UCSB Academic Senate
Academic Senate Council on Research and Instructional Resources

Application for 2021-22 Faculty Research Grant

APPLICANT INFORMATION

NAME
Arnab Mukherjee

RANK
Assistant Professor

DEPARTMENT
Chemical Engineering

EMAIL ADDRESS
arnabm@engineering.ucsb.edu

PROJECT INFORMATION

PROJECT TITLE
Engineering bioluminescent sensors for sulfation imaging

Amount Requested
$16,139.90

The Department/Unit that will be receiving/administering the funds:

Financial Coordinator
Cristina Wilson

EMAIL ADDRESS
cbwils@ucsb.edu

DEPARTMENT
CHNE

Previous Funding Record for this Project
This is a new project that has not received previous funding support.

Subvention
No.

Past Funding Support from the Academic Senate for Other Projects
I have received funding support from the Senate within the past three fiscal years, and the progress/results and current balance are as described here:

Other Research Support
I have start-up funds.

CHNE departmental startup funds

I do have other extramural research support.

NIH R35 MIRA, DoD Discovery Award, and ICBT (all for projects completely unrelated to the work proposed here)

I do not have other research support.

Recent Publications
I have recent publications.

Use of Human Subjects
This project does not involve the use of human subjects.

This proposal is not a request for the Pearl Chase Funds.

Table of Contents

A. SIGNIFICANCE

A.1| Overview of scientific problem: probing sulfation physiology in living cells

Sulfation is a major pathway for metabolizing drug molecules[1] and controlling the activity of steroid hormones[2-3] and monoamine neurotransmitters[4-5]. Imbalances in sulfation have been associated with serious skeletal, metabolic, neurological, and endocrine disorders as well as impaired activity of certain drugs (*e.g.*, apomorphine, commonly used to improve motor function in Parkinson's disease)[6-11]. Yet, no technology exists to dynamically probe sulfation processes inside living cells. Existing methods to monitor sulfation largely rely on assays involving chromatography and mass spectrometry, which require cells to be lysed before a measurement can be made[12]. *If a technology could be developed to probe sulfation directly and dynamically inside living cells, it would have a transformative impact in many areas of biomedicine.*

A.2| Scientific goal: developing genetically encoded sulfation indicators (GESIs)

To address the clear and immediate need for live-cell sulfation sensors, in this project, we propose to develop the first genetically encoded indicator for probing sulfation events as they occur inside living cells. Over the past two decades, reporter genes have become established as one of the most powerful techniques for studying biological processes in living cells. Genetically encoded phosphorylation indicators for instance, have proven to be of great value in enabling several fundamental scientific breakthroughs, even aiding the development of novel therapies such as kinase inhibitors[13-14]. However, analogous reporters for sulfation do not currently exist. *Our long-range goal is to develop this capability by co-opting molecular components from luminous organisms, optimizing them by protein engineering, and genetically expressing them in cells to monitor sulfation noninvasively with bioluminescence.*

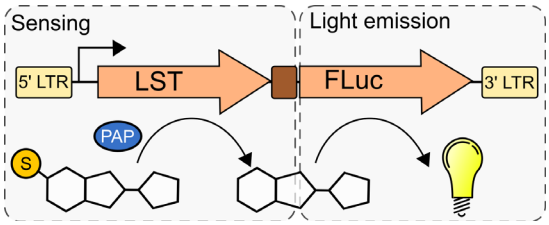

Figure 1. Principle underlying genetically encoded sulfation indicators (GESIs).

Our proposed approach for developing GESIs is based on connecting 3'-phosphoadenosine-5'-phosphate (PAP), a universal sulfation by-product, to light output, using components from firefly bioluminescence (**Figure 1**). Like most luminous species, fireflies express luciferase (FLuc), which can produce light by oxidizing luciferin. As a means to preventing auto-oxidation, luciferin is often stored as (non-luminogenic) sulfo-luciferin[15-18]. To produce light, sulfo-luciferin is first converted to luciferin by a luciferin sulfotransferase (LST), and PAP serves as a cofactor in this conversion[15, 18-20]. We therefore hypothesize that FLuc can be combined with LST and sulfo-luciferin to monitor sulfation by coupling PAP to light emission in 2 steps (**Figure 1**). In the first step, LST responds to PAP by converting sulfo-luciferin to luciferin. Next, luciferin molecules are oxidized by FLuc to produce light. Support from the Faculty Research Grant is thus requested to form and validate initial GESI constructs based on this principle, by pursuing the following aims.

Aim 1: Developing a sulfation sensor for bioluminescence imaging. To image intracellular PAP formed during sulfation, GESIs must be responsive in the 0.2 – 20 μM range of PAP concentrations[21]. In Aim 1, we will therefore pursue a directed evolution campaign to engineer new LST variants with optimum sensitivity and dynamic range.

Aim 2: Validating intracellular functionality of biosensors. The most promising LST identified in Aim 1 will be co-expressed with VLuc in a neuroblastoma cell line (SH-SY5Y) that exhibits high dopamine sulfation activity. After developing methods to deliver sulfo-luciferin in SH-SY5Y cells, we will establish proof-of-concept by using GESIs to monitor sulfation activity stimulated by treating cells with dopamine.

A.3| Scientific and biomedical significance

If successful, GESIs will provide the *first* technology to probe sulfation activities in living cells by combining the sensitivity, accessibility, and versatility of bioluminescence imaging with the accuracy and reliability of genetically encoded reporters. We expect three main outcomes from this project. First, we anticipate that GESIs will be broadly applicable in many areas of biomedicine, including neuroscience, molecular endocrinology, and drug development. Second, conceptual advances resulting from this work can be adapted to target more challenging sulfation pathways, for instance tyrosine and glycan sulfation, which occur in the Golgi apparatus and play key roles in immune signaling and skeletal development. Third, given the prevalence of bioluminescence in preclinical research, it should be fairly straightforward to extend GESIs to more sophisticated models of sulfation research involving small vertebrates.

B. INNOVATION

The *status quo* for monitoring intracellular sulfation is that invasive means are first required to prepare cell extracts, which can then be assayed for sulfated products and metabolic markers (*e.g.*, PAP) using liquid chromatography, mass spectrometry, and radioactive labeling. While recent advances in probe chemistry have made it possible in some cases, to detect sulfation by fluorescence, these probes have limited range and cannot be genetically encoded in cells. The sulfation sensors (GESIs) we propose here address these challenges by pursuing three principal innovations. First, we exploit recent advances in our understanding of molecular bioluminescence to develop the first genetically encoded reporter for detecting sulfation events. In particular, while the idea of detecting PAP to monitor sulfation has been previously explored, the mechanism of connecting PAP to a bioluminescent readout using FLuc and LST is new to this proposal, requiring innovations in protein design and engineering (Aim 1). Second, expressing GESIs in mammalian cells will require innovations in reporter gene construction, sulfo-luciferin delivery, and new lentiviral vectors for genetic encoding (Aim 2). Third, the proof-of-principle work we propose in Aim 2 will yield new measurements of intracellular sulfation that are currently inaccessible using any other experimental means.

C. SUMMARY OF EXPERIMENTAL APPROACH

C.1| Aim 1: Developing a sulfation sensor for bioluminescence imaging

Overview of study design. GESIs comprise 3 components, FLuc, LST, and sulfo-luciferin. Of these, LST is the key component connecting PAP (sulfation biomarker) to FLuc bioluminescence by enzymatic "uncaging" of sulfo-luciferin. Accordingly, our work in Aim 1 will involve a molecular optimization campaign to identify LST variants with PAP sensitivity in the 0.2 – 20 μM range. This design criterion is chosen to ensure that our sensors are responsive to typical concentrations of intracellular PAP formed during sulfation events.

Methodological details. Our optimization platform comprises generation of LST variants by site saturation mutagenesis, expression of mutant libraries in *E. coli* in multi-well plates, *in situ* chemical lysis such that each well contains a single clone, bioluminescence screening to test for promising mutants, further rounds of mutagenesis by DNA shuffling and bioluminescence testing to identify variants with synergistic (or additive) improvements. Although crystal structures are currently unavailable for LSTs, sequence alignment and homology modeling have identified conserved motifs that overlap with known PAP binding sites in human sulfotransferases. All 8 amino acids located within these motifs will serve as our initial targets for saturation mutagenesis. We will pick ~ 95 colonies per site to ensure full coverage of the mutation space, thereby increasing our chances of finding an improved mutation at any given position. For the screening step, lysates corresponding to each clone (as well as the parental LST) will be incubated with excess sulfo-luciferin and 5 different concentrations of PAP in the range of 0.1 – 20 μM, giving ~ 500 single–well screens per library. Bioluminescence will be initiated by injecting equal amounts

of purified FLuc to all wells. The dose-response characteristics of each clone will be used to guide selection of sensitive mutants based on the concentration of PAP required to achieve half-maximum bioluminescence. At the same time, we will also be alert for significant variations in peak bioluminescence among variants, which could indicate substrate or product inhibition preventing LST from achieving maximum sulfo-luciferin conversion. Viable clones from each library will be sequenced, unique mutants pooled, and further mutagenized by 3 – 5 rounds of molecular shuffling and bioluminescence screening to identify combinations of mutations that lead to additive (or synergistic) gains in sensitivity. To keep library sizes within the capacity of our medium-throughput pipeline, we will screen ~ 2000 clones in each round using a single low concentration of PAP that will be progressively reduced in successive rounds. Viable variants will be identified based on bioluminescence intensity.

Once an optimized LST has been identified, it will be sequenced, purified from *E. coli*, and assayed to establish Michaelis-Menten constants, k_{cat} and K_m. Next, we will test functional GESIs (formed by incubating purified components, *i.e.*, LST + FLuc + sulfo-luciferin) by acquiring time-resolved bioluminescence measurements after treatment with varying concentrations of one of several cellular metabolites, including PAP, PAPS, coenzyme A, adenosine, S-adenosyl methionine, adenosine tri-, di-, and monophosphates, and corresponding deoxy adenosine nucleotides. These experiments will define detailed performance characteristics of GESIs, including molar sensitivity, dynamic range, response kinetics, and molecular specificity.

Expected outcomes, possible pitfalls, and alternative strategies. The primary expectation in Aim 1 is to develop GESIs with PAP sensitivity in the 0.2 – 20 μM range. We expect the activation kinetics of GESIs to be limited by the k_{cat} of LST for sulfo-luciferin conversion (further discussed below), which is likely to be slower compared to k_{cat} of luciferin turnover by FLuc (> 100 min^{-1}). Turn-off kinetics will depend on the decay rate of FLuc bioluminescence, typically lasting a few minutes. All the techniques proposed in Aim 1 are already used in our lab for engineering fluorescence and magnetic resonance reporters. Thus, we do not expect any major technical hurdles. However, two possible challenges are anticipated. First, LSTs engineered for optimal sensitivity might exhibit slow turnover (*i.e.*, low k_{cat} values) for sulfo-luciferin conversion. Aside from limiting sensor responsiveness, a low k_{cat} could become particularly problematic if it leads to LST being kinetically out-competed by PAP phosphatase (k_{cat} ~ 27 min^{-1}) resulting in PAP hydrolysis and inaccurate GESI readouts in cells. If we face this conflict, a possible remedy might be to include additional rounds of "kinetic" screening in our molecular evolution pipeline. Here, mutants from each round will be screened using progressively shorter incubation times with PAP and sulfo-luciferin before injecting FLuc and measuring bioluminescence. The advantage of this approach is that it increases our chances of finding a desirable combination of sensitivity and kinetics, although at the cost of requiring many more rounds of mutagenesis and screening. Second, because the crystal structure of LST is unknown, the amino acids mutagenized in Aim 1 may not represent accurate targets for saturation mutagenesis. An alternative approach that does not require crystallographic information would be to generate the initial library of LST variants by error-prone PCR with a mutation frequency of 1 – 2 base changes per kb, resulting in ~ 1 random amino acid change per LST.

C.2 Aim 2| Validating intracellular functionality of biosensors.

Overview of study design. In Aim 2, we will apply GESIs to monitor dopamine sulfation activity in a human neuroblastoma cell line (SH-SY5Y), which endogenously expresses high levels of SULT1A3, the major enzyme responsible for sulfating monoamine neurotransmitters in the central nervous system. Metabolic sulfation of dopamine represents a highly studied system in the field. The work in Aim 2 will therefore allow benchmarking of GESIs by harnessing alternative established methods to probe biological sulfation in this well-studied system.

Methodological details. Gene sequences for FLuc and LST will be codon-adapted for mammalian usage and cloned polycistronically using a 2A ribosome-skipping peptide. Each gene will include distinct epitopes (FLAG and c-myc), allowing expression of FLuc and LST to be independently quantified by immunoblotting. The complete construct (~ 3 kb) will be packaged in lentiviral particles and used to transduce SH-SY5Y cells. After confirming intracellular expression and FLuc activity, our focus will shift to optimizing delivery of sulfo-luciferin. Unlike luciferin, which diffuses across the cell membrane, transport of sulfo-luciferin is likely to be slower due to its anionic property. Accordingly, we will first treat native SH-SY5Y cells with a wide range of sulfo-luciferin doses. For each dose, we will lyse cells at different time points and assay for sulfo-luciferin uptake by measuring bioluminescence after incubation with LST, FLuc, and PAP. These experiments will define the final dose and duration for delivering sulfo-vargulin to cells.

Following sulfo-luciferin delivery to stably transduced SH-SY5Y cells, we will treat cells with 50 – 100 μM dopamine to initiate sulfation activity. A second group of cells, also stimulated with dopamine, will be further treated with sodium chlorate, a broad-spectrum inhibitor of biological sulfation. Immediately after each perturbation, we will begin acquiring longitudinal measurements of whole cell bioluminescence. The interval between successive recordings will be determined by the incubation time required to re-supplement sulfo-luciferin in cells. All bioluminescence readouts will be converted to estimates of intracellular PAP from a standard curve of known PAP concentrations treated with purified GESIs (with each component adjusted to match intracellular concentrations of LST, FLuc, and sulfo-vargulin). In parallel, we will perform experiments involving alternative (non-genetic) methods to monitor sulfation: first, by immuno-assaying for un-sulfated dopamine in the culture media using an ELISA-based technique; next, by directly measuring the concentration of PAP in lysed cell extracts. For the latter measurements, cell lysates harvested at different time points will be dialyzed and treated with PAP phosphatase to release inorganic phosphate, which will be quantified using malachite green. Both these assays are established in the field and will yield complementary readouts of sulfation activity that will serve to validate our technique as well as aid in interpretation of our new bioluminescence readouts. All measurements will be controlled by performing identical experiments in SH-SY5Y cells where sulfation activity will be suppressed by CRISPR/Cas9 knockout of SULT1A3, the main gene whose product catalyzes dopamine sulfation.

Expected outcomes, possible pitfalls, and alternative strategies. We expect Aim 2 to result in new bioluminescence-based measurements of sulfation activity that are qualitatively consistent with results obtained using alternative approaches established in the field. The main techniques that we propose to use in Aim 2, including viral gene delivery, live-cell imaging, and gene editing are already established in our lab. Nevertheless, two challenges come to mind. First, if intracellular delivery of sulfo-luciferin becomes a crucial limiting factor, we will explore the possibility of increasing its uptake by co-expressing GESIs with an organic anion transporter known as Oatp1. Several recent reports have established the broad utility of OatP1 for imaging cells by enhancing uptake of poorly permeable molecular probes, including many luciferin derivatives. Another remedy that we might consider would be to mask the anionic charge in sulfo-luciferin by a labile ester that is removed by intracellular esterases upon delivery. Notably, this strategy has been fairly successful in delivering many sulfonated fluorophores to cells. Second, if we detect any indications of decreased cell viability or metabolic burden from over-expression of GESIs, we will attempt to mitigate this problem by switching to weaker promoters. Alternatively, we may move to a doxycycline-inducible system that would allow GESI expression to be precisely and temporally controlled to stay within toxicity limits.

C.3| Scientific rigor. The measurements proposed here will be performed in replicates of 6 and reported as mean ± SEM. For comparing 2 groups, we will use the t-test. For > 2 groups, we will use 1-way ANOVA. Q-Q plots will be used to inspect data for deviations from normality in which case nonparametric tests will be used. Statistical significance will correspond to $p < 0.05$.

REFERENCES

1. Mulder, G. J., *Sulfation of Drugs & Related Compounds*. CRC Press: 2020.
2. Gibbs, T. T.; Russek, S. J.; Farb, D. H., Sulfated steroids as endogenous neuromodulators. *Pharmacology Biochemistry and Behavior* **2006,** *84* (4), 555-567.
3. Smith, C. C.; Gibbs, T. T.; Farb, D. H., Pregnenolone sulfate as a modulator of synaptic plasticity. *Psychopharmacology* **2014,** *231* (17), 3537-3556.
4. Coughtrie, M. W., Catecholamine sulfation in health and disease. In *Advances in pharmacology*, Elsevier: 1997; Vol. 42, pp 339-342.
5. Roth, J. A., Sulfoconjugation: role in neurotransmitter and secretory protein activity. *Trends in Pharmacological Sciences* **1986,** *7*, 404-407.
6. Coughtrie, M., Sulfation through the looking glass—recent advances in sulfotransferase research for the curious. *The pharmacogenomics journal* **2002,** *2* (5), 297-308.
7. Kester, M.; Van Dijk, C.; Tibboel, D.; Hood, A.; Rose, N.; Meinl, W.; Pabel, U.; Glatt, H.; Falany, C.; Coughtrie, M., Sulfation of thyroid hormone by estrogen sulfotransferase. *The Journal of Clinical Endocrinology & Metabolism* **1999,** *84* (7), 2577-2580.
8. Foster, P. A.; Mueller, J. W., SULFATION PATHWAYS: insights into steroid sulfation and desulfation pathways. *Journal of molecular endocrinology* **2018,** *61* (2), T271-T283.
9. Kälsch, J.; Bechmann, L. P.; Heider, D.; Best, J.; Manka, P.; Kälsch, H.; Sowa, J.-P.; Moebus, S.; Slomiany, U.; Jöckel, K.-H., Normal liver enzymes are correlated with severity of metabolic syndrome in a large population based cohort. *Scientific reports* **2015,** *5* (1), 1-9.
10. Noordam, C.; Dhir, V.; McNelis, J. C.; Schlereth, F.; Hanley, N. A.; Krone, N.; Smeitink, J. A.; Smeets, R.; Sweep, F. C.; Grinten, H. L. C.-v. d., Inactivating PAPSS2 mutations in a patient with premature pubarche. *New England Journal of Medicine* **2009,** *360* (22), 2310-2318.
11. Oostdijk, W.; Idkowiak, J.; Mueller, J. W.; House, P. J.; Taylor, A. E.; O'reilly, M. W.; Hughes, B. A.; De Vries, M. C.; Kant, S. G.; Santen, G. W., PAPSS2 deficiency causes androgen excess via impaired DHEA sulfation—in vitro and in vivo studies in a family harboring two novel PAPSS2 mutations. *The Journal of Clinical Endocrinology & Metabolism* **2015,** *100* (4), E672-E680.
12. Paul, P.; Suwan, J.; Liu, J.; Dordick, J. S.; Linhardt, R. J., Recent advances in sulfotransferase enzyme activity assays. *Analytical and bioanalytical chemistry* **2012,** *403* (6), 1491-1500.
13. Lin, W.; Mehta, S.; Zhang, J., Genetically encoded fluorescent biosensors illuminate kinase signaling in cancer. *Journal of Biological Chemistry* **2019,** *294* (40), 14814-14822.
14. Oldach, L.; Zhang, J., Genetically encoded fluorescent biosensors for live-cell visualization of protein phosphorylation. *Chemistry & biology* **2014,** *21* (2), 186-197.
15. Fallon, T. R.; Li, F.-S.; Vicent, M. A.; Weng, J.-K., Sulfoluciferin is biosynthesized by a specialized luciferin sulfotransferase in fireflies. *Biochemistry* **2016,** *55* (24), 3341-3344.
16. Shimomura, O.; Johnson, F. H., Chemical nature of bioluminescence systems in coelenterates. *Proceedings of the National Academy of Sciences* **1975,** *72* (4), 1546-1549.
17. Nakamura, M.; Suzuki, T.; Ishizaka, N.; Sato, J.-i.; Inouye, S., Identification of 3-enol sulfate of Cypridina luciferin, Cypridina luciferyl sulfate, in the sea-firefly Cypridina (Vargula) hilgendorfii. *Tetrahedron* **2014,** *70* (12), 2161-2168.
18. Cormier, M. J.; Hori, K.; Karkhanis, Y. D., Bioluminescence of Renilla reniformis. VII. Conversion of luciferin into luciferyl sulfate by luciferin sulfokinase. *Biochemistry* **1970,** *9* (5), 1184-1189.
19. Anderson, J. M.; Hori, K.; Cormier, M. J., [28] A bioluminescence assay for PAP (3′, 5′-diphosphoadenosine) and PAPS (3′-phosphoadenylyl sulfate). In *Methods in enzymology*, Elsevier: 1978; Vol. 57, pp 244-257.
20. Stanley, P. E.; Kelley, B. C.; Tuovinen, O. H.; Nicholas, D., A bioluminescence method for determining adenosine 3′-phosphate 5′-phosphate (PAP) and adenosine 3′-phosphate 5′-sulfatophosphate (PAPS) in biological materials. *Analytical biochemistry* **1975,** *67* (2), 540-551.
21. Toledano, E.; Ogryzko, V.; Danchin, A.; Ladant, D.; Mechold, U., 3′-5′ phosphoadenosine phosphate is an inhibitor of PARP-1 and a potential mediator of the lithium-dependent inhibition of PARP-1 in vivo. *Biochemical Journal* **2012,** *443* (2), 485-490.

SCIENTIFIC IMPACT

The immediate output of our research will be the ability to dynamically monitor intracellular sulfation activity by recording bioluminescence readouts using genetically encoded sulfation indicators (GESIs). In the future, we expect researchers using GESIs the same way as they use genetically encoded phosphorylation indicators today, as versatile reporters applicable in many areas of biomedicine. Several general scientific goals may be achieved for the first time using GESIs:

(1) Understanding how sulfation affects central nervous system signaling. The activities of major monoamine neurotransmitters, including dopamine and serotonin is controlled by sulfation. Very little is currently understood about the integrated roles of neurochemical sulfation, synaptic activity, and neurotransmitter release on neural signaling. Using GESIs, it should become possible to compare sulfation measurements with complementary readouts obtained using genetically encoded calcium indicators and neurotransmitter probes to reveal much needed insights connecting these fundamental neurochemical processes.

(2) Probing sulfation mechanisms in hormone metabolism. The role of sulfation in controlling the activity of steroid hormones has long been known and much work has been done to identify steroid sulfotransferases and profile their tissue distribution by immunohistochemistry. However, it has been considerably harder to study the highly complex gene networks regulating sulfotransferase expression, involving multiple inputs, transcription factors, nuclear receptors, and feedback mechanisms. Exploring these mechanisms underlying sulfotransferase regulation could be greatly facilitated by the ability to accurately measure intracellular sulfation with GESIs.

(3) Investigating sulfation mechanisms in disease and treatment. Defects in physiological sulfation have been implicated in metabolic, musculoskeletal, and developmental disorders as well as endocrine-related cancers. The activities of many drug molecules are further profoundly altered by sulfation. For example, apomorphine, which is used to treat Parkinson's disease is rapidly ($t_{1/2}$ ~ 50 mins.) inactivated by sulfation. Pharmacological tools to modulate sulfation pathways are therefore of great interest both for drug development and basic research on disease mechanisms. The technology developed here could uniquely aid these efforts by allowing sulfation modulators to be examined and characterized directly in living cells.

A. JUSTIFICATION FOR FACULTY RESEARCH GRANT SUPPORT

The proposed project focuses on developing the first genetically encoded molecular reporter for bioluminescence imaging of sulfation, a key metabolic process controlling the activity of drugs, hormones, and neurotransmitters. This work represents a **fundamentally new direction** for our lab, both in terms of the imaging modality (our lab's specific expertise is in fluorescence imaging and MRI) and the scientific problem addressed (bio-sulfation). Needless to say, the project is in its early days and currently unfunded. Support from the Academic Senate Faculty Research Grant will thus be crucial to initiate this undertaking in two specific ways. First, while the scientific premise of the project is based on recently published work, much more preliminary data will be needed to become competitive for extramural support from agencies such as the NIH. Successful completion of the stated project goals will provide this vital springboard, leading to at least one (but possibly multiple) R21 applications within the next few months. Second, support from the Faculty Research Grant will go a long way in fortifying our (recently initiated) collaboration with the Oakley lab where the long-range goal is to construct a variety of functional biosensors by discovering novel molecular components from luminous marine organisms. This represents the union of two very complementary research thrusts on campus, *i.e.*, evolution of marine bioluminescence (Oakley, EEMB) and biomedical imaging (Mukherjee, ChE). The work described in the current project will provide a roadmap for this collaboration and aid our ongoing efforts in recruiting a jointly advised Ph.D. student (likely, from the BMSE program).

B. DETAILED BUDGET

Total amount requested: $16,139.90

Project personnel	% time	Hourly rate	Months	
1 Graduate Student Researcher	47 %	$29.34	3 mo. (summer)	$6619.10
	12.5%	$29.34	6 mo. (academic yr.)	$3520.80
Molecular biology, cell culture, and bioluminescence reagents				*$6000.00*
Total				*$16,139.90*

C. BUDGET JUSTIFICATION

Project personnel	Role
1 Graduate Student Researcher	Preliminary testing of purified LST + FLuc + sulfo-luciferin for monitoring PAP, establishing and optimizing workflow for protein engineering, detailed characterization studies on response properties of most promising LST variants, deploying and validating GESIs in human neuroblastoma cell lines.

Basic reagents requested above are specific to this project and currently not supported by other grants. These include neuroblastoma cell lines (SH-SY5Y), luciferin and sulfo-luciferin reagents, gene sequences corresponding to FLuc and LST, malachite green assay reagents for measuring PAP, and an ELISA-based test kit for quantifying dopamine.

PREVIOUS ACADEMIC SENATE SUPPORT

PI Mukherjee received a Faculty Research Grant in 2020 ($12,711, 06/30/20 – 07/01/21) to pursue work on engineering biosensors for imaging gut metabolites by magnetic resonance imaging (MRI). Key outcomes that were enabled by this funding are highlighted below:

1. We were able to acquire crucial preliminary data demonstrating our basic concept of imaging yeast cell biosensors by engineering them to express genetic reporters based on aquaporins. We were also able to assemble preliminary versions of our sensors by cloning bioresponsive GPCR(s) in yeast. This data provides us with a compelling basis to submit an R01 proposal (target date: June 5th, 2021) in response to the following request for application: https://grants.nih.gov/grants/guide/pa-files/PAR-18-434.html.
2. We also (unsuccessfully) submitted a Pew Research proposal based on the above results as well as the first phase submission to the Moore Inventor Fellows Program (pending).
3. We hosted an undergraduate summer scholar from Caltech (Isabella Hurwitz) who worked on this project, developing a detailed computational model describing the transit dynamics of particular metabolites in the vertebrate gut. Results from this work were presented at the Caltech Summer Undergraduate Research Fellowship/Student-Faculty Program seminar day (Oct. 17th, 2020, Pasadena, CA).
4. The Ph.D. student overseeing the above project (Emily Sun†) recently graduated with an M.S. in Chemical Engineering, successfully completing her thesis titled: "Noninvasive Biosensors for MRI-Based Imaging of Butyrate and Serotonin in the Gut".

Overlap: The work supported by the previous Faculty Research Grant does not have any overlap with the project proposed here.

Remaining balance: none

†COVID impact: Emily Sun decided to quit the Ph.D. program for reasons connected (in part) to the effects of COVID-19 on research activity, which led to a temporary hiatus in continued work on the project. We are currently in the process of recruiting a new graduate student (or postdoctoral scholar) to resume this work.

A. PUBLICATIONS IN LAST 3 YEARS

1. Miller, A.D.C., Ozbakir, H.F., **Mukherjee, A**. Calcium-responsive contrast agents for functional magnetic resonance imaging. *Chemical Physics Reviews*. (accepted). **Editor's pick for Featured Article**
2. Ozbakir, H.F., Miller, A.D.C., Fishman, K.B., Martins, A.F., Kippin, T.E., **Mukherjee, A**. A protein-based biosensor for detecting calcium by magnetic resonance imaging. (in preparation) Preprint: *bioRχiv*. (2021). doi: https://doi.org/10.1101/2021.02.04.429691
3. Anderson, N.T., Weyant, K.B., and **Mukherjee, A**. Characterization of flavin binding in oxygen-independent fluorescent proteins. *AIChE J*. 66(12). (2020) doi: 10.1002/aic.17083
4. Ozbakir, H.F., Anderson, N.T., Fan, K.C., **Mukherjee, A**. Beyond the Green Fluorescent Protein: Biomolecular reporters for anaerobic and deep-tissue imaging. *Bioconjugate Chemistry*. 31(2). 293-302. (2019) doi:10.1021/acs.bioconjchem.9b00688.
5. Lu, G.J., Farhadi, A., **Mukherjee, A.**, Shapiro, M.G. Proteins, air, and water: reporter genes for ultrasound and magnetic resonance imaging. *Current Opinions in Chemical Biology*. 45. 57-63. (2018) doi: 10.1016/j.cbpa.2018.02.011

B. RECENT HONORS

2018 NARSAD Young Investigator Award from Brain & Behavior Research Foundation
2019 Maximizing Investigators' Research Award (National Institutes of Health)
2020 Discovery Award (Peer-Reviewed Medical Research Program, Department of Defense)

C. PROFESSIONAL SERVICE

Campus service	Graduate Affairs Committee, 2017-present; Graduate Recruiting Committee, 2017-19; Faculty Advisor to ChE Graduate Students' Symposium, 2017-present; Harvey Karp Postdoctoral Award Committee, 2020; Lindros Postdoctoral Fellowship Award Committee, 2018; Errett Fisher Postdoctoral Fellowship Award Committee, 2018; UCSB Biosafety External Review Panel, 2019
Grant reviewer	NIH BRAIN Initiative, 2019, 20; Israel Science Foundation, 2020, National Science Center, Poland, 2018; Health & Medical Research Fund, Hong Kong Special Administrative Region, 2016.
Conference activities	**Conference chair/organizer**: International Conference on Biomolecular Engineering (*virtual*), Jan. 2021 **Reviewer and session chair**: Enabling Technologies for Immunotherapy, American Institute of Chemical Engineers Annual Meeting, PA, Nov. 2018; Combinatorial Techniques in Protein Engineering, American Institute of Chemical Engineers Annual Meeting, PA, Nov. 2018; Mammalian Cell Culture, American Chemical Society Annual Meeting, PA, Mar. 2020 (postponed due to COVID-19)