CPS: Frontier: Collaborative Research: bioCPS for Engineering Living Cells

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Goal and Motivation

We aim to create a next-generation biological cyber-physical system (bioCPS) in which desired global behaviors can be achieved in populations of living cells through the identification and characterization of local behaviors. The ability to synthesize systems that control biological patterning could lead to advances in manufacturing, amorphous computing, tissue engineering, and drug development. For instance, differentiation of pluripotent stem cells in tissue or organoid engineering can be mapped to a pattern formation control problem.

To achieve this goal, we equip cells with sensing, communication, and decision making capabilities using methods from synthetic biology. Additionally, micron-scale mobile robots assist in optimizing the formation of patterns by affecting communication through opto-genetic triggering of genetic circuits or by moving cells and signals.

Workflow Summary

Our workflow involves five main steps. First, experimental data is collected and fed into an analysis method that creates **mathematical models** for biological modules and their compositions. Simultaneously, a desired pattern or behavior is defined by a user and is converted into a formal specification using **machine learning** techniques. Next, the biological modules are composed using a structural specification, and **design space exploration** is performed on their associated models using **simulation** and model checking to identify the design that best realizes the user-defined behavior. The selected design's behavior is then verified using **experimental design**. Finally, **microrobotics** are utilized in the physical system to aid in communication and sensing and to provide precise top-down control of cellular patterning.

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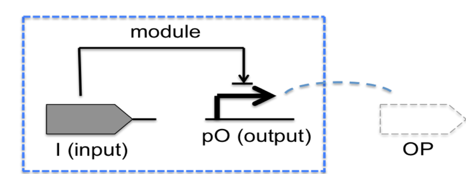
Workflow

Modeling

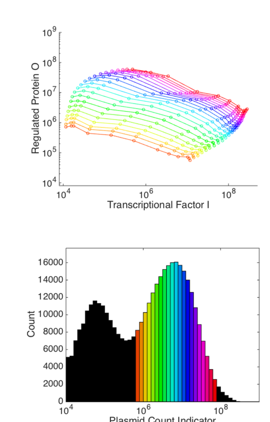
Biological Module



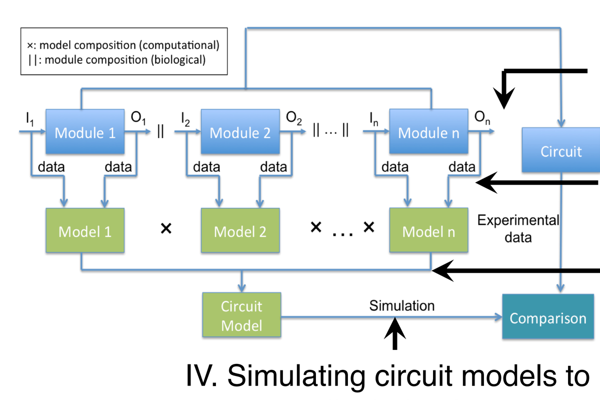
Bacteria cells Hill-function Based Model

Mammalian cells Bin-dependent Model

Creating composable models of biological modules:
- Characterize modules
- Build models
- Compose using Prüfer codes



Predictions of Circuit Behaviors



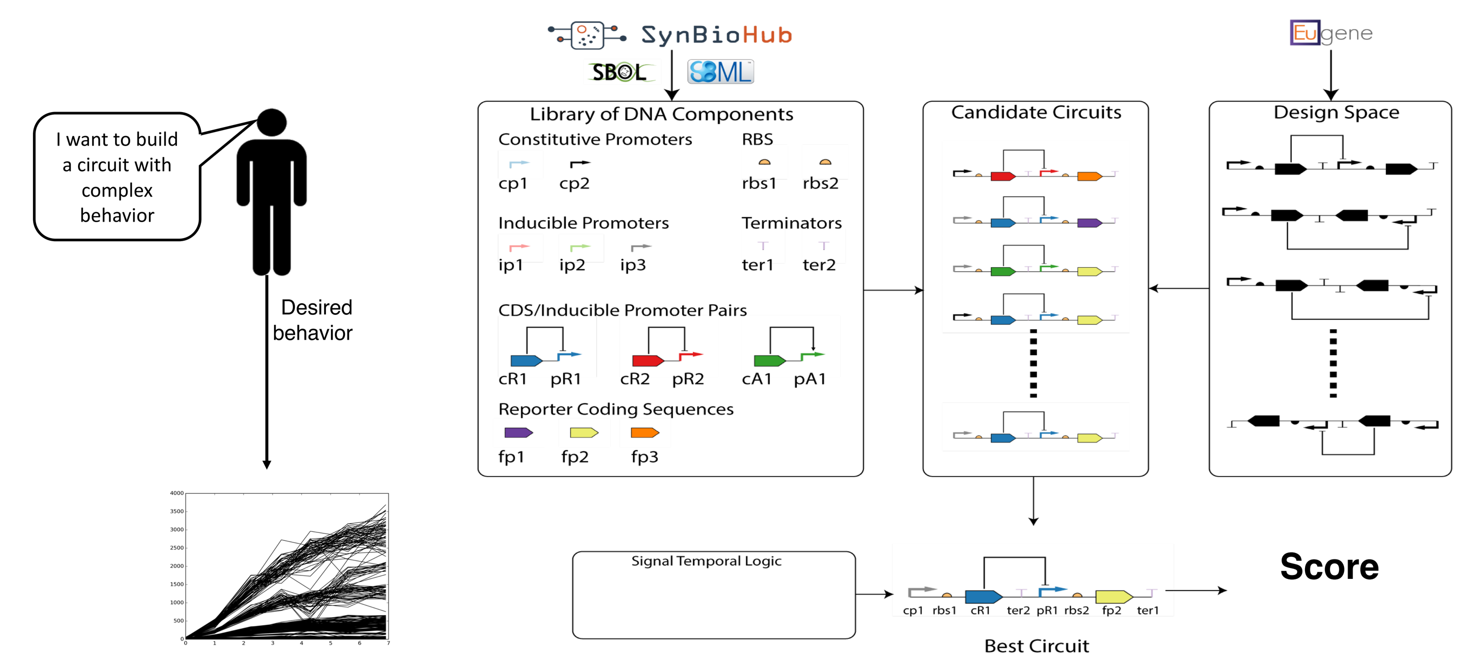
I. Building an in-silico model

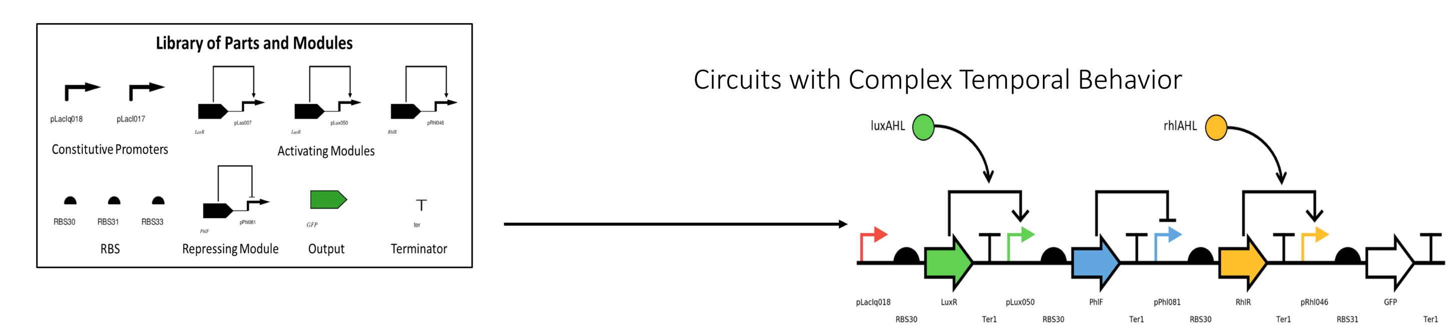
II. Fitting kinetic parameters to the experimental data.

III. Modular composition

IV. Simulating circuit models to predict behaviors

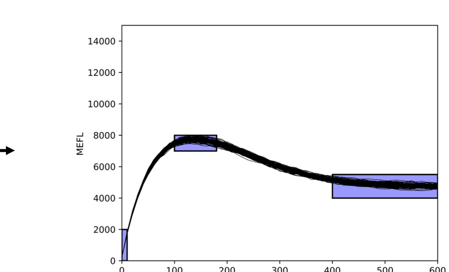
Design Space Exploration



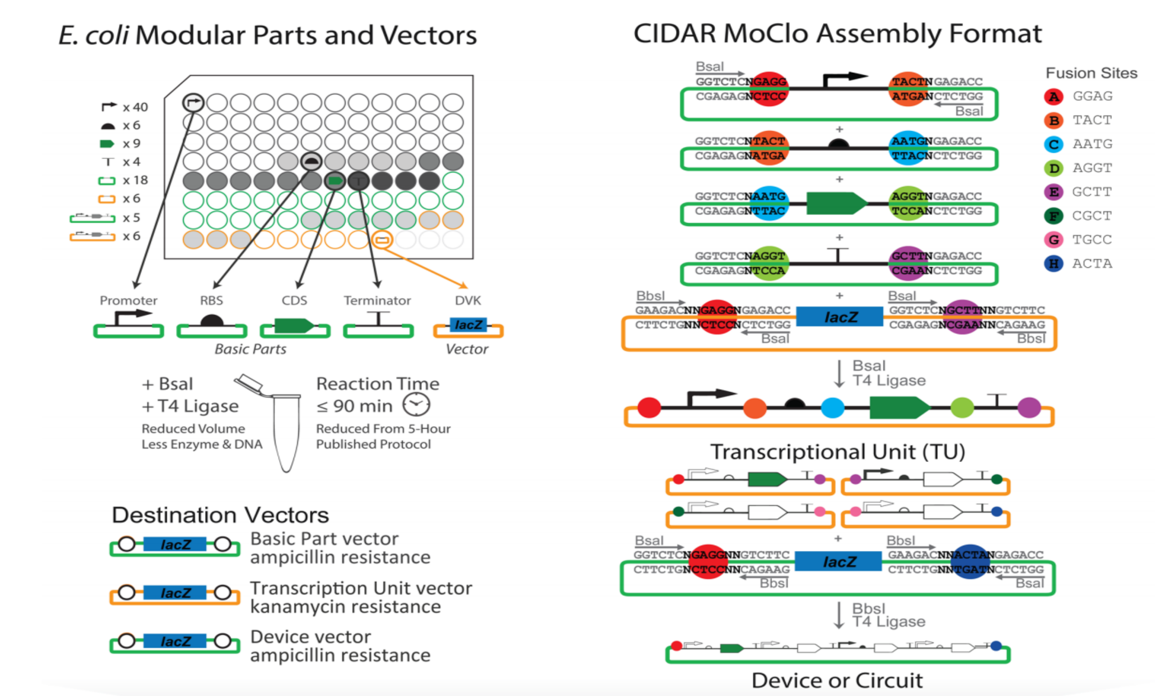


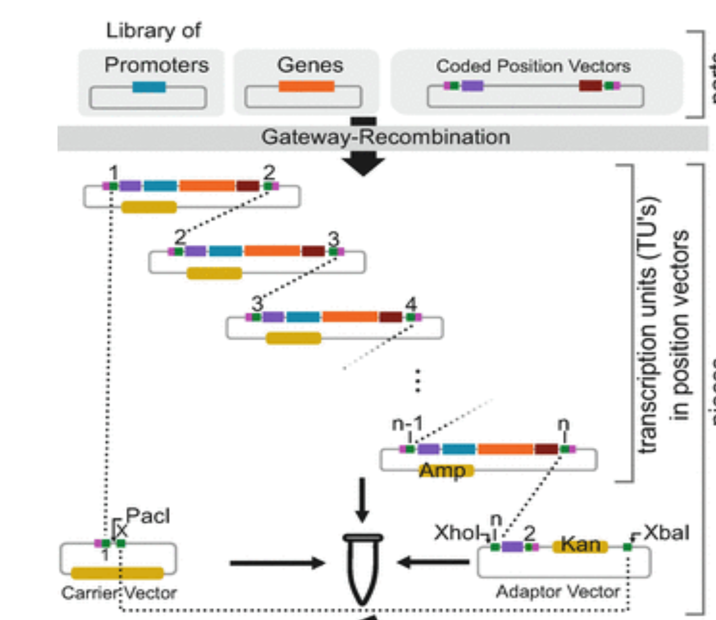
$G_{[0,10]}(out > 0 \wedge out < 2000)$
$\wedge$
$G_{[100,180]}(out > 7000 \wedge out < 8000)$
$\wedge$
$G_{[400,600]}(out > 4000 \wedge out < 5500)$

Circuit Specification



Building Genetic Circuits



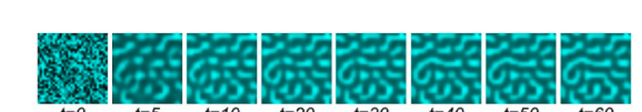


modular cloning strategies (MoClo) to help facilitate efficient and reusable construction of DNA constructs

Optimizing Patterns

Steady State Pattern Synthesis

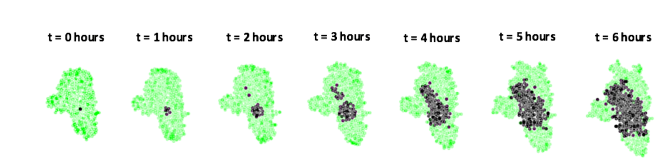
$\varphi : \forall_{B^*} \bigcirc ((\forall_{\{SW,NE\}} \bigcirc ((W \geq 1/2)) \wedge (\forall_{\{NW,SE\}} \bigcirc (W \leq 1/2)))$
$B^* = \{NW, NE, SW, SE\}$



Spatio-Temporal Pattern Synthesis

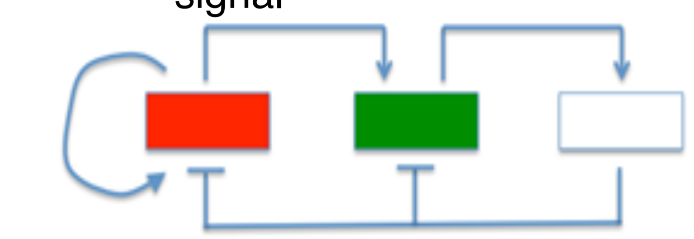
$\varphi : F_{[t_1,t_2]}\, G_{[0,T]}\ Pattern$

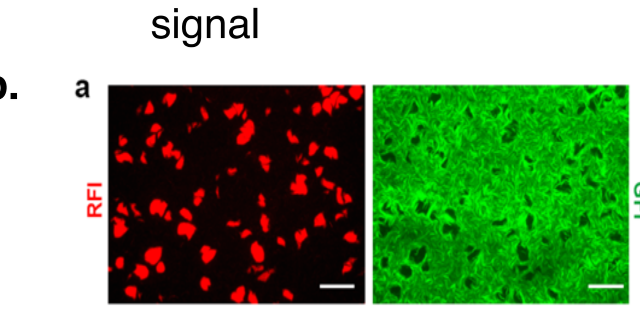
"Eventually reach the "Pattern" between time t_1 and t_2 and always remain in "Pattern" for T hours"



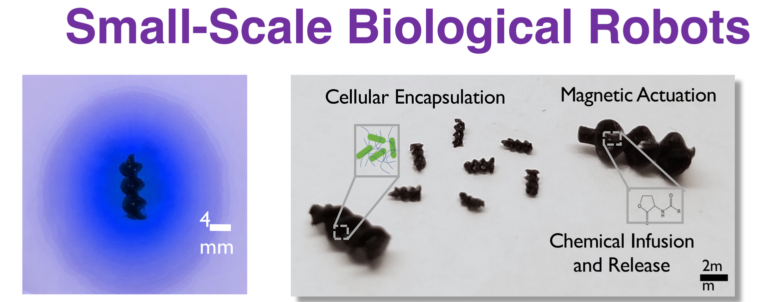
Experimental Design

Traditional Turing reaction-diffusion patterns are formed by the combined action of a short-range activator and a long-range repressor (a). Appropriate tuning of biological and physical parameters can give rise to patterns in cell response, such as the micrographs of fluorescent *E. coli* (b).



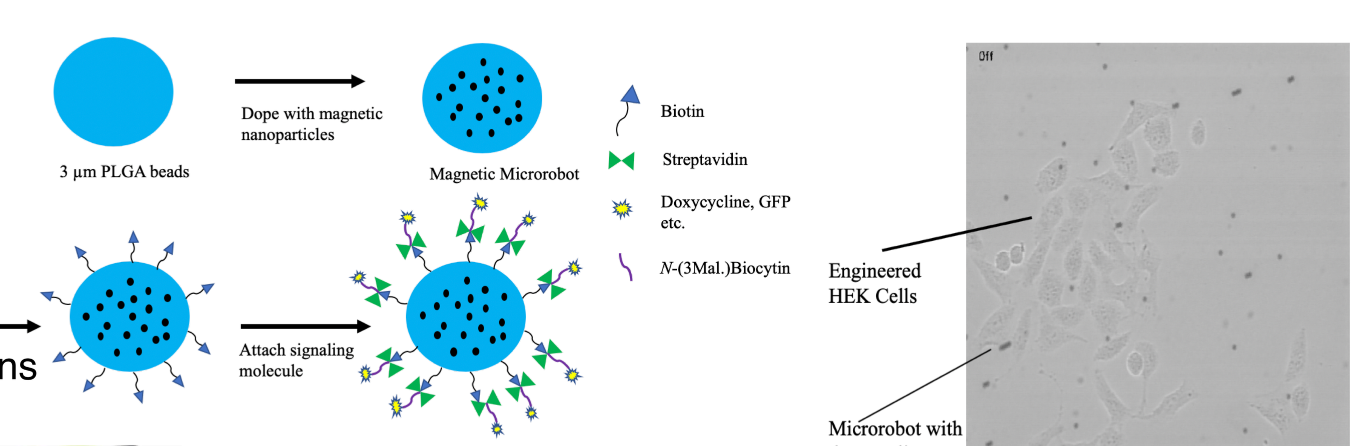


Small-Scale Biological Robots



Fabricated via 3D micromolding of hydrogels
Magnetically actuated and Biocompatible

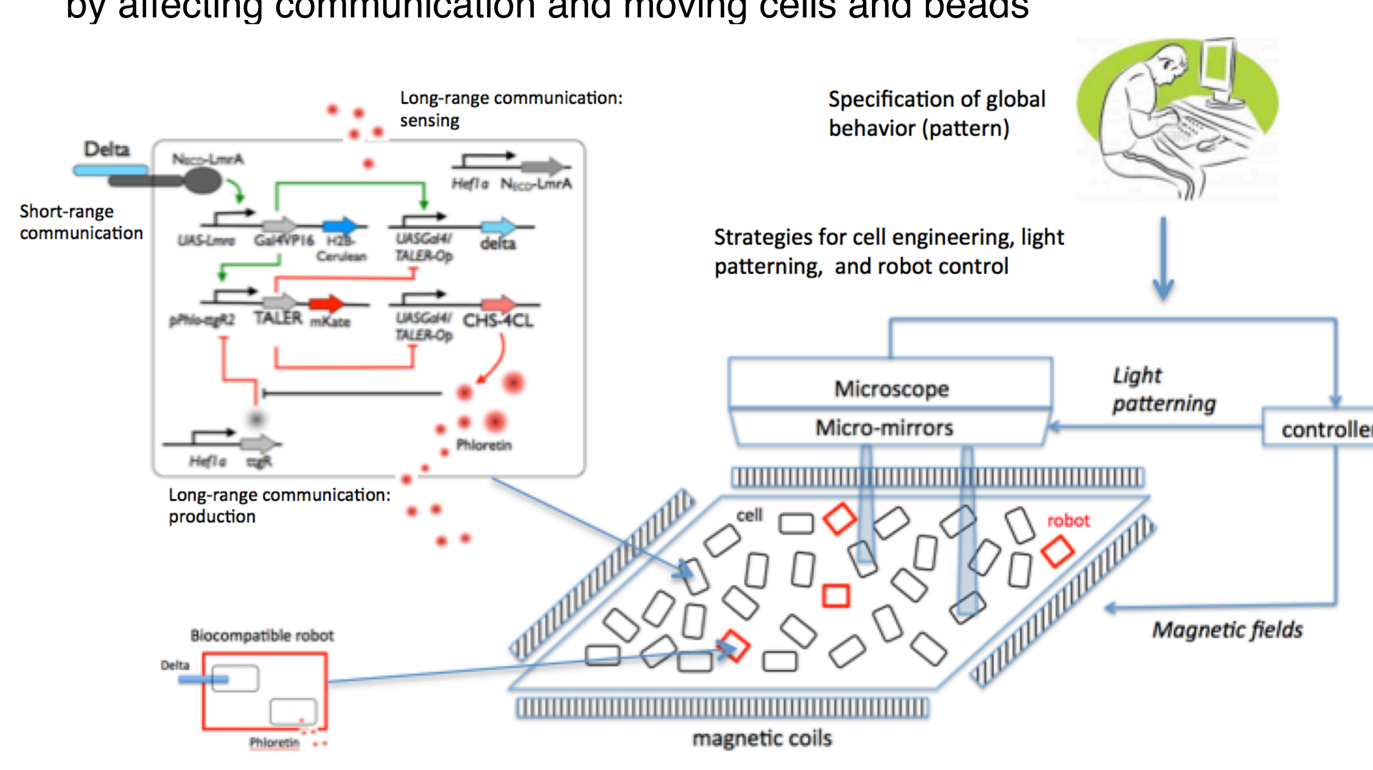
Encapsulating signaling molecules in microrobots



Delivery of signaling molecules in engineered cells

Biological Cyber-physical Systems

Micron-scale mobile robotics optimize the formation of patterns by affecting communication and moving cells and beads

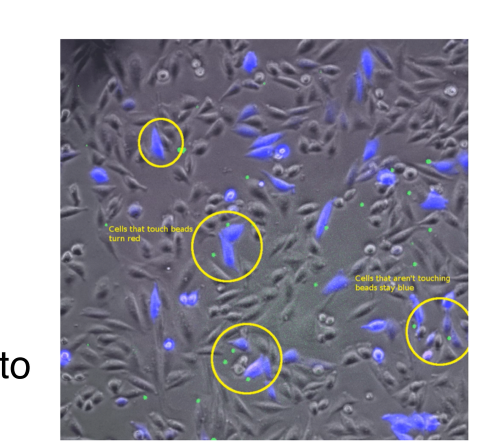


Robot Assistant for Communication, Sensing, and Control in Cellular Networks

Chinese Hamster Ovary cells, Engineered with a synthetic Notch system

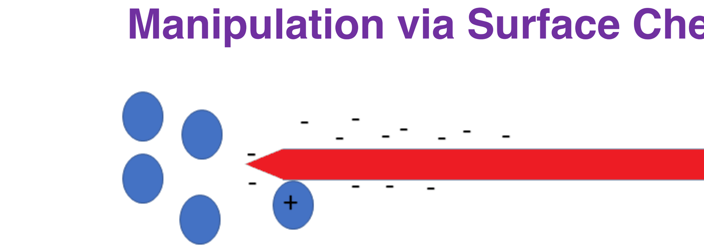
The cells with the circuit are blue

The cells change color from blue to pink on exposure to GFP

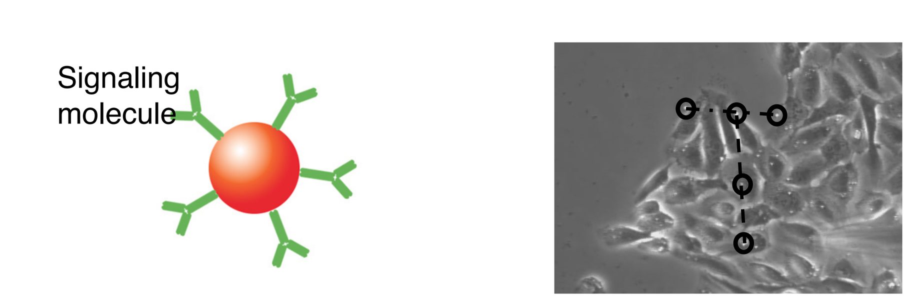


Cellular Response with Chemical Payloads

Manipulation via Surface Chemistry



attraction of bead with surface < attraction of bead with pipette
attraction of bead with pipette < attraction of bead with cells



For mammalian systems