A Model of a Synthetic Biological Communication Interface between Mammalian Cells and Mechatronic Systems

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***Abstract*— The creation of communication interfaces between abiotic and biotic systems represents a significant research challenge. In this work, we design and model a system linking the biochemical signaling pathways of mammalian cells to the actions of a mobile robotic prosthesis. We envision this system as a robotic platform carrying an optically monitored bioreactor that harbors mammalian cells. The cellular, optical signal is captured by an onboard fluorescent microscope and converted into an electronic signal. We first present a design for the overall cell-robot system, with a specific focus on the design of the synthetic gene networks needed for the system. We use these synthetic networks to encode motion commands within the cell's endogenous, oscillatory calcium signaling pathways. We then describe a potential system whereby this oscillatory signal could be outputted and monitored as a change in cellular fluorescence. Next, we use the changes resulting from the synthetic biological modifications as new parameters in a simulation of a well-established mathematical model for intracellular calcium signaling. The resulting signal is processed in the frequency domain, with specific frequencies activating cognate robot motion subroutines.**

***Index Terms*— Calcium signaling, gene circuits, robotics, synthetic biology.**

I. Introduction

THE engineering of interfaces between living (biotic) and nonliving (abiotic) systems represents a significant research challenge. Examples of abiotic-biotic relationships occur readily in nature, such as the response of a living organism to nonliving mass and energy sources (e.g., oxygen and sunlight) [1]. Alternatively, here we will consider the engineering of a communication link between living cells and a mechatronic system to be an abiotic-biotic engineering challenge. Many research efforts pursue such links, such as in the development of solid-state neural prostheses that can restore hearing [2], sight [3], and movement [4]. Similarly, the field of optogenetics has emerged to include a focus on optically monitoring genetically modified cells capable of changing their fluorescent light output [5].

Recently, we developed a model for an abiotic-biotic robotic system consisting of bacterial colonies that served as the central command and processing units for a mobile robot. The interactions between the bacteria and their robotic conveyance formed a biomimetic system representing an organism's gut microbiome and the host organism, respectively. Our previous model system was able to demonstrate that relatively complex host predation behaviors could emerge when a simple information processing architecture was genetically encoded in the bacteria living inside the host [6].

In the work described here, we have developed a different architecture to enable the fluorescent signaling output of engineered mammalian cells to command and control a mobile robotic prosthesis (i.e., the robotic platform). We chose to encode the envisioned cells' commands to the robot within their endogenous, oscillatory calcium signaling pathways. Using a range of recombinant biosensors, mammalian cells can be robustly engineered to report their calcium signal (i.e., their intracellular calcium concentration) by changing fluorescence [7]. As a result, this calcium signal can be optically captured and processed. We therefore designed a system architecture whereby changes in the dominant frequency of these calcium oscillations activated specific motion subroutines to direct the response of the mobile robotic prosthesis. This system is illustrated in Fig. 1.

A. Endogenous Calcium Pathway Capabilities and Previous Modeling Efforts

Our hybrid mammalian cell–robot system is based on simple and robust robot components, with its critical information processing steps based on cellular calcium signaling. Calcium is a ubiquitous 2nd-messenger in eukaryotic cells and encodes a range of spatiotemporally varying signals within the body by passing Ca^{2+} ions across a range of nanoscale channels, pumps, and buffers [8]. Rewiring the calcium signaling pathway therefore represents an opportunity for mammalian synthetic biology to encode information across multiple scales.

While calcium is a critical structural component in the body, it also serves a major role in information processing in mammalian cell physiology. Calcium responses can be induced using chemical agonists for G-protein-coupled-receptors (GCPRs) that activate efflux from endoplasmic reticulum (ER) calcium stores through a canonical $G_{\alpha,q/11}/IP_3$

Manuscript received July 15, 2016; revised September 24, 2016; accepted October 18, 2016. Date of publication October 25, 2016; date of current version January 12, 2017. This work was supported by the Office of Naval Research of the USA under award N00014-15-1-2502 and the Air Force Office of Scientific Research of the USA under award FA9550-13-1-0108. *Asterisk indicates corresponding author.*

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Digital Object Identifier 10.1109/TNB.2016.2620942

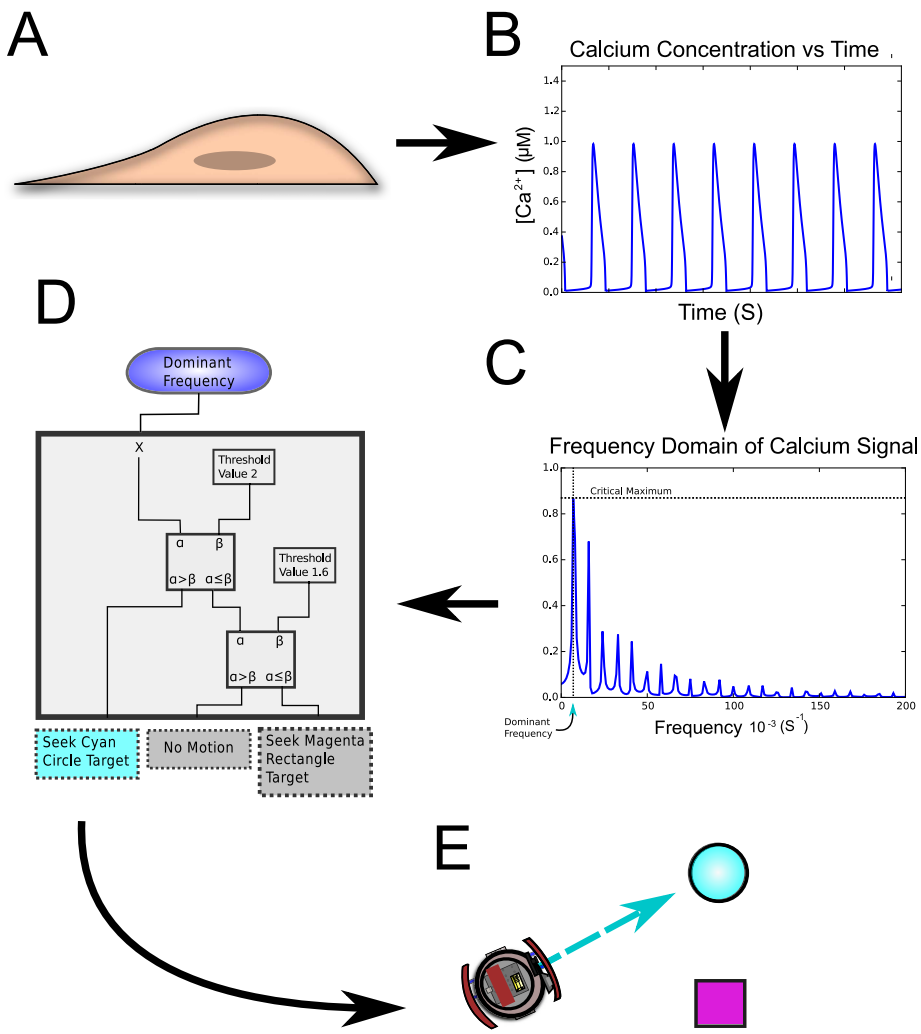

Fig. 1. Designing a Living-Nonliving Interface for Mammalian Cells. (a) There are a variety of mammalian cell types, from nerves to epithelial. Here we depict a common fibroblast. (b) Mammalian cells produce oscillatory calcium signals within the cytosol. These signals may be monitored optically. (c) Calcium signals may be transformed to understand the characteristic frequency, and underlying features, of the signals. (d) By using the dominant frequency as an information packet, robots may be programmed to respond to mammalian calcium signals. By setting thresholds, we are able to link specific robot subroutines with buckets of dominant frequency values. (e) These subroutines may initiate robot actuation, sensing protocols, manipulation, coordination, or locomotion, as depicted here.

second-messenger cascade shown in Fig. 2. In order to rapidly increase the intracellular calcium concentration (i.e., the intracellular calcium signal), the cell maintains a higher concentration of calcium within its intracellular store (i.e., the endoplasmic reticulum or ER), while also selectively permitting calcium entry from the extracellular space. Moreover, calcium signaling is often characterized by oscillations, and the period of these oscillations often encodes the key biological signal [8]. Fortunately, a robust four-state model for the IP_3-gated calcium channel that connects the ER to the cytoplasm was described Tang and Othmer [9] twenty years ago.

B. Synthetic Biology's Utility in Rewiring Mammalian Physiology

In our work here, we envision a system that uses the programmable gene circuits of synthetic biology to reprogram calcium signaling. Over fifteen years ago, the creation of two gene circuits, a bistable switch [10] and an oscillator [11], helped to accelerate the emergence of synthetic biology.

These networks have also provided significant insight into biological phenomena as well. For example, in reporting the first synthetic oscillator [11], termed the "repressilator," the authors made the point that constructing engineered gene networks provided insight into the biophysical design principles underlying oscillation. This premise has since been repeatedly demonstrated as different synthetic biological circuits have provided fundamental understanding of how biological networks support memory [10], [12], [13], detect sequences of events [13], and especially how biology utilizes noise and stochasticity to enable phenotypic diversity [14]–[20].

While most of these studies have occurred in bacteria [10], [11] or yeast [21], [22], synthetic biology is also moving rapidly into mammalian cells [23], [24], sometimes with bacteria themselves a delivery agent of the synthetic biological tool. In some of these studies, the potential to treat cancer has been highlighted. For example, Li and colleagues intravenously delivered engineered, cancer-invading bacteria to target a tumorigenic pathway *in vivo [25]*. They

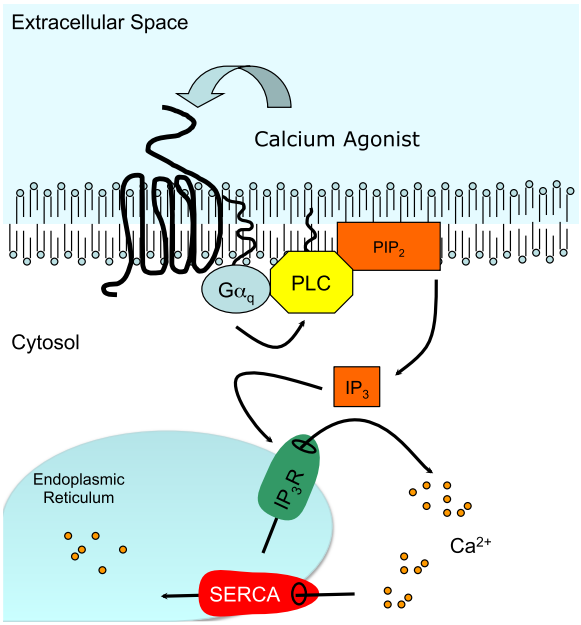

Fig. 2. Critical Components of the Calcium Signaling Cascade. After an external primary calcium agonist activates a membrane receptor, a G-protein activates phospholipase C, PLC, which results in the generation of the IP_3 small molecule. IP_3 causes release of calcium from the endoplasmic reticulum, ER, into the cytosol. The system is restored by sarcoendoplasmic reticulum calcium ATPases, which pump calcium back into the ER.

used RNA interference (RNAi) to create bacterial invaders that reduced expression of *CTNNB1* (encoding β-1 catenin), a gene that activates many epithelial cancers. The bacteria were engineered to produce short hairpin RNA (shRNA) that leverage endogenous mechanisms to inactivate *CTNNB1* transcripts. When this construct was delivered to mice with subcutaneously xenografted human colon cancer cells, the gene expression was knocked down in the tumor cells. These results demonstrate that significant physiological impact may be made by using RNAi-based synthetic biology to regulate intracellular pathways in mammalian cells.

II. System Design, Modeling, and Simulation

We envisioned creating the synthetic biological component of our system to alter calcium dynamics by using an RNAi system to alter the dynamics of calcium signaling. Previously, RNAi has also been used to screen the function of different players in the calcium signaling pathway [26], and the calcium pathway has been targeted by synthetic biologists [27]. However, the tunable expression circuits of synthetic biology have not been used to tune the dynamics of the calcium pathway. Moreover, analytical models have not been used to inform synthetic biological tuning of the dynamics of this frequently oscillatory pathway.

A. Gene Network Design

As noted, we used a widely cited, robust analytical model [9], [28] to guide our selection of calcium pathway targets for RNAi knockdown. Our initial modifications of this model, and resulting computational simulations, suggested two RNAi targets for either increasing or decreasing the period of cytosolic calcium oscillations.

Specifically, we recognized that phospholipase C (PLC) activity leads to IP_3 production (Fig. 2), and therefore we rationalized that decreasing IP_3 could result by knocking down PLC using RNAi. Next, we recognized that the sarcoendoplasmic reticulum calcium ATPase (SERCA) restores calcium storage in the sarco- or endoplasmic reticulum of cells, so knocking down SERCA would also alter the pathway's dynamics.

Therefore, we envisioned placing the shRNA necessary for RNAi targeting of each of these pathway components behind synthetic switches for tight expression gene expression [29] as shown in Fig. 3. These synthetic constructs can easily be activated by the application of antibiotics like erythromycin or pristinamycin. The resulting constructs could then each be stably transfected into a standard cell lined such as HEK 293. Calcium signaling would be monitored in individual cells simultaneously transfected with of recombinant, calcium-sensitive fluorescent reporters [30], [31].

To incorporate these cells into our previously described microfluidic bioreactor, linked to a mobile robotic platform [6], we envision that stably transfected HEK 293 cells (containing the RNAi construct) would be cultured within microfluidic chip-based bioreactors capable of manipulation with syringe pumps [32] until sub-confluent. These cells would also carry a robust calcium sensitive fluorescent probe such as ratiometric pericam [7], [33]–[35]. HEK 293 cells would then be induced to activate knockdown of either SERCA or PLC as shown in Fig. 3.

B. Calcium Pathway Modeling

Previous work has established a robust, analytical model for simulating and tuning cytosolic calcium oscillations [9]. By slightly modifying this model, we can derive two equations that simulate calcium (1) and IP_3R (2) concentrations respectively.

This model captures four possible binding states of the IP_3-sensitive calcium channel connecting the endoplasmic reticu-

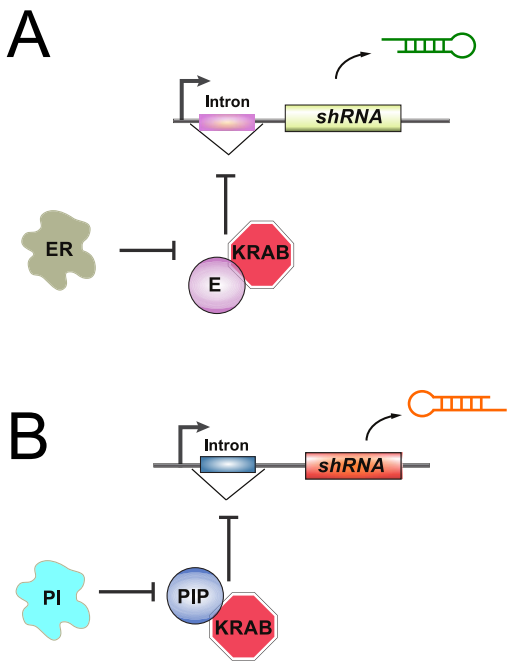

Fig. 3. Synthetic Tools for Altering Calcium Signaling. Antibiotics such as erythromycin and pristinamycin can be used to activate the production of short-hairpin RNA molecules, which result in knockdown of SERCA or PLC expression, and altering of calcium signaling dyanmics.

lum and the cytoplasm, also known as the IP_3 receptor [8]. As shown in Fig. 4(a), in the model, the receptor first binds to IP_3, which activates efflux from the ER to the cytosol. It next binds an activating cytosolic calcium ion, and finally binds to a second, inhibitory cytosolic calcium ion. The cytosolic calcium pool is constantly being pumped back into the ER by the sarcoendoplasmic reticulum calcium ATPase (SERCA) pump, which is itself activated by cytosolic calcium. Analytical modeling of the process can be reduced to the following expressions [9]:

$$\frac{d[IP_3R]}{dt} = -\varepsilon[IP_3R] + \beta_1[Ca^{2+}]^2\frac{(1-[IP_3R])}{([Ca^{2+}]+\beta_0)} \quad (1)$$

$$\frac{d[Ca^{2+}]}{dt} = \alpha_1(\beta-[Ca^{2+}]) + \alpha_2[Ca^{2+}]\frac{(\beta-[Ca^{2+}])(1-[IP_3R])}{([Ca^{2+}]+\beta_0)} - \beta_1\frac{}{[Ca^{2+}]^2}([Ca^{2+}]^2+\alpha_2^2). \quad (2)$$

Here, $[Ca^{2+}]$ represents the cytosolic calcium concentration and $[IP_3R]$ represents the concentration of receptor (i.e., gated channel) simultaneously bound to IP_3 and two calcium ions (i.e., all receptor binding sites occupied). The constants $\alpha_1, \alpha_2, \beta, \beta_0, \beta_1$, and ε, describe parameters relating to the binding kinetics depicted in Fig. 4(a).

This model is predicated on assumptions presented in previous literature [9] describing the behavior of cytosolic calcium interaction pathways. Crucially, this model makes the simplifying assumption that $[Ca^{2+}]$ is not significantly exchanged between the cytoplasm and extracellular environment [9]. Additionally, the model assumes that the calcium pumps associated with the endoplasmic reticulum may be modeled by a Hill function with a Hill coefficient of 2 [9]. Finally, the

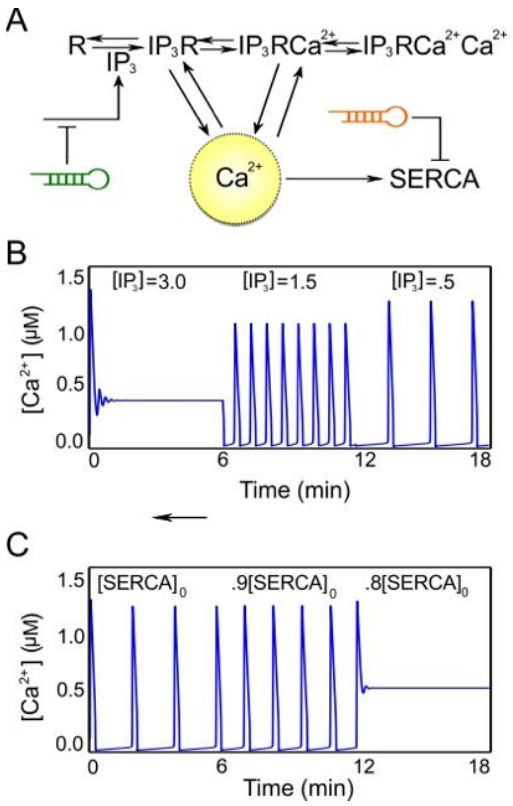

Fig. 4. Programmable Control of Mammalian Calcium Signals. (a) The calcium signal pathway is depicted in chemical form. R denotes an unbound IP_3 receptor. All arrows represent chemical binding events, modeled by first-order mass action kinetics. (b) By producing targeted shRNA, we can attenuate the value of $[IP_3]$ within the cell. This causes variations in the amplitude and frequency of the calcium oscillatory signal. It should be noted that at high $[IP_3]$ values, such as 3.0 μM, the calcium signal holds steady. (c) Similarly, by producing different shRNA, we can attenuate the value of [SERCA] within the cell. This causes an increase of in frequency while having a minimal affect on the amplitude of the calcium signal. However, increasing [SERCA] eventually results in a termination of calcium signal oscillations and calcium holding at a steady value.

model assumes that the rate of ion transport through channels affected by IP_3 is linearly related to the percent of open channels. Taking these assumptions into consideration, the governing equations (1, 2) may be formulated by employing first-order mass action kinetics to the reaction scheme depicted in Fig. 4(a).

C. Simulation

With the governing equations in place, we are able to simulate calcium oscillations using any commonly available numerical method technique for systems of ordinary differential equations. Herein, all numerical simulations were programmed in the MATLAB® 2015b. Simulations were processed using MATLAB's ode45 numerical method approach with a variable time step. Ode45 is a modified implementation of a Runge-Kutta 4^{th}-order algorithm.

Initial conditions and parameter values for the simulation were drawn from previous literature [9]. $[IP_3]$ and [SERCA] values were modified at the start of a simulation run where appropriate.

Total experimental simulations were conducted iteratively, with subordinate simulations lasting for 1 minute of runtime.

This iterative strategy was used so that single-sided power spectra could be calculated over rolling blocks of 5 minutes of runtime. The rolling, single-sided power spectrum was used to identify the dominant signal frequency resulting from each calcium signaling simulation. Double-sided power spectra were generated using MATLAB's Fast Fourier Transform function (fft) and then converted into single-sided power spectra.

The dominant frequency was calculated by taking the array containing the single-sided power spectrum and passing it through a maximum-value filter. This filter selected the two largest values in the frequency domain, and then compared the largest to the second largest. If the largest value was at least 25% greater than the second largest value, then the largest value was selected as the dominant frequency. If the largest value was not 25% larger than the second largest value, the filter produced an error message, indicating there was no dominant frequency.

D. Motion Conversion

The dominant frequency calculation was crucial for interfacing the engineered mammalian cells with the simulated robot. By parsing out the dominant frequency, we were able to convert a piece of information inherent in the oscillatory signal of the mammalian calcium network into a single-valued, analog piece of information. By taking this analog value, we could pass it through a set of filters that correspond to different robot behavior [6].

In principle, the filter that links the dominant frequency value to a discrete robot executable acts as a finite state machine. Furthermore, this finite state machine can be used to encode for any behavioral subroutine, sensing protocol, or mechatronic actuation, from the movement of wheels, to the positioning of manipulators. Here, we set a simple filter that commanded a robot to seek and move towards a cyan, circular target when the dominant frequency was above a threshold value, and seek a magenta, rectangular target when the dominant frequency was below a second threshold value. Between these thresholds, the robot was commanded to remain motionless, seeking neither target. The state machine for this robot's behavior is shown in Fig. 1(d).

III. Results

From our knowledge of the calcium-signaling pathway, we identified IP_3 as a tunable biological component that could be attenuated by the presence of shRNA, as shown by the green RNA loop in Fig. 4(a). By using the synthetic genetic construct presented in Fig. 3(a) in conjunction with the calcium pathway detailed in Fig. 4(a), we were able to simulate the effects of modulating the available $[IP_3]$ within the cytosol.

A. Parameter Sweep of [IP_3]

The results in Fig. 4(b) detail the nonlinear effects of $[IP_3]$ modulation on the calcium signal. When $[IP_3]$ is above a critical threshold, here characteristically depicted by $[IP_3] = 3.0\ \mu$M, the calcium signal holds at a steady value, elevated above baseline. However, upon lowering $[IP_3]$ below 2.0 μM, we observe periodic oscillations within the calcium signal, qualitatively characterized by peaks and periods of recovery. This characteristic feature is indicative of a nuanced frequency encoding mechanism, inherent within mammalian calcium signaling. Within Fig. 4(b), from time 6-12 min, we demonstrate this characteristic periodic oscillation by setting $[IP_3]$ to equal 1.5 μM.

Crucially, we are able to demonstrate that by producing shRNA, and thereby knocking down the concentration of IP_3, we are able to modulate the frequency of the resulting calcium signal. Once oscillations appear, decreasing the $[IP_3]$ further has the affect of decreasing the dominant frequency of the oscillation, and giving rise of a slightly higher peak calcium signal. From time 12-18m, we demonstrate this difference by lowering $[IP_3]$ to be equal to 0.5 μM.

In this characteristic example, it is crucial to note that by decreasing the concentration of $[IP_3]$ by a factor of three, we are able to attenuate the dominant frequency of calcium oscillations by a factor of close to two and half.

B. Parameter Sweep of [SERCA]

Fig. 4(b) demonstrates an ability to modulate the frequency of a mammalian calcium signals by producing shRNA that decreases the concentration of $[IP_3]$. In a similar manner, shRNA affords us the ability to selectively decrease the concentration of sarco/endoplasmic reticulum Ca^{2+}-ATPase, or SERCA within a cell. Fig 4(a) depicts this pathway with the orange RNA loop.

Starting with the basal levels of SERCA within the cytoplasm, denoted as $[SERCA]_0$, we can observe characteristic calcium signal oscillations, as shown in Fig. 4(c) from time 0–6 min. However, upon decreasing the cell's [SERCA], we observe an increase in the frequency of calcium oscillations, as shown in Fig. 4(c) from time 6–12 min. By decreasing the amount of available SERCA by 10% from basal levels, we see an increase of 68% of the dominant signal frequency.

Interestingly, unlike $[IP_3]$, [SERCA] has a minimal affect the amplitude of the calcium spikes as long as oscillations are present. However, when the value of [SERCA] is decreased by 20% from the basal state, there is a collapse in the oscillatory signal, and the calcium signal holds at a steady value. This steady signal is similar to the affect seen when there are elevated levels of $[IP_3]$.

Taken together, shRNA gives us an ability to selectively tune the oscillations of calcium signaling. By attenuating the concentrations of $[IP_3]$ and [SERCA], we have two knobs by which we can tune the calcium signal.

C. Cell-Robot Interface

As demonstrated by Fig. 4, shRNA give us an ability to modulate the dominant frequency of calcium signals produced by mammalian cells. By optically measuring this calcium signal, and passing it through thresholds that correspond to a finite state machine, shown in Fig. 1(d), we can experiment with robot behavior directly from simulated mammalian cells.

First, we chose a $[IP_3]$ value of 1.66 μM. By simulating the calcium signal with this $[IP_3]$ value, and linking it to

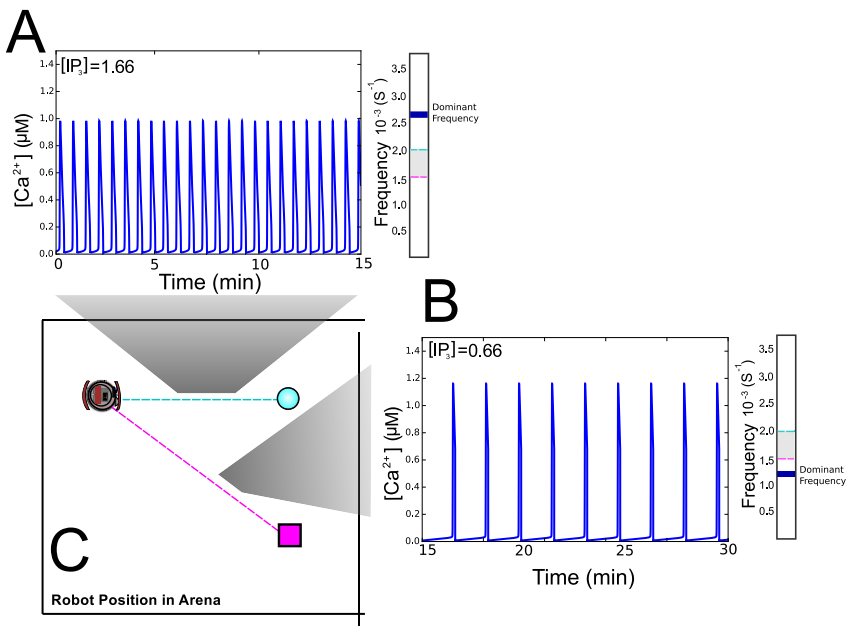

Fig. 5. Using Tunable Calcium Signals to Control Mobile Robotics with a Mammalian Cell. (a) By setting the [IP_3] value at 1.66 μM, we produce a calcium signal with a characteristic dominant frequency of 2.6×10^{-3} s^{-1} (b) By producing shRNA, we attenuated the [IP_3] value within the cell to 0.66 μM. This produced a calcium signal with a dominant frequency of 1.3×10^{-3} s^{-1}. (c) The dominant frequencies from Fig. 5(a) caused a simulated robot to seek and move towards the cyan circular target. The dominant frequencies from Fig. 5(b) caused a simulated robot to seek and move towards the magenta, rectangular target. These behaviors were controlled by the finite state machine presented in Fig. 1(d).

our rolling Fast Fourier Transform operator, and a robot simulation, we observed how a calcium signal could cause a robot to seek the cyan, circular target. This was because the calcium signal produced, shown in Fig. 5(a), had a dominant frequency above 2.0×10^{-3} s^{-1}. Under these conditions, the finite state machine, presented in Fig. 1(d), controlling robot subroutines dictates that the robot should seek and move towards the cyan, circular target. The robot's physical motion is shown in cyan within Fig. 5(c).

However, by inducing shRNA production that attenuated the levels of [IP_3] within the mammalian cell, we were able to simulate a calcium signal that had a dominant frequency significantly lower, as shown in Fig. 5(b). Under these conditions, an [IP_3] value of 0.66 μM produced a calcium signal with a dominant frequency of 1.3×10^{-3} s^{-1}. This dominant frequency was below the lower threshold of 1.6×10^{-3} s^{-1}, and as a consequence the finite state machine controlling robot behavior indicated that the robot should seek the magenta, rectangular target. The corresponding robot behavior for this signal is depicted in magenta in Fig. 5(c).

Thus, by modulating the value of [IP_3] within the mammalian cells, two distinct robot subroutines were enacted. By modifying levels of [SERCA] within the cell, similar results may be attained. Importantly, these locomotive subroutines serve as proxies for any mechatronic actuation, modification, or variation that a robot might enact. Crucially, we were able to show that by using calcium oscillation frequencies as an information packet, we were able to create a bridge linking mammalian cellular behavior with mechatronic systems.

IV. Discussion

In the work presented here, we designed and modeled an approach for using synthetic biology to couple mammalian calcium oscillations to mechatronic systems. While we used a mobile robot as a tractable mechatronic system, as noted, our system converts information encoded in biological oscillations and passes it to a finite state machine that could be used to encode for any behavioral subroutine, sensing protocol, or mechatronic actuation, from the movement of wheels, to the positioning of manipulators. In principle, this means that this approach could be extended to optogenetically link implanted engineered cells to external prosthetic behaviors.

Importantly, we based our system on a ubiquitous 2^{nd}-messenger system. This system regulates processes ranging from muscle contraction to fertilization to the rapid release of synaptic vesicles in neural firing, to the significantly slower phenotypic remodeling, and gene transcription observed in cardiac hypertrophy [8], [36]. It serves to relay the information from a primary signal sensed at the extracellular membrane to intracellular kinases. As a result of this 2^{nd}-messenger function, calcium is a critical regulator of multiple cellular functions [37], [38]. In some cases, calcium exhibits crosstalk with another major 2^{nd}-messenger, cAMP. In fact, the mathematical model we modified here was previously expanded to describe calcium crosstalk with cAMP molecules by Gorbunova and Spitzer [28]. Calcium signaling is also critical in mechanotransduction [39], [40] and can be robustly activated by both mechanical stimuli and chemical agonists [8], [41]–[45].

Because calcium's role is so varied and serves multiple purposes, tools that regulate this signal could have significant impact. Moreover, because the cell naturally has the capacity vary this signal across internal and external spatiotemporal scales, manipulating and leveraging part of the signal with the tools of synthetic biology may have less deleterious effects to overall cell physiology. As a result, the tools and

approaches we have presented here may outline a new way to robustly link synthetic biology, mammalian cell physiology, and mechatronic systems.

ACKNOWLEDGMENT

The authors thank R. S. Schwartz for helpful comments during the conceptualization of the calcium modeling approach. The authors also thank S.-H. Paek for help with figure schematics.

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