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 have assembled orthogonal transcription and translation systems employing dedicated machinery, independent from the equivalent host cell machinery. In parallel, we are developing test systems and metrics to measure the interactions between an engineered system and its chassis. Lastly, we are exploring methods to "port" a simple engineered system from a prokaryotic to a eukaryotic organism so that the system can function in both organisms.

2 Introduction - engineering biological systems: past and future

It is becoming possible to engineer simple multi-component systems in living organisms based on transcriptional logic [1, 3, 5]. While today's engineered biological systems hint at a future ability to design and build complex systems with many components, 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, 6].

The future engineering of biological systems will be greatly facilitated by adopting some of the concepts that have proved useful in other engineering disciplines. Central among these concepts are the ideas of 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].

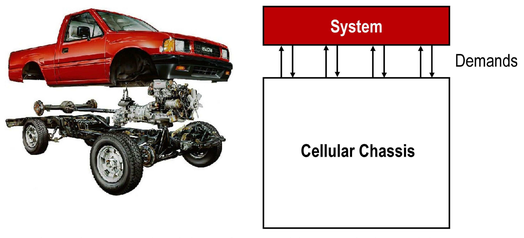


Figure 1 - Just as the power supply and chassis of an automobile support the driver and the accessory systems, so the cell supports an engineered biological system.

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 standard chassis/system interface will allow interchangeability of systems and chassis.
- The chassis/system interface should be simple to improve predictability of system function.

4 References

[1] M. B. Elowitz and S. Leibler. A synthetic oscillatory network of transcriptional regulators. *Nature*, 403(6767):335–8, 2000.

[2] M. B. Elowitz, A. J. Levine, E. D. Siggia, and P. S. Swain. Stochastic gene expression in a single cell. *Science*, 297(5584):1183–6, 2002.

[3] T. S. Gardner, C. R. Cantor, and J. J. Collins. Construction of a genetic toggle switch in escherichia coli. *Nature*, 403(6767):339–42, 2000.

[4] B. He, W. T. McAllister, and R. K. Durbin. Phage rna polymerase vectors that allow efficient gene expression in both prokaryotic and eukaryotic cells. *Gene*, 164(1):75–9, 1995.

[5] S. Hooshangi, S. Thiberge, and R. Weiss. Ultrasensitivity and noise propagation in a synthetic transcriptional cascade. *PNAS*, 102(10):3581–3586, 2005.

[6] N. Rosenfeld, J. W. Young, U. Alon, P. S. Swain, and M. B. Elowitz. Gene regulation at the single-cell level. *Science*, 307(5717):1962–1965, 2005.

5 Dedicated systems

- Transcription and translation systems that are dedicated to an engineered system can be used to separate the demands of the system and the chassis.
- Dedicated systems can be used as a standard interface between systems and chassis.

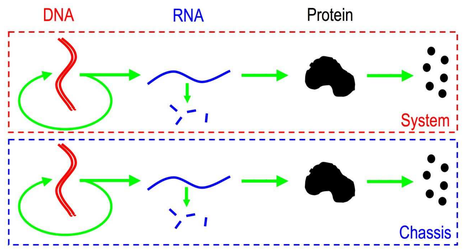
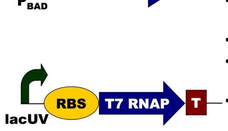


Figure 2 - Dedicated systems separate the gene expression of an engineered system from that of the cellular chassis. This means that the behavior of the engineered system should become decoupled from the behavior of the chassis.

6 A biological virtual machine

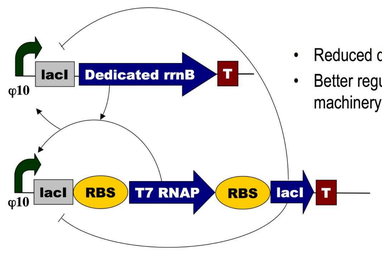
- A software virtual machine allows an application to function as expected regardless of the computer architecture running the virtual machine.
- A biological virtual machine allows an engineered biological system to function as expected regardless of the cellular chassis running the virtual machine.
- A biological virtual machine can be assembled from a set of dedicated systems, orthogonal to the cellular chassis' own systems.

VM1.0



- Combine simple dedicated transcription and translation systems
- T7 transcription system, BL21(DE3)
- Plasmid borne dedicated rrn operon, pCH1497-ASD1
- BL21(DE3)+pCH1497-ASD1 = **BL21(VM1.0)**

VM2.0



- Reduced demand on chassis
- Better regulation of dedicated machinery synthesis

Figure 3 - Two early versions of a virtual machine. VM1.0 has been assembled and shown to function as described below. VM2.0 is an improved design with better regulation of the dedicated systems and which should be less dependent on host resources than VM1.0.

7 Reporter Devices

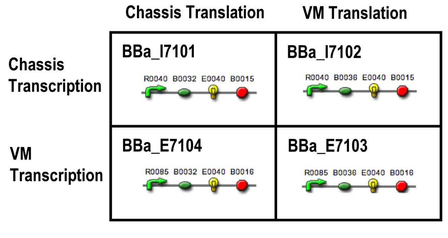


Figure 4 - BL21(VM1.0) contains two transcription systems and two translation systems. For both transcription and translation there is the native chassis system and the virtual machine's dedicated system. We have built reporter devices for each combination of transcription and translation system. These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

8 VM1.0 performance

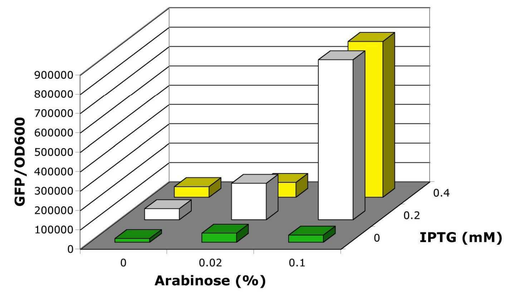


Figure 5 - The performance of the virtual machine was examined by measuring the fluorescence output of BL21(VM1.0) containing the reporter device, BBa_E7103. GFP fluorescence was measured under varying concentrations of IPTG to induce the dedicated transcription machinery and arabinose to induce the dedicated translation machinery. The addition of both inducers is necessary for the expression of GFP.

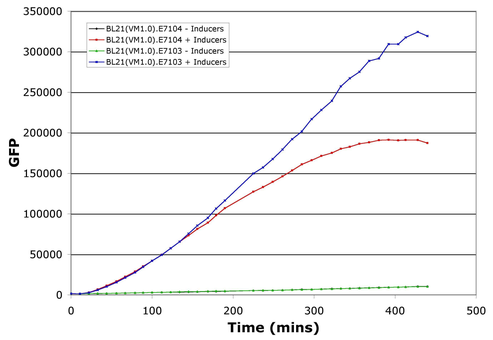


Figure 6 - The reporter device using both dedicated transcription and translation produces GFP at a constant rate for longer than the reporter device using dedicated transcription and chassis translation.

9 Measuring chassis response to an applied demand

- Can we measure the demand a system places on a chassis and the response of the chassis?
- How does a cellular chassis respond to an applied demand for machinery, materials or energy?
- Can this demand be specified quantitatively?
- Can we predict *a priori* the demand placed by a system on the cellular chassis?

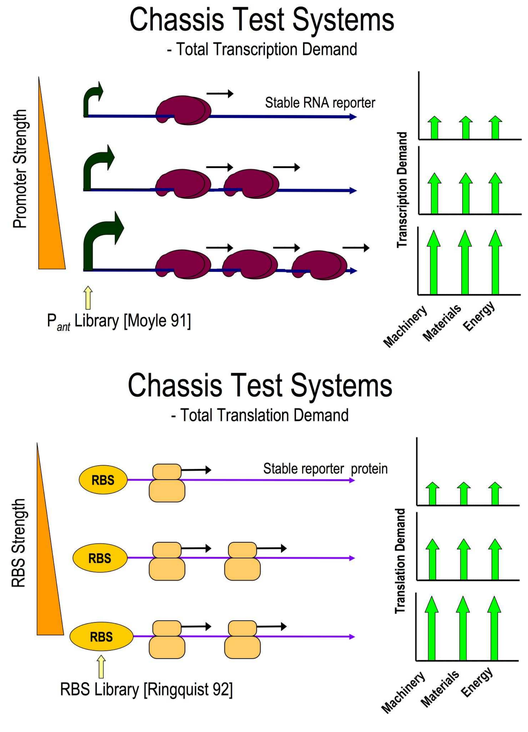
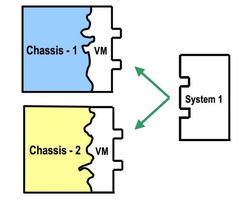
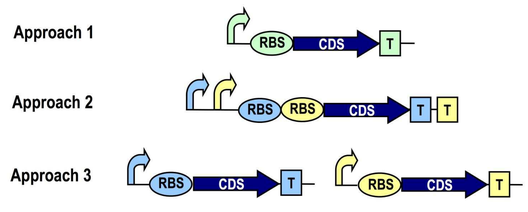


Figure 7 - Methods to place a demand that can be specified on a cellular chassis. The response of the cellular chassis to these different demands can be measured by growth rate or ppGpp levels etc.

10 System portability

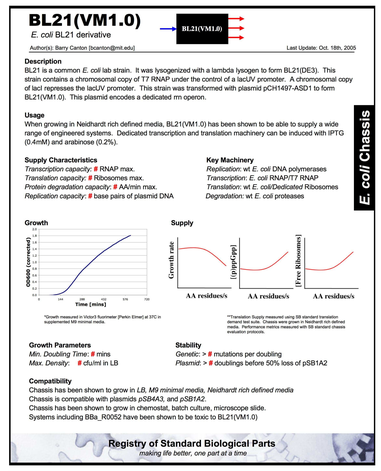
- Desirable to be able to move systems from one chassis to another
- Ideally, system performance should be the same in each chassis
- Requires a virtual machine in each chassis

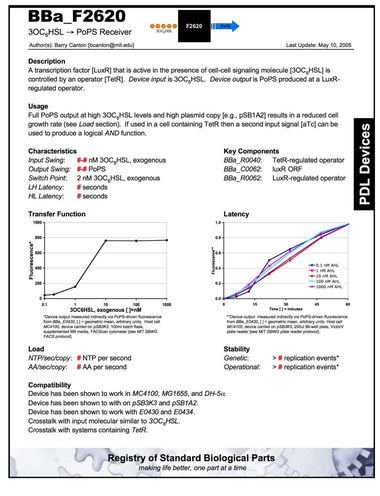




[4]

11 Chassis and system data sheets





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- `http://openwetware.org/wiki/Dedicated_systems`