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NRL/6910/MR--2021/1

Multimodal Remote Control of Gene Expression

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February 10, 2021

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REPORT DOCUMENTATION PAGE

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1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
10-02-2021	NRL Memorandum Report	October 2019 – October 2020

4. TITLE AND SUBTITLE

Multimodal Remote Control of Gene Expression

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
NISE

6. AUTHOR(S)

Tanya Tschirhart

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER
N2V6

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Naval Research Laboratory
4555 Overlook Avenue, SW
Washington, DC 20375-5320

8. PERFORMING ORGANIZATION REPORT NUMBER

NRL/6910/MR--2021/1

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Naval Research Laboratory
4555 Overlook Avenue, SW
Washington, DC 20375-5320

10. SPONSOR / MONITOR'S ACRONYM(S)

NRL-NISE

11. SPONSOR / MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

DISTRIBUTION STATEMENT A: Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

Karle Fellowship

14. ABSTRACT

The objective of this research is to integrate both light-driven (optogenetic) and electronically-driven (electrogenetic) control of gene expression in bacterial cells for precise spatiotemporal control of multiple genes. Non-chemical modalities such as electronics and light offer more precise spatiotemporal control of genes than traditional chemical methods and can be used to remotely control cell function. There are a number of optogenetic and electrogenetic systems that have been developed that work well in bacteria that were evaluated for a multimodal system. Precisely controlling when multiple genes in a pathway turn on and off can be a key technology in enabling optimal biosynthesis of materials and molecules of interest.

15. SUBJECT TERMS

Synthetic biology, Genetic engineering, Bioelectronics
Biotic-abiotic interface, Biotic-abiotic communication

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			Tanya Tschirhart
Unclassified Unlimited	Unclassified Unlimited	Unclassified Unlimited	Unclassified Unlimited	9	**19b. TELEPHONE NUMBER** *(include area code)* (202) 404-2360

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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NRL Memorandum Report

Multimodal remote control of gene expression

Tanya Tschirhart (NRL, CBMSE, Code 6910)

Multimodal remote control of gene expression
Tanya Tschirhart

Objective

The objective of this research is to integrate both light-driven (optogenetic) and electronically-driven (electrogenetic) control of gene expression in bacterial cells for precise spatiotemporal control of multiple genes. Non-chemical modalities such as electronics and light offer more precise spatiotemporal control of genes than traditional chemical methods and can be used to remotely control cell function. There are a number of optogenetic and electrogenetic systems that have been developed that work well in bacteria that were evaluated for a multimodal system. Precisely controlling when multiple genes in a pathway turn on and off can be a key technology in enabling optimal biosynthesis of materials and molecules of interest. This project benefits both biohybrid systems and synthetic biology. High fidelity information flow across the biotic/abiotic interface necessitates a signaling mode that does not depend on diffusible chemicals alone, and takes advantage of abiotic signaling modalities such as light and current flow. A fieldable biohybrid device that controls cells using those modalities will not only be more robust, but also allow for greater overall complexity with the combined biological and non-biological capabilities. Biohybrid implants could similarly benefit from this research. Non-chemical inputs that function together also present a significant advancement in synthetic biology – allowing greater spatiotemporal accuracy in cellular perturbation and programming. These circuit and gate building blocks will be used to design more complex systems and programs in cells.

Technical Approach

Standard DNA design and assembly methods were used to construct one or two vector genetic circuits for either optogenetic, electrogenetic, or multimodal induction. *Escherichia coli* cells were used to test the circuit and gate function. Specially-designed setups and devices were used to expose cells to light and electronic signals that induce the circuits, and cell fluorescence was measured using flow cytometry or fluorescent microscopy.

Methods

Standard DNA assembly methods (restriction cloning, Gibson Assembly, MoClo) were used for plasmid construction, and were then transformed into chemically-competent *Escherichia coli* cells, NEB10β. The fluorescent reporter proteins sfgfp (green fluorescent protein) and RFP (red fluorescent protein) were used as outputs for the electrogenetic and optogenetic systems, respectively. Cell fluorescence was measured using a flow cytometer (BD Accuri C6), fluorescent microscope (Olympus BX53), or a plate reader (Spectramax M3). For optogenetic induction, the previously-reported light plate apparatus (LPA)[1] was used to program various intensities of blue light exposure to cells in different wells of a 24-well plate. For electrogenetic

Manuscript approved February 4, 2021.

induction, an in-house potentiostat-controlled three-electrode two-vessel setup was used to electronically generate hydrogen peroxide in-situ in a cell culture. These are shown in Figure 4. A new setup that combines blue LED light and the in-house electrogenetic setup was designed for testing the multimodal system.

Results

Design and initial testing of electrogenetic system for blue-light interference

The electrogenetic circuit responds to either solution-based on electronically-generated hydrogen peroxide. The OxyR protein is produced from the *OxyR* gene under the control of the constitutive strong promoter *proD*. Peroxide oxidizes –SH moieties on OxyR, forming disulfide bonds and changing the protein's conformation so that it binds to and promotes transcription from the *PoxyS* promoter[2, 3]. In our circuit, this results in production of the sfgfp protein and visible green fluorescence of cells (Figure 1a,b). We have previously characterized the function of this system in detail, and know that between 25-50 μM peroxide results in maximum sfgfp production within about 45-60 minutes[4]. For our multimodal gate, we investigated the effect of the application of blue light on electrogenetic gate function. It is known that bright light can generate various radicals or oxidants in solution. We used the LPA apparatus to doubly expose cells to both peroxide and various blue light intensities, the results of which are seen in Fig1c. Our initial results show that there may be some attenuation of the circuit with blue light exposure. Additional combinations of peroxide and blue light will need to be tested, as will different cell concentrations, lengths of exposure, and media, in order to minimize blue light effects on the electrogenetic response.

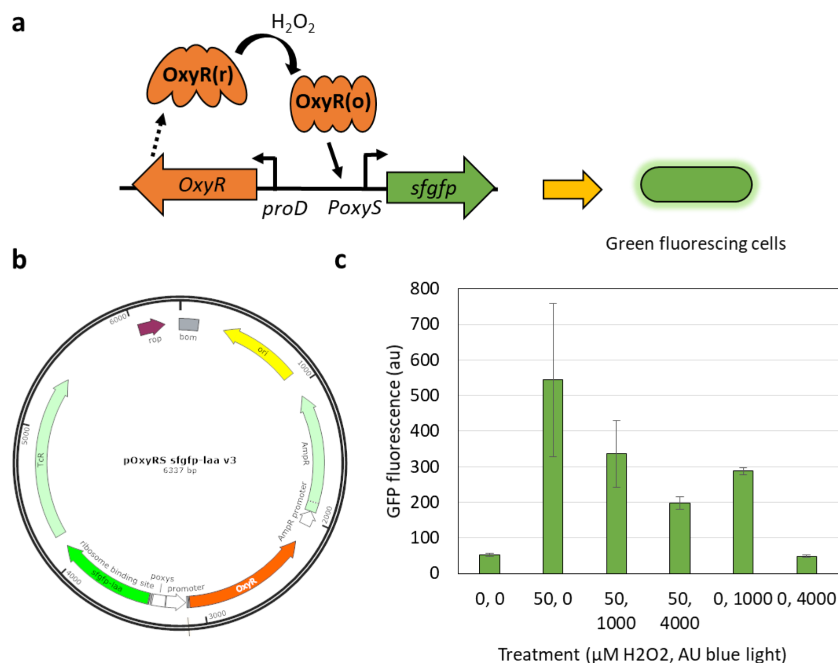

Figure 1. a. Diagram depicting the OxyR/PoxyS-based electrogenetic circuit. **b.** Plasmid map containing the electrogenetic circuit in a. **c.** Initial results investigating the effect of the combination of various peroxide concentrations in addition to various blue light intensities on the function of the circuit in a, as measured by GFP fluorescence.

Design and initial testing of optogenetic system for peroxide interference

The optogenetic circuit responds to constant application of blue light at 450 nm. The EL222 protein is produced from the EL222 gene under a constitutive promoter. Blue light activates the LOV domain on EL222, which promotes transcription from the pEBlind promoter of the rfp gene and production of the RFP protein and visible red fluorescence of cells (Figure 2a, b). This optogenetic has previously been characterized and responds well to application of blue light of >2 W/m^2 [5]. For our multimodal gate, we investigated the effect of addition of 50 µM peroxide for 45 minutes. We used the LPA apparatus to expose cells with the optogentic gate to both peroxide and different light intensities. Red fluorescence was measured after 60 minutes of exposure. Our initial results show inconsistent RFP production with increasing light intensity both with and without peroxide (Figure 2c). We will need to further optimize the testbed for consistent increased RFP production with increasing light intensity by changing the media, test duration, light intensities, and peroxide levels.

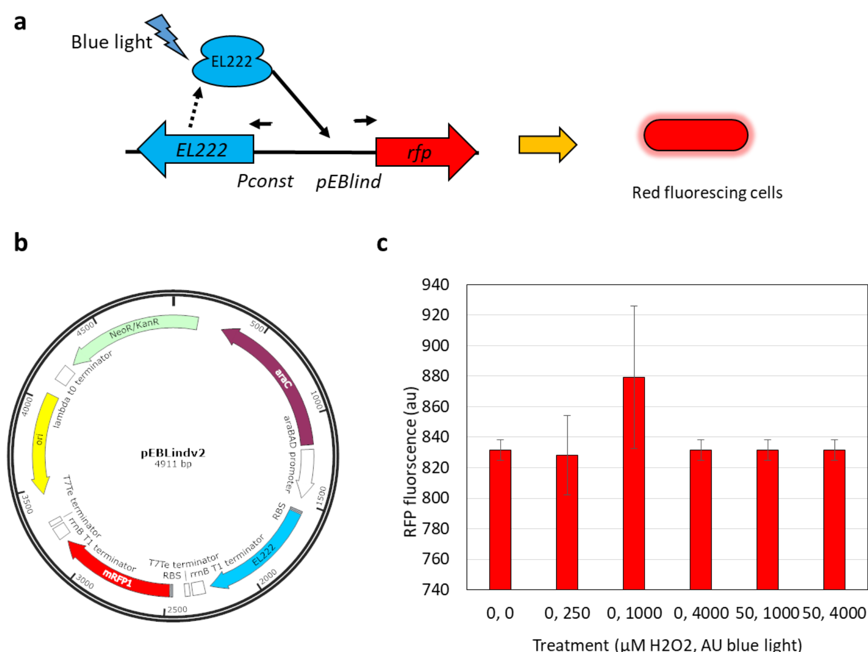

Figure 2. a. Diagram depicting the EL222/pEBlind-based optogenetic circuit. **b.** Plasmid map containing the optogenetic circuit in a. **c.** Initial results investigating the effect of the combination of various blue light intensities and peroxide on the function of the circuit in a, as measured by RFP fluorescence.

Multimodal genetic circuit design

The initial design for the multimodal AND gate is presented in Figure 3a. We will use the OxyR/PoxyS and EL222/pEBlind systems in addition to the split T7-RNA Polymerase AND gate. The split T7-RNA Polymerase AND gate has previously been well characterized and offers customization in terms of the pT7 promoter variants and thus different response functions[6]. This is important since the EL222 and OxyR responses will be completely novel when tested in this context. The AND gate also offers a fast and "concentration"-based response based on the two inputs, and we have previously worked with this gate in our lab. In the proposed design, a

fluorescent protein such as sfgfp will indicate the final output levels, which will be controlled by both peroxide and blue light. We expect that higher blue light intensities in combination with high peroxide levels will result in the highest activation and sfgfp production (mock data in Figure 3b). This is based on our previous results and those found in literature. Actual induction levels will depend on results from thorough testing of the electrogenetic and optogenetic circuits separately, and take interference into account.

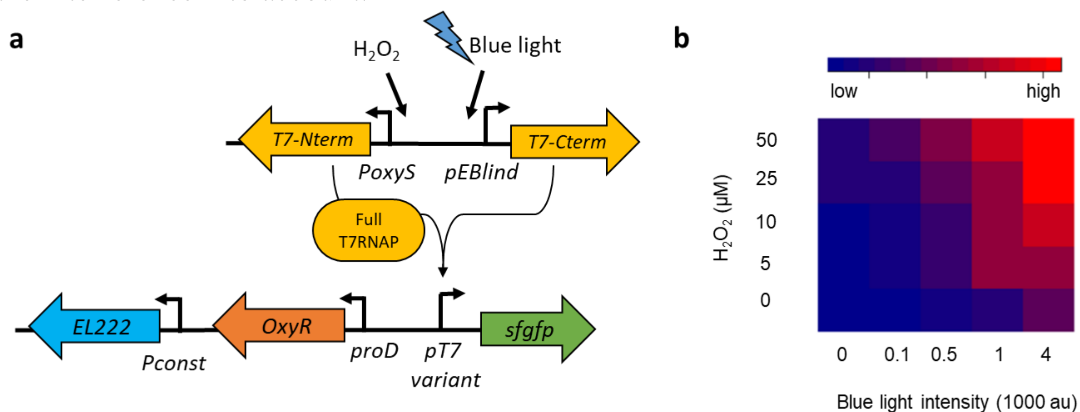

Figure 3. a. Diagram depicting the T7-RNA Polymerase-based AND gate based on peroxide and blue light inputs. **b.** Mock data showing the type of expected results (sfgfp production) upon induction with various levels of peroxide and blue light.

Device design for multimodal circuit testing

To induce cells with non-chemical stimuli, specialized devices were designed in order to accommodate electronic and light interfacing with aqueous cell cultures.

For optogenetic induction, a specialized light plate apparatus (LPA) was built, based on previous reports[1] (Figure 4a). This device allows the placement of a 24-well culture plate on top of a circuitboard-controlled LED array. This is housed in a 3D-printed enclosure and allows for the whole device to be placed in a shaking incubator, which is necessary for optimal cell growth and protein production. We have used the LPA extensively in our lab for optogenetic experiments. The light intensity, duration, and even trends over time can be controlled through a web-based program and loaded on-board through an SD card.

For electrogenetic, we have a specialized in-house setup that allows the testing of single cultures of cells at a time (Figure 4b). Unlike optogenetics though, electrogenetic induction (peroxide generation) can be brief and intermittent, in order to produce the maximal response. A potentiostat is used to control the voltage at a gold electrode immersed in the cell culture. This allows in-situ charge-based peroxide production (typically 5 minutes or less). A counter electrode in a separate vessel is connected by a salt bridge. Typically, after the initial electrogenetic induction, cells are removed from the induction vessel and cultured in a shaking incubator.

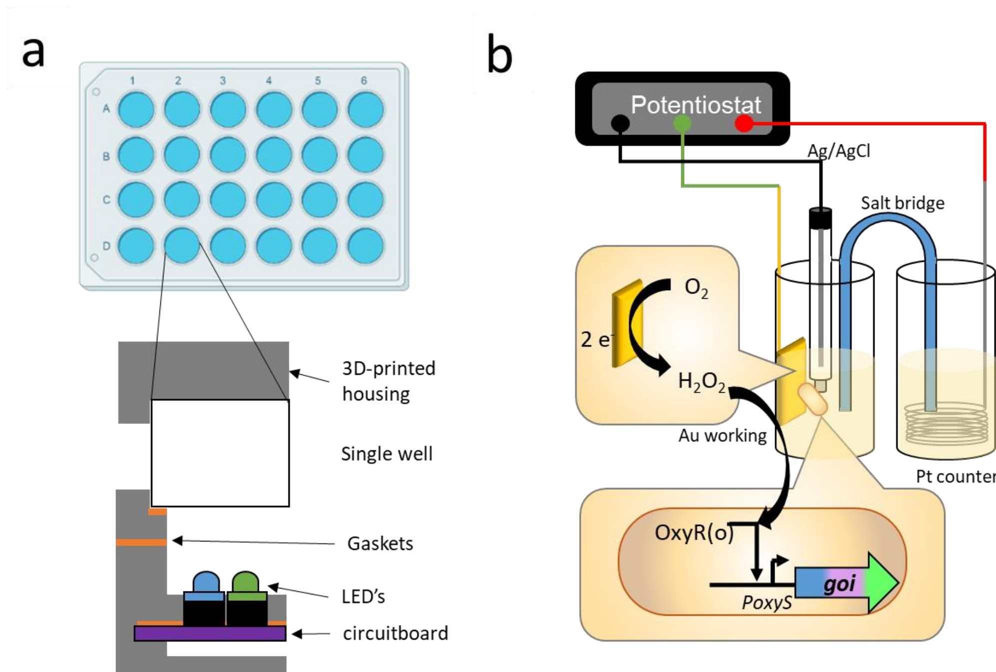

Figure 4. a. Diagram of the 24-well Light Plate Apparatus. **b.** Diagram depicting the setup used for electrogenetic induction of cells.

For a multimodal device, we need to both apply intermittent electronic stimuli as well as continuous blue light stimuli. The electrogenetic induction amplitude is controlled by the signal length, mixing of the culture (oxygenation), cell amount, media, electrode size, and voltage. The optogentic induction amplitude is controlled by media, oxygenation of the culture, LED light intensity, and length of blue light exposure to some degree. After thorough testing in the LPA with exogenously-added peroxide and blue light induction, we will first test cells for electrogentic induction in the original setup and then the removal of those cells and their placement into the LPA for light induction. This will allow for a sequential induction of the electrogenetic and then optogenetic components. Next, a setup such as one depicted in Figure 5 will be used. The basis will be the electrogenetic setup with a potentiostat, with the addition of a programmable LED that will shine underneath the culture tube at the same time. This will allow induction of both optogenetic and electrogenetic systems concurrently. Based on the results from this setup, a device that integrates electrodes into something similar to the LPA will be designed and used to test multiple samples at once. This will allow for efficient characterization of the multimodal AND gate and open doors for investigating other gates and circuits that are induced by both light and electronics.

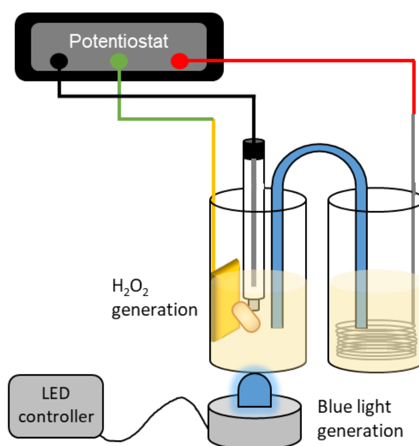

Figure 5. Diagram depicting a setup for concurrent exposure of cell culture to electronically-generated peroxide and LED-based blue light.

Summary

Non-chemical stimuli can offer many benefits over chemical stimuli when it comes to activating engineered cell function. Light-based (optogentic) and electronics-based gene induction systems offer the greatest promise and flexibility in that they are fast, programmable, and have been used in a number of cells and situations. This project explored a novel multimodal gene induction system that would use both optogenetic and electrogenetic components to build a genetic AND gate to control cell behavior. We found that further experiments will be necessary to fully take advantage of this system and have designed both the genetic AND gate and a multimodal device/setup for the necessary inductions. This project benefits both biohybrid systems and synthetic biology and S&T focus areas such as Autonomy & Unmanned Systems, Power & Energy, and Warfighter Performance. High fidelity information flow across the biotic/abiotic interface necessitates a signaling mode that does not depend on diffusible chemicals alone, and takes advantage of abiotic signaling modalities such as light and current flow. A fieldable biohybrid device that controls cells using those modalities will not only be more robust, but also allow for greater overall complexity with the combined biological and non-biological capabilities. Biohybrid implants could similarly benefit from this research. Non-chemical inputs that function together also present a significant advancement in synthetic biology – allowing greater spatiotemporal accuracy in cellular perturbation and programming. These circuit and gate building blocks will be used to design more complex systems and programs in cells.

Publications

Refereed:

1. Terrell J**, Tschirhart T,** Jahnke J, Stephens K, Liu Y, Tsao CY, Dong H, Hurley MH, Pozo M, McKay R, Tsao CY, Wu HC, Vora GJ, Payne GF, Stratis-Cullum D, Bentley B. "Bioelectronic control of a microbial community using surface-assembled electrogenetic cells to route signals". Accepted.

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