Flexible Implementation of the BASIL CURE

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Abstract

Course-based Undergraduate Research Experiences (CUREs) can be a very effective means to introduce a large number of students to research. CUREs are often an extension of the instructor’s research, which may make them difficult to replicate in other settings because of differences in expertise or facilities. The BASIL (Biochemistry Authentic Scientific Inquiry Lab) CURE has evolved over the past 4 years as faculty members with different backgrounds, facilities, and campus cultures have all contributed to a robust curriculum focusing on enzyme function prediction that is suitable for implementation in a wide variety of academic settings. © 2019 International Union of Biochemistry and Molecular Biology, 00(00):1–8, 2019.

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Introduction

Course-based undergraduate research experiences (CUREs) have been suggested as a way to introduce large numbers of undergraduate students to research and attain both short-term learning objectives related to specific courses as well as long-term goals of increasing retention of students in STEM careers, especially students from underrepresented groups [1–4]. Many CUREs originated as local experiments when faculty members sought to liven up laboratory courses with their own research projects. While not a direct substitute for a research experience accomplished within a research laboratory, these localized CUREs leverage the expertise of faculty members to allow scaling up of undergraduate research. Subsequently, some CUREs have grown into nationwide projects that engage thousands of students and, in addition, provide central support and professional development to faculty. One of the earliest nationwide CUREs emerged from the laboratory of Sarah Elgin to engage faculty and students around the country in the Genomics Education Partnership and focused on using bioinformatics tools to annotate eukaryotic genomes [5, 6]. Similarly, the Science Education Alliance (SEA) grew out of Graham Hatful’s bacteriophage characterization project, which led to engagement of thousands of first-year students in introductory biology courses in both computational and bench research [7, 8]. The Small World Initiative is an example of a CURE that focuses on using bench research to solve an important societal problem (antibiotic resistance and discovery of new antibiotics) and spans different curricular levels [9, 10]. A 2017 article in BAMBED pointed out that there
are few CUREs that focus on the study of proteins [11]. Since the publication of that article, two protein-based CUREs have emerged: the Malate Dehydrogenase Cure Community (MCC [12]; mdh-cures-community.squarespace.com) and Biochemistry Authentic Scientific Inquiry Lab (BASIL), the focus of this article.

Development of BASIL

In 2004, Grell and Parkin from Rochester Institute of Technology (RIT) created EZ-Viz [13] to provide a more intuitive interface to the PyMOL molecular graphics environment [14]. The following summer, Hanson and Westin from RIT transformed EZ-Viz into ProMOL [15], which enabled users to create and recognize enzyme catalytic sites in PyMOL. ProMOL underwent many revisions with significant input from Herbert Bernstein and his students at Dowling College. Once ProMOL was mature, research students from RIT and Dowling College created a library of over 800 enzyme active sites in ProMOL based on annotated data from the Catalytic Site Atlas [16]. As the students made predictions of protein function based on statistical and visual comparisons, they wanted to test their predictions in the wet lab. The students began their in vitro testing and, in short order, began using additional bioinformatics tools such as BLAST [17], Dali [18], and Pfam [19] to refine their searches and make better predictions. The students developed a workflow that included the computational analyses followed by literature searches for potential enzyme activity assays on predicted substrates, expression and purification of the protein, and ultimately confirmation of enzyme function with the assay. Through this process, students demonstrated remarkable growth as scientists:

1. They were moving beyond their instructors’ suggestions.
2. They were creating testable hypotheses.
3. They started asking “what if” questions.
4. They started identifying protocols and asking to order chemicals to test their hypotheses.

Based on these observations, we proposed that this growth could be observed on a larger scale with students in a biochemistry laboratory course in which students acquire biochemistry skills within an open-ended investigation.

We expanded our team from RIT and Dowling College to include colleagues who were part of our personal/professional network. The initial NSF proposal from this team received very helpful suggestions from NSF reviewers, so we created a poster for ASBMB 2014 in San Diego [20] to recruit additional participants, who contributed to a subsequent successful proposal.

Development of the BASIL curriculum began in summer 2015, with initial contribution and implementation on six very different campuses: St. Mary’s University San Antonio (a minority serving institution), Ursinus College, Hope College, Oral Roberts University (three liberal arts PUIs), Cal Poly San Luis Obispo (a large public institution), and the Rochester Institute of Technology (a comprehensive university transitioning to a Carnegie class 2 Ph.D.-granting institution). Throughout this process, we also had the constant presence and influence of a biochemistry education focus spearheaded by Trevor Anderson from Purdue.

In 2017, BASIL began expanding to other campuses, first when one of our faculties transitioned to a position at a different college. Others joined as the result of conversations at national conferences (Biophysical Society, ASBMB) or simply by reading our articles [21–23] or online materials (basiliuse.blogspot.com). One clear theme has been present from the beginning until now - the need for flexibility to enable instructors to implement the BASIL curriculum on different campuses.

**BASIL Curriculum**

BASIL is currently composed of five computational and six wet lab modules (Table I). All are available to download free of charge from our website: https://basilbiochem.github.io/

<table>
<thead>
<tr>
<th>Module</th>
<th>Title</th>
<th>Computational</th>
<th>Wet Lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Structure alignment with ProMOL and PyMOL</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Protein BLAST search</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Using Pfam to predict protein function</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Structural alignment with Dali</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Molecular docking with Autodock Vina [29] and PyRx [30]</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Expression of proteins from lactose-inducible vectors</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Protein purification</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Determination of protein concentration</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Using SDS-PAGE to assess the purification of a protein</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Protein activity assays</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Enzyme kinetics assays</td>
<td>X</td>
<td></td>
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</tbody>
</table>

**TABLE I**

BASIL Modules. All modules are freely available at https://basilbiochem.github.io/basil/
basil/ [24]. Each Student Module contains the following sections: Learning Goals and Objectives, Introduction, Protocol (including sections on purpose, supplies, safety considerations, procedures, and clean up), Interpreting Results, and References. Instructor resources include an Instructor Guide featuring prerequisite student knowledge, prerequisite instructor knowledge, a teaching discussion, experimental design considerations, laboratory preparation notes, and data interpretation considerations. Moreover, we have developed and made available assessment questions for each module that are aligned with the learning objectives. Student modules are freely accessible whereas instructor resources require permission to access (instructions to request access are available on the website). In our experience, the entire curriculum takes approximately 42 hours of in-laboratory time to complete. However, as we discuss below, BASIL has been designed with great flexibility so the time commitment can vary greatly.

In the BASIL curriculum, a team of 2–3 students selects (or is assigned) a PDB entry that is listed as having “unknown function” and studies it over the course of an academic term. Using the modules the students attempt to confirm a function for the protein. While the modules are numbered from 1–11 for convenience, the order of their use is flexible depending on the goals of the course and choices of the instructor. Three different implementations that have been used with BASIL are presented in Fig. 1.

As a starting point in our lab courses, we have focused on analysis of structures that align well with serine hydrolase templates in ProMOL as reported in McKay et al. [25]. Our students have done well in both the computational and wet lab modules with the following PDB entries, which all appear when searching the PDB for “unknown function:” 4EZI, 3DS8, 3L1W, 4Q7Q, 2014, 3B7F, 2QRU, 3HO4, 3CBW, and 3FEQ. All of these have been easily overexpressed and purified, and they all pass the test for a probable hydrolyase (the assay is described below). However, it is more difficult to narrow down the substrate specificity for some than for others. The DNASU Plasmid Repository (dnasu.org/DNASU) has created the BASIL starter pack V1 which contains plasmids for most of these proteins for a modest price.

As an example of a protein that is easy for students to study, PDB entry 4Q7Q has been particularly fulfilling. The researchers who submitted the crystal structure of 4Q7Q to RCSB identified it as a possible lipase [26]. It aligns well with a number of serine hydrolase templates in PromOL/PyMOL. A simple BLASTp search gives a long list of similar proteins, the most similar being SGNH lipases, so named for the amino acids found in the active sites of this class of esterases. In addition, 4Q7Q is fairly easy to express and purify, making it especially suitable for a CURE lab. The DNASU plasmid repository [27] offers the plasmid containing the coding sequence of 4Q7Q. The coding region has a T7 promoter, a nus-tag, and a 6xhistag. The nus-tag allows large quantities of soluble 4Q7Q to be produced, and the 6xhistag facilitates easy purification on nickel- or cobalt-chelated affinity chromatography resins. The protein expresses well, purifies easily and shows a large band with MW ~ 30 K by SDS-PAGE. While no group has yet determined the “best” substrate for 4Q7Q, every year this protein has afforded the students working on it some excellent experience in enzymology. These experiments are usually done with the artificial substrate para-nitrophenyl acetate (PNPA) [28]. PNPA is colorless yet when hydrolyzed to p-nitrophenol yields a bright yellow color that absorbs at 405 nm. Enzyme 4Q7Q is the most active at PNPA hydrolysis of the unknown proteins yet studied. In fact,
4Q7Q can be used as a positive control enzyme for the entire project because it is more reliable at PNPA hydrolysis than even chymotrypsin. Students have been able to study the activity of 4Q7Q at different pH values, at different ionic strengths, and in the presence and absence of magnesium, calcium, and zinc ions. They have tested its ability to hydrolyze p-nitrophenyl esters of longer carbon chains, from p-nitrophenyl butyrate to p-nitrophenyl dodecanoate.

For proteins other than lipases, p-nitrophenyl derivatives can be purchased that may identify a specificity for peptides, glycosides, or oligonucleotides, narrowing the specificity of the unknown protein. Examples of proteins that have not been found to hydrolyze these PNP derivatives include 2QRU and 3CBW, making it difficult to form hypotheses about these proteins’ native activities. Depending on the amount of time a course has for assaying other possible substrates, studying these proteins might be less or more desirable. Since BASIL is a discovery-based curriculum, progress on many fronts will be continually discussed on the BASIL discussion forum: https://www.basilbiochem.org/.

Flexible Implementation of BASIL

As a team, we have been using different approaches to the BASIL curriculum on our campuses, based on curricular goals, available resources, scheduling, and instructor expertise. We used a Qualtrics survey with 17 Likert-style questions to catalog the various ways that practitioners thought about and implemented BASIL. Fourteen instructors completed the survey. Six were new to the BASIL curriculum, having taught it for one year, while six were more seasoned, having taught it for at least three years. Instructors overwhelmingly chose the BASIL CURE as a means to increase student excitement for science, improve students’ learning, and provide more students with a research experience (Fig. 2).

BASIL has been implemented in diverse higher education settings, from liberal arts colleges to research universities, and has also been carried out successfully at the high school level. Institutions varied in class size and availability of teaching assistants or other support (Fig. 3). Departments offering

FIG 2

Why did instructors implement the BASIL CURE? Instructor responses to a series of questions beginning with “I implemented BASIL because…” Responses were collected using a Likert scale. The widths of the bars reflect the absolute number of responses to each Likert option. [Color figure can be viewed at wileyonlinelibrary.com]
objectives or expertise. The additions/alterations fell into three main groups: protocols, assessments, and research questions (Table II). Several instructors dedicated lab time to develop the technical skills needed for their student populations, for example a pipet skills lab or buffer preparation lab. One had students explore techniques using traditional cookbook-style labs during the first part of the semester before approaching BASIL. In this way, students had experience with techniques, such as SDS-PAGE, before having to apply them to their BASIL research project. One instructor added in a Western blot analysis using anti-His tag antibodies since this technique had not been covered yet in the curriculum at that institution. In some cases, the change wasn’t an addition but a substitution, for example, using an alternate expression method that worked well in the hands of the instructor and for which the infrastructure was already in place.

Each BASIL module contains Learning Goals, Learning Outcomes, and Assessment Questions aligned with these goals and outcomes, all of which are available on the BASIL website. The flexibility of BASIL facilitated the ability of all

### TABLE II

The BASIL CURE was extended in unique ways at various institutions through additional protocols/lab techniques, assessments, and research questions

<table>
<thead>
<tr>
<th>Protocols</th>
<th>Assessments</th>
<th>Research Questions</th>
</tr>
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<tbody>
<tr>
<td>Pipet skills</td>
<td>Lab report/journal article</td>
<td>Analyzing Nudix hydrolases</td>
</tr>
<tr>
<td>Buffer design</td>
<td>Primary literature discussions</td>
<td>Analyzing kinases</td>
</tr>
<tr>
<td>Buffer preparation</td>
<td>Short writing assignments</td>
<td>Inhibitor analysis</td>
</tr>
<tr>
<td>Plasmid map analysis</td>
<td>Future directions assignments</td>
<td>Plasmid restriction digests</td>
</tr>
<tr>
<td>Restriction digests</td>
<td>Homework questions</td>
<td>Structural analysis with topology diagrams</td>
</tr>
<tr>
<td>Western blots</td>
<td>Poster presentations</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mid-semester progress presentations</td>
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</tr>
</tbody>
</table>
instructors to design further assessments aimed at their specific student population and course goals (Table II). These included writing assignments and presentations in both poster and oral formats. Several institutions required students to present their research at campus-wide events.

The flexible design of BASIL easily allows fresh research questions to be developed (Table II). One of BASIL’s major strengths is flexibility of the enzyme class under investigation. Instructors Jeff Mills and Suzanne O’Handley at RIT decided to use the BASIL model to study members of the Nudix hydrolase Superfamily, which is the focus of the O’Handley research lab. Bioinformatic tools provided a wealth of data. Students found that the conformationally heterogeneous active sites of Nudix hydrolases were not conducive to active site alignments with ProMOL/PyMOL or molecular docking with PyRx/Autodock Vina. After induction and lysis, crude extracts were saved for activity analysis. The students spent several weeks optimizing enzymatic substrates and conditions using the crude cell extract, ultimately purifying the enzyme using metal ion affinity chromatography (Fig. 1). The students successfully demonstrated the power of combining in vitro and in silico techniques to identify substrate specificity for this enzyme class. Moreover, on three campuses students continued working on independent research based on the work they started during the lab course. It is our hope that such research projects will lead to discovery-focused publications by BASIL students in the future.

As with all research, whether course-based or not, challenges arise. We found that challenges for the BASIL CURE fell into three main categories: technical problems, instructor challenges, and student difficulties. They ranged from short-term minimal issues to long-term impacts (Fig. 4). Technical problems included lack of access to equipment, despite the fact that the BASIL modules were devised to employ commonly-available laboratory equipment. We hope that as the BASIL community grows, frozen cell pellets and already purified protein will be made available from BASIL collaborators to allow institutions without culturing facilities to participate in BASIL. Sometimes there was a bottleneck for students if, for example, only a single spectrophotometer was available. However, flexibility is increased because protein activity assays (Module 10) can be carried out qualitatively if no spectrophotometer is at hand. Many faculty members found that software installation or use (especially with ProMOL and PyRx) was frustrating and sometimes this was dependent upon the availability of Information Technology support at the institution. To support wet lab and computational implementations of BASIL a discussion community is available at https://www.basilbiochem.org/.

Some instructors found that the time required to learn a new curriculum was a barrier to implementation. We appreciate that there is a learning curve associated with some of the computational software, and accordingly we have included video tutorials for the computational modules. On some campuses the lack of a TA or other laboratory preparation personnel increased the time commitment by the faculty member, however, many still carried out BASIL successfully (Fig. 3).

The learning curve and technical issues discussed above hold true for the students as well as the instructors. BASIL instructors reported that this could lead to frustration for

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**FIG 4**

Instructor responses to the question “How much of an impact do the following challenges have on your implementation of BASIL?” X-axis Impact scale: 1 = Insignificant (minimal), 2 = Minor (short-term), 3 = Moderate (Significant), 4 = Major (Considerable short-term impact), 5 = Catastrophic (Extensive long-term impact). Diamonds represent the mean, the thicker vertical line in each box the median, and any statistical outliers are represented as dots. [Color figure can be viewed at wileyonlinelibrary.com]
some students. Some of the students were not interested in the challenge of a CURE, preferring a cookbook-style lab simply to fulfill a graduation requirement. Others were frustrated by the lack of success at intermediate points during the academic term. In the wet lab portion of the BASIL curriculum, students frequently need to troubleshoot problems with expression levels (though not with 4Q7Q) and protein purification (some of the proteins include maltose binding protein tags that interfere with 6xHistag binding to metal ion affinity chromatography resins). They also encounter challenges with identifying potential substrates and, for putative lipases, getting the longer chain molecules to dissolve in aqueous buffer. Several students were challenged by the more advanced computational activities and the different perspective required for bioinformatics work, where they need to think in terms of “possible answers” to the question being asked, rather than a “right answer”. Finally, the nature of the BASIL curriculum was problematic for some students, as it did not line up neatly with the corequisite biochemistry lecture course. Nonetheless, we believe that the frustrations encountered and oftentimes overcome provide a framework that may assist in the development of characteristics of a scientist such as resilience and persistence.

Conclusion

The combination of in vitro and in silico work in the BASIL curriculum has been implemented in many different ways on the broad spectrum of campuses that participate in the project. The instructors come from a variety of backgrounds, yet are able to implement this CURE despite differences in facilities, student populations, and institutions. The curriculum has been designed (through much trial and error) with the following features to ensure flexibility and straightforward implementation:

- The students learn and use wet bench techniques that are common in most biochemistry lab courses and readily available on most campuses.
- In cases where instruments or resources are not available on a given campus, workarounds are provided in the instructor versions of the modules.
- While a variety of proteins is usually studied in a single lab section, the expression and purification of these proteins follow the same patterns.
- The modules are interdependent, but it is possible to omit some of the modules and still provide a robust learning experience for the students.
- Faculty members who use the modules can focus on their own strengths in choosing modules.
- Three of the bioinformatics modules are based on web applications that can be accessed from most web browsers.
- We have prepared videos to facilitate students and faculty in completing the two more challenging bioinformatics modules (ProMOL/PyMOL and PyRx/Autodock Vina).
- We have an online discussion community ready to help.

We believe that the BASIL CURE can be implemented on most college campuses to expose students to open-ended research questions with the opportunity for them to create and test hypotheses and sometimes fail in scientific pursuits, an experience common in a research lab but not often afforded in a traditional undergraduate classroom laboratory. Additionally, it allows faculty and teaching assistants to expand their technological skill set and to broaden their pedagogical base.

Acknowledgments

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