APPLICATION CHECKLIST
Presidential Faculty/Student Collaboration and Publication Grant

Deadline February 25th

Please print and complete this checklist and attach it as the cover page of your grant application. For more information about Presidential Faculty/Student Collaboration and Publication grants, please see http://gustavus.edu/facdev/GrantOpportunities/PresidentialGrant.php.

Faculty information

Name:____Jeff Dahlseid_____ Dept:____Biology & Chemistry_____

Email: ___dahlseid@gustavus.edu_____ Rank:____Associate Professor_____

Student Information

Name:____Xiao Xiu_____ Year:____Junior (class of '12) _____

Email: ___xxiao@gustavus.edu_____ Major:___Biology_____

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
- ☑ Explanation of how this project fits into the educational trajectory of the student (include year of graduation; student eligibility is limited to full-time returning students)

☑ **Presidential Budget Proposal Form attached as last page of application**

☑ **Nine (9) copies of completed application (including this checklist) to be submitted to the John S. Kendall Center for Engaged Learning (SSC 119)**

If successful, my proposal can be used as an example to assist future faculty applications. This decision will not in any way influence the evaluation of my application.

(Yes) No (please circle one)

Presidential Faculty/Student Collaboration Grant
BUDGET INFORMATION

Faculty Stipend ($300 per week, up to $2,400)

Student Summer Stipend ($400 per week, up to $3,200)

Student Summer Campus Housing ($43.75 per week, up to 8 weeks)

Total Budget Maximum ($6450 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)		$1000
1: lab supplies	Cost: $1000	
2:	Cost:	
3:	Cost:	
Travel Costs (cannot include conference travel; see http://cms.usu.edu/finance/travel.php for allowable travel expenses)		$
Airfare:		
Mileage: Number of miles ____ @ $0.55/mile		
Lodging:		
Meals:		
Stipends & Housing		$5950
Faculty Stipend	$300 per week, up to $2,400	$2400
Student Summer Stipend	$400 per week, up to $3,200	$3200
Student Summer Campus Housing	$43.75 per week, up to 8 weeks, $350	$350
TOTAL EXPENSES		$6950
AMOUNT REQUESTED		$6450

Have you applied for, or received funding from, another source to help support this project? No.

Funding Source:

Amount:

Please explain how the Presidential will be used in addition to the other funding.

Function of *Nereis diversicolor* metalloprotein II (MPII) in cadmium resistance

Jeff Dahlseid
Xiao Xiu

I. Project Details

A. Brief description of the proposed project and collaboration:

This request is for funds to support the research collaboration of Jeff Dahlseid (Biology and Chemistry Departments) and Gustavus student Xiao Xiu ('12) in the summer of 2011. The overarching goal of the proposed project is to investigate the potential function of metalloprotein II (MPII) from *Nereis diversicolor* in conferring resistance to the biologically toxic metal cadmium. Support of the project will provide the opportunity to complete planned experiments, present the results in symposia and in manuscript for publication, and to provide mentoring through collaboration that will help Xiao further develop her critical and independent thinking and scientific communication skills.

Xiao Xiu enrolled in my 2008 Biomolecular Research (IEX) course for first year students the first time I taught it. New to working with first years in research, I was impressed by Xiao's resilience and eagerness to learn. She found laboratory research fun and so sought support to collaborate with me from our Howard Hughes Medical Institute (HHMI) undergraduate research program for rising sophomores. Xiao won the award and thus began our collaboration. That first summer, she continued work on the project she started in the IEX course. While largely a methodological project, Xiao demonstrated great enthusiasm, highly effective technique (also known as "good hands"), persistence in repetitive work, and productiveness. I had not worked with a rising sophomore before and was not sure what to expect, but I was impressed. Furthermore, when I agree to collaborate with a student in summer research, I consider it a serious and long-term commitment. So, I invited Xiao to consider a second summer of research in my laboratory to begin work on her current project. A second summer and a new project added breadth to her laboratory training, provided opportunity to learn new science and more deeply, and further developed her ability to read the primary literature and present her work. Xiao has continued work on her project through the fall and January terms, and is presently enrolled in BIO 392 Biology Research for 1.0 credit to work on her project. Continued work on this project through the summer will allow Xiao to see it come full circle to completion, to present a full story at symposia, and to prepare and submit a manuscript for publication. These experiences will deepen her preparation and increase her desirability as an applicant for whatever she pursues after Gustavus.

B. Project Description:

Nereis diversicolor is a marine worm of the North Atlantic that exhibits a remarkable adaptability to environmental changes, including a tolerance to heavy metals sufficient to permit survival in contaminated environments otherwise toxic to living systems[1]. The molecular mechanisms for this tolerance are not yet clear. Demuynck *et al.*[2] reported the isolation of a *N. diversicolor* protein, metalloprotein II (MPII), which bound to the heavy metal cadmium. They

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[1] Pook, C. *et al.* (2009) Marine Pollution Bulletin **58**: 1063-1071.
[2] Demuynch, S. *et al.* (1993) Eur. J. Biochem. **217**: 151-156.

determined the MPII amino acid sequence, which revealed that it was 81% identical to *N. diversicolor* myohemerythrin (myoHr), a known iron-binding protein. While the amino acids in myoHr that bind iron are also present in the same locations in MPII, natural samples of MPII bind cadmium, not iron[2]. Thus, the authors speculated that the specific cadmium binding property of MPII might provide a molecular mechanism for part of the high metal tolerance of *N. diversicolor*. Further evidence comes from a report that the levels of MPII protein increased in worms exposed to cadmium, a response consistent with it having a role in metal tolerance[3].

The goal of our project is to investigate the potential function of *N. diversicolor* MPII in conferring tolerance to cadmium. We are pursuing three lines of investigation. If MPII functions directly in cadmium tolerance, we would expect to be able to collect evidence that correlates the presence of MPII with cadmium tolerance. The first two lines of investigation are to explore possible correlations. For the first, we are experimentally testing whether introducing high levels of MPII into baker's yeast confers increased tolerance to cadmium. For the second, we are isolating the DNA sequence for the MPII gene from *N. diversicolor* samples isolated from both natural and high-metal containing environments. We reason that worms that have become metal tolerant so as to survive in high-metal environments may contain changes to the sequence of MPII that improve its function in cadmium tolerance compared to worms in more natural environments. Finding such changes would support a role for MPII in cadmium tolerance and, should such changes be found, we can test directly the functional consequence of the changes to cadmium tolerance by testing growth of baker's with high levels of these MPII variants.

Additional support for the function of MPII in cadmium tolerance includes higher levels of MPII observed in worms exposed to cadmium[3]. In particular, researchers noted that the levels of MPII protein were increased, but the levels of the corresponding messenger RNA (mRNA), the informational molecule that instructs protein production, were unchanged[2]. Ordinarily, the levels of mRNA for a particular protein generally dictate the level of the corresponding protein. Thus, the reported results suggest that regulation of MPII may be at the level of protein production. Previous research has described other metalloproteins that regulate their own expression (autoregulate) as a function of the availability of the metal they bind, essentially serving as a biosensor[4]. We speculated that this might also be true for MPII and are interested in testing whether MPII autoregulates its own expression. For the third line of investigation, we wish to measure MPII mRNA and protein levels that exist in MPII-containing yeast grown in the presence or absence of cadmium. If we reproduce the results in yeast that were previously observed in worms, it would suggest that MPII and cadmium are sufficient to regulate its MPII protein levels, confirming autoregulation, and provide us with an experimental system to further investigate the molecular nature of the phenomenon.

For our first line of investigation, we sought to isolate the effect of the MPII protein in cadmium tolerance from the many other potential mechanisms for metal/cadmium resistance that *N. diversicolor* worms have. Thus, instead of testing the metal tolerance of worms, we are analyzing the metal tolerance conferred by MPII to another organism (baker's yeast) that is ordinarily quite sensitive to cadmium and does not have its own MPII, a so-called heterologous system. This system has several advantages; yeast can be readily manipulated so as to produce high levels of MPII; cadmium tolerance is easily assessed by a simple growth assay; the growth rate of yeast allows for the experiments to be done quickly; and testing variants of MPII is readily accommodated. We have had a DNA chemically synthesized that will promote the

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[3] Demuynck, S. *et al.* (2004) J. Exp. Biol. **207**: 1101-1111.

[4] Cairo, G. and S. Recalcati. (2007) Expert. Rev. Mol. Med. **9**: 1-13.

production of high levels of MPII protein when introduced into yeast, and Xiao has introduced this DNA into appropriate strains of yeast. She has placed these yeast and appropriate controls on standard media with and without cadmium, and assessed their growth. Thus far, she has seen no discernable increase in tolerance due to the presence of the MPII protein. Xiao still needs to repeat this experiment and some related controls, which essentially include measuring the levels of MPII mRNA and/or protein to insure that there is a high level of MPII protein in appropriate yeast strain but not in the control strain, as expected. If this result holds true, it is consistent with something else, and not MPII, having a significant role in cadmium tolerance in *N. diversicolor*.

For our second line of investigation, we have sought to isolate and sequence the DNA for the gene for the MPII protein for worms isolated from both a natural and high-metal (including cadmium) environments. We have identified collaborators at the University of Exeter, Professor Tamara Galloway and her graduate student Christopher Pook, who have provided us with homogenates of the appropriate worms. Three Gustavus biochemistry majors previously isolated RNA from the homogenate of worms from a high-metal environment and isolated a fragment of the MPII DNA. Xiao has obtained the DNA sequences for several independent isolates, which encompass over half of the gene. She has isolated RNA from the homogenate of worms from a natural environment and isolated a similar fragment of MPII DNA. Xiao has additional work before this part of the project will be completed, but she has made good progress. She needs to obtain DNA sequences for the second fragment of DNA, and isolate and sequence DNA fragments for the remainder of the MPII gene for both types of worms. Once we have these DNA sequences, we can analyze them for any differences and, if found, make DNAs to test the MPII variants for function in cadmium tolerance as described above.

For our third line of investigation, we seek to address the possibility that the MPII protein autoregulates its own production. For the same reasons outlined for our first line of inquiry, we will use baker's yeast as a heterologous system to address this question. Xiao will measure the levels of MPII mRNA and protein in baker's yeast strains grown in the presence or absence of cadmium. In order to do this properly, she will need two things. First, Xiao will need to determine how to appropriately expose yeast to cadmium, without compromising the experiment due to its toxic effects. This may involve a short term but acute exposure, or a sub-toxic but chronic exposure. It most certainly will involve some optimization. Second, she will need to generate reagents to detect the MPII protein. Presently Xiao and I are working with Professor Lammert to raise antibodies in rabbits for this purpose. We have rabbit sera in hand that may contain the antibodies, but we need to verify that. Xiao can then grow yeast strains under the appropriate conditions, extract their RNAs and/or proteins, and measure the levels of MPII mRNA and protein using methods standard to my laboratory. This will expand Xiao's experimental training and repertoire.

C. Anticipated completion date:

Having started on the project last summer, Xiao is already well underway with some of the experiments and has the corresponding techniques well in hand. Her work this semester should allow her to develop the reagents and learn the remaining techniques, so I anticipate most of the experimental work and identification of an appropriate journal for publication will be completed by the end of the summer. Xiao intends to continue the project during the academic year, and this will include completing any remaining experiments, presenting her results at two or more symposia, and working with me to prepare a manuscript for publication. It is my expectation that we can realistically expect to get this done by the end of May 2012.

D. Anticipated outcomes:

The outcomes we anticipate from the funding of this project include:

1) Completion of the experiments described above,
2) Two to three presentations (by Xiao) of the results at symposia, and
3) Preparation and submission of a manuscript for publication.

E. Placement for dissemination:

Xiao will present the results of this project at the Gustavus Fall Research Symposium, the Midstates Consortium for Math and Science Symposium, and possibly an appropriate national meeting (if sufficient funds are available). During the summer of 2011, Xiao will identify the national meetings and journals that would serve as appropriate places for presentation and publication, respectively. If funding can be secured, Xiao will present her results at a national meeting. An example of a possible meeting would include the American Society for Biochemistry and Molecular Biology meeting. In either case, we will work together to prepare a manuscript for publication, which we intend to submit in May of 2012, or earlier if possible. Potential journals may include the Journal of Biological Chemistry or Biochemistry, a journal of the American Chemical Society.

II. Participant Details

A. Biographies:

Xiao Xiu

I am a Junior Biology Major, and planning to graduate in May 2012.

As a freshman, I wanted to explore and learn through my college experience. I knew that I wanted a science major, but I wasn't sure of what kind of science I would like. J-term arrived, and the course "Biomolecular Research" attracted my attention. At first, I just wanted to experience what research was like. As I was drawn more and more into the course (like how to use the lab instruments and techniques), my interest in research grew. Because I had fun through the research experience, I decided to apply for the summer research opportunity provided through the HHMI program. Having no confidence that I would get in due to the competitiveness, I still tried because the opportunity was just too special to me to pass it up. I got my feet wet during J-term research, and the first summer permitted me to "float" in water (gained more knowledge, but still far from the end). The first summer research provided a more in-depth research environment because there were practice presentations and more literature readings. From the summer experience, my interest in research grew even more because it allowed me to work independently on a topic that interests me instead of a class or lab that introduces a variety of topics but that do not necessarily interest me.

Spring of my sophomore year, I decided that I would switch from a Biochemistry major to a Biology major because it would provide me with more room in my schedule to do research. I like how research enables me to think in a creative way and plan my own experiments instead of a class where most facts are laid out and labs are set up. Research brings more freedom for me to explore the topics that interest me than a classroom environment.

I applied to do research again during the summer of my sophomore year. The second summer, I started a different project, and it gave me a new perspective of the variety of topics that are being studied by scientists. From the second summer I learned to do more than just float in the water, I learned to swim (I learned new techniques that were not used in the previous one,

and I gained more knowledge about literature reading and using the literatures to create new methods for experiments). The new project diversified my research experience and knowledge, which has prepared me to transition into the upcoming summer project if I get nominated for this grant. With the second summer on this project I want to *swim in a race and strive to win an award* whether it's a real award or self-accomplishment. I would like to present the new knowledge to the field of molecular biology, write a paper, publish my hard work, and make an impact in the scientific community.

Jeff Dahlseid

I earned my Bachelor of Arts degree from Gustavus, with a major in chemistry and a minor in biology. I conducted research with Professor John Lammert on the immunosuppressive effects of phenytoin, an anticonvulsant drug used to treat epilepsy, on mucosal antibody production. Inspired by my professors and my experience as a laboratory teaching assistant at Gustavus, I thoughtfully considered how to apply my deep interest in the molecular life sciences to the world's needs and decided to pursue a career as a teacher/scholar in a liberal arts setting. I went to graduate school at Northwestern University and studied with Professor Susan Pierce, an immunologist (now at the National Institutes of Health), in the department of Biochemistry, Molecular Biology & Cell Biology. My thesis focused on the biochemical events (formation of peptide-protein complexes) in late endocytic compartments (vesicles) that are necessary to activate so-called B cells to produce antibodies. During this time, I also pursued and developed my interests in teaching through teaching assistantship opportunities and by auditing a course on teaching in higher education from Northwestern's School of Education and Social Policy.

Following completion of my doctorate, I accepted a post-doctoral fellowship with Professor Mike Culbertson at the University of Wisconsin, Madison, in the Department of Genetics and Laboratory of Molecular Biology. I left immunology to study the regulation of gene expression at a post-transcriptional level in a model genetic organism. Specifically, my research focused on a specialized biochemical pathway for RNA degradation in the simple eukaryote, baker's yeast. This was a conscious decision, to move to a system that was more affordable, genetically tractable, and better suited for collaborating with undergraduates in a liberal arts setting. I secured funding from the NIH to support the last two years of this three and a half year experience. I also began teaching, yeast genetics at the University and undergraduate biochemistry at Edgewood College, a small Catholic liberal arts institution in Madison.

I started a tenure-track position in the Chemistry Department at St. Olaf College, where I taught mostly biochemistry and started my independent research program. I helped secure external funding from the Merck/AAAS Undergraduate Science Research program and won a major award from the National Science Foundation for the research in my laboratory. After four years, I separated from St. Olaf to accept a position with the biochemistry program at Gustavus, for which I serve as program director. At Gustavus, I have taught courses in biochemistry, biology and January term (both BIO and NDL). I re-established my research program and have helped secure external funding to support development of our programs and undergraduate research, from the Howard Hughes Medical Institute (HHMI), LiCor Corporation, Project Kaleidoscope's Keck Consultancy, and the Merck/AAAS program (twice). I have seven peer-reviewed publications, including two since I came to Gustavus, plus I have one manuscript in preparation and anticipate another handful to emerge upon completion of projects in progress, including the one proposed here.

B. Career statements:

Xiao Xiu

I have a lot of motivation to continue with research for the third summer, and the second summer on the same project. The Presidential Faculty/Student Collaboration and Publication Grant will provide me with a second year on the same project, and it is key to my success because I will have the opportunity to see and work on a research project from beginning to end. My commitment to the research project is like an extracurricular activity on a resume, so it will show my personality.

The second summer of research will allow me to develop my project enough to present it at the Midstates Consortium for Math and Science and possibly a national meeting, and submit the results of the project for publication. After learning the techniques and becoming familiar with it in the first summer, I will be more efficient and effective at producing quality data in the second summer. The second summer will also provide me with more practice on communication and writing skills that are useful skills wherever I go. Then, when I do have some quality data, I will also gain the experience of putting it together for publication. The experience from start to finish is hard to attain with just one summer of research experience, so the finish product will mean a lot to me because its like "I have experience and done all of this as an undergraduate?!"

Then on the other hand, the research experience will not only teach me new knowledge and experiences but it will also make me a stronger candidate for a graduate school. I will be more prepared for the process of research from beginning to end even though there is more to the process in graduate school. Also, I will have better communication and writing skills so I don't have to spend as much time in graduate school to learn those skills.

Jeff Dahlseid

The projected described in this proposal is part of collaboration between my laboratory and Dr. Brandy Russell's (Chemistry), a major collaborative undertaking with a colleague at my own institution. Dr. Russell and I were able to initiate this collaboration with support we helped secure from the Merck/AAAS Undergraduate Science Research program. While we included our collaborative project in the proposal because it was good and interesting science that we wanted to pursue, another major motivation was to secure support for interdisciplinary and/or cross-department research and to promote undergraduate research (stipends and supply money), which the grant provided. Xiao was one of five students who worked on aspects of my and Dr. Russell's collaboration; at least ten others were supported to work on other Gustavus research projects. We also sought funds form Merck/AAAS to further develop our summer research program; we sought a stronger community of learners. The grant was the second in a series of two from Merck/AAAS that have materially improved our summer undergraduate research programming, helped us further develop the community of learners, and quite likely also positively impacted the successful review of our last HHMI proposal.

By those motivations, we have succeeded. Yet, for all we have poured into the project thus far (grant writing, mentoring students, broadening the focus of our attention on research to support interdisciplinary and undergraduate research on campus, etc.), we have little beside the grant to show for it. Completion of the project described here, specifically publication, represents the natural achievement that should follow the effort invested, and will document our productivity from the support provided by the Merck/AAAS program. This will be both personally gratifying, but will also help in securing funding for our work in the future.