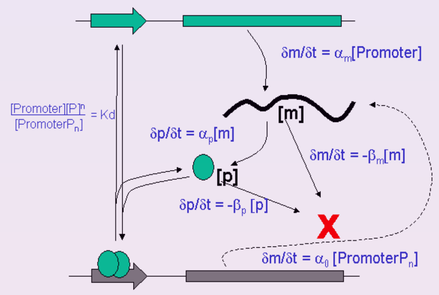
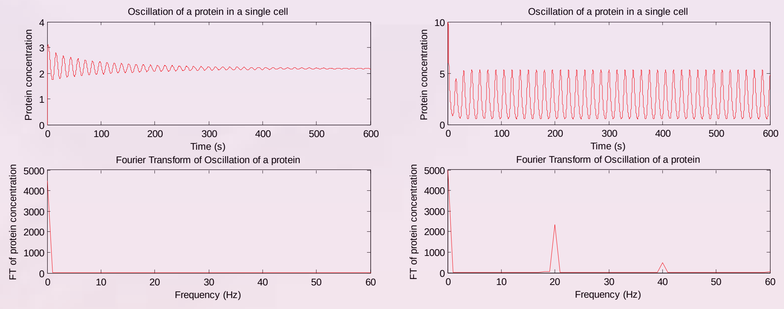
MATHEMATICS

Basic Elements

A rigorous analysis starts from an understanding of the basic parameters that describe each genetic element and its interactions with the system. Transcription and translation rates, decay rates, and promoter strength and leakiness are some of the variables involved.

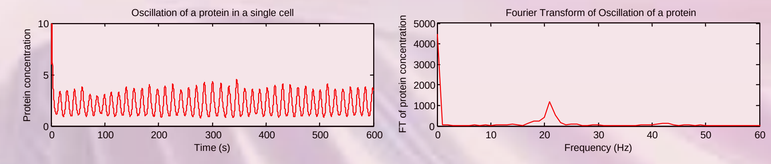




The desired oscillatory behavior is exhibited only after careful selection of component values.

Stochasticity

Biological systems are inherently noisy and complex. The major source of noise is the stochastic, discrete nature of cellular events.



Abstract

In synthetic biology, simulation informs the design process, so that appropriate parts can be chosen and they can be arranged in an appropriate topology. Our initial target, a three-state coupled oscillator, can be described in terms of simple equations describing aspects of each of its constituent parts. These equations interact to produce a periodic output for the circuit over time. A stochastic element can then be included to account for the discrete, random nature of individual cells - which produces a noisier output. This is still periodic, but individual oscillators move out of phase with each other. To overcome this problem we investigated additional circuitry to synchronize the system. A major challenge is to understand the best topology for this added branch - our results demonstrate that the degree of coupling is strongly dependent on the topology of the synchronization circuit. We have thus been able to make important recommendations to the biology team about how to construct the synchronization plasmid.

mRNA Equations:

$$\frac{dm_i}{dt} = -m_i + \frac{\alpha_1}{1 + p_k^n} + \alpha_0 + \frac{kS_{int}}{1 + S_{int}}$$

$P_{\lambda m}(n) = \lambda^n e^{-\lambda}/n!$

$\Delta m/\Delta t = \text{rand}(P_{\lambda m}(n))$

$$\frac{dm_j}{dt} = -m_j + \frac{\alpha_1}{1 + p_i^n} + \alpha_0 + \frac{kS_{int}}{1 + S_{int}}$$

$P_{\lambda m}(n) = \lambda^n e^{-\lambda}/n!$

$\Delta m/\Delta t = \text{rand}(P_{\lambda m}(n))$

$$\frac{dm_k}{dt} = -m_k + \frac{\alpha_1}{1 + p_j^n} + \alpha_0 + \frac{kS_{int}}{1 + S_{int}}$$

$P_{\lambda m}(n) = \lambda^n e^{-\lambda}/n!$

$\Delta m/\Delta t = \text{rand}(P_{\lambda m}(n))$

Protein Equations:

$$\frac{dp_i}{dt} = -\beta(p_i - m_i)$$

$P_{\lambda m}(n) = \lambda^n e^{-\lambda}/n!$

$\Delta m/\Delta t = \text{rand}(P_{\lambda m}(n))$

$$\frac{dp_j}{dt} = -\beta(p_j - m_j)$$

$P_{\lambda m}(n) = \lambda^n e^{-\lambda}/n!$

$\Delta m/\Delta t = \text{rand}(P_{\lambda m}(n))$

$$\frac{dp_k}{dt} = -\beta(p_k - m_k)$$

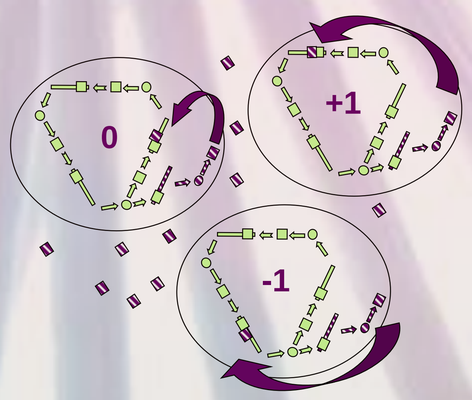
$P_{\lambda m}(n) = \lambda^n e^{-\lambda}/n!$

$\Delta m/\Delta t = \text{rand}(P_{\lambda m}(n))$

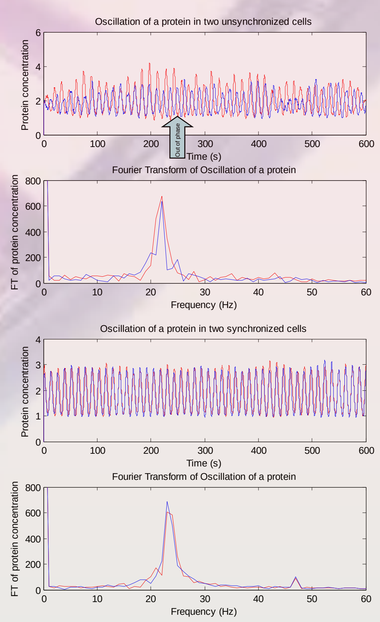
$$\frac{dS_{int}}{dt} = -k_{s0}S_{int} + k_{s1}p_x - \eta(S_{int} - S_{ext})$$

$$\frac{dS_{ext}}{dt} = -k_{se}S_{ext} + \eta\sum_{n=1}^{N}(S_n - S_{ext})$$

Synchronization

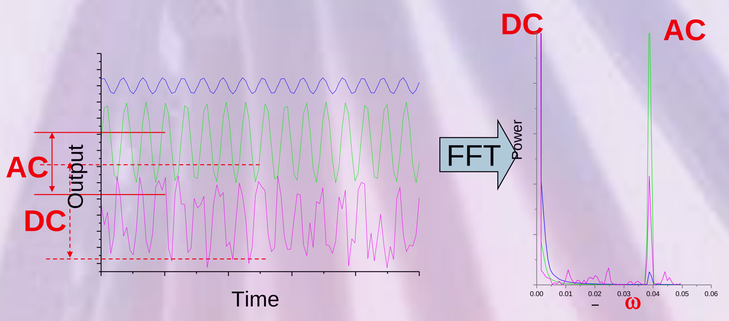


Synchronization is based on an additional branch that links individual cells though a common, extracellular molecule – a system naturally used by bacteria to sense their population density. An important consideration is the topology with which this branch leaves and reenters the system.

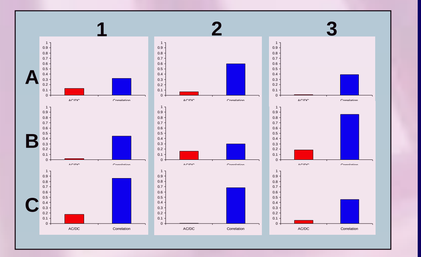


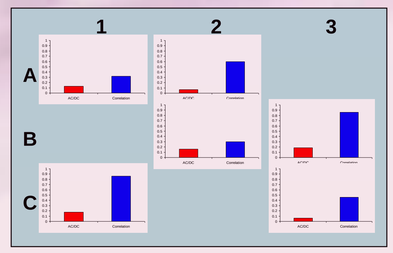
Analysis

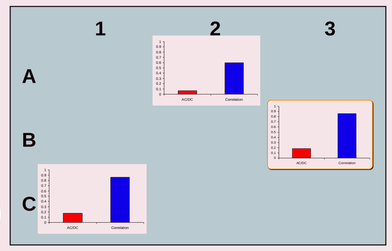
A higher AC/DC ratio indicates stronger and more stable oscillations.



The lux gene and its promoter must be inserted in the 3B configuration for best results.



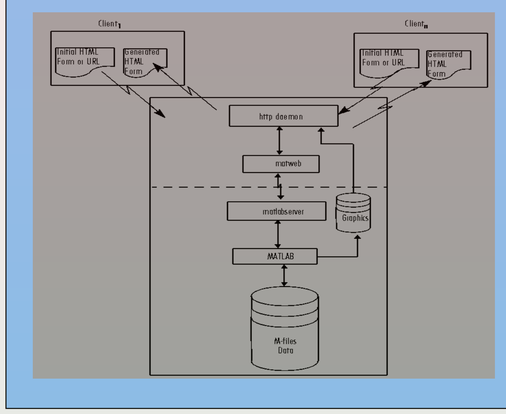




Future Work

- Expand the system to multiple (>2) cells in a 3-dimensional organization
- Perform a more complex 3-dimensional diffusion analysis of the coupling molecule based on defined spatial arrangements

Anyone can simulate this!



The entire simulation and analysis package is online on our website: http://www.iit.edu/~ipro302s06

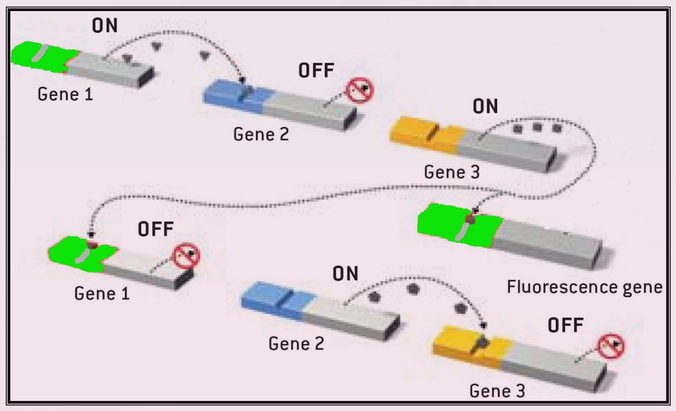
The Matlab Web Server collects input data on a web page, transmits it through a CGI application (*matweb)* to a process running on the server (*matlabserver)* which calls Matlab to execute the appropriate file, generate the results, and send back the information to the client browser in html.

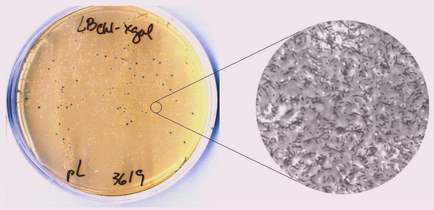
BIOLOGY

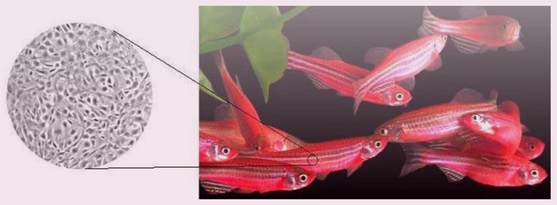
Abstract

The biological aspects of this project involve providing basic operational parameters to the design team and the construction of the system by the cloning team in the lab. The implementation of the system involves the synthesis of 4 plasmids. Each plasmid contains between 5 and 15 individual genetic elements of several types. This includes coding genes that dictate the proteins produced, promoters that control the expression of these genes, and terminators that control transcription.

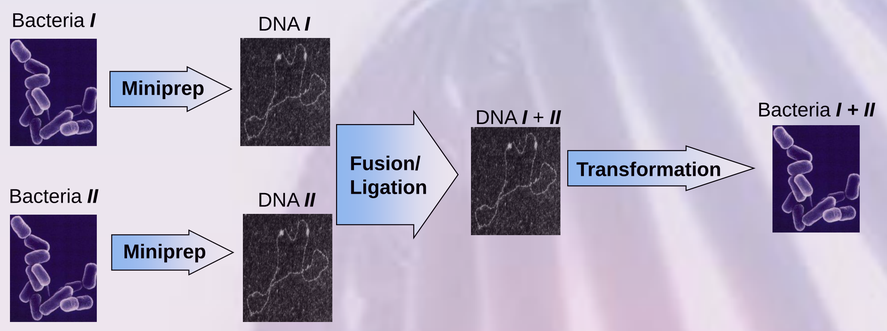
At this point, the system has been implemented in bacteria. However, in principle it should be possible to transfer this system to any organism. In coming semesters, we hope to move this to more complex multocellular organisms – our initial target is a lower vertebrate, the Zebrafish, *Danio rerio*.

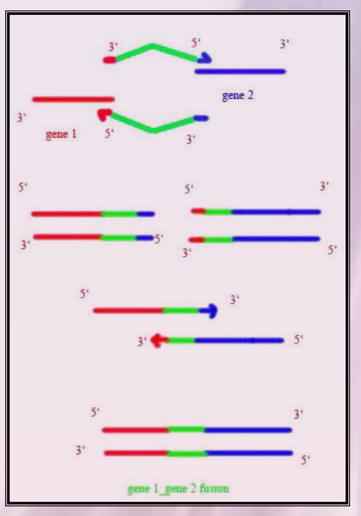






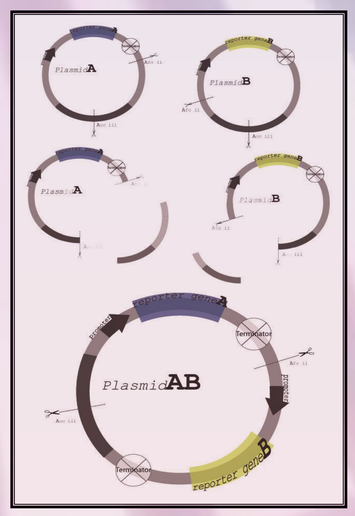
Methods





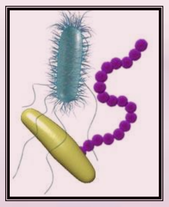
◄Fusion PCR

Ligation►



Where do we get these genes?

- Virus/Phage
  - –pR
  - –cI



- Bacteria
  - – lacI
  - – TetR

- Synthetic
  - –pLTet
  - –pLlac

- Jelly Fish
  - –XFPs

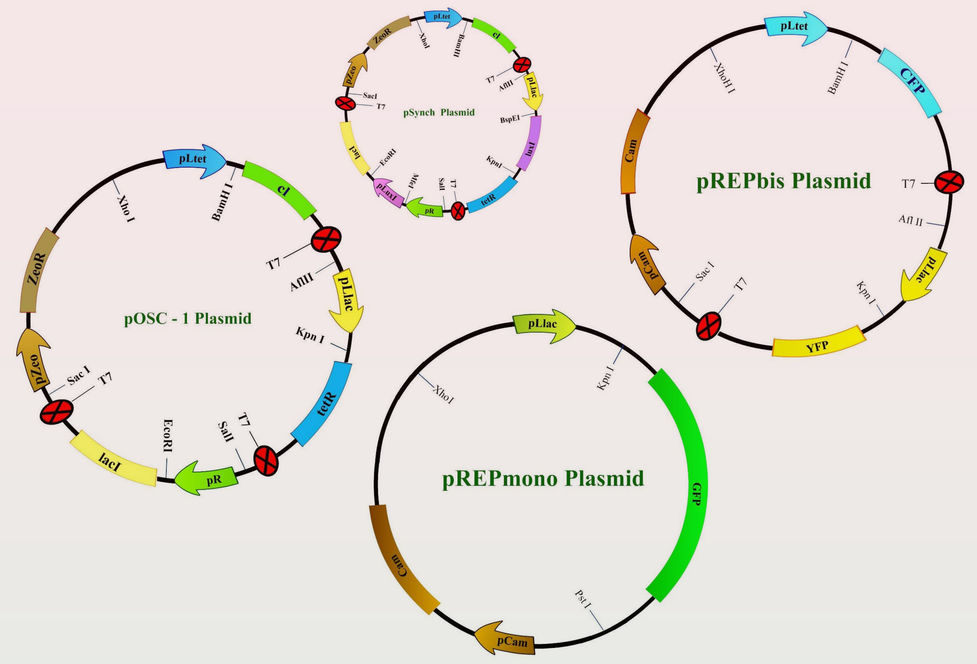
Construction status

Module	Plasmid	Status
pLac-GFP	pREPmono	Plasmid completed
pLac-YFP-t7	pREPbis	Confirmed with sequencing, ready for ligation
pTet-CFP-t7	pREPbis	Confirmed with sequencing, ready for ligation
pR-lacI-t7	pOSC	Requires confirmation with RE analysis and sequencing
pLac-tetR-t7	pOSC	Requires confirmation with RE analysis and sequencing
pTet-cI-t7	pOSC	Module completed
pLux	pSynch	Amplified
LuxR	pSynch	Amplified
LuxI	pSynch	Amplified

Our plasmids…

These four plasmid targets are the ultimate realization of our project. pOSC contains the three sets of linked repressors and promoters; this plasmid controls the system and is one of the the most complex, containing 15 elements. We have developed two auxiliary reporter plasmids, pREPmono and pREPbis, which report the state of pOSC in a way that is easy to detect. These three plasmids have been under development for the past 2 semesters, and we have completed one and very nearly completed two others.

We have also developed a new target plasmid, pSYNCH, a modified version of pOSC. The design for this plasmid was only recently developed since it is dependent on the results of modeling analysis obtained this semester, so the implementation of this target is just beginning



SYNTHETIC BIOLOGY:
Engineering Novel Organisms

IPRO 302

Advisor: Nick Menhart

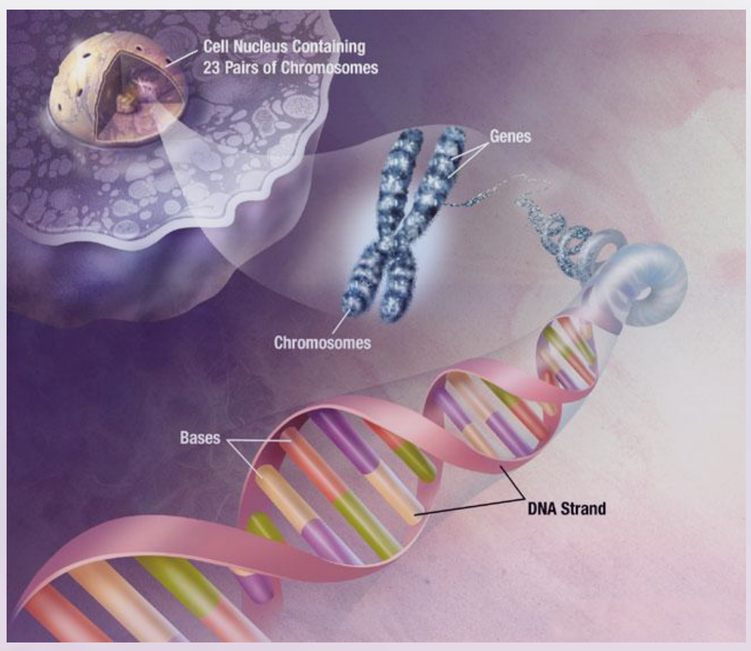
Team Leaders: Emad Allam, Bryan Bridgeman, Heather King, Thien Le, Lily Liu

Team Members: Hua Chen, Faraz Hussain, Daniel Hutchinson, Soo Lim, Saba Mahmud, Edward Maltby, Siddhartha Patel, Hazel Ramirez, Trillian Ross

ILLINOIS INSTITUTE OF TECHNOLOGY

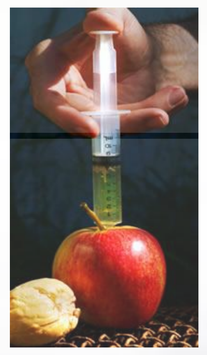
The Big Picture

The purpose of this project is to understand how genes interact with each other within a living organism, and potentially to modify or even synthesize novel organisms. In order to understand this, a simple dynamic genetic system was constructed.



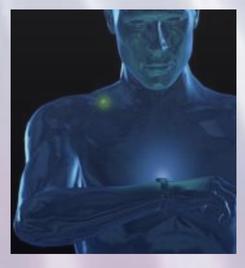
This may seem like a fantasy, with techniques from synthetic biology, it is possible to create organisms that deviate from nature. Simple transgenic organisms are already being produced, such as transgenic food plants or the Glo-Fish shown below, which involve only 1 or 2 genetic elements. Our system incorporates about 25 genetic elements, a step toward the simplest organisms which include 50 genes.

Applications

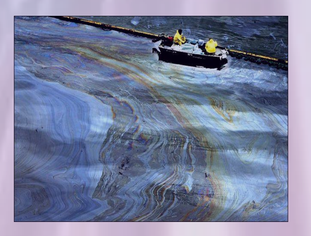


Genetically Modified Crops

Insulin Production



Biosensors



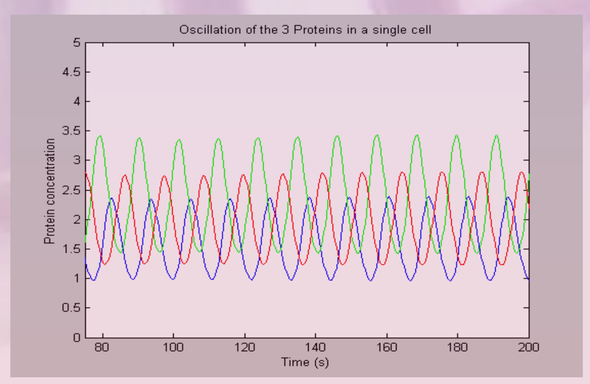
Bioremediation

Biofuel

Starting from the Bottom Up

Simpler systems must be understood before more complex systems can be constructed. If the approach to how genes interact is understood, our own biological system can be designed.

Modeling and analysis of the system is crucial to this effort, since as the complexity of the system increases it is not immediately apparent if it will behave as hoped - or how it will at all.



All the functions of our circuit are carried out by biological machines – even output: here fluorescent proteins controlled by genes we inserted report about the state of the genetic system.

In Our Project…

A biological circuit in bacteria was developed that changes the organism's heir characteristic behavior. This was done by assembling simple biological machines according to a specific plan. A synchronized oscillatory system was chosen since it encompassed dynamic behavior, communication and environmental sensing – all hallmarks of living systems. We are now taking the first steps toward moving into higher organisms – and eventually even humans.