EDUCATIONAL PROPOSAL

The goal of this educational plan is to implement a discovery-based research experience in an undergraduate organic chemistry laboratory. Engaging the undergraduate population within their first two years in science, technology, engineering and math (STEM) fields is paramount for retention of students for careers in science and technology.[25,26] An estimated 40% of students who enter STEM fields fail to achieve their STEM degree.[26] This attrition is inadequate to meet the forecasted demand of 1 million new STEM professionals needed within the coming decade.[25] Traditional lecturing methods are not as effective at retention.[27,28] However, long duration research experiences, have been shown to not only increase retention in STEM fields, but also boost perceived knowledge gain, personal confidence, and self-identification as a scientist.[26-27]

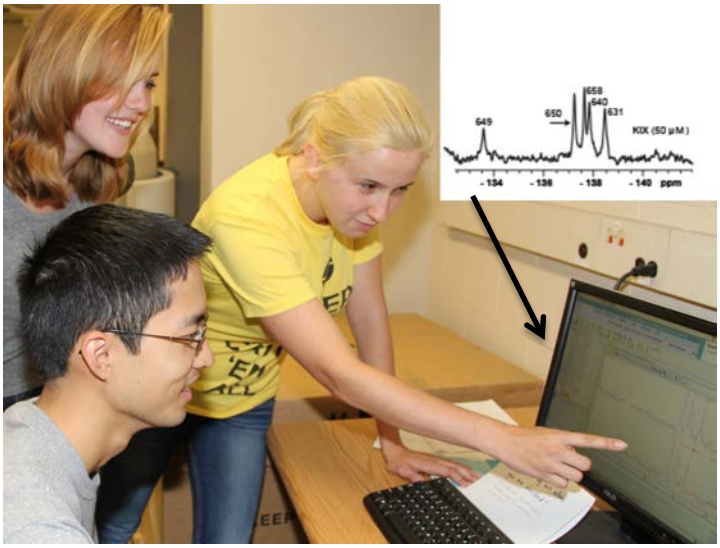


Fig. 1 Two GAC students and UMN trainer analyze a PrOF NMR spectrum of a fluorinated protein and small molecule

At both the University of Minnesota (UMN) and primarily undergraduate institution Gustavus Adolphus College (GAC), over 40% of chemistry majors fail to receive an independent research experience in their home department. Engaging a large number of students in a discovery-based research experience in the undergraduate teaching laboratory can be scaled to help meet this need. Although challenging, successful cases such as the Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) course,[26] "The Chemistry and Biology of Everyday Life (CBEL),[29] and the University of Texas at Austin's Freshmen Research Initiative[30] have garnered enthusiasm. In this proposal, a new organic chemistry laboratory will be implemented, designed around discovery of bio-active small molecules, based on multi-step reaction design and "fragment-based" synthesis, and using a readily analyzed biomolecular nuclear magnetic resonance spectroscopy (NMR) experiment. Fragment-based synthesis with protein NMR analysis has yet to be incorporated in an organic chemistry laboratory, but offers a route for independent molecular design, tractable syntheses, and exposure to macromolecule:small-molecule analysis. A winter "J-term" pilot course of 17 students at GAC in 2015 and follow-up research experience this summer with three students hosted jointly by UMN and GAC, has generated considerable enthusiasm prompting further curriculum development.

Background and Motivation

I recently developed a chemical biology boot camp for graduate students that takes place over two weeks and contains six teaching modules. This course was designed to reinforce concepts covered in class and enable students to hit the ground running in their respective research groups. By having a senior graduate student assist in the laboratory, valuable teaching experience and mentorship is provided. I have run the boot camp for two years with 14 students. Working with education specialist, Dr. Kris Gorman, I developed pre- and post-boot camp attitudinal surveys to assess students' enthusiasm, background, and proficiency with chemical biology techniques. Survey input led to using new biophysical methods (e.g., fluorescence polarization) and non-linear regression analysis. Portions of this bootcamp have now been integrated into our undergraduate chemical biology course, implemented by Cottrell Scholar Prof. Erin Carlson. I now aim to translate a chemical biology research experience into an undergraduate organic chemistry lab in collaboration with Prof. Scott Bur at GAC. For training the next generation of scientists, the American Chemical Society has recently changed the chemistry degree certification to include a macromolecular (MSN) course requirement. Molecular recognition at protein surfaces using small molecules is one theme to address a portion of this requirement, and is well-aligned with this organic/chemical biology laboratory course proposal.

Overview of the lab.

Research in my laboratory explores the fluorination of protein surfaces for characterizing protein:small-molecule interactions using ^{19}F NMR spectroscopy. One method to reduce the challenge of small molecule discovery for protein interfaces is to reduce the size and complexity of small molecules that are employed. However, a sensitive detection method is required to quantify the interaction. My lab screened 508 low complexity small molecules called fragments and characterized their interactions along the protein surface.[14] Due to the low complexity of the molecules, students at GAC will design analogs of these small organic molecules, each taking ownership of their individual scaffold and fostering creativity in the design process.

Derivatives of small molecule fragments can be made in short two to four step syntheses with only a small amount of material needed for testing (1 to 5 mg), making multi-step synthesis tractable in an undergraduate laboratory. Small molecules will be tested against my lab's fluorinated proteins. The ^{19}F NMR is acquired rapidly and requires low amounts of protein for characterizing large libraries of compounds. All students will receive the NMR spectral data to analyze and assess the success of their designs. Due to the proximity of GAC and UMN (70 miles), several students will come to UMN and learn how to carry out the protein binding assays as was performed this summer.

To our knowledge, a fragment-based, small-molecule discovery research experience has not been demonstrated in an undergraduate laboratory curriculum. However, a discovery-based lab using molecular recognition and small-molecule synthesis has been developed by Prof. Stephen F. Martin at the Univ. of Texas - Austin, supporting the feasibility of this approach. The learning objectives to be met by this research experience in an undergraduate teaching laboratory are designed to train students to plan a multistep synthesis, troubleshoot reaction conditions using literature precedent rather than follow a prescribed set of conditions, analyze biomolecular NMR data for comparing experimental results to a positive control, identify important parts of small molecules for binding, and develop new synthesis proposals for these small molecules. Assessment will include pre-and post-knowledge and attitudinal surveys developed with the UMN Center for Educational Innovation, and online assessment using the Classroom Undergraduate Research Experience (CURE) survey.[29] Finally, analysis of three reflective writing assignments on the students' perception of their research experience (pre-, mid-, and post semester) will be used to assess the change in of their impression of the scientific process (e.g., What is an experiment, a theory, a hypothesis, and research?[27]). A one-year baseline survey will be given to students taking the current organic chemistry laboratory at GAC and an inquiry-based organic chemistry laboratory at UMN for comparing student learning outcomes and self-perception from the discovery-based research experience.

Preliminary Data

The KIX domain of the CREB binding protein (CBP) has been a useful model protein for characterizing protein-protein[31,32] and protein-small molecule interactions.[33-35] KIX protein:protein interactions serve important roles in fatty acid metabolism, long-term memory formation, as well as hematopoesis.[31,36,37] My lab developed the protein-based fluorine NMR method, referred to as PrOF NMR, as a tool to discover molecules that bind to KIX.[13,14] The PrOF NMR screen used 508 low complexity molecules, called fragments, to investigate the sensitivity of the PrOF NMR method for ligand discovery.[14] As the first demonstration of using PrOF NMR in this format, several new small molecules that bind to KIX have been discovered. Structure-function data elucidated the importance of aryl acetic acids and biaryl acids for recognizing the KIX protein. The fragments identified through the PrOF NMR screen fell into four structural types, represented by thiazole **3F5**, phenylacetic acid **11E7**, quinoline **8E2**, and benzothiophene **2G11** (**Fig. 2**). The proposed laboratory will explore analogs of these scaffolds, including elaborated biaryl small molecules. Analysis of these compounds will provide valuable insight into their role as transcription factor mimics, and ability to bind to a protein surface. Many proteins are amenable to fragment methods, expanding the scope of this course for future iterations.

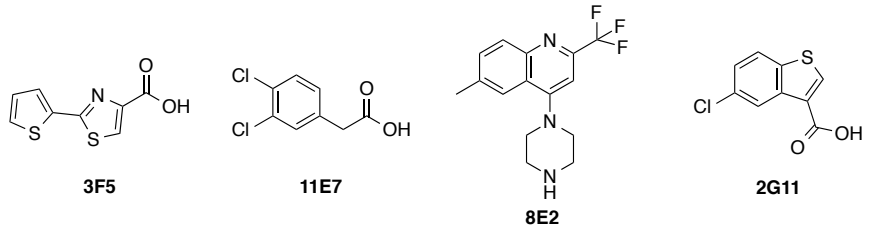


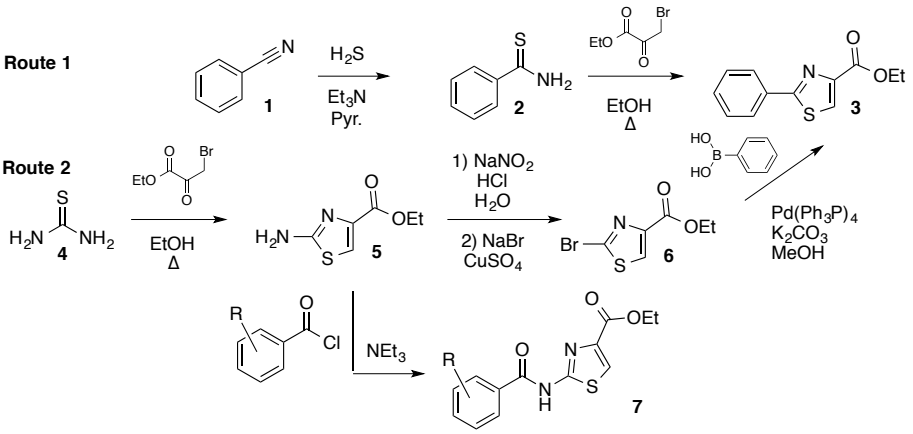
Fig. 2. Fragments identified by PrOF NMR KIX-binding experiments.[14]

An initial pilot program carried out by Prof. Bur developed the synthesis and literature skill building exercises this past winter with 17 students. As an example of the type of syntheses the students would conduct, the students developed two similar, yet complimentary synthetic routes to fragments such as **3F5** (**Scheme 1**). These two complementary routes allow for the use of a variety of commercially available starting materials to make several analogs (vide infra). A new route for the biaryl amide, **7**, was validated this summer.

Three students, including one from the J-term, continued working with Prof. Bur this summer to evaluate the synthetic routes pursued in the winter course. These students joined my laboratory at the end of July, learning how to express the fluorinated protein KIX and carry out the binding assay. Their first day focused on carrying out a series of five-minute protein-ligand binding experiments. The second day, they expressed the fluorinated protein using protocols developed in my chemical biology boot camp and submitted manuscript (Nature Protocols, *submitted*). The final day was spent purifying the protein, and assessing fluorine incorporation using electrospray ionization mass spectrometry. To enrich their experience, undergraduates stayed with either a postdoctoral fellow or graduate student from my laboratory.

Laboratory Class

One significant aspect of the collaborative educational project, is the translation of some of the research into



Scheme 1. Synthetic routes to thiazole derivatives developed during J-term and evaluated this summer with Prof. Bur. Hydrolyzed products of **3** and **7** were tested by PrOF NMR

the curricular laboratories at Gustavus, particularly in the second semester organic chemistry course (CHEM 251 which includes a weekly laboratory component). During the first class of the semester, I will provide the students with a recent publication from my lab, and describe our research method to provide context for their organic chemistry laboratory experience, and introduce them to protein visualization software Pymol. By design, the synthetic routes pursued are short and the chemistry is relatively simple, making them ideal for incorporation into teaching laboratories for sophomores. Materials generated will directly contribute to the research project and train students at an early stage to generate interest in chemistry-based research. Interested students could continue working on the research project after the course finishes. Drawing from the successful model developed by the Center for Authentic Science in Education (CASPiE) project,[27] we would translate our research into the classroom using an approach that includes a "skill building" project, where everyone makes a known analog so as to introduce students to the research problem and to build competence and confidence with standard research techniques. This will include training in how to use SciFinder Scholar to both find chemical information and get a sense of what experimental conditions have been used in similar reactions. Following the skill building exercise, students will engage in experimental design of a new molecule with some freedom to explore the parameters of the experiment (e.g. which boronic acid to use) from a list of common building blocks (e.g., **6**). We expect this to provide a constructivist model of learning[38] and students will benefit from this Kolb-like cycle.[39] Specifically, they will: have the opportunity to reflect upon and then repeat experimental techniques and instrumental methods; demonstrate learning gains from the skills building exercise; and develop stronger ownership over the outcome of the investigative experiment. Leaving time for reaction trouble-shooting provides the students with a realistic perspective on a research experience and allows for critical thinking for exploring a new reaction condition.

Students are anticipated to finish their designed small molecules, near the last quarter of the semester. Compounds will be sent to UMN for binding analysis with the fluorinated protein KIX, and a positive control compound will be added. The final data is a 1D NMR spectrum that the students can analyze remotely using the program TopSpin and the instrumental skills learned throughout the semester. By analyzing the NMR data, students will be able to assess which parts of the molecules (if any) are important for binding. I will join the students again for a discussion of this data. As a final project, students will be asked to propose a new synthesis of a molecule which would be expected to bind to the protein. Data obtained from this laboratory will be further utilized for instructional purposes in my Chemical Biology course at UMN (4411/8411), focusing on small-molecule:protein interactions and biophysical analysis.

In summary, the Pomerantz and Bur labs have collaborated to develop new synthetic routes for rationally designed small-molecule fragments as protein ligands. The training benefit for undergraduates is high, including development of scientific literacy and implementation of advanced synthetic organic techniques. A broader impact on the undergraduate population at both UMN and GAC will be met through implementation of the synthetic approach and macromolecule analysis into the undergraduate laboratory. Within the structure of an organic curriculum, the discovery-based format brings a research experience to students who may not otherwise have an opportunity to partake in an independent research experience during their undergraduate studies. A potential long-term outcome is the increased retention of students enrolled in graduate-level STEM research programs, as well as continued research experiences at GAC and UMN. This program will also lead to experimental data to be incorporated in the chemical biology laboratory (4423W) and theory course (4411/8411), laying the foundation for future implementation into the organic laboratory curriculum at the UMN. If successful, the results in this research-based laboratory experience will be described in a pedagogical journal and potentially a research journal if the small molecules are highly active.

ASSESSMENT PLAN. *Define expected outcomes of your educational plan. How will your evaluation design provide information to improve your project as it develops and progresses? How will you determine whether your stated project objectives are being met according to the proposed timeline?*

Expected outcomes of this educational proposal are a research-based laboratory experience aligned with the new ACS macromolecular/supramolecular guidelines. A research-based experience at the sophomore level is anticipated to help fulfill the needs of undergraduate students in obtaining a research experience where departmental resources are not currently sufficient and increase retention of STEM discipline students. Moving away from a traditional laboratory experience, we will evaluate learning gains through practical application of concepts learned in course work, self-identification as a scientist, and a broadened appreciation of research-based science. Allowing time for troubleshooting chemical reactions that are not guaranteed to work and analyzing molecules that are not guaranteed to interact with a protein is anticipated to provide students with specific ownership of their small molecule synthesis and assay results as well as a realistic view of the research experience. A strong educational and research relationship is anticipated to be built between GAC and UMN.

Assessment: To aid in a baseline comparison with traditional and inquiry-based labs, in the coming year students at UMN and GAC will each take pre- and post- 10-15 minute Classroom Undergraduate Research Experience (CURE) surveys provided and analyzed by Prof. Lopatto and data analyst Ms. Leslie Jaworski at Grinnell College. The survey includes questions to assess student learning gains, scientific attitude, and overall impression of the course. Students enrolled in the research-based laboratory experience in Fall 2017 will take the same surveys for comparative analysis.

Throughout the research-based laboratory course, the students will be required to write three short reflective papers on their perspectives of the research experience or expectations in the laboratory. Questions that will be included based on the successful CASPiE program for differentiating student's perception of research from traditional laboratory and discovery-based laboratory experiences,[27] will also include their opinions on: What an experiment is, what a theory and hypothesis are, and what research means to them. Prof. Bur and myself, with the UMN Center for Educational Innovation, will analyze the writing to help assess student learning gains and scientific attitudes. Surveys mid-way through will allow for course corrections and future curriculum changes.

Prior to implementation, a second winter J-term will be implemented to further assess the feasibility of running the course on a larger scale and refining the reflective writing questions. The current retention of students staying on to carry out a summer research experience with both Prof. Bur and visitation to my own laboratory highlights significant student interest. Similar retention in research will be carefully monitored.

Identify departmental or institutional colleagues who might play a role in this educational endeavor (as mentors, collaborators, etc.) as appropriate and describe the role they will play.

Prof. Jane Wissinger (UMN) has agreed to have her organic chemistry laboratory with several inquiry-based experiments to be surveyed. (See letter of support)

Drs. Paul Baepler and Kris Gorman (Center for Educational Innovation) have helped evaluate our proposal and are providing support in developing and assessing the reflective writings assignments. (See letter of support)

Prof. Scott Bur (GAC) is a current collaborator and is helping implement the organic chemistry laboratory (See Letter of Support)

Profs. Dwight Stoll and Amanda Nienow (GAC) co-chair the chemistry department at GAC and are supporting the curricular developments. (See Letter of Support)

LETTER OF SUPPORT. *Include a letter of support from your Departmental Chair, Dean or Provost that endorses your educational proposal and indicates why you are the appropriate faculty member to undertake this project. Please insert the letter following the ACADEMIC LEADERSHIP STATEMENT.*

LIST OF REFERENCES. *Annotate the proposal with a list of references from the primary literature. Include all authors and titles. If more space is required, attach a maximum of one additional page. Use Arial 10 or 11 point font.*

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