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Fragment-Based Ligand Discovery Using Protein-Observed ^{19}F NMR: A Second Semester Organic Chemistry CURE Project

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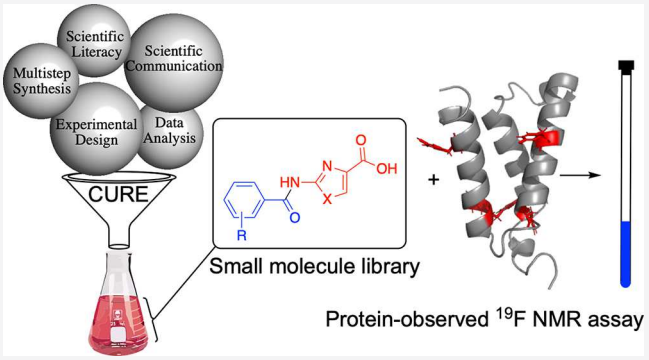
ABSTRACT: Curriculum-based undergraduate research experiences (CUREs) have been shown to increase student retention in STEM fields and are starting to become more widely adopted in chemistry curricula. Here we describe a 10-week CURE that is suitable for a second-semester organic chemistry laboratory course. Students synthesize small molecules and use protein-observed ^{19}F (PrOF) NMR to assess the small molecule's binding affinity to a target protein. The research project introduced students to multistep organic synthesis, structure−activity relationship studies, quantitative biophysical measurements (measuring K_d from PrOF NMR experiments), and scientific literacy. Docking experiments could be added to help students understand how changes in a ligand structure may affect binding to a protein. Assessment using the CURE survey indicates self-perceived skill gains from the course that exceed gains measured in a traditional and an inquiry-based laboratory experience. Given the speed of the binding experiment and the alignment of the synthetic methods with a second-semester organic chemistry laboratory course, a PrOF NMR fragment-based ligand discovery lab can be readily implemented in the undergraduate chemistry curriculum.



KEYWORDS: *Laboratory Instruction, Organic Chemistry, Inquiry-Based/Discovery Learning, NMR Spectroscopy, Proteins/Peptides*

INTRODUCTION

There is a demonstrated and critical need for new science, technology, engineering, and math (STEM) professionals,[1] yet 40% of U.S. students who enter studies in STEM fields fail to achieve their STEM degree.[2] Retaining students in STEM fields depends upon engaging the undergraduate population within their first two years.[1,2] While traditional lecture-based pedagogies are not as effective at retention,[3,4] 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.[2,3,5−7] In addition, the links between experiential pedagogies and student learning gains are well documented,[8,9] and curriculum-based undergraduate research experiences (CUREs) are now established pedagogical approaches,[10] because they more closely align the learning outcomes of laboratory curricula with the outcomes from research experiences.

CUREs provide authentic research experiences on a scale that is difficult to achieve with traditional student−faculty collaborative research. While several successful examples have garnered enthusiasm, such as the Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) course,[2] the Chemistry and Biology of Everyday Life (CBEL),[8] and the University of Texas at Austin's Freshmen Research Initiative,[11] there remains a need for more CURE and CURE-like courses at primarily undergraduate institutions (PUIs) to provide broad and inclusive access to research early in the undergraduate experience. Here, we describe the implementation of a 10-week fragment-based ligand discovery (FBLD)[12−16] project that uses protein-observed ^{19}F (PrOF) NMR[17,18] to assess protein−ligand binding in a second-semester organic chemistry course at a PUI.

To our knowledge, FBLD has not yet been applied to a CURE in the published literature, though it offers many attractive features for modifying an organic chemistry lab. This ligand discovery approach includes short modifiable syntheses and an introduction to quantitative biophysical measurements. Protein NMR is not typically introduced in an organic chemistry lab course, yet the accessibility of PrOF NMR provides a connection between students' small-molecule characterization tools and a macromolecular recognition

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event. The selection of the project was influenced by a desire to increase the research readiness of our students as well as to leverage the scale of the curricular laboratory for advancing faculty research projects. The adaptability of critical components for implementation in other contexts is also discussed.

Project Background

Over the last 20 years, FBLD programs have been readily adopted in both academic and industrial settings.[19] The successful outcome of such programs can be seen in the FDA approval of four drugs originating from FBLD screens across diverse protein classes including protein−protein interactions (PPIs).[16,20−22] The chemical space surrounding drug-like molecules of approximately 500 MW is immense, estimated to be on the order of 10^{63} molecules.[23] The premise behind FBLD is the screening of low complexity, low molecular weight molecules (<300 MW), to more effectively sample the chemical space necessary to bind a given target.

The challenge associated with screening low molecular weight small molecules is their low binding affinity due to a reduced number of potential interactions with the drug target. As such, sensitive biophysical techniques are necessary to detect these interactions. Robust synthetic chemistry is then required to improve the affinity and determine a structure−activity relationship (SAR) of the fragment binders.

In their seminal report, Shuker et al. applied protein-observed $^{1}H-^{15}N$ HSQC NMR to both detect fragment binding and guide the SAR for inhibitor development.[12] Since that time, many biophysical methods have been developed for screening fragments, though NMR remains one of the most readily adopted.[24] Most fragments exhibit fast exchange kinetics, which manifests as a perturbation in the chemical shift of the signal for nuclei near the binding event in the NMR spectrum of the protein versus protein challenged with ligand.[25,26] This experiment also provides structural information about where the ligand binds to the target.

A difficulty in using $^{1}H-^{15}N$ HSQC NMR is the cost associated with producing large amounts of isotopically labeled proteins. As an alternative, PrOF NMR uses proteins containing amino acid side chains that are labeled with bioorthogonal ^{19}F.[27] The labeling choice of aromatic amino acids in this research is important as these amino acids are predominantly found at PPI interfaces.[28] One advantage of using ^{19}F NMR is the reduced cost of protein production due to the 100% isotopic abundance of ^{19}F. In addition, the hyperresponsiveness of the ^{19}F nucleus to changes in the chemical environment leads to simplified, well-resolved 1D NMR spectra which readily report on ligand interactions. In some cases, the fluorinated resonances are 6−20 fold more responsive than ^{1}H NMR to ligand binding.[29] Of particular importance for our purposes, high-field magnets are not a requirement, because ^{19}F has an 83% similar signal sensitivity as ^{1}H. The 400 MHz magnet and RT broadband probe used in the following experiments highlight the suitability of using PrOF NMR in many different research environments.

The first reports of PrOF NMR fragment screening were conducted by Pomerantz et al.[30] and Gee et al.[18] with the transcriptional coactivator CREB Binding Protein (CBP) using a 3-fluorotyrosine-labled KIX domain (3FY-KIX). KIX is involved in PPIs with over a dozen transcription factors through two distinct binding sites, each containing at least one tyrosine residue. This protein domain has also been proposed as an anticancer drug target due to interactions with CREB, c-MYB, and MLL.[30−32] Initial leads from the original fragment screens provide an opportunity for future SAR studies through qualitative analysis of chemical shift perturbations in the NMR spectra and quantitative analysis of the dissociation constants (K_d) of new KIX ligands. Given that fragment synthesis can be achieved in relatively few synthetic transformations and the NMR measurements build upon a sophomore-level introduction to spectral analysis, here we sought to translate our research screen into a CURE.

STRUCTURE OF THE COURSE

Our course employed a three-step process that was modeled upon the Center for Authentic Science Practice in Education (CASPiE) approach:[33] First, the students were introduced to the project and received the training needed to support the research. Second, each student made the same molecule, in this case a lead fragment known to interact with the protein of interest, to learn the techniques needed for the synthesis. Following the synthesis, the students learned to measure the binding interaction with the fluorinated protein using PrOF NMR. Third, students repeated the synthesis with one starting material varied to create an analogue of the lead fragment. If their synthesis was successful, they measured the analogue's binding interaction for comparison with the lead fragment. The course was built with four critical areas in mind: chemical literacy, experimental design, data analysis, and scientific communication.

The 4 h laboratory course was generally divided into a 1 h prelab and a 3 h wet lab. Throughout the course, prelab was used to discuss scientific literacy, writing, data analysis, and troubleshooting of reactions. Table 1 shows the timing of various components. The following discussion of the course provides both a general description of the course components and a specific research project that we used. This course structure is amenable to a variety of proteins, so adaptation to other institutions is not limited to this specific research.

Part 1: Introduction to the Research Project

The first week of the course was a dry lab used to provide an overview of the project, training in how to search the chemical literature using SciFinder Scholar, and training in lab safety. In a 45 min seminar-style presentation, the three major features of a project to find small-molecule ligands that can disrupt PPIs were outlined for the students. PPIs were discussed as important for a wide variety of biological functions,[34] though they are difficult to target with small molecules because of their large and plastic binding area when compared to traditional protein−small-molecule interactions.[35−38] FBLD was introduced as a strategy for targeting PPIs where there are no endogenous small molecules to begin SAR studies. Finally, the use of NMR, particularly PrOF NMR, to guide an FBLD strategy was discussed.

Next, the details of the research project were introduced. In our case, the KIX domain of CBP was presented, and students were shown the initial data obtained from a PrOF NMR screen (vide supra).[18] A "hit" molecule, GAMLE1, was revealed, and students worked on an exercise to both familiarize them with various search capabilities in SciFinder Scholar and to lead them to propose a retrosynthetic analysis of GAMLE1 (Scheme 1). The synthesis of GAMLE1 is not reported in the literature, so students found similar molecules upon which to base their syntheses. For GAMLE1, the key step is a

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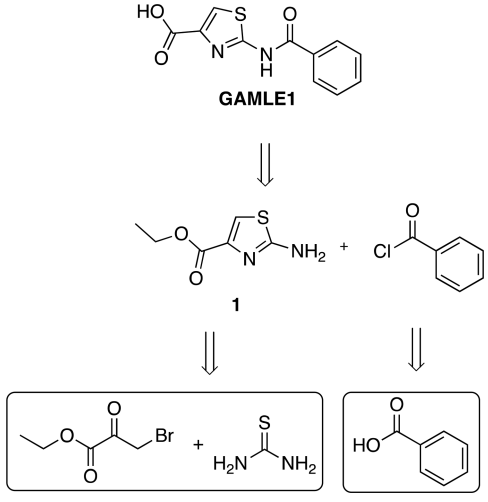
Table 1. Outline of the Course

Dates	Discussion (D)/Activities (A)[a]
Week 1	Introduce the project (D)
	Chemical safety (D/A)
	Scientific literacy (D/A)
Week 2	Kinds of literature (D/A)
	The "Introduction" section (D)
	Thiazole synthesis
Week 3	The "experimental" section (D)
	Acid chloride synthesis
	Amide coupling
Week 4	Peer review (D)
	Product purification
	Ester hydrolysis
	Docking (D/A)[b]
Week 5	Compound characterization
	PrOF NMR (D/A)
	Experimental design (D)
	Choose target
Week 6	Peer review (D)
	Analogue synthesis
Week 7	Analogue synthesis
Week 8	The "abstract" section (D)
	Analogue synthesis
Week 9	Product purification
	PrOF NMR of analogues
Week 10	Clean up and check out

[a]D and A designate discussion topics or activities for prelab; items without designation were laboratory activities. [b]Optional, depending upon the project.

Scheme 1. Retrosynthetic Analysis of GAMLE1



coupling of amino thiazole **1** with an aromatic carboxylic acid derivative. The first assignment, due for the second week, was to find literature procedures for making **1**. Invariably, students found the condensation/cyclization of thiourea with ethyl bromopyruvate, though there are several different sets of conditions that are used.

Laboratory safety is a critical concern when each student in the class is following a different procedure and using reagents that are more hazardous than those found in most introductory organic chemistry teaching laboratories. Accordingly, the first week also included safety training that was originally designed for summer research students. In addition, students filled out a safety card (see Supporting Information) for each new chemical they intended to use. The format of the card was based upon the abbreviated safety data used for the Gilbert and Martin textbook[39] and contained a collection of essential information from the Safety Data Sheets for each chemical.

Part 2: Synthesis and Binding Analysis

Weeks two through five were dedicated to the synthesis and binding analysis of GAMLE1. During prelabs, discussion focused on the various conditions that were uncovered in the students' literature searches, particularly on elements of experimental design (such as scale, solvent, temperature, time), different kinds of literature (e.g., reviews, patents, communications), writing (e.g., what is the purpose of an introduction or abstract?), and troubleshooting when things go wrong (not all of the conditions they find work well in their hands and on their scale). After completing their synthesis of GAMLE1, students learned how to prepare NMR samples and conduct the appropriate PrOF NMR experiments to determine if GAMLE1 binds to the 3FY-labeled KIX protein. With GAMLE1, students observed the change in the chemical shift of the signal at −139.45 ppm, which corresponds to fluorine-labeled Y631 (Figure 1). This specific tyrosine is involved in a PPI with MLL. Students were provided with raw data from a ligand titration experiment, and they determined the K_d for GAMLE1 (vide infra).

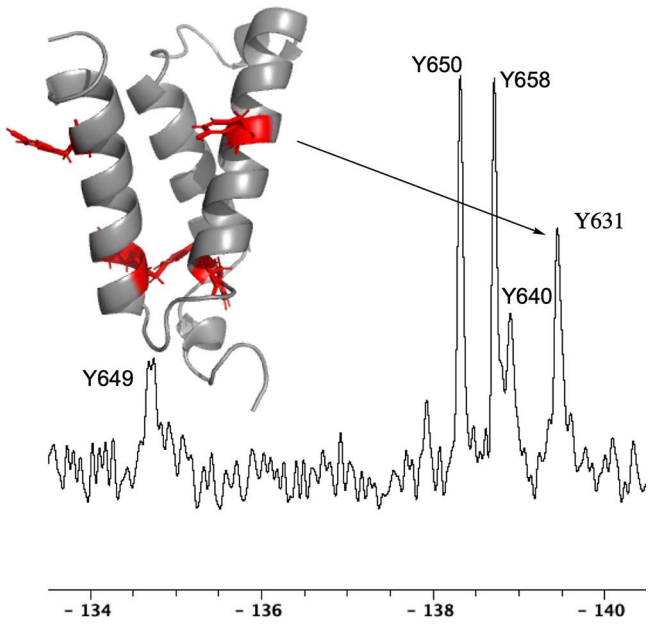


Figure 1. KIX domain (tyrosines in red) and PrOF NMR spectrum with resonance assignments.

Part 3: Analogue Synthesis

Students repeated the synthesis in weeks six through nine, this time changing one of the starting materials. In our example, students produced a series of GAMLE1 derivatives, represented by the general structure **5** (Scheme 2). Students could make a thiazole (X = S), oxazole (X = O), or imidazole (X = N), and they could change the substitution pattern on the aryl acid. They were not given specific guidance regarding which derivative to make. With a different project, computational docking was used to help students pick a derivative (vide infra). If their synthesis was successful, they tested the

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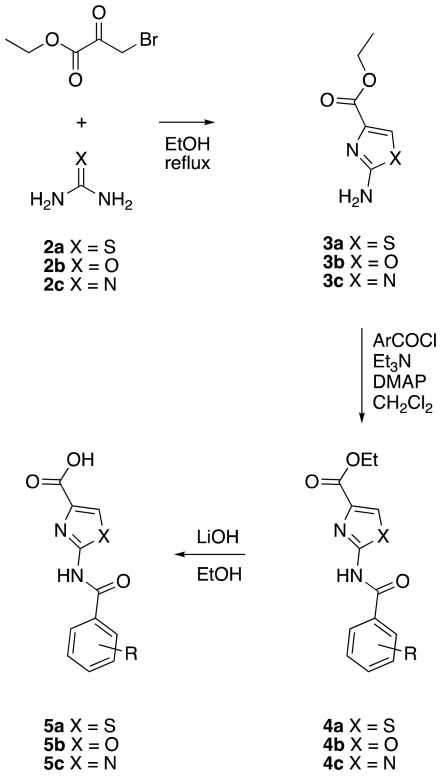
Scheme 2. General Synthesis of the GAMLE1 Analogues



derivative against 3FY-labeled KIX using PrOF NMR. Figure 2 shows a representative sample of student data covering a range of possible outcomes. Entry (e), for example, shows the signal of interest disappearing, suggesting intermediate exchange kinetics; entry (b) shows a typical change in the chemical shift for the signal of interest; and entry (a) shows the spectrum for a molecule that facilitates the precipitation of the protein from the sample. Points of diversity in the SAR are represented by the comparison of (c) and (e) with differences in the substituent on the aromatic ring, whereas comparisons of (b) and (c) or (d) and (e) show differences in the heterocycle.

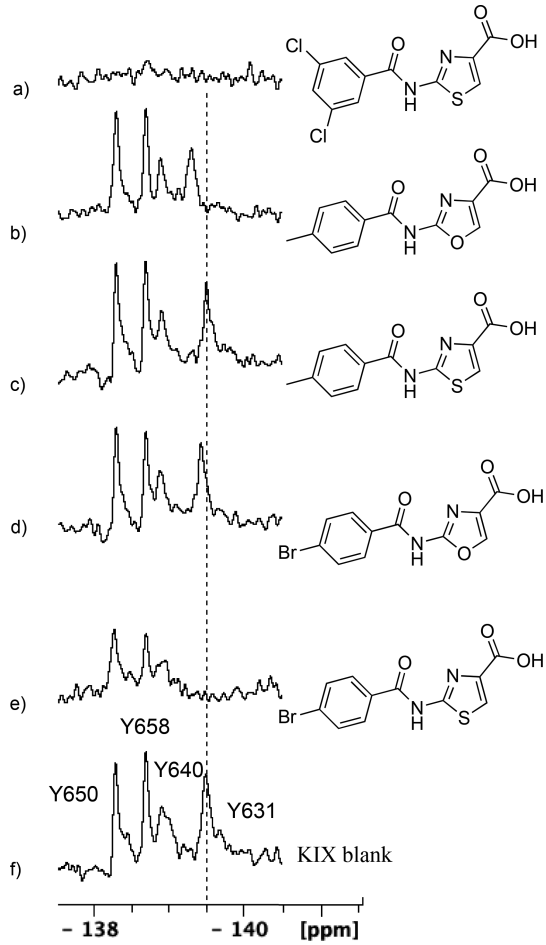


Figure 2. Representative data collected by students. Examples (a)–(e) are the ^{19}F NMR spectra of the 3FY-KIX protein (80 μM) plus a student derivative (1.5 mM). Spectrum (f) corresponds to the 3FY-KIX protein without added ligand.

CORE COMPONENTS

Scientific Literacy

In the first week, students were introduced to SciFinder Scholar. Various SciFinder activities have been published in this journal over the years,[40−43] and we adapted the approach and activities of Swoger and Helms[44] by changing the search terms to be relevant to elements of this project. The final SciFinder activity was for students to find a literature precedent for the synthesis of a molecule similar to GAMLE1, as well as a precedent for making each of the materials needed for the synthesis. In essence, they conducted a retrosynthetic analysis.

In a subsequent week, students were provided with a review article, a patent, a full paper, and a media report, each of which was relevant to some aspect of the project. Adapting Swoger and Helms's approach,[44] students engaged in a discussion about primary and secondary literature, as well as popular writings that helped them to distinguish the differences between the types of literature, the strengths and weaknesses of each type, and the contexts in which one may be more appropriate to cite than another.

Finally, one prelab period was devoted to reading a full paper and discussing the various sections. What, for example, is the purpose of an abstract? An introduction? By this time, students were receptive to the concise and complete style used to communicate experimental details. Particular attention was paid to specific stylistic aspects, such as the citation format and how these differ from writing in other disciplines.

Experimental Design

Experimental design was discussed over several weeks during prelab time. Initially, the conversation focused on retrosynthetic analysis and was grounded in the references they found during the SciFinder activity. In the second week, students provided an idealized experimental procedure based upon a literature report before they were allowed to begin their synthesis. They shared the experimental conditions discovered in the literature, and then the class discussed similarities and differences among the literature references they found. For example, two different approaches were discovered, with some variation within each, for the synthesis of **1**: heating the reagents to reflux in ethanol[45] and a solvent-less procedure where the reagents are ground together in a mortar and pestle.[46] It should be noted that, in the first year of the CURE, students tried to rely on the abbreviated experimental conditions presented in SciFinder, rather than finding the experimental section in the abstracted paper. In the second

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year, the importance of finding the experimental section in the original paper was emphasized, and this misunderstanding was less of a problem.

In the third week, students discussed the results of the various methods they tried in the lab and converged on a single set of conditions to use for the analogue synthesis. Discussion included the identification of experimental variables (e.g., time, temperature, concentration, solvent) as well as how each might affect the outcome of the reaction. Students often pointed out the differences in aqueous workups between the procedures, and this led into a discussion about the purpose of a workup.

Data Analysis

In week five, students learned how to quantify binding events using PrOF NMR. Much like week one, this was primarily a dry lab. Students learned how to process previously acquired NMR data for a titration of GAMLE1 against the KIX protein. In practice, PrOF NMR experiments generate a spectrum of the internal reference (trifluoroacetic acid) and a spectrum of the fluorinated protein.[17] Students used the reference spectrum to find a correction factor to calibrate the chemical shift axis and then apply that correction to the fluorinated-protein spectrum. A spreadsheet template was provided to facilitate this work (see the Supporting Information).

The chemical shifts in the spectra obtained at different ligand concentrations were calibrated, and the difference in the chemical shift ($\Delta\delta$) was plotted against the ligand concentration. Using nonlinear regression tools (e.g., SigmaPlot, which requires the purchase of a license), students fit the data to eq 1 (where P is the protein concentration and L is the ligand concentration) to find K_d and derive a maximal chemical shift ($\Delta\delta_{max}$). Figure 3 shows a representative binding isotherm. Again, a template was provided to facilitate data analysis.

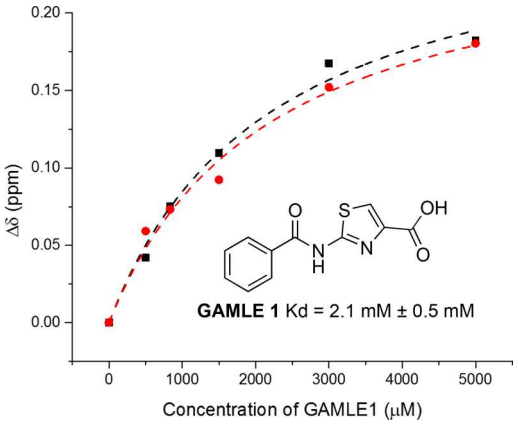

Figure 3. Data for the two titration experiments of GAMLE1 against the 3FY-labeled KIX protein following the resonance for Y631. The dashed curves represent the nonlinear regression fit from eq 1.

Near the end of the lab, students learned how to prepare and submit their samples of GAMLE1 for PrOF NMR analysis. Thus, by the end of week five, students had learned how to process and analyze experimental data, use nonlinear regression software, and quantify a fragment:protein dissociation constant. This prepared them to submit, process, and analyze the appropriate experimental data for their analogue in the last week.

$$\Delta\delta = \Delta\delta_{max} \times \frac{(K_d + P + L) - \sqrt{(((K_d + P + L)^2) - 4PL)}}{2P} \quad (1)$$

Scientific Communication

The student work culminated in a report written in the style of an ACS journal manuscript, and assignments throughout the semester provided the opportunity for students to work on parts of the report and get feedback for revision. During prelab activities, various components of the report (e.g., introduction, results and discussion, experimental details) were discussed, and the students received assignments to complete all or part of the discussed section. By week three, for example, students had run their first reaction and isolated, purified, and characterized the product. Students wrote a detailed experimental procedure for the reaction that was due at the beginning of week 4, including full characterization of the isolated molecule. Instructor feedback was provided, and students had the opportunity to revise their assignment. By the end of the semester, all of the sections were written, had received comment, and had been revised, facilitating the final assembly and grading.

Figures, equations, schemes, and charts were elements that frequently confused students. Preparation and insertion of these graphical elements into their papers got special attention during prelab discussions. Students used a ChemDraw template (ACS Document 1996) for preparing equations and schemes and PyMol[47] visualization software for preparing figures that highlight the location of binding sites and fluorinated residues in the target protein. Other software packages, such as ChemDoodle and Chimera,[48] could also be used. Activities that involve drawing schemes, equations, and figures that incorporate use of these software packages provided students the opportunity to work with graphical elements and receive feedback on drawing size, composition, and consistency.

■ ADAPTABILITY

PrOF NMR

The concentration of the protein in PrOF NMR samples is relatively low compared with other NMR samples students may prepare, and extensive signal averaging is required at field strengths typical of NMR spectrometers at PUIs. For comparison, a sample with approximately 40 μM of the 3FY-KIX protein on a Bruker 500 MHz spectrometer equipped with a Prodigy cryoprobe (~2000:1 S:N) takes less than 10 min to generate useful spectral data. The same sample on a 400 MHz spectrometer equipped with a broadband room-temperature probe (~300:1, S:N) takes nearly 3 h to acquire data of similar quality. Doubling the protein concentration shortens the experiment, but not enough to make it useful in a curricular laboratory experiment. The relaxation delay time for a single NMR scan accounts for most of the experiment time. Delay times can be relatively long based on the longitudinal relaxation time of the fluorine resonances. Using a Ni-DTPA complex in the NMR samples reduces the longitudinal relaxation time, and thus the relaxation delay time,[49] significantly shortening the experiment time. NMR experiments on samples containing 90 μM protein and 40 mM Ni-DTPA can run in approximately 30 min (~3000 scans with a

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relaxation delay of 0.5 s), which is comparable to the ^{13}C or DEPT NMR experiments that are often run in undergraduate laboratories (Figure 4). For specific parameters used to acquire PrOF NMR spectra, please see the Supporting Information.

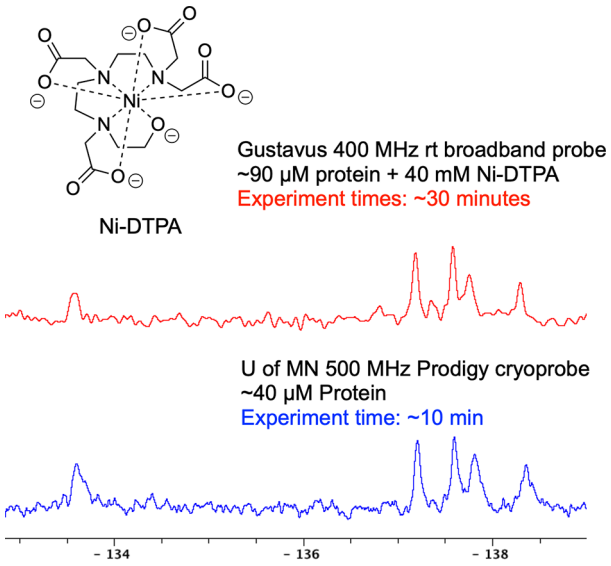

Figure 4. Comparison of the experimental spectra for the PrOF NMR experiments. A spectrum acquired with 40 μM 3FY-KIX protein sample on a 500 MHz spectrometer with a Prodigy cryoprobe took just under 10 min from sample insertion to ejection (blue). A spectrum acquired with a 90 μM 3FY-KIX protein sample on a 400 MHz spectrometer with a room temperature broadband probe and Ni-DTPA added to the sample took approximately 30 min (red).

Protein

The efficiency of protein expression, isolation, and purification and the stability of the protein can pose significant challenges for adapting this kind of project to the PUI environment. A variety of proteins are amenable to metabolic fluorine labeling, especially with 5-fluorotryptophan,[50] and many fluorinated amino acids are commercially available. Our initial course design used 3FY-KIX and focused on GAMLE1 as the molecular scaffold for the SAR work. Recently, we have begun using a 5-fluorotryptophan-labeled bromodomain of the *Plasmodium falciparum* GCN5 homologue protein (5FW-PfGCN5).[51] In our experience, this bromodomain is easier to express and is more stable than the 3FY-KIX domain. Figure 5 shows the PrOF NMR spectrum of 5FW-PfGCN5 with and without a ligand added.

Both the 3FY-KIX domain and 5FW-PfGCN5 bromodomain proteins could be recycled after the NMR experiments. Pooling of the NMR samples (excluding those samples wherein a ligand analogue precipitated the protein) and buffer exchange using a commercially available desalting column provided protein samples that were usable in new experiments. We were able to recycle the protein samples up to three times without a significant loss of material.

Computational Docking

In recent iterations of the course, we incorporated a computational docking activity to give students experimental results upon which they could base analogue selection. Docking-based virtual screening has become an important tool in finding small molecules that bind to PPI interfaces,[52] though identifying the site where small molecules may interact

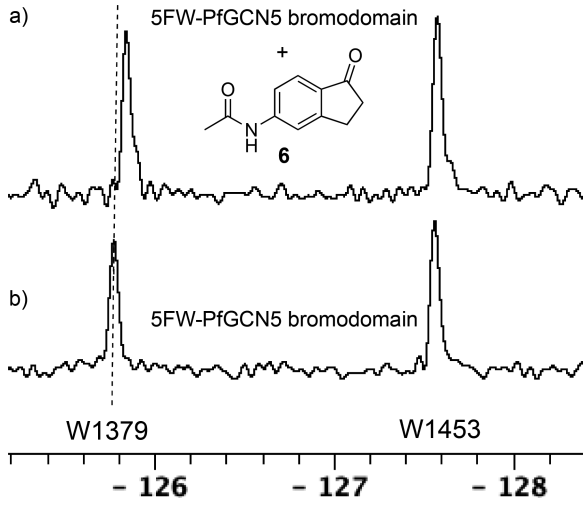

Figure 5. (a) PrOF NMR spectrum of 5FW-PfGCN5 (45 μM) and ligand **6** (800 μM). (b) Spectrum of the 5FW-PFGCN5 without added ligand.

can be challenging.[53] The free online tool FTMap[54,55] could help identify "hot spots" where small molecules may bind (Figure 6). In the docking activity, students picked a derivative of the lead molecule **6**, and then submitted a .mol2 file of their derivative (e.g., **7**) and a .pdb file of the PfGCN5 bromodomain to SwissDock, a freely accessible Web site.[56,57] In these experiments, students did not need to define a "binding box" where they think the analogue will bind; blind docking worked well. The results were visualized with PyMol and compared with the binding of the hit molecule. The energy difference between the hit and the derivative could be compared to predict if the derivative should bind better or worse than the original hit.

Challenges to Implementation

The ability to tailor the components of this CURE to suit a particular project (e.g., selection of the protein to study, computational docking) facilitates the implementation of this project at a variety of institutions, though there are still significant logistical challenges that warrant comment. First, for each student to conduct a titration experiment of their derivative using PrOF NMR requires significant spectrometer time (>4 h/student). In practice, few students have been able to do more than the initial binding assessment within the time frame of the laboratory course. Many, however, have continued their work on the project as part of an independent study. Summer research students are also able to verify and extend the initial results. This highlights the synergy between the course project and student/faculty collaborative research.

A second challenge involves the expression, isolation, and characterization of the fluorine-labeled proteins. The Supporting Information contains detailed instructions for the expression and isolation of the proteins, including how to prepare buffer solutions, growth medium for the cells, and sources for some specific consumables. In general, the expression, isolation, and characterization of the ^{19}F-labeled proteins takes approximately a week. For those unfamiliar with protein expression, collaborations with colleagues who are more familiar with protein expression and isolation can be productive avenues for both obtaining sufficient quantities of protein and for learning the basic techniques required. Building collaborations with other courses to expand the impact of the

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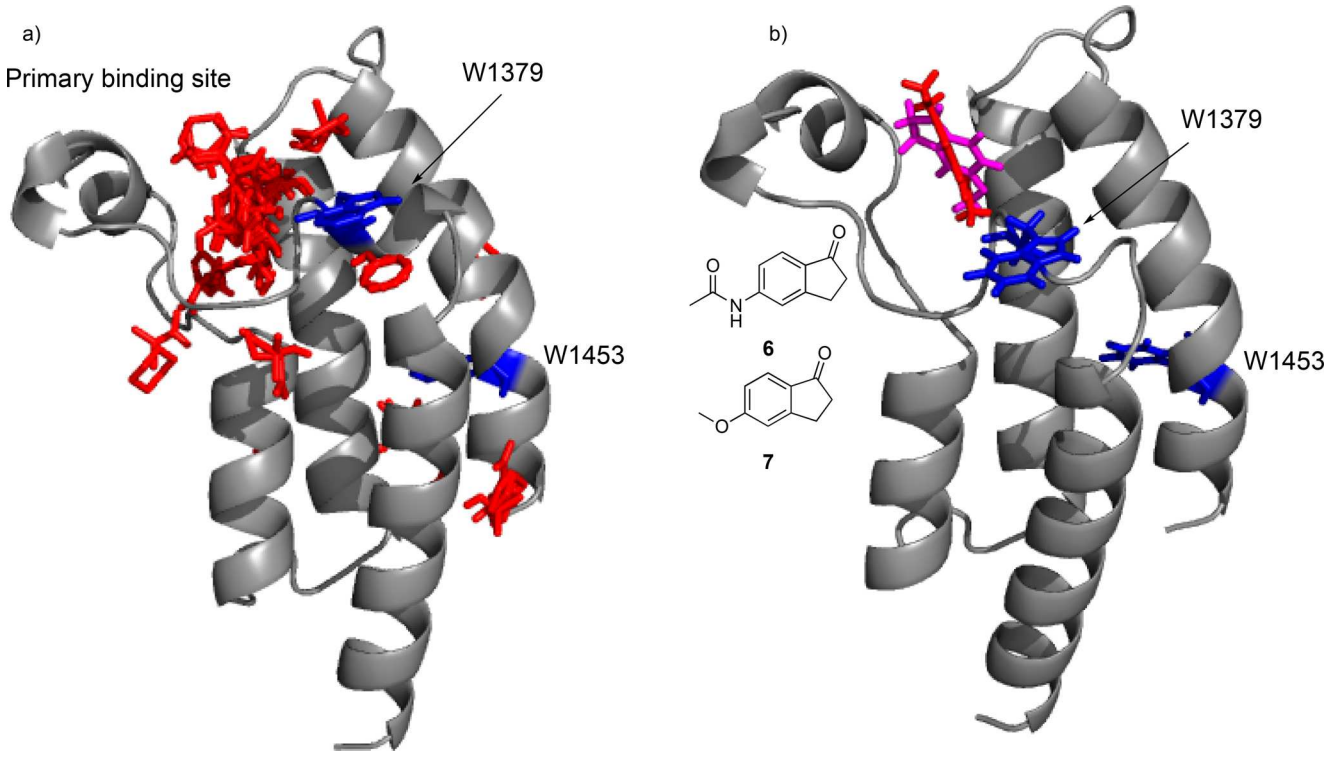

Figure 6. (a) FTMap results for the PfGCN5 bromodomain (tryptophans in blue) with clusters of small molecules (red) revealing the primary binding site. (b) Binding poses for "hit" compound **6** (red, ΔG = −6.48) and derivative **7** (magenta, ΔG = −6.34) against the PfGCN5 bromodomain (tryptophans in blue) predicted using SwissDock.

project is another option at some institutions. The basic techniques required for expression, isolation, and characterization of the ^{19}F-labeled proteins make this suitable for biochemistry and protein-centered advanced courses. Finally, many proteins can be snap-frozen and stored for later use, opening the possibility of using undergraduate research students, who are quite capable of producing sufficient quantities of protein as part of either independent study activities or summer research programs if the project aligns with a faculty member's research goals.

OUTCOMES

Implementing the research-based course requires considerable investment in faculty time, so determining if there were any significant student outcomes would justify the investment. We were also curious to see both how our research-based course compared with other organic chemistry laboratory experiences and how the research-based course compared with other research and research-like experiences. We compared our research-based course with the traditional second-semester organic laboratory course at Gustavus and with the inquiry-based laboratory at the University of Minnesota.

Comparison Groups

Gustavus Adolphus College is a private undergraduate institution with an enrollment of approximately 2400 students. The traditional second-semester organic chemistry laboratory at Gustavus is a 10-week course that meets for 4 h once per week. As opposed to the research-based course described here, it focuses on technique development, includes laboratory experiments where both students and instructors know the outcome, and provides clear directions for students to complete the experiments. Students write laboratory reports that are highly templated, and they give at least one oral presentation of their work to the class. Total enrollment for the traditional laboratory course at Gustavus is approximately 50 per year, with sections of 18−20 students, each. Students did not know if they were enrolling in the research-based course or the traditional course, and the research section had a different faculty instructor than the traditional sections.

The University of Minnesota—Twin Cities campus (U of MN) is a public institution with approximately 35,000 undergraduate students. The inquiry-based organic chemistry laboratory course at the University of Minnesota is a two-semester-in-one course that meets once a week for a 50 min lecture with the instructor and twice a week for 4 h under the supervision of a teaching assistant. Each experiment begins with one or more questions related to the learning outcomes and green chemistry concepts. The semester begins with building laboratory techniques skills which are applied to various types of reactions, separations, purifications, and analysis. Though products are generally predictable, questions focus on determining ratios of possible product mixtures, the identification of the major product, or comparing "greenness" of two processes. Approximately, 630 students are enrolled in the U of MN course each year with section sizes between 16 and 18 students.

Survey Data

Learning outcomes of curriculum-based undergraduate research experiences have been assessed,[2,3,5−9] although which specific components contribute to a given learning gain remains unclear.[58] The CURE survey has been used fairly extensively for assessing the self-perceived learning gains of students enrolled in curricular-based undergraduate research experiences,[59] providing aggregate results for students in the

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form of means and standard deviations for responses to each question. Response frequency would provide for nonparametric statistical analysis of the ordinal data from the Likert-scale questions, and the small class sizes at PUIs limits the analysis of the sample. Nevertheless, the CURE survey has provided interesting insights,[7,8] and it offered a relatively easy way to determine if our research-based course was delivering learning outcomes similar to other research experiences. The CURE survey was given to students in the "traditional" laboratory course at Gustavus (three years of data), the research-based course at Gustavus (two years of data), and the University of Minnesota's (U of MN) inquiry-based laboratory (one year of data).[60]

As expected, students in the research section showed an increase in several self-assessed skill gains compared to the traditional section. Table 2 provides results from a subset of CURE survey questions that highlight the significant increases; more complete survey results and analysis are available in the Supporting Information. In each entry, the difference in means between the traditional and the research sections was significant using a two item t test. A normal distribution was assumed, though the data provided only means and standard deviations. The t test comparisons used the multiple-significance-test correction for significance at the 95% confidence level. Data from the survey, including survey questions, are included in the Supporting Information. The strongest differences between the traditional and the research sections are their "understanding of the research process in [their] field" ($\Delta = 0.95$, entry 3), "tolerance for obstacles faced in the research process" ($\Delta = 0.88$, entry 1), and "ability to read and understand primary literature" ($\Delta = 0.87$, entry 6). As a negative control, entry 7, "skill in how to give an effective oral presentation", captures the fact that the traditional section is assigned a significant oral presentation, while the research section is not.

Table 2. Means and Significance for Select Questions from the CURE Survey[a]

Entry	Self-reported skill gain	Research[b]	Traditional[c]	Inquiry[d]
1	Tolerance for obstacles faced in the research process	4.16	3.28**	3.37**
2	Readiness for more demanding research	3.77	3.09*	3.01**
3	Understanding of the research process in your field	3.92	2.97**	2.88**
4	Understanding of how scientists work on real problems	3.97	3.22*	3.42*
5	Understanding that scientific assertions require supporting evidence	3.94	3.33*	3.57[f]
6	Ability to read and understand primary literature	3.46	2.60**	2.79*
7[e]	Skill in how to give an effective oral presentation	1.54	3.13**	2.06[f]
8[g]	This course was a good way of learning about the process of scientific research	4.57	3.79**	3.55**

[a]Mean values on a Likert scale where 1 is no gain and 5 is very large gain. * indicates p-values which are significant at the 95% confidence level for the multiple-test comparison (<0.0025) when compared to the research section. ** indicates p-values which are significant at the 99% confidence level for the multiple-test comparison (<0.0005) when compared to the research section. [b]Respondents = 46. [c]Respondents = 94. [d]Respondents = 132. [e]Negative control. [f]Significance value does not meet the multitest comparison threshold. [g]Mean values on a Likert scale where 1 is strongly disagree and 5 is strongly agree.

While the only significant difference between the inquiry section at the U of MN and the Gustavus traditional lab section was the negative control (entry 7), comparison of the research section at Gustavus to the inquiry section at U of MN showed differences in most of the same skill gains as the comparison of the research and traditional sections at Gustavus. The means in entries 5 and 7 did not show a statistical difference between the research-based course and the U of MN inquiry course. In addition, students in the research section responded more favorably to the statement "this course was a good way of learning about the process of scientific research" than students in either the traditional section at Gustavus or the inquiry section at the University of Minnesota (99% confidence ($p < 0.0025$); $\Delta = 0.78$, and $\Delta = 1.02$, respectively).

From this data, it appears that the scientific literacy component of the research-based course produced a noticeable improvement in students' perceived outcomes. Specific CURE questions about students' perceived gains in data analysis and scientific communication (specifically scientific writing) do not show a statistical difference. Overall, the gains observed are in line with the outcomes of other curriculum-based undergraduate research experiences,[58] and they provide evidence to support the continuation of this research-based course.

CONCLUSION

PrOF NMR is a technique that is amenable to undergraduate organic laboratories at PUIs. The FBLD project described above provides students with an introduction to authentic research experiences, exposes students to key elements of scientific literacy and communication, and demonstrates how the tools of chemistry can be used to address questions in disciplines such as biology. The project is adaptable to a variety of research questions through the selection of the target protein, and the results from the project can be used to advance a faculty member's research program.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.1c00028.

Sample activities, notes on procedures, and protein expression/isolation protocols (PDF, DOCX)

Template file for the PrOF NMR chemical shift analysis (XLSX)

Sample NMR data (ZIP)

CURE survey data (PDF)

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Notes

The authors declare no competing financial interest.

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(60) This work was exempted from IRB requirements, because no identifying information was collected from students in the CURE survey.

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