

Digital Control and Communication in Living Cells

MIT9904-10

Progress Report: July 1, 1999 December 31, 1999

Thomas F. Knight, Jr and Gerald Jay Sussman

Project Overview

Genetic regulatory networks form one of the basic computational infrastructures of life. The science of such systems has been well established over the past twenty years through pioneering work of Monod and Ptashne, among others.

This project undertakes to transfer that scientific knowledge into engineering practice, by starting the serious work of characterizing components, engineering interfaces, simplifying the technology, and educating a set of students who can easily cross the boundaries between biological science and computational engineering. Stated more directly, the project is to learn how to engineer life.

Our approach has been to start our efforts with very simple structures, using well characterized organisms and genetic regulatory elements. In particular, we are working with standard laboratory strains of *E. coli* and standard promoter and reporter gene constructs.

An important initial goal was the construction and outfitting of a microbiology laboratory within the computer science building at MIT. This effort was largely complete by September, 1999. Another important initial goal was attracting and educating a core of motivated and educated graduate students and staff to perform the research. Early on we decided that it was easier to educate researchers with a CS background about biology than the other way around. Today, we have two full time graduate students, a post doctoral student, and two staff, all of whom are trained as computer scientists, but, equally, are trained in molecular biology theory and practice.

Our initial experiments primarily centered around the development of our skills in gene transfer, plasmid construction, and bacterial transformation. In these experiments, we focussed on simple gene promoter and reporter systems, such as the LacZ promoter and Green Fluorescent Protein (GFP).

Progress Through December 1999

Some important laboratory equipment was installed and debugged during this period, including a Biotek FL-600 fluorescent plate reader, a Nikon E800 fluorescent microscope, and an ABI 394 DNA synthesizer. All of these are now in daily use within our laboratory. In addition, an evaluation and upgrade of our deionized water system was performed to solve recurrent problems with cell transformation efficiency.

During this period we had several important technical results. Our strategy of isolating useful genetic components from natural systems paid off with the isolation and sequencing of the bioluminescence genes from two distinct bacterial organisms. One of these organisms, *Vibrio fischeri* lives natively in a symbiotic relationship with the Japanese pinecone fish, *Monocentris japonica*, where it exhibits density dependent induction of the bioluminescence cassette. A second bioluminescent system was also isolated and sequenced from the terrestrial bacterium *Photobacterium luminescens*. The *Vibrio* system has been used in our recently reported experiments on engineering cell to cell communications., while the *Photobacterium* system shows promise as a bioluminescent system because, in *E. coli*, the system is active at 37C, unlike the *Vibrio* system.

Our most recent work, detailed in a paper which has been submitted to the Sixth International Conference on DNA Computing, demonstrates that we can digitally control a genetic circuit in one cell, create an intercellular communication chemical, transmit that signal through the cell membranes of the sender and receiver, and activate a genetic circuit in a receiver cell. These same mechanisms can be used to produce gradient dependent behavior in large assemblies of cells.

We are also improving our ability to engineer intracellular genetic circuits. Recent, but incomplete work in that area focuses on the implementation of simple flip flop storage mechanisms built out of DNA binding protein genetic circuits.

Research Plan for the Next Six Months

Our work over the next six months will center on continued interactions with the biology community. Specifically, we have begun to work closely with Prof. Hazel Sive, who studies the genetic control of development of the Zebrafish. We anticipate that this collaboration will lead to important insights both in biology and in computer science, as we learn in more detail what kinds of computation are performed in living cells during development.

Our work on constructing simple genetic circuits and characterizing their components will accelerate. We are now planning the isolation of a second genetic intercellular communications system, the LasI/LasR system from *Pseudomonas aeruginosa*. We believe that having a second intercellular communications system will enable us to perform a series of patterned development experiments, in which we engineer local cell behavior to create, e.g. polka-dot structures.

Another goal is to codify what we have learned about the difficulty (or ease) of constructing our laboratory and educating our students. We believe that much of what took us months to learn could be transferred to others much more quickly with the right set of educational tools. The creation of such a curriculum will be an important next step.

Several experimental procedures will become essential to our work, and we need more effort in creating the educational and experimental infrastructure in those areas. Specifically, genetic regulatory networks are now best studied using gene chip arrays. Most such arrays are now focused on human or mammalian genetics. We must develop similar technology for much simpler genetic systems found in bacteria, and become expert at using it.

Another key enabler, which also lowers the barrier to entry for other engineering oriented researchers, is the development of efficient, largely automated plasmid construction tools. We believe that this process, which currently occupies a good student or technician for two weeks can be performed over a two day period in a largely automated way. Such a change could dramatically improve the efficiency of not just this research, but also that of the larger medical and biological community.