Presidential Faculty/Student Collaboration and Publication Grant
Deadline Monday, February 24th

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: Brandy Russell | Email: brussell@gustavus.edu

Department: Chemistry | Rank: Associate Professor

STUDENT INFORMATION

Name: Colleeen Caldwell | Email: ccaldwe2@gustavus.edu

Major(s): Biochemistry | Graduation Year: 2015

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)		**$ 0**
1:	Cost:	
2:	Cost:	
3:	Cost:	
Materials (e.g., books, printing, software, lab supplies)		**$650**
1: protein concentration supplies	Cost: $250	
2: gel electrophoresis supplies	Cost: $300	
3: Schlenck line supplies	Cost: $100	
Travel Costs (cannot include conference travel, see http://gustavus.edu/finance/travel.php for allowable travel expenses)		**$ 0**
Airfare:		
Mileage: Number of miles____ @ $0.56/mile		
Lodging:		
Meals:		
Stipends & Housing		**$7,600**
Faculty Stipend	$300 per week, up to $3,000 for a maximum of 10 weeks	3,000
Student Summer Stipend	$400 per week, up to $4,000 for a maximum of 10 weeks	4,000
Student Summer Campus Housing	$60 per week, up to 10 weeks	600
Total Expenses		**$8,250**
Amount Requested (Total Expenses + Requested Stipends + Housing)		**$8,100**

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: Chemistry department
b. Amount: about $400
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**.**

   Our materials expenses far exceed the $500 allowed by this budget, even beyond the $650 estimated above. Brandy has access to about $400 for lab supplies through the chemistry department to help pay for the more routine items that we will use up in this project (chemical reagents, sample tubes, pipette tips, gloves, etc).

Presidential Faculty-Student Collaboration Grant Proposal

Characterizing Cadmium Coordination in Metalloprotein II

Brandy Russell & Colleen Caldwell
February 24, 2014

Project Details

1. Overview

In this project, we will study a cadmium-binding protein from the marine worm *Nereis diversicolor*. Investigations into the mechanisms by which this worm defends itself from cadmium poisoning in its habitat led to the discovery of a cadmium binding protein (called metalloprotein II, or MPII) that belongs to the myohemerythrin family.[1] This is unexpected because prior to this finding, all known myohemerythrins were iron-binding proteins. Even stranger is the finding that this cadmium-binding MPII has a very similar amino acid sequence to an iron-binding myohemerythrin (simply called myohemerythrin, myoHr) in the same worm.[1-3]

It is quite unusual for a single organism to contain such similar proteins that do not bind the same metal atoms. How can a small difference in amino acid sequences lead the proteins to bind different metal atoms? The myoHr/MPII system is thus a unique model system that has the potential to reveal much about metal binding selectivity in proteins. In the proposed work, Colleen Caldwell (’15) and Brandy Russell (associate professor of chemistry) will work together to gather information about the structure of the cadmium-bound MPII.

2. Feasibility and preparation

Colleen began working on and reading about this project in January 2014, and has already familiarized herself with the project background and several of the relevant lab methods. Her internship/work experiences have prepared her well for independence in the laboratory. Her prior coursework supports her understanding of both the theory and the practice of this research. Colleen and Brandy will continue working together through the spring 2014 semester, further increasing Colleen’s expertise.

Brandy has been working on this project for several years, along with several Gustavus student. She has gained valuable experience with the idiosyncrasies of this particular protein, and has also learned a great deal about how to most effectively collaborate with students on her research. Several of Brandy’s past students have presented their research at national American Chemical Society meetings, and several are pursuing graduate study in biochemistry, as Colleen wishes to do.

Past work by Brandy and her student collaborators (including Colleen) have paved the way for us to make excellent progress this summer:

- We developed a system to “grow” the needed protein samples using *Escherichia coli* bacteria, which is much more efficient than collecting the protein from the *N. diversicolor* worms. (Laura Secor, Jordan Makela, Mandy Halfen)
- We modified existing protocols for removing and replacing iron from myoHr[4-6] for removing iron and adding cadmium to MPII. (Veronica Taylor, Mandy Halfen, Sarah Lucht)

- In the process of the above, we have demonstrated that cadmium indeed binds to MPII in a specific way. (Veronica Taylor, Sarah Lucht, and students from the 2012 CHE 385 class)
- We learned about and started to develop specific protocols for monitoring the structure of cadmium-bound MPII by NMR spectroscopy[7] (Veronica Taylor) and by native gel electrophoresis (Sarah Lucht).
- We identified a challenge with some samples degrading in storage and forming dimers (pairs of individual molecules linked together). We learned how avoid and minimize this problem. (Colleen Caldwell, and students from CHE 360 will be working on this further in spring 2014)

3. Collaboration style

Colleen has substantial laboratory experience, but this will be her first time in the role of research collaborator. Brandy has spent a lot of time learning to collaborate effectively with students in the research laboratory, but each new collaborator is different. After some discussion, we have outlined how we think the collaboration will work, but we are prepared to adjust as needed throughout the project.

The tentative plan is that we will each work simultaneously on separate aspects of the project, teaching and consulting as needed to bring the project together. Of course, Brandy has many more years of experience, so she will always be available to teach techniques and theory, help troubleshoot problems, ensure methods comply with disciplinary standards, and generally oversee the entire project. This is not a one-way relationship, however, and Brandy has intentionally organized the work flow so that Colleen can develop expertise focused on certain areas of the project and become a full intellectual contributor as she goes. We have tried to make the plan for collaboration clear in our project design below.

4. Project design

Developing protocols and learning new techniques

The first phase of any research project is determining and learning the methods to be used in the research. There are three such areas where we need to spend some time: cadmium binding experiments, native protein gels, and ^{113}Cd NMR spectroscopy. Brandy and Colleen will "divide and conquer" the methods to be developed:

The Russell lab has established basic protocols for binding metals to myoHrs, but for technical reasons, we need to tweak these and use a buffer that does not contain sulfur. Colleen will begin by practicing these complex, multi-day techniques on "known" systems. Once she can reproduce past researchers' results, she will adapt the procedure to use a sulfur-free buffer.

The Russell lab has been using native protein gels, but for this project we will need to identify several proteins to use as size standards. Brandy will read papers, consult with colleagues, and conduct experiments to find a good set of standards.

The basic ^{113}Cd NMR experiments have been set up on the chemistry department's new NMR spectrometer. NMR methods require significant expertise to develop: undergraduate students could do so, but it would likely take an entire summer to complete what an experienced person could do in a week. To allow Colleen to focus on

learning in other areas, Brandy will work out the NMR experimental details and teach them to Colleen only as needed.

Characterization of cadmium-bound MPII

The big questions as we characterize cadmium-bound MPII are 1) How many cadmium atoms are bound to each protein molecule? 2) Is there a large structural change upon cadmium binding to MPII? 3) Where, within the protein structure, does the cadmium bind? Colleen and Brandy will work together to design experiments that address as many of these questions as possible, although we adopt the scientists' habit of describing our plan in the third person and in passive voice below.

1) The number of cadmium atoms per protein molecule can be measured using ICP-MS (inductively coupled plasma-mass spectrometry) with the help of Jeff Jeremiason. We hope to be able to use ICP-MS to quantitate both cadmium and sulfur; since we know that each protein molecule contains one sulfur atom, we can then obtain a ratio of cadmium atoms to protein molecules. Sulfur detection by ICP-MS is very challenging, though, so we may need to send our samples to another lab or turn to another analytical method to quantitate the protein.

2) We will employ native protein gels along with fluorescence, UV/visible, and NMR (^{1}H, ^{113}Cd) spectroscopies to compare MPII with and without cadmium bound. These combined methods can, relatively quickly, give basic information about major structural changes upon cadmium binding. We expect to be able to determine, for example, whether MPII largely maintains its shape, loses all defined shape, changes to different but still well-defined shape, changes shape in one area but remains the same in another, or pairs up to form dimers.

Depending on the specific results and on the amount time available, we will pursue aspects of this question more deeply. For example, while native protein gels are a respected method to detect the presence or absence of dimers, it can be tricky to clearly establish whether one is observing dimer formation as opposed to some other size change. A reliable set of size standards will help, but it is possible that we will wish to follow up with an additional method like size exclusion chromatography.

3) If there is sufficient time, we will begin to address the question of *where*, within the protein structure, the cadmium binds. ^{113}Cd NMR may provide some insight into what types of amino acid are binding to cadmium.[7] Site-directed mutagenesis could be used to produce new versions of MPII that lack one or more suspected cadmium-binding amino acid. ^{1}H NMR experiments can be used provide highly detailed structural information, though this analysis would likely take longer than the grant period.

5. Anticipated outcomes & placement for publication

In terms of scientific outcomes, this project will yield the first information about cadmium-bound MPII structure beyond its amino acid sequence. Such information contributes to an understanding of how (and whether) *N. diversicolor* uses MPII to protect itself from cadmium toxicity. In the bigger picture, this kind of work advances our understanding of protein structure and our ability to engineer new proteins with novel industrial or home uses or to predict the structure of many proteins that cannot be isolated or easily studied.

Colleen will present her research results in a talk or a poster on campus (Fall Research Symposium, spring Sigma Xi Symposium, Celebration of Creative Inquiry), and if possible, at a regional undergraduate venue such as the Midstates Consortium for Math and Science Undergraduate Research Symposia or the Winchell Symposium. If the results warrant it, Colleen and Brandy may present the results of our work at a regional or national conference such as the American Chemical Society meeting.

If we are very lucky, we may be able to submit our results as an article to a well-respected chemistry or biochemistry journal, such as *Biochemistry* or *Journal of Biological Inorganic Chemistry*. In reality, it is very rare in this subdiscipline of chemistry to produce a publishable body of results in a single year, and this project likely needs a few more years to mature. Still, the work proposed here is designed to be likely to produce results significant enough to be included eventually when the work is published. A small portion of the results are very likely to be included in a manuscript that Jeff Dahlseid and Brandy have been planning to write about a project from his laboratory about another aspect of MPII.

In terms of outcomes for Colleen, this summer will provide a deep experience with a biochemistry research project that will help prepare her for graduate study. In addition to the laboratory work, Brandy and Colleen will read and discuss related literature articles and have informal conversations about graduate school. As part of a summer research community, Colleen will have regular opportunities to practice presenting her research, to hear about her peers' research, and to learn about and discuss science research ethics.

6. Anticipated research completion date

Although this project will likely continue for a number of years, we believe it is feasible to complete the pieces of this project proposed above during summer 2014 and the 2014-15 academic year. Our completion date is therefore May 30, 2015.

Participant details

1. Biography for Colleen Caldwell

I graduated from Maple River High School with honors in 2011. I developed a strong interest in biochemistry and protein science after an experience with seizures induced by a possible protein channel disorder. This strongly influenced the path I chose for my continued education. I am currently a junior Biochemistry and Molecular Chemistry major and Neuroscience minor. I have found strong academic interest in biology, chemistry, and neuroscience, especially as related to the chemistry of proteins. At Gustavus I have worked in the Dining Services as well as the Chemistry Department. I have especially enjoyed working with the Chemistry department where I held positions both as a tutor in Chemistry and as a teaching assistant which allowed me to share my interest with other students considering a similar path. In addition to working on campus I have also worked full time in Mankato between positions at a hotel and the Mankato Water Treatment laboratory. Besides my work experience, I have participated in extracurricular activities including Chemistry Club, various musical groups, and volunteering as a tutor.

Following graduation from Gustavus I plan to attend graduate school to study Biochemistry and Protein science and participate in research. I hope that I will eventually

be able to research the intricacies of protein function as associated with various neurological diseases. Participating in summer research with Dr. Russell would allow to me be better prepared for the next step in my education as well as allowing me to explore my career and vocational interests.

2. Career/educational trajectory statement by Colleen Caldwell

Throughout my time at Gustavus, I have taken several opportunities to explore different career options. I have found a strong interest in all of my Biochemistry related classes and labs, as well as working as both a tutor and teaching assistant for the Chemistry Department. This has helped me confirm to myself that Biochemistry is certainly a field I would like to enter. Outside of Gustavus I have also held several jobs, including an internship testing water and the Mankato Water Treatment laboratory. The job was mainly analytical. While I was able to learn a lot about important laboratory techniques which have been beneficial in both biology and chemistry, I found the work to be repetitive with little forward progress.

Beginning January of 2014, I have been able to work with Dr. Russell on research in metalloprotein II and myohemerythrin, two metal binding proteins. During my time working in and out of the lab I have learned a lot, not only about these two metal binding proteins, but about research in general. In contrast with my other work experience in an analytical setting, I found that research took hold of my interest in a whole new way. With each new day there was always the feeling of moving forward, unlike my previous work experience. Even with the challenges that come along with research, new accomplishments continue to be made. Research allows helps the development of strong critical thinking skills as well as creativity. Working with Dr. Russell has been great for me and strengthened my interest in Biochemical research, especially relating to proteins.

The opportunity to work with Dr. Russell over the summer would allow for a more extensive experience in Biochemical research. Because there are less time restraints than during the regular semester, we would be able to make much more progress on our project. Further, I would be able to have a more representative experience of what a full time career in Biochemical research would be like. The experience would certainly prepare me to continue my education after I graduate from Gustavus to obtain a PhD in Biochemistry. In pursuing my goal I would be able to study a subject that I love and continue on to a career that I find both enjoyable and rewarding.

3. Biography for Brandy Russell

I graduated with a Bachelor of Arts degree in chemistry from Alfred University in 1998. My extremely positive experience as an undergraduate researcher under Prof. Johanna Crane sparked my interest in teaching at a primarily undergraduate institution. I completed my doctoral work in the chemistry department at the University of Rochester in 2003. During graduate school I received an award for excellence in teaching and several named graduate research fellowships. My thesis research in Prof. Kara Bren's group centered on nuclear magnetic resonance (NMR) investigations of protein folding, conformational dynamics, and stability and resulted in seven peer-reviewed articles and several invited seminars and poster presentations. After my graduate studies, I accepted a Howard Hughes Medical Institute postdoctoral fellowship in the chemistry department at the University of Illinois at Urbana-Champaign. In my postdoctoral work, I focused on

engineering metal binding sites into proteins to tune their reactivity. In addition to laboratory research, I collaborated with my advisor, Prof. Yi Lu, on a new teaching project: a unique, multi-level, multidisciplinary course for science majors (supported by the Howard Hughes Medical Institute). Both my laboratory research and my education research led to seminars and poster presentations (some invited) and peer reviewed articles, including a research article in *Nature* and an article in the Education Forum of *Science* magazine. I joined the faculty of the chemistry department at Gustavus Adolphus College and was awarded tenure in spring 2012. During my time here, I have mentored 18 students in my research laboratory and traveled to two national American Chemical Society conferences with three of those students to present our work.

3. Career statement for faculty member Brandy Russell

I am very excited about the prospect of working collaboratively with Colleen Caldwell this summer. I have a great deal of confidence that this collaboration will be fruitful, both in terms of making progress on my research and providing Colleen with an educational experience in which she will experience firsthand what a research-based career is like and learn biochemistry more deeply.

I consider collaboration with undergraduate students to be a vital part of my scholarly work. One of my chief goals as an educator is to cultivate students' curiosity about science, in addition to helping students develop the creativity, self-confidence, knowledge and skills to act on that curiosity. The research laboratory is an ideal arena for this kind of mentoring. I designed my research program with the intent of including undergraduate student collaborators at every step.

The specific subjects of my research have evolved over time, but all relate to my interests in metalloprotein structure, folding, dynamics, and function. In all of my research projects, I have employed spectroscopy to probe the metal-coordinating environment of metalloproteins. When I joined the Gustavus faculty in the fall of 2005, I started a brand-new research project, applying my favorite methods and questions to a protein I had never worked with before, myohemerythrin (myoHr).

More recently, I launched the project in this proposal. I worked with several Gustavus students to get the project started, and chose to spend my sabbatical research time continuing the work. I reached a frustrating and disappointing roadblock during my sabbatical but between my efforts alone during my sabbatical and a collaboration with Colleen this January, the project is back on track. Colleen and I work very well together – having the opportunity to work with a talented and independent research student like her will have an enormous impact on my research progress this summer.

References

1. Demuynck, S., et al. (1991) *C. R. l'Academie. Sci., Ser. III* 312(7): 317.
2. Demuynck, S., et al. (1993) *Eur. J. Biochem.* 217: 151.
3. Nejmeddine, A., et al. (1997) *C. R. l'Academie. Sci., Ser. III* 320(6): 459.
4. Zhang, J. H., et al. (1991) *Biochemistry* 30(2): 583.
5. Zhang, J. H. and Kurtz, D. M. (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89(15): 7065.
6. Zhang, J. H., et al. (1992) *Inorg. Chem.* 31(8): 1359.
7. Oz, G. L., et al (1998) *Biochem. Cell Biol.* 76(2-3): 223.