Sensing and Actuation for Micro Bio Robots

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I. Introduction

Scaling down the size of robots enables new ways of exploring and manipulating microscale environments. Specifically in the field of biology, microrobots can be a tool for single cell analysis; such as for manipulating cells [1], measuring cell interactions [2] and cell surgery [3].

To create microrobots, we need to miniturize actuation and sensing capability to the cell scale. Bacteria have been integrated into microrobots for actuation as an on-board power source [4, 5]. To integrate sensing onto the same platform, the sensor must be able to sense an environmental cue, generate a physical change detectable using standard microscopy equipment and be small enough to fit onto a microscale robotic platform. Advances in synthetic biology have enabled different behaviors and responses to be programmed into biological cells, the result is a biosensor packaged into a living cell.

In separate experiments, we have shown that bacteria can be used for sensing as well as for propelling microstructures to make micro bio robots (MBRs). This poster will detail on the sensing side, experimental results of an MBR capable of sensing cell damaging environments and on the actuation side, a stochastic dynamical model of the motion of bacteria propelled MBRs using a simplified chemotaxis molecular model. This work is toward creating microrobots that integrate inorganic with organic materials for sensing and actuation.

II. Sensing

We integrate synthetically engineered *Escherichia coli* (*E.coli*) cells capable of detecting and reporting DNA damaging environments with a magnetic microrobotic platform. UV light is used as a proxy to induce reversible DNA damage, this causes a change in gene expression which is reported by the expression of green fluorescent protein (GFP); this can be detected using fluorescence microscopy[6]. The genetic circuit, experimental characterization of the cell and results of micro bio robot with sensing capability are discussed in this poster.

A. Method

Low Power Toggle Cells

The sensor cells used in these experiments are *E.coli* JM2.300 synthetically engineered to include plasmids pLPTa and pCIRa. The gene circuit is bistable because it involves two repressible promotors that are arranged in a mutually inhibitory network allowing the cell to have two stable states [7], Fig.1(a). A chemical inducer, isopropyl-beta-thiogalactopyranoside (IPTG), forces the cells into a low-state when the activity of lacI is low and the activity of λcI increases. The cell switches to high-state by induction with a transient pulse of UV light. This causes the degradation of

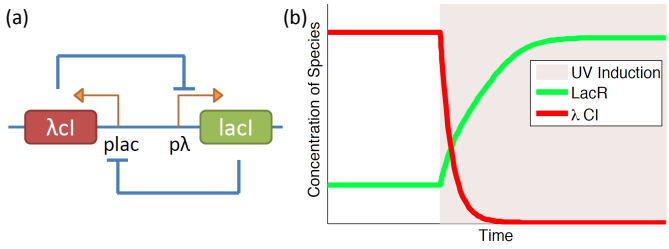

Fig. 1. Schematic of the genetic engineered circuit showing mutually repressive genes λcI and lacI. (b)Mathematical model of the toggle switch dynamics qualitatively showing the transition of species LacR and λCI after UV induction. Images from [6]

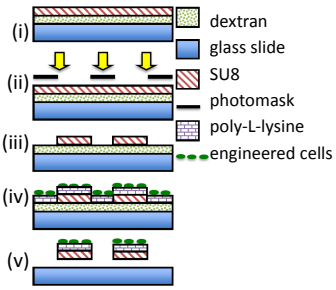

Fig. 2. Fabrication of biosensors. (i) Spin coating of sacrificial layer of dextran and then SU8 for the substrate of biosensor. (ii-iii) Photolithography patterning of microstructures. (iv) Cell attachment into microstructures using a layer of poly-L-lysine. (v)Release of biosensor by dissolving the dextran layer. Image from [6]

protein λCI and increases activity of lacI, and is linked to GFP production. These two states are stable even after the inducer is removed.

The interactions between the components in the genetic toggle circuit can be described by a pair of coupled dimensionless nonlinear differential equations [7]:

$$\frac{dU}{dt} = \frac{\alpha_1}{1+V^\beta} - U \ , \ \frac{dV}{dt} = \frac{\alpha_2}{1+U^\gamma} - V$$

where U is the concentration of repressor protein LacR, V is the concentration of repressor protein λCI, α_1 is the effective rate of synthesis of repressor protein LacR, α_2 is the effective rate of synthesis of repressor protein λCI, β is the cooperativity of repression of promoter pλ and γ is the cooperativity of promoter plac. Fig.1(b) shows qualitatively how the differential equations govern the change in species concentration as the cell transitions from a low-state to a high-state. This effect is modeled by decreasing the effective rate of synthesis of repressor protein λCI, α_2, and decreasing the cooperativity of repression of promoter, pλ, β.

Fabrication

Methods that we propose for transporting sensor cells are (1) to attach them to micro magnetic robots integrating the sensing onto the manipulable robot, Fig.3bi; (2) to attach cells onto several microplates and use a micro magnetic robot to manipulate the plates, Fig.3bii. Both proposed solutions involve attaching cells onto a substrate, however *E.coli* cells

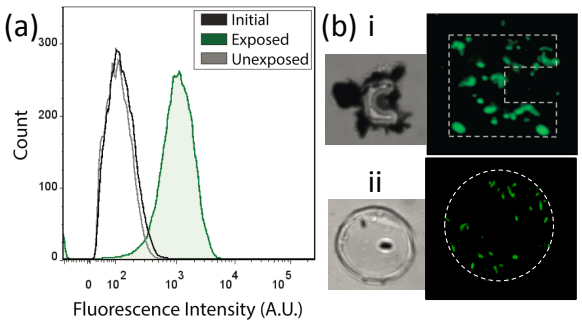

Fig. 3. Experimental results. (a)A histogram of fluorescence intensity from flow cytometry analysis of engineered low power toggle switch cells. Cells are exposed to 50μJ/cm^2 UV irradiation. (b) Phase contrast and fluorescence images of cells attached to (i) 80μm x 80μm micro magnetic robot, (ii) 60μm diameter microplate Image from [6]

are not naturally adherent. We treat surfaces that we want cells to attach to with 0.1% poly-L-lysine, a protein which promotes binding of biological cells. The fabrication of micro magnetic robotics is a single-exposure process using standard photolithography techniques, details are discussed in our previous work [1] and shown in a schematic in Fig.2.

B. Results

Integrating MBRs with cells for sensing is challenging because the behavior of cells are affected by physical changes in their environment, such as attaching them to hard surfaces such as an SU8 substrate. Standard biology protocols involve culturing cells in solution and the use of flow cytometer to analyse the fluorescence of the cell population. Fig.3a shows a histogram of fluorescence intensity for 10,000 cells using this method. However, the fluorscence of cells attached to MBRs cannot be analysed in this way. Moreover, it is advantageous to beable to determine fluorescense using standard lab equipment. The fluorscence intensity of cells can be measured using fluorescence microscopy - taking images with a camera, identifying cells attached to the MBR and then measuring the fluorescence intensity of each cell.

Fig.4 shows phase contrast and fluorescence images of both biosensor cells integrated onto micromagnetic robots and on an SU8 microplate. In both cases, the cells have been exposed to UV irradiation and it is apparent that there are cells expressing GFP. By attaching cells to SU8, there is a large number population of cells which have been exposed but are not expressing GFP. Therefore, it is important for micro bio robots to use a large population of cells as sensor to more reliably determine the correct readout for UV exposure.

III. Actuation

Flagellated bacterial cells can also be used to actuate microstructures to make bacteria propelled MBRs, in our experiments we use *Serratia Marcescens*. We endeavor to control these devices, to do this we need to understand the dynamics. We propose a bottom-up model for the motion of bacteria propelled MBRs that incorporates the distribution of bacteria, models the bio-molecular network governing the bacteria flagellar motor to capture the stochastic switching between run and tumble state of each bacterium and a force

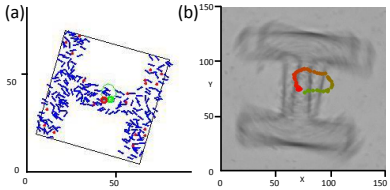

Fig. 4. Preliminary results for a predominantly rotating MBR, trajectory starts at green and ends at red. (a) Simulation trajectory, (b) Experimental trajectory.

balance. The main focus in the poster will be the simplified chemotaxis model used to simulate the run and tumble behavior of cells. Preliminary simulation results validated by experimentally observed clockwise angular velocity bias are shown.

A. Stochastic Model of Bacteria

From observations of the steady state behavior of *E.Coli* in the absence of external driving forces such as chemical gradients, we can make the extension that the propulsive force exerted by each bacterium is a stochastic process [8]. This process can be modelled in each cell using a Gillespie algorithm [9]. Assuming that cells do not interact with one another, an independent Gillespie algorithm is used to predict the state, run or tumble, of each cell attached to an MBR. By using a Gillespie algorithm, each generated trajectory will be unique as a different combination of run-tumble sequences will emerge. The result will be one possible trajectory for the MBR, by running may trials for the same system it is possible to obtain a distribution of trajectories.

To model the stochasticity of the flagella, a simplified chemical model of the chemotaxis network in *E.coli* is used. Since the specific interest is in the flagellum behavior in steady-state in the absense of chemical attractants, a simplified model is used which involves kinases CheA and protein CheY and the state of the flagella (1) to (5), where each equation has a specific rate of reaction denoted by the greek letters.

$$CheA \xrightarrow{\alpha} CheA - p \tag{1}$$

$$CheA - p + CheY \xrightarrow{\beta} CheY - p + CheA \tag{2}$$

$$CheY - p \xrightarrow{\gamma} CheY \tag{3}$$

$$Tumble \xrightarrow{\mu} Run \tag{4}$$

$$CheY - p + Run \xrightarrow{\rho} CheY - p + Tumble \tag{5}$$

The state of CheA and CheY are enough to model the state of the cell because the motor behavior is directly dependent on its interaction with CheY; moreover, the phosphorelation state of CheY depends on CheA. CheZ, CheW, CheB and CheR are omitted as well as methylation reactions even though they are involved in the chemataxis network. This is done to maintain simplicity of the model and because response to chemical stimuli are not considered here.

B. Preliminary Results

Fig.4 shows similar trajectories between experiment and simulation, however the orientation does not match well. This suggests that there may be additional factors affecting the motion of the MBR not captured in this model. This may include the forces from bacteria running into the sides of the MBR and forces exerted on the MBR while the cell is in a tumble.

IV. Conclusion

We have demonstrated the implementation of sensing capabilities on micro bio robots made possible by advances in synthetic biology. In our experiments, UV light is used as a proxy for UV light detection for experimental ease, however, other sensors can also be programmed into the cells via genetic engineering. Secondly, we discuss work in a bottom-up model of bacteria propelled microstructures which is able to capture the stochasticity of cell behavior. This is work toward micro bio robots with on-board sensing and actuation realised through interfacing organic cells in biology and inorganic structures.

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