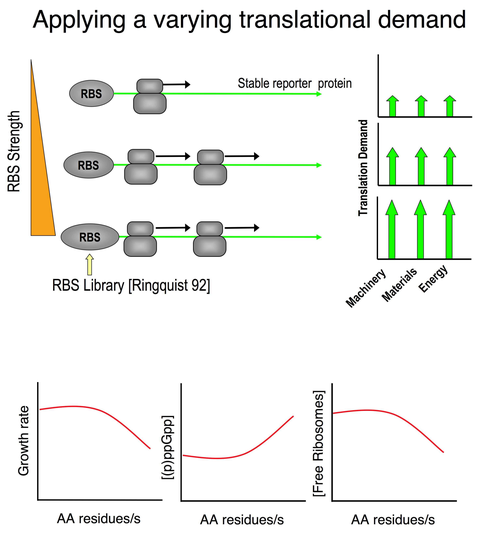


Figure 7 - Methods to place a translation demand that can be specified on a cellular chassis. The response of the cellular chassis to these different demands can be measured by growth rate or ppGpp levels etc. Similar experiments have been planned to measure response to transcription demand.

11 Modeling system demand

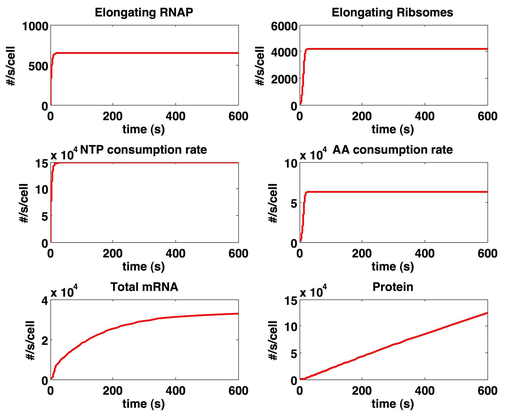
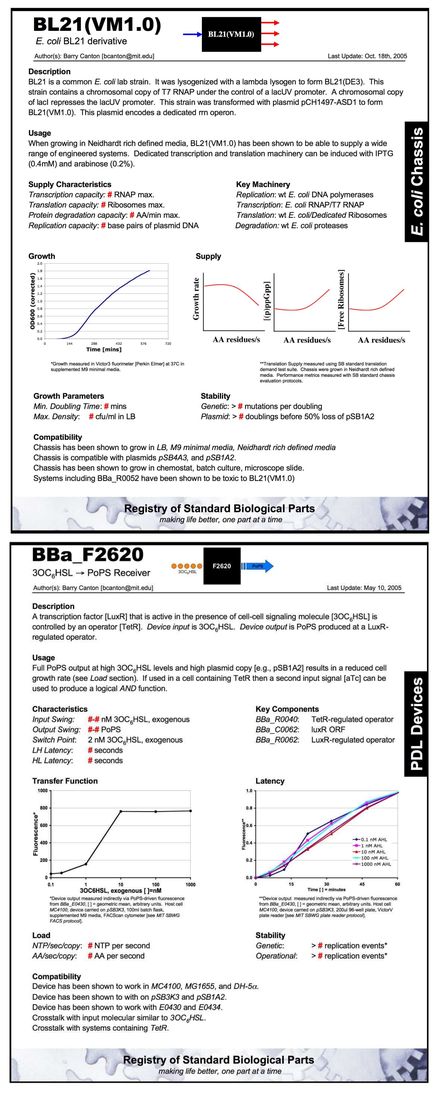


Figure 8 - Simulation results from a 9 species ODE model of transcription and translation demand of BBa_E7104. The model is parameterized using data from literature. This simple model crudely predicts the demands for machinery and materials that the system places on the chassis.

12 Chassis and system data sheets



BL21(VM1.0)

E. coli BL21 derivative

Author(s): Barry Canton [bcanton@mit.edu] — Last Update: Oct. 18th, 2005

Description

BL21 is a common *E. coli* lab strain. It was lysogenized with a lambda lysogen to form BL21(DE3). This strain contains a chromosomal copy of T7 RNAP under the control of a lacUV promoter. A chromosomal copy of lacI represses the lacUV promoter. This strain was transformed with plasmid pCH1497-ASD1 to form BL21(VM1.0). This plasmid encodes a dedicated rrn operon.

Usage

When growing in Neidhardt rich defined media, BL21(VM1.0) has been shown to be able to supply a wide range of engineered systems. Dedicated transcription and translation machinery can be induced with IPTG (0.4mM) and arabinose (0.2%).

Supply Characteristics

Transcription capacity: # RNAP max.
Translation capacity: # Ribosomes max.
Protein degradation capacity: # AA/min max.
Replication capacity: # base pairs of plasmid DNA

Key Machinery

Replication: wt *E. coli* DNA polymerases
Transcription: E. coli RNAP/T7 RNAP
Translation: wt *E. coli/Dedicated* Ribosomes
Degradation: wt *E. coli* proteases

Growth

OD600 (corrected) vs Time (mins)

*Growth measured in Victor3 fluorimeter [Perkin Elmer] at 37C in supplemented M9 minimal media.

Supply

Growth rate; [(p)ppGpp]; [Free Ribosomes] — AA residues/s

**Translation Supply measured using SB standard translation demand test suite. Chassis were grown in Neidhardt rich defined media. Performance metrics measured with SB standard chassis evaluation protocols.

Growth Parameters

Min. Doubling Time: # mins
Max. Density: # cfu/ml in LB

Stability

Genetic: > # mutations per doubling
Plasmid: > # doublings before 50% loss of pSB1A2

Compatibility

Chassis has been shown to grow in *LB, M9 minimal media, Neidhardt rich defined media*
Chassis is compatible with plasmids *pSB4A3,* and *pSB1A2.*
Chassis has been shown to grow in chemostat, batch culture, microscope slide.
Systems including BBa_R0052 have been shown to be toxic to BL21(VM1.0)

E. coli Chassis

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BBa_F2620

3OC6HSL → PoPS Receiver

Author(s): Barry Canton [bcanton@mit.edu] — Last Update: May 10, 2005

Description

A transcription factor [LuxR] that is active in the presence of cell-cell signaling molecule [3OC6HSL] is controlled by an operator [TetR]. *Device input* is 3OC6HSL. *Device output* is PoPS produced at a LuxR-regulated operator.

Usage

Full PoPS output at high 3OC6HSL levels and high plasmid copy [e.g., pSB1A2] results in a reduced cell growth rate (see *Load* section). If used in a cell containing TetR then a second input signal [aTc] can be used to produce a logical *AND* function.

Characteristics

Input Swing: #-# nM 3OC6HSL, exogenous
Output Swing: #-# PoPS
Switch Point: 2 nM 3OC6HSL, exogenous
LH Latency: # seconds
HL Latency: # seconds

Key Components

BBa_R0040: TetR-regulated operator
BBa_C0062: luxR ORF
BBa_R0062: LuxR-regulated operator

Transfer Function

Fluorescence/Absorbance vs 3OC6HSL, exogenous [ ]=nM

*Device output measured indirectly via PoPS-driven fluorescence from *BBa_E0430*. [ ] = geometric mean, arbitrary units. Host cell *MC4100*, device carried on *pSB3K3*, 100ml batch flask, supplemented M9 media, FACScan cytometer [see *MIT SBMG FACS protocol*].

Latency

Fluorescence/Absorbance vs Time [ ] = minutes — 0.1 nM AHL; 1 nM AHL; 10 nM AHL; 100 nM AHL; 1000 nM AHL

**Device output measured indirectly via PoPS-driven fluorescence from *BBa_E0430*. [ ] = geometric mean, arbitrary units. Host cell *MC4100*, device carried on *pSB3K3*, 200ul 96-well plate, Victor*V plate reader [see *MIT SBMG plate reader protocol*].

Load

NTP/sec/copy: # NTP per second
AA/sec/copy: # AA per second

Stability

Genetic: > # replication events*
Operational: > # replication events*

Compatibility

Device has been shown to work in *MC4100, MG1655,* and *DH-5α.*
Device has been shown to with on *pSB3K3* and *pSB1A2.*
Device has been shown to work with *E0430* and *E0434.*
Crosstalk with input molecular similar to *3OC6HSL.*
Crosstalk with systems containing *TetR.*

PDL Devices

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13 Acknowledgments

I Support OWW openwetware.org

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- Christopher Hayes & Kathleen McGinness
- National University of Ireland
- http://openwetware.org/wiki/Dedicated_systems