

BIO-I/O Components and Design Tools

16 March 2001

Dr. Rob Carlson, Dr. Roger Brent, Dr. Alejandro Coleman Lerner, Dr. Drew Endy
The Molecular Sciences Institute

Dr. Milan Mrksich, U Chicago

Contact: Dr. Rob Carlson, The Molecular Sciences Institute
(rcarlson@molsci.org)

Summary

Using biological components as building blocks for synthetic systems is an obvious potential outgrowth of an improved understanding of biology. Many examples of hybrid biosensors and rudimentary engineered systems have already been demonstrated. The further development of biology as technology will require, and provide, mechanistic rules for biological systems. The creation of a synthetic biology will proceed in an analogous fashion to the creation of synthetic chemistry, which required a mechanistic description of chemical systems. Assaying the function of systems of biological components will further require the ability to get information into and out of those systems. During the project period, we will develop a biological input/output (Bio-I/O) capability consisting of experimental tools and predictive models that can be used to interact with biological systems, all based on the well understood and easily manipulated yeast a factor signal transduction pathway. This pathway is complex enough to serve as a model for higher eukaryotic systems but is accessible to experiment and theory. While the Bio-I/O components will be developed in yeast they can and will be ported to mammalian and plant cells. Our efforts to develop these capabilities will require us to work with a complex system consisting of many components. Quantitative, predictive models of biological systems, and of the yeast a factor pathway in particular, will serve as the basis of eventual design tools to aid this explicit biological engineering. Success in these efforts would result in more than an improved ability to build sensors for toxins, pathogens, or other entities we wish to detect in the environment. Achievement of a mature BIO-I/O capability would also yield components and design tools that would aid the design and construction of more complex biological systems.

We will initially use electrical manipulation of cells and their biochemistry for input and changes in engineered optical properties as outputs. In later years of the project we will engineer input pathways (recognition molecules coupled to cellular events) that can be made to detect binding of any specified molecule. We will also develop outputs that result in changes in electrical properties of the cell that couple directly to electronics.

A likely prerequisite in approaching the general problem of building devices with biological components is an interface between those biological components and the existing information manipulation infrastructure based on silicon. Moreover, any attempt to build quantitative models of biological systems will require the capacity to perturb those systems in a determined manner. Electrical manipulation of immobilized ligands to provide input to signal transduction systems fills both these requirements.

For initial input we will utilize biochemical signals that can be manipulated by standard IC technology, and the “signal” will be the α -peptide that controls the yeast α -factor pathway. The pathway is initiated by the binding of the 13 amino acid α -peptide to a receptor present on the membrane of a yeast cell. This binding results in the dissociation of a heterotrimeric G protein and sets in motion a series of reactions that prepare the cell for mating. Our approach to presenting the peptide to yeast cells will utilize technology that has been developed in the laboratory of our collaborator, Dr. M. Mrksich[1]. Dr. Mrksich and coworkers immobilize ligands (in our case the ligand will be a peptide) on electrodes using a redox-active group. The application of an electrical potential selectively releases the ligand. This release activates the ligand, providing a means to study quantitatively the interaction between a cell-surface receptor, the ligand, and a signaling pathway, all in real time. Such quantitative control over the input signal is key both to understanding the molecular basis of the transduction process and to constraining the model of the pathway.

We are already building a model of the α -factor pathway. It is clear from our work that development of increasingly constrained models into full-fledged design tools will require us to utilize new computational strategies and algorithms that are themselves deliverable goods. The traditional approach to modeling biochemical networks involves a defined set of molecular species whose interactions are defined by *average* rate constants. These constants are in general determined by measuring the behavior of many molecules of a given species. However, many interesting biochemical systems, including the yeast α -factor pathway, consist of only a few molecules of each species where most reactions occur at times explicitly different than the average. These reaction times are governed by a distribution. Therefore, models of these systems must be simulated stochastically, an approach that requires development of a new computational strategy capable of producing quantitative descriptions of systems of small numbers of molecules wherein reaction times are drawn from a distribution. We are constructing a model of the α -factor pathway along these lines, where each run, or simulation, of the model describes the response of the α -factor pathway in a single cell. Multiple runs produce the expected behavior of a population of cells where the resultant distribution constitutes a prediction testable through measuring many individual cells. Thus interacting with a system intrinsically stochastic in nature requires experimental tools capable of determining distributions rather than simple averages. In this case, the experimental tools must be capable of measuring a quantity across a population of cells with single cell resolution.

One example of the utility of this approach is in the elucidation of “non-genetic individuality” in *E. coli* chemotaxis[2], where a population of genetically identical *E. coli* displays a distribution of chemotactic responses. The performance of the flagellar rotary motor is quite uniform and the distribution of responses among individual bacteria seems to be a function of the very sensitive response of the motor to variations in the signal processing network [3]. This was demonstrated by simultaneously measuring the activity of individual motors and the concentration in CheY-P, whose concentration controls the amount of time the motor rotates one way versus the other. The variation in behavior may be due to developmental differences between individuals, but it is likely due in some part to fluctuations in the small number of molecules participating in the network. This latter feature will be present in many organisms and pathways of general interest. Accurate models of such systems must clearly reproduce the distribution of behaviors on a cell-by-cell basis, and any experimental test of those models must be

capable of resolving differences at the level of individual cells. Thus the general problem of quantitatively understanding protein networks within cells requires the ability to simulate and measure single cells.

We are currently developing techniques that can be used to measure distributions at the level of single cells. These measurements will be based upon transcription of reporter genes, changes in FRET as proteins change association or conformation during signal transduction, direct physical monitoring of the state of a protein network, and eventually electrically via ion channels designed to change conductance in response to given environmental conditions. Detection and measurement with reporter genes has the advantage of relying upon existing technology, but is relatively slow, whereas FRET based measurements, direct physical monitoring of a protein network, and designed ion channels are all potentially very fast, essentially instantaneous, but are technologies requiring further development. Along these lines, we will develop new optical techniques to monitor the state of protein networks based upon changes in light scattering by colloidal metal particles coated with protein affinity reagents. We will embark on a program of designing ion channels to provide electrical monitoring of the state of a protein network. These technologies will provide a toolbox of test and measurement techniques for building models and for the eventual design process.

These new experimental tools will first be used to constrain the model sufficiently that it can be used to make predictions. When the model is sufficiently constrained, the experimental tools will be used to test predictions of the model. When the model produces accurate predictions it will become useful as a design tool. At that point, many of the biological and computational tools developed under this project will become the input/output infrastructure for the querying and monitoring the state of synthetic systems.

Our general strategy to building new systems will be to utilize existing cellular and molecular infrastructure where possible to produce a set of working components, such as general molecular sensors, and then to begin more ambitious engineering of components based on what we learn in initial efforts. We will begin by modifying the input of the a-factor pathway (the a-factor receptor) so that it can be used as a general receptor molecule, providing a way to trigger the existing transduction cascade with any desired molecular input. This will be accomplished by first modifying the a receptor with an adapter that accepts protein aptamers. We have considerable experience developing these aptamers as biomolecular affinity reagents and we are confident they are a good initial platform for our biological engineering effort.

In order to empower true biological engineering, it is necessary to have computational frameworks that are capable of accurately representing biological system behavior. Furthermore, these computational frameworks need to be able to interface with existing representations of other physical systems (electrical, mechanical, fluidic, et cetera) so that true integrated systems can be represented. Existing biological systems provide significant challenges for the development of biological simulations. These challenges derive from the facts that the components of most (if not all) biological systems are incompletely characterized, and the actual behavior of the systems is difficult to measure. Thus the concomitant development of Bio-I/O technologies capable of defining, observing, and manipulating biological systems is necessary for the development of useful computational frameworks that can be used to support biological design tools. The combined effort we propose here is aimed at producing an integrated set of Bio-I/O components and design tools.

To our knowledge, no current effort to combine construction of input/output components and construction of design tools for these systems now exists.

References:

1. Mrksich, M., *Tailored substrates for studies of attached cell culture*. Cell Mol Life Sci, 1998. **54**(7): p. 653-62.
2. Spudich, J.L. and D.E. Koshland, *Non-genetic individuality: chance in the single cell*. Nature, 1976. **262**(5568): p. 467-71.
3. Cluzel, P., M. Surette, and S. Leibler, *An ultrasensitive bacterial motor revealed by monitoring signaling proteins in single cells*. Science, 2000. **287**(5458): p. 1652-5.