AWARD NUMBER: W81XWH-20-1-0101

TITLE: Can Altered Bioenergetics Drive Antibiotic Persistence in Low-Oxygen Cond

PRINCIPAL INVESTIGATOR: Arnab Mukherjee

CONTRACTING ORGANIZATION: University of California, Santa Barbara

REPORT DATE: MARCH 2021

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
MARCH 2021	ANNUAL	03/01/2020 - 02/28/2021

4. TITLE AND SUBTITLE

Can Altered Bioenergetics Drive Antibiotic Persistence in Low-Oxygen Conditions?

5a. CONTRACT NUMBER
W81XWH-20-1-0101

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Arnab Mukherjee

E-Mail: arnabm@ucsb.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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

UNIVERSITY OF CALIFORNIA, SANTA BARBARA
OFFICE OF RESEARCH
3227 CHEADLE HALL
SANTA BARBARA CA 93106-0001

8. PERFORMING ORGANIZATION REPORT NUMBER

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

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

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

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The goal of this project is to engineer the first set of anaerobically visible fluorescent ATP indicators and apply them to examine how intracellular bioenergetics differs between aerobic and anaerobic states for bacterial cells treated with antibiotics. While several ATP sensors are currently available, these require oxygen to produce fluorescence. To address this issue, we propose to develop our sensors based on fluorescent proteins that do not require oxygen. In this project period, we established the key conceptual and methodological framework for constructing these sensors. Significant findings include: (1) identification of 14 viable positions in the reporter backbone where binding domains may be inserted to modulate fluorescence properties based on the principle of allostery (2) establishing a linker mutagenesis and screening pipeline for high-throughput testing of candidate sensor constructs for ATP-induced changes in fluorescence (3) validation of methods for titrating intracellular ATP without causing changes in overall reporter fluorescence (4) identification of terminal amino acids that may be truncated to shorten overall size of the reporter construct by 12 amino acids (potentially making this the smallest reporter gene currently available).

15. SUBJECT TERMS

Fluorescent reporters, genetically encoded ATP sensors, flavin-binding fluorescent proteins, anaerobic imaging, anaerobic microorganisms, antibiotic response, persister

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	13	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION

The goal of this project is to engineer the first set of anaerobically visible fluorescent ATP indicators and apply them to examine how intracellular bioenergetics differs between aerobic and anaerobic states for bacterial cells (*E. coli*) treated with antibiotics. While several ATP sensors are currently available, these universally require oxygen to produce fluorescence. To address this issue, we will develop our sensors based on flavin-binding proteins (*e.g.*, CreiLOV, iLOV, *etc*.) that do not require oxygen to produce fluorescence. Our work will therefore yield a new technology that will be broadly useful for basic research in anaerobic microorganisms in addition to applying this technique to study how antibiotics affect ATP concentrations and viability in anaerobically cultured *E. coli* cells.

2. KEYWORDS

Fluorescent reporters, genetically encoded ATP sensors, flavin-binding fluorescent proteins, anaerobic imaging, anaerobic microorganisms, antibiotic response, persister cells

3. ACCOMPLISHMENTS

What were the major goals of the project?

Aim 1: Engineer an oxygen-independent, genetically encoded ATP sensor

- Major Task 1: Engineer a 4-HT-inducible allosteric fluorescent sensor based on AFP
- Major Task 2: Engineer an ATP-inducible fluorescent reporter based on the AFP- ERLBD fusion
- Major Task 3: Use AnViAS to detect ATP in bacteria treated with various chemical and environmental modulators of metabolism

Aim 2: Image/quantify ATP distribution in antibiotic-treated bacteria.

- Major Task 4: Optimize time lapse fluorescence imaging for single cell studies
- Major Task 5: Time lapse fluorescence imaging of bacterial bioenergetics and persistence in different oxygen levels

What was accomplished under these goals?

In this project period, we made several significant advances towards completing Major Task 1 and initiating work on Major Tasks 2 and 3. A summary of main outcomes is enumerated below, and full details are provided in subsequent sections.

- We completed our initial search for allosteric hotspots throughout the reporter (iLOV, a brighter homolog of our previously published CreiLOV reporter) backbone comprising 108 unique positions. From this work, we identified a loop region ($_{92}$DQKG$_{95}$) as the most viable location for developing the proposed ATP biosensors. In principle, this location should also allow for constructing other sensors, thereby establishing the first prototype for developing genetically encoded indicators for research in anaerobic microbiology.
- We established a robust methodology to decrease intracellular ATP by ~ 3 orders of magnitude without affecting reporter fluorescence. This technique provides a platform to rapidly screen whole cell libraries for ATP-dependent changes in fluorescence, while avoiding the need for cell lysis. In addition, the cyanide and arsenate dose-response profiles generated here will be used to benchmark our sensors by validating fluorescence-based measurements of intracellular bioenergetics under defined perturbations.

- We developed a single-step Gibson assembly procedure to generate 3-5 (randomized) amino acid linkers connecting the ATP-sensing domain (ATP-ε) to the reporter backbone at any location. Using this method, we developed 14 distinct linker libraries corresponding to insertions of ATP-ε at various positions in the reporter backbone. These libraries are currently being screened to identify variants with optimal ATP response properties.
- Finally, we optimized medium-throughput screening via multi-well whole cell fluorescence assays. We applied this platform to test 180 clones from 4 linker libraries, discovering some promising variants along the way. We also developed methods to massively increase our screening capacity with fluorescence activated cell sorting (FACS). Using this approach, we were able to sort 236,948 individual clones from 4 linker libraries with the goal of enriching for most fluorescent variants, which will be subsequently tested for their response to ATP perturbations.

In the following sections, we provide a detailed description of major research activities, significant results, important findings, and key outcomes pertaining to this project period.

Towards engineering ATP biosensors: searching for allosteric hotspots in iLOV

Our goal in this project period was to identify viable positions in the iLOV backbone where an ATP binding domain (or more generally, any ligand binding domain) could be inserted to allosterically modulate fluorescence signals without diminishing peak intensity. We implemented two approaches towards this goal:

Approach 1. In the first approach, we constructed protein chimeras by inserting the estrogen receptor ligand binding domain (ERLBD) at various positions in iLOV. ERLBD was chosen as a suitable binding motif to test for allostery based on the ability of this domain to convert from an open to a closed state in the presence of a cognate ligand, 4-hydroxytamoxifen. Our aim was to identify insertion sites in iLOV that satisfy two criteria: (1) amenability to incorporation of ligand-binding motifs without irreversible loss of fluorescence (2) ability to allosterically couple ligand-binding to an increase in iLOV fluorescence. We completed the construction of 108 individual clones, expressed them in *E. coli*, and tested variants for an increase in fluorescence (δF/F > 1) following overnight incubation with 4-hydroxytamoxifen. Key results from these screens are summarized below:

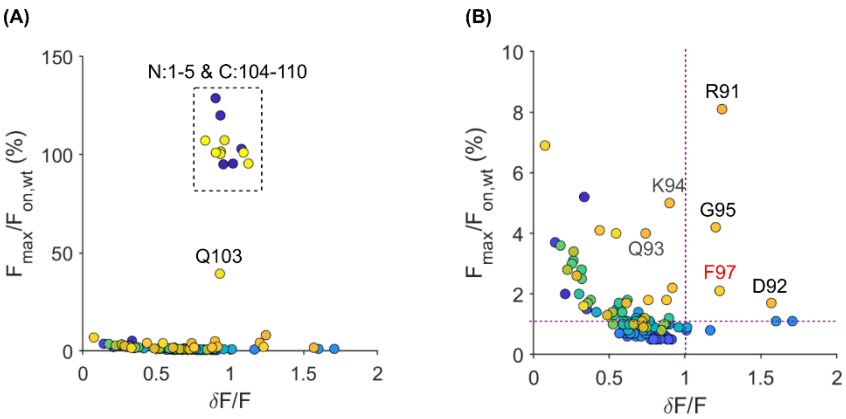

Figure 1. Library screening of ERLBD-iLOV chimeras to search for allosteric hotspots. (A) Testing results for all 108 chimeras. N and C terminal insertions that do not perturb fluorescence are boxed. (B) Expanded view of weakly-fluorescing variants. Positions of interest in the iLOV backbone are indicated.

1. 12 constructs involving insertions at the N and C termini of iLOV were found to retain fluorescence close to that of native iLOV. However no ligand-induced change in fluorescence was observed for these constructs (δF/F ~ 1). (**Fig. 1A**)

2. A handful of constructs (7/108) was found to exhibit a moderate increase in fluorescence (*i.e.*, δF/F > 1) in response to saturating amounts of ligand (**Fig. 1B**). Of these, 3 constructs have negligible peak fluorescence (~ 1 % that of native iLOV), while a fourth construct (insertion before F97) was found to exhibit high variability. The remaining 3 constructs, involving insertions before R91, D92, and G95 exhibit δF/F > 1 without incurring drastic losses in peak intensity. **These sites represent the most promising locations for engineering allosteric activity in iLOV**.

Approach 2. In the second approach, we relied on the crystallographic information identify potential positions for engineering allosteric function in iLOV. These positions were selected based on multiple criteria including (1) location within protein loops (2) low sequence conservation among other flavin-binding fluorescent proteins (3) high crystallographic B-factors. We identified 14 promising sites, which we grouped into 4 ranked tiers (**Fig. 2**). Notably, amino acids in tier 1 ($_{92}$DQKG$_{95}$) overlap closely with the residues identified in the ERLBD-scanning experiments above, reinforcing their amenability for allosteric coupling with ligand-binding motifs.

The above experiments allowed us to predict that the $_{92}$DQKG$_{95}$ loop in iLOV could serve as a viable position for developing an allosteric ATP indicator. To do so, one would need to (1) identify a suitable ATP-binding motif for engineering allosteric response (2) insert the motif at each of promising sites identified in our work here (**Fig. 2**) (3) optimize the composition and length of amino acid linkers connecting the ATP-binding motif to the iLOV backbone at each junction (4) develop a medium or high throughput screening pipeline to test linker libraries for a change in fluorescence in response to intracellular ATP perturbation. The remainder of our activities in this period focused on developing methods to accomplish these tasks.

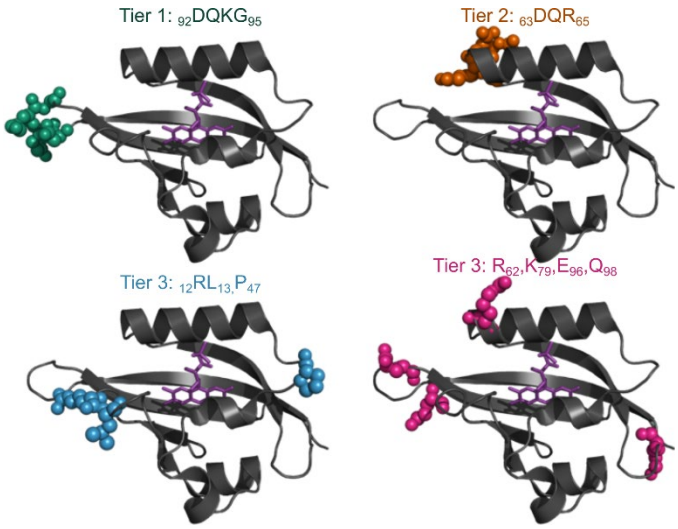

Figure 2. Structure-guided identification of viable sites in iLOV for engineering allosteric ATP biosensors.

ATP-binding motifs to allosterically modulate iLOV fluorescence

We surveyed 5 potential ATP-binding domains that were previously used in the field to develop GFP-based (thus, O_2 dependent) ATP biosensors (**Table 1**). These domains are derived from the ATP-ε subunit of *Bacillus sp*. and differ in their relative sensitivities to ATP. From these domains, we selected the *B. subtilis* ATP-ε subunit as our sensing motif, based on its ability to response to ATP at low milimolar concentrations, which is within the dynamic range of our bacterial ATP perturbation methodology.

Table 1. Binding motifs for allosterically coupling ATP to changes in iLOV fluorescence

Sensor	ATP binding domain N terminal component	ATP binding domain C terminal component	K_D (mM) (25 °C)	K_D (mM) (37 °C)
ATeam 1.03	*B. subtilis*	*B. subtilis*	~0.6	3.3
ATeam 3.10	*Bacillus sp. PS3*	*Bacillus sp. PS3*	–	0.0074
QUEEN-NA	*B. subtilis*	*B. subtilis* R122K R126K	non-binding	
QUEEN-2m	*B. subtilis*	*Bacillus sp. PS3*	4.5	2
QUEEN-7μ	*Bacillus sp. PS3*	*Bacillus sp. PS3*	0.0072	0.014
This study	*B. subtilis*	*B. subtilis*	TBD	TBD

Constructing iLOV chimeras incorporating ATP-ε inserts

Based on the work described above, we developed chimeric constructs incorporating the *B. subtilis* ATP-ε subunit at each of the 14 positions identified in Tiers 1-4 (**Fig. 2**). We incorporated these constructs in a modular low-copy plasmid (pJUMP) allowing their expression from a strong promoter (BioBricks J23100). *Throughout this project, these (**Table 2**) constructs will serve as our parent molecules for engineering allosteric ATP sensors using linker mutagenesis coupled with high throughput screening for fluorescence changes in response to ATP perturbation*.

Table 2. iLOV-ATP-ε constructs for allosteric testing by linker mutagenesis

Controls	Tier 1	Tier 2	Tier 3	Tier 4
pJUMP-MT (empty vector)	iLOV-ATPε-D92	iLOV-ATPε-D63	iLOV-ATPε-R12	iLOV-ATPε-R62
pJUMP-iLOV	iLOV-ATPε-Q93	iLOV-ATPε-Q64	iLOV-ATPε-L13	iLOV-ATPε-K79
	iLOV-ATPε-K94	iLOV-ATPε-R65	iLOV-ATPε-P47	iLOV-ATPε-E96
	iLOV-ATPε-G95			iLOV-ATPε-Q98

Developing methods for intracellular ATP perturbation

Our next goal was to develop a method for decreasing intracellular ATP from mM to low μM concentration, which would allow us to screen iLOV-ATP-ε linker libraries for allosteric activity. At the same time, we would need to ensure that ATP perturbation by itself does not cause any changes in iLOV

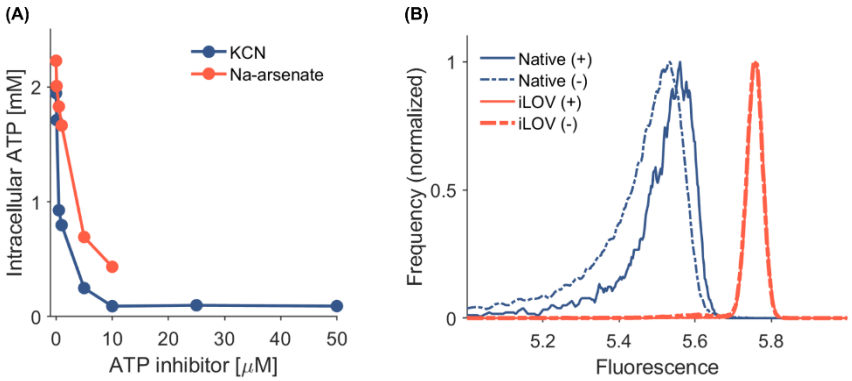

Figure 3. ATP perturbation in *E. coli*. (A) KCN and sodium arsenate dramatically lower intracellular ATP concentration without (B) significantly affecting iLOV fluorescence.

fluorescence, which would confound our fluorescence-based assays. We tested two methods to reduce intracellular ATP in *E. coli* – one using potassium cyanide and the other, sodium arsenate**.** Out of these methods, the former technique was found to dramatically lower intracellular ATP (**Fig. 3A**) without any noticeable effect on overall fluorescence in native or iLOV-expressing cells (**Fig. 3B**). *Moving forward, we will use potassium cyanide challenge as our perturbation methodology for lowering intracellular ATP in preparation for the screening experiments*.

Construction and medium-throughput screening of linker libraries

Linker mutagenesis. A modified Gibson assembly procedure was used to concurrently incorporate the ATP-ε motif at selected locations (**Table 2**) as well as randomize the composition and length of each linker connecting the motif to iLOV. In particular, Gibson primers were designed to contain three distinct sections: a 5' region that is complementary to the ATP-ε domain, a central region containing 3 – 5 degenerate codons (NNK for forward primers, MNN for reverse primers) to introduce randomized 3 – 5 amino acid linkers at each junction, and a 3' region that is complementary to the vector backbone at each junction of the corresponding insertion site. Primers harboring 3, 4, and 5 degenerate codons were mixed in a ratio of 1:32:1024 to ensure that linkers of all lengths are represented with equal probability in the randomized library. The resultant vectors were sed to transform *E. coli* cells in preparation for ATP perturbation and screening.

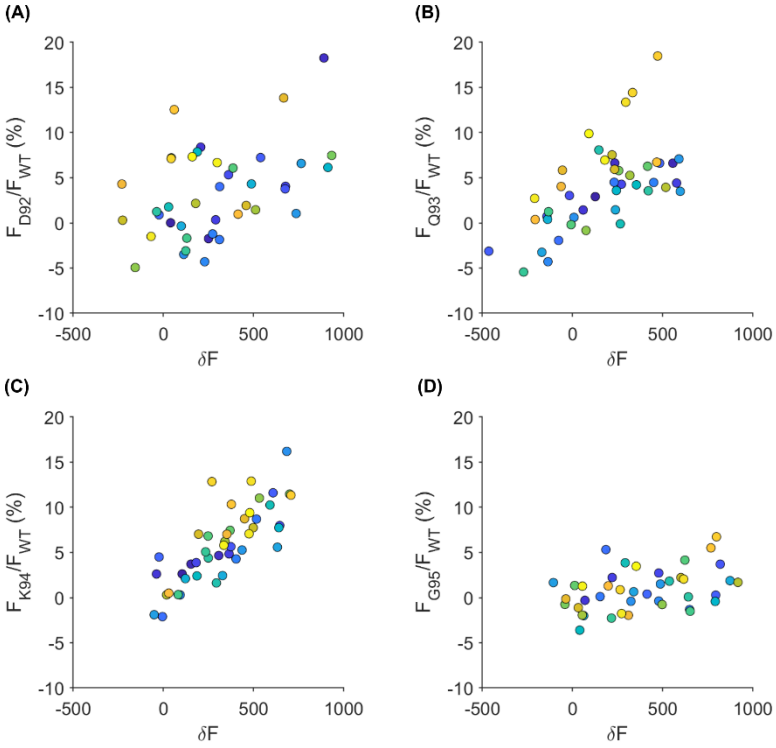

Figure 4. Multi-well screening of $_{92}$DQKG$_{95}$ linker libraries for ATP-induced changes in fluorescence. N = 45 individual clones per library were tested for changes in fluorescence (δF) triggered by ATP depletion using potassium cyanide.

Screening. *E. coli* cells expressing linker libraries were grown in multi-well plates and treated with potassium cyanide (as described in Section A.4) to reduce intracellular ATP. We acquired whole-cell fluorescence measurements in the on and off (*i.e.*, ATP-deficient) states and calculated the net ATP-triggered change in fluorescence (δF). Using this procedure, we screened 180 colonies (**Fig. 4**) from linker libraries corresponding to the $_{92}$DQKG$_{95}$ loop (**Fig. 2**), identifying several promising clones exhibiting significant δF/F without major loss of fluorescence loss. These variants have been submitted for sequencing and the first set of results is enumerated below (**Table 3**).

Table 3. Promising linker variants from multi-well screening of $_{92}$DQKG$_{95}$ libraries

Site	5' linker	3' linker	F/F_{WT} (%)	δF
D92	VWGSL	TEGYL	9.78	627
D92	PRSMA	YYAEL	6.08	924
G95	QGRYH	SQPWS	2.40	1173

Developing a high-throughput approach for testing linker libraries

During the course of this study, we realized the need to increase our screening capacity in order to search through a larger diversity of linker compositions for their ability to confer allosteric function in iLOV. To this end, we are currently in the process of developing methods for bacterial fluorescence activated cell sorting (FACS) with the goal of testing > 10^4 (ideally, 10^5) clones from each linker library for ATP-induced changes in iLOV fluorescence. This requires a corresponding increase in the size of linker libraries obtained after Gibson assembly, leading us to use electroporation (6.811 x 10^5 transformants/reaction) instead of chemical transformation (2.2 x 10^3 transformants/reaction) as the optimal method for preparing libraries moving forward.

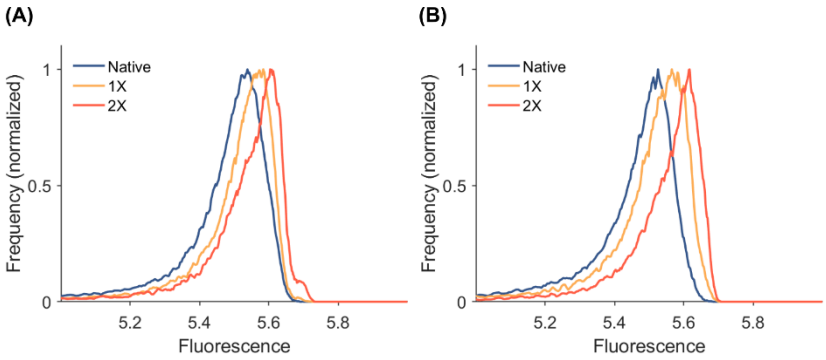

Figure 5. FACS-based screening of linker libraries for ATP-induced changes in fluorescence. Initial efforts were directed at optimizing enrichment of bright linker variants in (A) D92 and (B) Q93 libraries, prior to cyanide induced ATP depletion.

What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?

The project has so far resulted in one publication, which is described below (Section 6).

What do you plan to do during the next reporting period to accomplish the goals?

- Our immediate goal is to optimize FACS-based screening of $_{92}$DQKG$_{95}$ linker libraries to identify ATP responsive mutants via 2 separate rounds of sorting involving positive sorting for fluorescent variants under conditions of physiological ATP followed by negative sorting to identify variants that lose fluorescence after cyanide-induced depletion of ATP. The main challenge we face now is the occurrence of several non-fluorescent variants in initial libraries from the 1st positive sort, likely due to *E. coli* cells (~ 1 µm) being substantially smaller than the narrowest chip (70 µm) currently available for sorting, which reduces selectivity. These non-fluorescent cells, if present in large numbers, can overwhelm

downstream, efforts to identify ATP-responsive mutants. We will attempt to remedy this problem in two main ways. First, initial libraries will be sorted multiple times (≥ 2 positive sorts) to enrich populations for fluorescent cells to the maximum extent possible. Second, the negatively sorted libraries will be taken through an additional round of sorting to identify clones that recover fluorescence once ATP levels are restored.

- After a viable ATP-responsive variant has been identified, our next goal will be to optimize it further by saturation mutagenesis of amino acids located near the optimized linker peptides at each junction. Alternatively, we may randomize the full construct by error-prone PCR and screen for improved variants using the FACS procedure optimized above. We believe that these additional rounds of mutagenesis and screening will be necessary to identify the best-performing construct for the antibiotic studies proposed in this work as well as enable our sensors to be broadly used to probe anaerobic bioenergetics.
- Our final goal will be to express these sensors in *E. coli* cells and examine single cell ATP distributions in aerobic and anaerobic conditions, in response to antibiotic treatment.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

The activities accomplished during this project period have laid the conceptual and methodological foundations for developing fluorescent biosensors that can be used to probe microbial bioenergetics in anaerobic (as well as aerobic) growth states. To our knowledge, the activities reported above represent the *first systematic approach towards of constructing allosteric biosensors based on flavin-binding fluorescent proteins (also known as LOV proteins)*. If our efforts in the remainder of this project prove successful, this will lead to a one-of-its-kind methodology for probing bioenergetics in anaerobic bacteria in addition to revealing new information on the interplay between energetics and antibiotics. In addition, the paradigm established here can (in principle) be extended to develop analogous sensors targeted towards any physiological analyte for which anaerobic sensors do not currently exist.

What was the impact on other disciplines?

In addition to serving as fluorescent reporters under anaerobic conditions, certain variants of flavin-binding LOV proteins are also widely used as optogenetic tools where the goal is to control biological processes using light. *In the course of this work, we realized that the methodology developed above could be extended to inventing a new class of optogenetic LOV constructs that are responsive to a cell's internal state (e.g., bioenergetic status).* This would then establish an entirely new paradigm in synthetic biology where light could be used to manipulate biological functions depending on the internal biochemical state of a cell. For instance, ATP-responsive optogenetic LOV proteins could be used to selectively activate kill switches in exhausted cells experiencing low levels of energy. This general principle can have wide-ranging impact in disciplines ranging from bioproduction to cell-based biomedicines. We are keen to build on the conceptual and methodological advances ensuing from this project to pursue the idea delineated here (pending successful securing of research funds).

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS

Changes in approach and reasons for change

We have not implemented any major changes to the workflow as originally proposed except for a few relatively minor modifications as noted below:

- Instead of optimizing linkers in the context of estrogen-receptor (*i.e.*, ERLBD) insertions, we have chosen to optimize linkers directly in ATP-ε bearing reporter constructs. The reason for this is that our initial ERLBD studies made it clear to us that iLOV fluorescence is highly sensitive to insertion of ligand-binding domains. In other words, insertions at most points in the iLOV resulted in irreversible loss of fluorescence (**Fig. 1**). This led us to hypothesize that linkers that work for ERLBD may not translate to corresponding ATP-ε constructs (**Table 2**). As a result, mutagenesis and screening are now performed directly on the ATP-ε constructs (**Figs. 4 – 5**) instead of the precursor ERLBD clones.
- For the same reasons as above, we chose to optimize linker length and composition by randomization rather than work with variations of fixed sequences such as flexible G_4S and/or rigid EA_3K repeats. *The basic underlying principle of optimizing allosteric constructs by linker mutagenesis, however remains unchanged from our original goals*.
- Instead of searching all 110 positions in the reporter backbone for allostery, we were able to perform our initial screens on 108 unique positions using the ERLBD scanning approach. The reason for this is that we were unable to successfully construct the remaining 2 ERLBD-iLOV chimeras despite multiple efforts.

Actual or anticipated problems or delays and actions or plans to resolve them

On account of COVID-19, all research activities in our lab (and campus) were completely suspended for a period of 3 months from March 19th to June 22nd in 2020. Between June 23rd and August 28th, our lab was permitted to function in a highly reduced capacity involving pre-scheduled 1 or 2-person shifts, restricted work timings, and night curfews, leading to very limited research activity overall (≤ 25 %) for an additional 2 months. From August 28th till date, we have been allowed to increase to 3-person shifts although the requirement for advanced scheduling and restricted work timings (*i.e.*, no research allowed from midnight – 8 AM) continue to remain in place. In addition, access to student offices remains closed and we are not allowed to have undergraduate researchers in the lab (our original goal was to recruit at least one undergraduate researcher to assist with sub-tasks in Aim 1 of this project). Under these conditions, we estimate that our research activity in the past 7 months has been at ~ 50 % of our normal (non-pandemic) throughput. Based on these estimates, the net loss in research activity in this period (due to the pandemic) corresponds to ~ 8.5 months (out of 12 mo.), although this estimate is only a very rough approximation. That said, we are still completely aligned with the original goals of this proposal and optimistic that we can expand and accelerate research further in the coming months as campus, county, and state policies are modified. Our optimism is also further reinforced by the significant findings described in this progress report.

6. PRODUCTS

Publications, conference papers, and presentations

Journal publications.

Anderson, N.T., Weyant, K.B., Mukherjee, A. Characterization of flavin binding in oxygen-independent fluorescent reporters. *AIChE J*. 66(12). (2020) doi: 10.1002/aic.17083 (acknowledgement of federal support: yes)

The above work characterizes basic characteristics of fluorescence response in iLOV and determines how this response may be modulated based on flavin-binding. This concept is related to the mechanistic principles underlying the development of allosteric ATP biosensors and is important for the characterization of sensor constructs as proposed in the SOW (Major Task 2).

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on this project?

Name	Nolan Anderson
Project Role	Graduate Student
Nearest person month worked	6
Contribution to project	Standardization of ATP depletion assays, structural studies of iLOV, linker mutagenesis and multi-well screening, preliminary FACS studies
Funding Support	UCSB Chancellor's fellowship, NIH

Name	Kang-Ching Fan
Project Role	Graduate Student
Nearest person month worked	3
Contribution to project	Initial screening experiments with ERLBD, structural studies
Funding Support	NIH

Name	Harun Ozbakir
Project Role	Postdoctoral Scholar
Nearest person month worked	6
Contribution to project	Establishing and optimizing procedures for reporter protein expression and purification (*e.g.*, ultrasonication, FPLC), insertion library experiments, establishing general protein engineering and library protocols (*e.g.*, linker mutagenesis, high efficiency electroporation), preliminary FACS optimization, provision of training to Graduate Student (above) in these procedures
Funding Support	

Name	Arnab Mukherjee
Project Role	PI
Nearest person month worked	1.2
Contribution to project	Overall supervision, data analysis & reporting
Funding Support	NIH, UCSB startup funds

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

In this period, the PI received two seed fundings on unrelated projects

- $12,711 from the UCSB Academic Senate Faculty Research Grant
- $90,233 from Institute of Collaborative Biotechnologies, Advanced Scientific Research Task Order, a University Administered Research Center (UARC)

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report

9. APPENDICES

Nothing to report