Presidential Faculty/Student Collaboration and Publication Grant
Deadline Monday, February 20, 2017

Please use this checklist and budget. Include with your completed application. For more information about Presidential Faculty/Student Collaboration and Publication grants, please see https://gustavus.edu/kendallcenter/grant-opportunities/presidential-grant.php.

FACULTY INFORMATION

Name: Scott Bur | Email: sbur@gustavus.edu

Department: Chemistry | Rank: Professor

STUDENT INFORMATION

Name: Emma Santa | Email: esanta@gustavus.edu

Major(s): BMB | Graduation Year: 2019

CHECKLIST

Project Details

√ Brief description of the proposed project including its collaborative nature
√ Clear statement of anticipated outcomes
√ Likely placement for publication or performances
√ Anticipated research completion date

Participant Details

√ Names and brief biographies of all participants
√ Explanation of how this project fits into the career of the faculty member
Note: Applications from faculty at all career stages are encouraged
√ Explanation of how this project fits into the educational trajectory of the student
Note: Statement should be written by the student; include year of graduation; student eligibility is limited to full-time returning students

√ **Presidential Budget Proposal Form**

√ If successful, my proposal can be used as an example to assist future applications. Check to give permission. This decision will not influence the application evaluation.

Submit electronically as a PDF to cblaukat@gustavus.edu at the John S. Kendall Center for Engaged Learning.

Presidential Faculty/Student Collaboration Grant

Budget Information

Faculty Stipend ($300 per week, up to $3,000 for a maximum of 10 weeks)
Student Summer Stipend ($400 per week, up to $4,000 for a maximum of 10 weeks)
Student Summer Campus Housing ($60 per week, for a maximum of 10 weeks)
Budget Maximum ($8,100 for all categories)

Item		**Amount**
Equipment (e.g., transcription machine, camera, cassette recorder – but not to include computer hardware)		**$**
1:	Cost:	
2:	Cost:	
3:	Cost:	
Materials (e.g., books, printing, software, lab supplies)		**$387.50**
1: Chemicals	Cost: $200	200.00
2: Consumables	Cost: $100	187.50
3:	Cost:	
Travel Costs (cannot include conference travel, see http://gustavus.edu/finance/travel.php for allowable travel expenses)		**$ 112.50**
Airfare:		
Train fare: $4.50/day Number days: 25 days (total of 5 weeks)		112.50
Lodging:		
Meals:		
Stipends & Housing		**$7600.00**
Faculty Stipend	$300 per week, up to $3,000 for a maximum of 10 weeks	3000.00
Student Summer Stipend	$400 per week, up to $4,000 for a maximum of 10 weeks	$4000.00
Student Summer Campus Housing	$60 per week, up to 10 weeks	$600.00
Total Expenses		**$500**
Amount Requested (Total Expenses + Requested Stipends + Housing)		**$8100**

Have you applied for or received funding from another source to help support this project? (If no, skip a, b, and c below.)

☑ Yes ☐ No

a. Funding Source: NSF (2016-2017)
b. Amount: $34,041
c. Please explain how the Presidential grant will be used in addition to the other funding, and (if relevant), how the Presidential grant project would be impacted if external funding is not approved**. The NSF funding supports my sabbatical at the University of MN. Of particular importance, this grant covers travel through June. Accordingly, Emma's travel (except train fare) is already covered. (We will park at the 28th Ave public park-and-ride, then ride the train into campus.) The Presidential grant will be used to cover Emma's summer stipend and housing. It will also be used for various consumables and chemicals that are not typically provided by the Chemistry Department.**

Using Fragment-Based Ligand Design for Inhibiting PfGCN5-mediate Gene Expression

Abstract

This proposal seeks to start a new research program in Dr. Bur's laboratory and revise laboratory curriculum for CHE-251. The project is based upon research methods that Dr. Bur acquired during his sabbatical at the University of Minnesota. Specifically, Dr. Bur and Emma Santa will express and purify a fluorine-labeled bromodomain of the malarial PfGCN5 protein at Gustavus, then design, synthesize, and test small molecules for binding to the protein. The synthesis and testing of the small molecules will then be included in sections of CHE-251 laboratory.

Background and Significance

Malaria is a protozoan infection caused mainly by two species: *Plasmodium falciparum* and *P. vivax*.[1] In sub-Saharan Africa, over 99% of the disease is caused by *P. falciparum*, which is transmitted through the bites of infected female *Anopheles* mosquitoes. While world-wide rates of infection are decreasing, approximately 429,000 deaths were attributed to malarial infections in 2015, and 70% of these deaths were in children under the age of five. In the same year, approximately 212 million new infections were reported. More that 90% of deaths and new infections are reported in Africa, specifically sub-Saharan Africa. Insecticide-treated netting, mosquito population control through insecticide spaying, and artemisinin-based combination therapies are the front line prevention and treatment methods. As a result of their use, infection rates fell 21% and mortality rates by 29% from 2010 - 2015. Unfortunately, in that same time frame, 60 of the 73 reporting countries reported resistance to at least one insecticide, and 50 reported resistance to two or more insecticide classes. In addition, *P. falciparum* resistance to artemisinin has been reported in several countries.

A protein called GCN5 is known to play a critical role in human gene expression by mediating protein-protein interactions (PPIs) that are critical for knowing what part of the DNA to transcribe. *P. falciparum* has a similar protein called *Pf*GCN5 that has also been shown to be critical for gene expression.[2] Selective disruption of this PfGCN5 protein, therefore, is an attractive new therapeutic approach to malaria. This could, for example, kill the parasite or hinder its ability to infect.[3]

Our approach to finding molecules that selectively disrupt PfGCN function will use fragment-based ligand design (FBLD) as a strategy.[4] Protein-protein binding domains are large. Molecules that are larger than typical small-molecule drugs are needed to effectively bind the region. The idea behind FBLD is to find smaller molecules that would be fragments of a potentially larger molecule. After identifying and optimizing several fragments, they can be linked together to give a larger molecule that selectively binds with high affinity.

Dr. Will Pomerantz's research group at the University of Minnesota has developed methods to incorporate fluorine atoms (^{19}F) into the protein-protein binding domains.[5] This gives a relatively easy way to use fluorine NMR spectroscopy to observe and quantify binding of the small molecule to the protein. In a proof of concept study, a series of these **Pr**otein-**O**bserved **F**luorine (PrOF) NMR experiments was conducted using a fluorine-labeled protein-protein interaction domain, called the KIX domain, of the Creb Binding Protein as a model, and a screen of over 500 small molecules yielded several compounds that were identified as potentially useful

fragments.[6] We propose to express fluorine-labeled versions of the PfGCN5 protein at Gustavus and analyze small molecule binding to the protein with PrOF NMR using the Gustavus NMR spectrometer.

Previous Work

Over the last three years, Dr. Bur has collaborated with Dr. Pomerantz to make analogues of the fragments identified in the small molecule screen against KIX. As part of this collaboration, Dr. Bur has run three January-term research courses focused on analog synthesis for fragments that bind the KIX protein. He also supervised three Gustavus students in the summer of 2015 to continue work on analog design and synthesis. He has planned the inclusion of analog synthesis within the Fall 2017 CHE-251 laboratory. (This was the focus of a recent, successful Cottrell Scholars grant from the Research Corporation submitted by Dr. Pomerantz in collaboration with Dr. Bur.)

To further the collaboration, Dr. Bur has spent the 2016-2017 academic year working in the Pomerantz laboratory to learn the cellular and molecular biology techniques needed to provide fluorinated KIX protein. In addition, he has worked on the expression and isolation of the malarial protein PfGCN5 so that he can bring it back to Gustavus as the seed of a new research project (the subject of this proposal). As part of his sabbatical, he worked out PrOF NMR conditions for the Gustavus NMR spectrometer so that high-quality data can be obtained in a reasonable amount of time. This last January term, he expanded the course to include PrOF NMR analysis of small molecules with PrOF NMR using the Gustavus instrument. During the 2017 January term, he also co-supervised an independent study student who helped initiate the protein expression and isolation project at Gustavus. This still requires significant work. Finally, Dr. Bur has used PrOF NMR to screen a chemical library at the U of MN and discover several fragments that bind to PfGCN. From these fragments, we will begin designing and synthesizing analogs to test against the protein.

Proposed Research

There are three main areas that Emma and Dr. Bur will work on to begin this research program at Gustavus. **The highest priority** is to optimize the expression and isolation of our protein by trying various cell lysis methods, including simple freeze/thaw, lysozyme, sonication, or detergent methods. We will also try various purification methods, such as affinity chromatography or "batch binding" methods (both of which rely on immobilized metal to pull the desired protein out of the cell lysate), and various buffer exchange conditions, such as dialysis or size-exclusion filters to get the protein in a buffer where it is stable.

The second highest priority is to design and synthesize analogs of the fragments that have been discovered to bind to the PfGCN5 protein. There are at least three general classes of compounds that we wish to analog (Figure 1), and Emma will help design all three. Specifically, we will try to develop synthetic routes that are able to be incorporated into the CHE-251 laboratories.

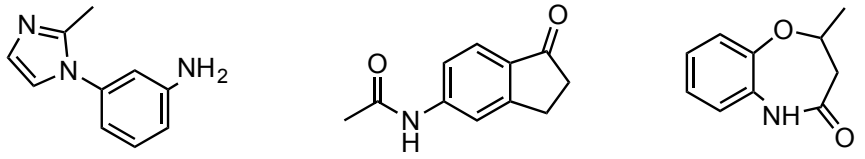

Figure 1

The third highest priority is to develop tools to gain structural information about how the fragments are binding to the protein. This may be beyond what we can do during the summer, but if everything else goes better than expected, Emma will help with site-directed mutagenesis to help assign the eight signals in the PrOF NMR of 3-fluorotyrosine labeled protein. This will give us a better understanding of where fragments bind, but will require significant work to establish what signal corresponds to which residue of the protein. Figure 2 shows a ribbon diagram of the protein (green) with all of the tyrosine amino acids highlighted in red; the top of the figure show the five tyrosine residues (red) on the protein-protein binding site.

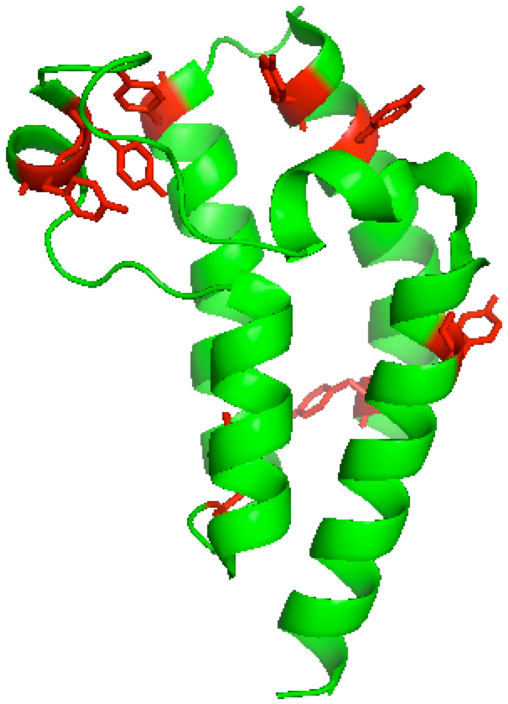

Figure 2

Collaborative Nature and Student Involvement

This is a new line of research at Gustavus, and it includes several disciples, not just organic chemistry. More specifically, this is a new area of research for Dr. Bur, and working with Emma will be very different than other projects where he's worked with students. Early in the summer, he will be working to teach Emma all the cellular and molecular biology that she will need. This will be done in the Pomerantz labs at the University of MN as part of Dr. Bur's sabbatical arrangement with Dr. Pomerantz. Emma will learn the general principles in a setting where there is significant expertise. Dr. Bur is still learning more of the nuances of this work; he is, therefore, as much of a student as Emma. Once we bring the work back to Gustavus, Emma and Dr. Bur will be at about the same level of knowledge for the first part of the project. They will be working closely together, each trying different conditions, and consulting with each other almost constantly about what they should try next.

For the second part of the project, Emma will do literature searches for syntheses of similar compounds, and Emma and Dr. Bur will decide together which synthetic route they will attempt. Emma will take the lead on the synthesis of the compound, and Dr. Bur will begin

scaling up the protein expression and isolation. There is a lot of "hurry up and wait" in the protein expression and isolation, so Dr. Bur will still be able to spend most of the time working with Emma. Emma was part of the 2017 January term, and she demonstrated that she can handle the independence on this part of the project.

If they get to the third part of the project, they are back to being equal partners in working out the polymerase chain reaction (PCR) conditions for site-directed mutagenesis. They will have guidance from Dr. Pomerantz and a number of people in the Gustavus community (Jeff Dahlseid, Brook Shields, Colleen Jacks, and Laura Burrack).

Feasibility and Completion Date

The largest hurdle to accomplishing this work is having a working analytical protocol for testing the binding of the small molecules with the protein. The fact that this is already worked out and that the concept was proven in the January-term courses provides confidence that this project is feasible at Gustavus.

The expression and isolation of the protein are things that other people at Gustavus have accomplished in other systems, and it is even part of two courses in the chemistry department (CHE-255 and CHE-360). For Emma and Dr. Bur, it's finding the specific conditions that work for the PfGCN5 protein. Although time consuming, this part of the project should be easily accomplished by mid summer, and they can be working on parts of the second priority during down times. As mentioned above, Dr. Bur will be traveling with Emma to the University of Minnesota during June to work in the Pomerantz lab. From there, it is a matter of modifying conditions to fit the equipment available at Gustavus. Dr. Bur expects that this part of the project will be done by the end of June.

The design, synthesis and testing of fragment analogs will take several years. Emma and Dr. Bur will be able to design the synthesis of one fragment and make considerable progress toward the synthesis in one summer. They will especially be looking for reaction conditions that are conducive to the CHE-251 laboratory course. The design will be done by mid-June, and synthetic efforts will be underway by the beginning of August.

If time allows, they can begin exploring the third priority by changing the fluorine-labeling scheme in the protein. Currently, Dr. Bur labels the two tryptophan residues, and the assignment of both of the signals in the PrOF NMR to the specific tryptophan residues in the protein are known. An alternate labeling scheme, such as using fluorinated tyrosine, will allow a more granular look at which residues are closer to the small molecule when it is bound. (There are eight tyrosines, and five of them are in the protein-protein binding site.) Much of this work is similar to the expression and isolation work they are proposing to do in the first priority activities. The difference is simply in the choice of fluorinate amino acid used when expressing the protein. The added work here is in a site-directed mutagenesis program to be able to know what signals in the PrOF NMR correspond to the specific residues in the protein. This is a standard molecular biology procedure, so they will have a number of resources on campus to help trouble shoot.

Overall, this is a several year, multiple-student project that has strong precedent in standard biochemistry and molecular biology protocols. Substantial progress (e.g. protein expression and isolation, some analog synthesis) will be made by the beginning of August, as measured by the completion of anticipated outcomes.

Anticipated Outcomes

This is, of course, a much bigger project that can be fully accomplished in one summer. Nevertheless, Dr. Bur anticipates enough work can be done to have some concrete outcomes to report:

- *Scholarly presentations:* We will give a preliminary poster presentation at the MCMS undergraduate research symposium in November.
- *Curricular enhancements:* Within the Gustavus curriculum, we expect that this project will give us another protein model and more molecular frameworks to incorporate into the CHE-251 laboratories, making this a more sustainable educational project. Work on the third priority may also provide some projects for the independent research portion of the CHE-255 (Biochemistry) and CHE-360 (Proteins) laboratories. This work is well suited to publication in pedagogical journals such as the Journal of Chemical Education. To the best of our knowledge, Gustavus is the only PUI currently using FBLD, and our incorporation of it into the undergraduate curriculum is already attracting much attention.
- *Competitive proposal for external funding:* The progress made will provide preliminary data for a competitive external grant proposal, most likely to either the NSF or the NIH. The work Emma and Dr. Bur will accomplish this summer provides a firm footing for the feasibility of this kind of work at Gustavus and will help demonstrate that they are competent and committed to this project.
- *Research experience for Emma Santa:* Emma's time will be split over the summer between Gustavus and the University of Minnesota, though she will be working directly with Dr. Bur in both cases. This will help Emma see what working in a graduate lab is like and will have a significant impact on her career discernment. In previous year's where Dr. Bur took students to work with Dr. Pomerantz's students, the Gustavus students benefited tremendously from the interactions.
- *Enhanced collaboration with internal and external partners:* This project continues and broadens an established collaboration between Dr. Bur and Dr. Pomerantz. It also has potential for building internal collaborations, especially curricular. As mentioned, some of the research would be very suitable to independent projects in CHE-255 and CHE-360. Dr. Bur and Dr. Dahlseid have already begun conversations to start this collaboration.

Participant Information:

Dr. Bur Bur is a professor in the chemistry department. He graduated from the University of Michigan in Ann Arbor with a BS in 1994. He worked for approximately one year at Parke-Davis Pharmaceuticals in Ann Arbor making drug analogues in support of an oncology project. He earned his PhD in organic chemistry from the University of Texas at Austin in 2000 after working with Stephen F. Martin in the field of synthetic methodology and natural products synthesis. From the fall of 2000 to the summer of 2003, he was a National Institutes of Health postdoctoral fellow at Emory University where he continued training under the direction of Albert Padwa. He joined the faculty at Gustavus Adolphus College in the fall of 2003.

Dr. Bur has been very involved in the planning process for new and renovated science facilities, and he directed the Nobel Conference for three years. He stepped down from the Conference because of the reinvigoration of the facilities planning process and the beginning of this collaboration with Dr. Pomerantz.

Emma Santa ('19) is a sophomore at Gustavus Adolphus College majoring in biochemistry. After Gustavus, she plans to attend graduate school and obtain a Ph.D. Emma is interested in pharmacology and plans on working in this field in the future. She is a teaching assistant for general and organic chemistry as well as a member of the Gustavus Chemistry Club.

Career and Educational Trajectories

Scott Bur My previous work has been rooted in traditional medicinal chemistry. Although not specifically focused on drug discovery, the methods we developed to make molecules were directly useful for drug discovery efforts. I've worked with over 30 students in independent research, either during the summer or academic year, in my 14-year career at Gustavus.

The pharmaceutical world has changed since my work at PArke-Davis. The methods used to examine drug candidates have also changed. In light of these changes, I used my most recent sabbatical to reinvent my research. Accordingly, this proposal represents a significant change in my research program, but it is a change that is both personally fulfilling and still appropriate for an institution like Gustavus. More than anything else I've pursued, this project represents the merging of my life as a scholar and my life as an educator.

This project is especially appropriate for Gustavus given the recent changes to the American Chemical Society's Committee on Professional Training guidelines, which mandate increased coverage of macroscale, mesoscale, and nanoscale chemistry. This project fits solidly in the macroscale domain, because it includes discussion of the synthesis and characterization of biological polymers (*i.e.* proteins) and includes alternate analytical techniques (*i.e.* PrOF NMR).

Emma Santa ('19) This research opportunity would enhance my undergraduate education. I took Dr. Bur's chemistry research methods course over the month of January. Throughout the month I gained an interest for the project and the research process, mainly from a synthetic organic chemistry perspective. I am excited by the chance to also delve into the biology side of this project. Being able to work on both sides of the project will give me a broader view of the project that not many research students get to experience. Participating in research with a faculty member will give me valuable laboratory skills that, for the future, set me apart from others when applying for graduate school, and allow me to get a well-rounded educational experience.

References

[1] World Malaria Report 2016. Geneva: World Health Organization; 2016.
[2] Cui, L.; Miao, J.; Furuya, T.; Li, X.; Su, X.-z; Cui, L. *Eukayot Cell* **2007**, *6*, 1219 - 1227.
[3] Josling, G. A.; Petter, M.; Oehring, S. C.; Gupta, A. P.; Dietz, O.; Wilson, D. W.; Schubert, T.; Längst, G.; Gilson, P. R.; Crabb, B. S.; Moes, S.; Jenoe, P.; Lim, S. W.; Brown, G. V.; Bozdech, Z.; Voss, T. S.; Duffy, M. F. *Cell Host Microbe*, **2015**, *17*, 741-751.
[4] Shuker, S. B; Hajduk, P. J.; Meadows, R. P.; Fesik, S. W. *Science* **1996**, *274*, 1531 - 1534.
[5] Gee, C. T; Arntson, K.E.; Urick, A. K.; Mishra, N. K.; Hawk, L. M. L.; Wisniewski, A. J.; Pomerantz, W. C. K. *Nature Protocols*, **2016**, *11*, 1414 - 1427.
[6] Gee, C. T; Koleski, E. J.; Pomerantz, W. C. K. *Angew. Chem. Int. Ed.,* **2015**, *54*, 3735 - 3739.