Grant-in-Aid of Research, Artistry and Scholarship

Title: Chemical Biology Infrastructure at UMD

Abstract: Matching funds are requested to purchase equipment fundamental to research at the interface of chemistry and biology (centrifuge and a -80°C freezer). Funding is sought to serve the specialized need of equipment that will serve chemical biology. In keeping with the ongoing support the department has invested in the development and growth of the chemical biology program within a once traditional chemistry department, matching support of 33% will be provided from Department and College of Science and Engineering at UMD. Due to the spatial separation of those invested in chemical biology from the rest of the department (Swenson Science Building contains chemical biology while the majority of the department resides in the Chemistry building) as well as the diversity in needs, core facilities are stretched thin. We seek to address this gap by requesting shared and fundamentally necessary equipment that will be dedicated to protein expression and purification.

Category No. 2: Partial support for major capital equipment to be shared by all members of the Department of Chemistry and Biochemistry that engages in chemical biology research. The requested equipment will fill a dire need that is currently unmet. The fulfillment of this critical need for capital equipment dedicated to a common and general use will increase the probability of new sources of external funding being obtained by a research active and currently funded cohort.

Equipment Requested: Centrifuge and -80°C Freezer

PI: Anne Hinderliter, Co-PI: Berry and Grundt

Department of Chemistry and Biochemistry University of Minnesota Duluth (UMD)

Dr. Hinderliter will maintain the -80°C freezer. Dr. Berry will maintain the centrifuge. Funds from the Department of Chemistry and Biochemistry will provide service and repairs in accordance with its commitment to research.

Anne Hinderliter (Department of Chemistry and Biochemistry) will be Principal Investigator and Primary User. Her research is focused upon elucidating why signaling complexes form at the membrane.

Steven M Berry (Department of Chemistry and Biochemistry): Primary user. The focus of Dr. Berry’s research is to understand the fundamental principles of metalloprotein design through the modeling, design and redesign of copper centers in existing protein scaffolds.
Peter Grundt (Department of Chemistry and Biochemistry): Primary user. The focus of Dr. Grundt’s research is to understand the role of the parasite *T. gondii* in the etiopathogenesis of schizophrenia.

**Category Justification**

Partial support is requested for the purchase of a centrifuge and a -80°C freezer. This equipment will be shared by a number of faculty members in the Department of Chemistry and Biochemistry whom are united in achieving a common goal in advancing chemical biology at the University of Minnesota Duluth.

**Present State of Knowledge**

*Centrifuge:* The Sorvall Evolution RC floor model centrifuge provides unmatched productivity with numerous features, including ergonomic operation, low noise levels, and a wide variety of compatible rotors. Most importantly, the centrifuge has critical feature denoted as the DuraFlex gyro system, which allows the highest imbalance tolerance possible. Many of our key research applications require the centrifugation of multi-liter volumes using large bottles. These bottles are known to fail with routine use and many centrifuges on the market are not robust enough to handle the resulting imbalance. In the last three years we have replaced the motor and drive board on our current centrifuge twice as a consequence of this problem. Also included in the purchase of the centrifuge are several lightweight aluminum rotors with carbon canisters that will enable effective processing of bacteriological samples. As the purchase of the centrifuge includes rotors, bottles, and the centrifuge itself, the package is being offered at a significant (~35%) discount to the university.

*-80°C Freezer:* An upright freezer (220V) is sought that is large enough to support multiple users yet retain enough space to be retained as emergency storage for the existing (and full) Biochemistry -80°C. Importantly, the freezer will contain thirteen cu. ft. of storage space which will be adequate for storage requirements of three users. An important component of freezer system will be the sensaphone emergency dial out system to warn of either power failure or system failure that could endanger valuable freezer contents. Similarly to the centrifuge, the -80°C is being offered to the university at a 30% discount.

**Plan of Work**

Anne Hinderliter, Assistant Professor

Principal Investigator and Primary User
Membranes offer infinite possible docking combinations onto which to assemble proteins dedicated to a specific signaling outcome. Little appreciated is by the very act of ‘docking’ the underlying pattern of membrane to which the molecule docked will be altered. Membranes are a self-organized system with unique biological properties. To understand membrane localized docking interactions, a strong research program that has an emphasis in binding interactions, of how to measure experimentally and model binding, is critical. My work has a common theme of having predictive power in the how and why things bind and has an emphasis in interactions localized on or near the membrane. This body of work has evolved in the past few years from elucidating how signal transduction complexes may be formed at the membrane to applying these defined principles to systems of pharmacological and material science interest. My long-term goal is to define those interactions that both lead to pathological interactions at the membrane, and to use such understanding to formulate unique organized systems mimicking these interactions to both treat and diagnose disease states.

The membrane is more than a barrier to drug delivery, it has still little understood properties that can transmute and modify the influence of drugs, signaling molecules, and proteins. In eukaryotes, a plethora of lipid species exists, with small differences in lipid chemical structure. The synthesis of such a variety of lipids argues against a simple barrier function for cellular membranes. We have suggested (1-3) that the role of lipids is that of signal amplification through multiple nearest neighbor interactions and of regulation of assembly of proteins in signaling complexes. The portion of my research program dedicated to basic questions utilizes a variety of experimental techniques and an equally varied selection of proteins that are modified, expressed and purified at UMD to advance these ever evolving ideas.


Steven M. Berry, Assistant Professor

Primary user

Our research group is interested in metalloprotein structure-function relationships with the ultimate goal of designing and engineering novel metal binding sites into proteins. Our main
goal is to design metal binding sites that impart new functionality to a protein. We accomplish this by altering existing metal binding sites as well as designing new sites at other locations in the protein scaffold. Advancements in protein mutagenesis and protein synthesis technologies have made protein manipulation routine. However, we require a centrifuge and a freezer that are capable of meeting our protein purification, protein manipulation, and protein storage needs.

The primary aim of our research group is to construct protein metal binding sites that achieve catalytic status. Study of native metalloenzymes is useful for determining the specific structural characteristics that give rise to a particular function. But, designing a protein to gain new functions is an even more powerful way of testing this relationship (1). We plan to accomplish this through the mutagenesis of azurin as described briefly in summaries of four major avenues of the project. First we have modified the redox potential for the azurin copper site by adding non-polar residues near the active site (2). Importantly for our long-term goals, the tuning of the redox potential will allow us to tune reactivity. Second, we have proposed to assay for the formation of radical containing amino acids and cross-linked amino acids near the copper site in azurin. These naturally occurring unusual residues are found in many catalytic metalloproteins. Third, we propose to examine the phosphate ester hydrolysis activity of a normally unreactive copper protein with the goal of producing activity. Finally, we have created and continue to examine models of catalytic Type 2 Cu sites such as those in nitrite reductase and peptidylglycine α-hydroxylating monoxygenase (3). The successful completion of all these projects requires modern protein purification, concentration, and separation techniques by the means of a floor model centrifuge. Long term storage of the proteins is made possible with a -80°C freezer.


Peter Grundt, Assistant Professor

Primary user

Toxoplasma gondii is an obligate intracellular parasite in the phylum Apicomplexa. Although its life cycle can only be completed only in cats, it infects a wide variety of intermediate mammalian hosts. In animals, infection with T. gondii may alter behavior and neurotransmitter function. In humans, acute infection with T. gondii can produce psychotic symptoms similar to those displayed by persons with schizophrenia. The link between T. gondii and schizophrenia
can be expanded further. Epidemiologic studies have demonstrated that individuals that have antibodies for *T. gondii* show a significantly higher probability to be affected by schizophrenia than seronegative controls. Thus, establishing the role of *T. gondii* as contributing environmental factor in the onset might lead to new medications for its prevention and treatment. (1)

Recently, we have found that compounds based on the natural products indirubin and tryptanthrin potently disrupt the life cycle of *T. gondii*, while displaying low host cytotoxicity. (2) At this point, the structure-activity relationships of these derivatives as well as the underlying pharmacological mechanisms remain unclear. Currently, we are designing and synthesizing analogs to a) improve the bioavailability of these classes of compounds and b) to devise pharmacological probes for mechanistic studies. Our research relies heavily on cell-based pharmacological evaluation, which at this point is performed off-site resulting in several months of waiting time. With the equipment requested in this application we intend to pursue in-house *T. gondii* assays. Due to *T. gondii* life cycle limitations adequate storage and testing equipment (such as -80°C freezer and centrifuge) for tissue culture components, samples, and parasite are very important for conducting these experiments properly. Furthermore it opens up possibility to develop new protocols, which are currently unavailable, due to technological limitations. Thus, to pursue this project more efficiently, both pieces of equipment in this application are pivotal for the outcome of these studies.


**Budget and Need Justification**

For the faculty dedicated to chemical biology there is some basic infrastructural equipment that is common in need, in use, and if obtained will enable continued and greater success in achieving external research funding. The primary users have had and currently hold funding from the National Institute of Health, Research Corporation, Dreyfus Foundation, Petroleum Research Corporation, and the Stanley Medical Research Institute. We recognize that the equipment requested represents an investment by the University of Minnesota to achieve the goal of greater and more varied funding. None of our current funding has been committed to major equipment within our budgets, eliminating this source of funding for the requested equipment. The Principal Investigator, A. Hinderliter, began her appointment in August 2007. Her start-up was spent entirely on the purchase of a lifetime spectrometer, a differential scanning calorimeter, and an isothermal titration calorimetry (the use of which she shares with
She transferred a research grade steady-state fluorimeter and a working wet laboratory that were all purchased from past and current research grants.

The equipment requested is a robust centrifuge and a -80°C freezer. Currently, we (Hinderliter, Berry, and Grundt) store our extensive transferred and UMD generated collection of purified proteins, plasmids, primers, cell lines, lipids, and fluorescence probes in the -80°C freezer within the undergraduate Biochemistry laboratory and scattered amongst various shared freezers in Swenson Science Building. This is a recipe for disaster as these freezers could be accidently left open by the many users whom are not accountable to the primary users of this proposal. The contents of the freezer represent years of work in the construction of mutants and cell lines, and thousands of dollars of reagents.

The centrifuge that is currently shared by the chemical biology faculty is disturbingly prone to unbalancing which bends the spindle and renders the centrifuge unusable for extended periods. This creates havoc in numerous research groups as this centrifuge is extensively used for protein purification. This proposal seeks to address this limitation by trading in and replacing this flawed equipment.

Word Count: 1,390