EVALUATION OF A NOVEL BIOCHEMISTRY COURSE-BASED UNDERGRADUATE RESEARCH EXPERIENCE (CURE)

by

Stefan M. Irby

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THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Trevor R. Anderson, Chair

Department of Chemistry

Dr. Kari L. Clase

Department of Agricultural and Biological Engineering
Department of Technology, Leadership, and Innovation

Dr. Chittaranjan Das

Department of Chemistry

Dr. Marcy H. Towns

Department of Chemistry

Approved by:

Dr. Christine Hrycyna

Head of the Graduate Program

This dissertation is dedicated to my uncle Steven "TT" McMahan

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LIST OF ABBREVIATIONS

Acronyms	Meaning
ALO(s)	Anticipated Learning Outcome(s)
BASIL	Biochemistry Authentic Science Inquiry Lab
CIAP	Calf Intestinal Alkaline Phosphatase
CoP	Community of Practice
CRM	Conceptual-Reasoning-Mode
CURA(s)	Course-based Undergraduate Research Ability(ies)
CURE(s)	Course-based Undergraduate Research Experience(s)
ER(s)	External Representations
KEC	Knowledge, Experience, and Confidence
LO(s)	Learning Outcome(s)
LPP	Legitimate Peripheral Participation
LR	Lower-rated CURA statement
MR	Middle-rated CURA statement
NUDIX	Nucleoside Diphosphate X (NUDIX) Hydrolase
PICURA	Process for Identifying Course-based Undergraduate Research Abilities
PNPA	p-Nitrophenyl Acetate
PPI	Participant Perception Indicator
TR	Top-rated CURA statement
VLO(s)	Verified Learning Outcome(s)
WR	Weighted-relevance

ABSTRACT

Author: Irby, Stefan, M. PhD
Institution: Purdue University
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Title: Evaluation of a Novel Biochemistry Course-based Undergraduate Research Experience (CURE)
Committee Chair: Trevor R. Anderson

Course-based Undergraduate Research Experiences (CUREs) have been described in a range of educational contexts. Although various learning objectives, termed anticipated learning outcomes (ALOs) in this project, have been proposed, processes for identifying them may not be rigorous or well-documented, which can lead to inappropriate assessment and speculation about what students actually learn from CUREs. Additionally, evaluation of CUREs has primarily relied on student and instructor perception data rather than more reliable measures of learning. This dissertation investigated a novel biochemistry laboratory curriculum for a Course-based Undergraduate Research Experience (CURE) known as the Biochemistry Authentic Scientific Inquiry Lab (BASIL). Students participating in this CURE use a combination of computational and biochemical wet-lab techniques to elucidate the function of proteins of known structure but unknown function. The goal of the project was to evaluate the efficacy of the BASIL CURE curriculum for developing students' research abilities across implementations. Towards achieving this goal, we addressed the following four research questions (RQs): **RQ1**) How can ALOs be rigorously identified for the BASIL CURE; **RQ2**) How can the identified ALOs be used to develop a matrix that characterizes the BASIL CURE; **RQ3**) What are students' perceptions of their knowledge, confidence and competence regarding their abilities to perform the top-rated ALOs for this CURE; **RQ4**) What are appropriate assessments for student achievement of the identified ALOs and what is the nature of student learning, and related difficulties, developed by students during the BASIL CURE? To address these RQs, this project focused on the development and use of qualitative and quantitative methods guided by constructivism and situated cognition theoretical frameworks. Data was collected using a range of instruments including, content analysis, Qualtrics surveys, open-ended questions and interviews, in order to identify ALOs and to determine student learning for the BASIL CURE. Analysis of the qualitative data was through inductive coding guided by the concept-reasoning-mode (CRM) model and the assessment triangle, while analysis

of quantitative data was done by using standard statistical techniques (e.g. conducting a parried t-test and effect size). The results led to the development of a novel method for identifying ALOs, namely a process for identifying course-based undergraduate research abilities (PICURA; RQ1; Irby, Pelaez, & Anderson 2018b). Application of PICURA to the BASIL CURE resulted in the identification and rating by instructors of a wide range of ALOs, termed course-based undergraduate research abilities (CURAs), which were formulated into a matrix (RQs 2; Irby, Pelaez, & Anderson, 2018a,). The matrix was, in turn, used to characterize the BASIL CURE and to inform the design of student assessments aimed at evaluating student development of the identified CURAs (RQs 4; Irby, Pelaez, & Anderson, 2018a). Preliminary findings from implementation of the open-ended assessments in a small case study of students, revealed a range of student competencies for selected top-rated CURAs as well as evidence for student difficulties (RQ4). In this way we were able to confirm that students are developing some of the ALOs as actual learning outcomes which we term VLOs or verified learning outcomes. In addition, a participant perception indicator (PPI) survey was used to gauge students' perceptions of their gains in knowledge, experience, and confidence during the BASIL CURE and, therefore, to inform which CURAs should be specifically targeted for assessment in specific BASIL implementations (RQ3;). These results indicate that, across implementations of the CURE, students perceived significant gains with large effect sizes in their knowledge, experience, and confidence for items on the PPI survey (RQ3;). In our view, the results of this dissertation will make important contributions to the CURE literature, as well as to the biochemistry education and assessment literature in general. More specifically, it will significantly improve understanding of the nature of student learning from CUREs and how to identify ALOs and design assessments that reveal what students actually learn from such CUREs - an area where there has been a dearth of available knowledge in the past. The outcomes of this dissertation could also help instructors and administrators identify and align assessments with the actual features of a CURE (or courses in general), use the identified CURAs to ensure the material fits departmental or university needs, and evaluate the benefits of students participating in these innovative curricula. Future research will focus on expanding the development and validation of assessments so that practitioners can better evaluate the efficacy of their CUREs for developing the research competencies of their undergraduate students and continue to render improvements to their curricula.

CHAPTER 1. INTRODUCTION

1.1 Motivation

Recent calls to action have advocated for the inclusion of more authentic research practices within student courses across various science disciplines and educational levels (e.g. Brewer & Smith, 2011; NASEM, 2015, 2017; NRC, 2003, 2012; PCAST, 2012). At the undergraduate level, this has been addressed by designing and implementing innovative courses called Course-based Undergraduate Research Experiences (CUREs). Additionally, CUREs have been proposed by educational researchers as ways to implement more effective teaching strategies into classrooms and as a way to pose "messy problems" for students to grapple with (Dolan & Collins, 2015). CUREs afford students the opportunity to develop their knowledge and competence to solve problems and do more discovery-type research in the lab, as compared to traditional recipe-driven labs which focus more on following protocols and performing technical procedures (Auchincloss et al., 2014). In addition to being an opportunity to incorporate more authentic research practices, a review of the CURE literature (Corwin, Graham, & Dolan, 2015) shows a variety of outcomes students may experience from participating in a CURE (Table 1.1). These outcomes highlight the potential for CUREs to impact learning within the cognitive, affective, and psychomotor domains (Table 1.1). However, although many published CUREs and proposed student benefits exist, most CUREs have not been rigorously studied and often rely only on students' self-reported data to make claims about the success of CURE curricula (Corwin, Runyon, Robinson, & Dolan, 2015; Kinner & Lord, 2018; Kloser, Brownell, Shavelson, & Fukami, 2013; Lefurgy & Mundorff, 2017; Shaffer et al., 2014; Shanle, Tsun, & Strahl, 2016). Because of this, there is a dearth of literature documenting the true effects CUREs have on actual student learning.

Table 1.1 Short-, medium-, and long-term outcomes documented in the CURE literature identified by Corwin, Graham, et al. (2015).

Short-term Outcomes	Medium-term Outcomes	Long-term Outcomes
Increased analytical skills	Increased self-efficacy	Enhanced science identity
Increased content knowledge	Increased motivation in science	Career Clarification
Increased technical skills	Increased tolerance of obstacles	Persistence in Science
Increased project ownership	External validation from a scientific community	
Increased communication skills		
Increased collaboration skills		
Sense of belonging to a larger community		

Though there are documented student outcomes, Corwin, Graham, et al. (2015) conclude their study by calling on the discipline-based research community to work toward developing instruments and methods for studying CUREs and their learning outcomes. Brownell & Kloser (2015), proposed a conceptual framework for measuring the effectiveness of CUREs and puts forth some examples of assessments for addressing the components of a CURE as outlined by Auchincloss et al. (2014). One key component for this framework is to identify student, course and faculty outcomes (Brownell & Kloser, 2015) which are aligned with the tenets of sound evaluation design. Work by Anderson (2007), has documented the importance of having alignment between course objectives, teaching, learning and assessment. However, the literature lacks detailed suggestions or processes to help instructors or education researchers to define learning outcomes, which in this project we term anticipated learning outcomes (ALOs) as they are usually speculated about by instructors but haven't been verified by student assessment.

Some CURE studies do define learning outcomes (LOs; e.g., Gray et al., 2015; Makarevitch, Frechette, & Wiatros, 2015; Olimpo, Fisher, & DeChenne-Peters, 2016; Staub et al., 2016) around which to center their evaluation. Generally speaking, learning outcomes (LOs) that have been documented for a CUREs fall into two categories: either they are adapted from the abovementioned five components of a CURE (Auchincloss et al., 2014) or another similar published source (e.g. Brownell et al., 2015; Olimpo et al., 2016), or they are actually based on the content or the activities within the actual CURE of interest (Gray et al., 2015; Kowalski, Hoops, & Johnson, 2016; Makarevitch et al., 2015; Staub et al., 2016). The focus tends to be on broadly applicable skills and not on specific research abilities. These might be good for assessing whether a course is CURE-like or not and for establishing broad goals for the students or faculty members to strive toward, but does not get to grips with the ultimate goal of CUREs of establishing the actual nature

of research abilities being developed. Thus many researchers rely on studying CUREs using self-report surveys about general aspects of their CURE of interest, and not what specific research abilities their students are developing. In this project we propose that by being able to identify specific ALOs will allow us to develop specifically tailored assessment measures and determine actual student learning (NRC, 2001).

This dissertation seeks to make contributions to both the CURE and assessment communities, by creating a process to rigorously determine what the specific course-based research abilities (CURAs) students should be developing from participating in a biochemistry CURE. These CURAs will be used as ALOs and the basis for assessment design to determine if they are verified learning outcomes (VLOs), meaning there is evidence that students are developing these abilities. This will give insight into what abilities students are actually developing within a CURE and will provide tools that can be applied to other courses, to assess student learning.

1.2 Goals and Overarching Research Questions for this Dissertation

The goal of this dissertation project was to evaluate and assess student learning in a novel biochemistry CURE. This dissertation aims to fill an important gap in the CURE literature about how to identify key CURAs and assess student leaning during a CURE, which could in turn advance research into CUREs in general. The components of this dissertation (Figure 1.1) are focused on developing and employing data-driven methods that allow for a rigorous assessment of student learning within a biochemistry CURE. Towards these goals, this dissertation set out to answer the following research questions:

- **RQ1)** How can ALOs be rigorously identified for the BASIL CURE?
- **RQ2)** How can the identified ALOs be used to develop a matrix that characterizes the BASIL CURE?
- **RQ3)** What are students' perceptions of their knowledge, experience, and confidence regarding their abilities to perform the top-rated ALOs for this CURE?
- **RQ4)** What are appropriate assessments for student achievement of the identified ALOs and what is the nature of student learning, and related difficulties, developed by students during the BASIL CURE?

1.3 Dissertation Organization

The dissertation is divided into four parts (Chapters 2-5; Figure 1.1):

- **Chapter 2)** Addresses RQ1: How to Identify the Research Abilities Instructors Anticipate Students Will Develop in a Biochemistry Course-Based Undergraduate Research Experience (CURE; Irby, Pelaez, & Anderson, 2018b);
- **Chapter 3)** Addresses RQ2 and RQ4: Anticipated Learning Outcomes for a Biochemistry Course-based Undergraduate Research Experience Aimed at Predicting Protein Function from Structure: Implications for Assessment Design (Irby, Pelaez, & Anderson, 2018a);
- **Chapter 4)** Addresses RQ3: Student Perception of Course-based Undergraduate Research Abilities Identified as the Anticipated Learning Outcomes for a Biochemistry CURE;
- **Chapter 5)** Addresses RQ4: VLOS Revealed by Assessment of Top-rated CURAs.

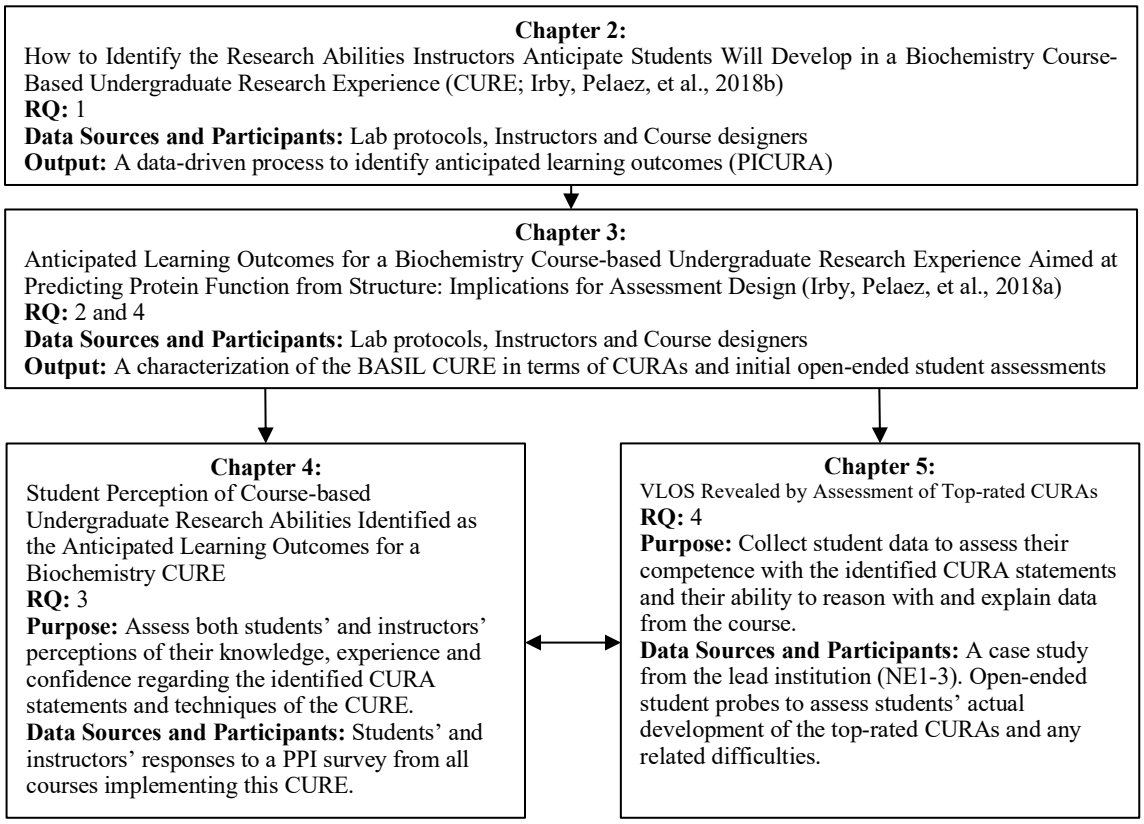

Figure 1.1 Flow chart of this dissertation together with details relating to the status, purpose, RQs, outputs, data sources and participants of each part.

The four dissertation parts build upon and inform each other. Chapter 2 describes the development of a novel process to use data to identify relevant abilities. Then, Chapter 3 shows how the ability statements generated by this process are used to construct a matrix of all the relevant abilities and to rank them in order to identify the most important CURAs. These CURAs are the focus of assessment development for the investigation of student learning (Chapter 5).

Chapter 4 focuses on collecting data about students' perceptions of their gains in knowledge, experience, and confidence regarding the top-rated CURAs, identified in Chapters 2 and 3, as well as of the computational and biochemical techniques they encounter in their BASIL courses.) Chapter 5 shows how the top-rated CURAs (Chapters 2 and 3), and the ALO matrix (Chapter 3) were used to design open-ended probes (or assessments), as well as semi-structured interviews to assess students' actual development of the top-rated CURAs. Therefore, Chapters 4 and 5 give insight into the nature of student learning and related difficulties from the BASIL CURE as well as how students perceive their learning.

1.4 Overarching Literature

Given the structure of this dissertation, the literature of specific relevance to each study is presented and discussed in each individual results Chapter 2-5. Literature of more general relevance is discussed in this section. Below is a brief overview of what constitutes a CURE and how this dissertation fits into the CURE literature.

CURE stands for **C**ourse-based **U**ndergraduate **R**esearch **E**xperience. Several groups of researchers have proposed ways to classify a course as a CURE (Auchincloss et al., 2014; Brownell, Kloser, Fukami, & Shavelson, 2012; Corwin, Runyon, et al., 2015; Lopatto & Tobias, 2010a; Weaver, Russell, & Wink, 2008). In general, a CURE can be thought of as an authentic inquiry (Buck, Bretz, & Towns, 2008) course that allows students to participate in research activities within their course, instead of engaging with other teaching lab formats (Table 1.2; Domin, 1999). Over the years, publications in the literature have demonstrated a progression of teaching lab styles or formats in chemistry from traditional "cookbook" labs, to open-inquiry, to full-on research internships (Table 1.2; Buck et al., 2008; Domin, 1999; Weaver, Russell, & Wink, 2008). As illustrated by the following operational definition used by Irby, Pelaez, et al. (2018b), CUREs are best situated between open-inquiry and research internships (Table 1.2): "a CURE is a lab course where students engage in activities resembling those done by scientists in a particular field, to conduct novel investigations about relevant phenomena that are currently unknown." Most commonly, a course is generally accepted as a CURE if it incorporates the five components identified by Auchincloss et al. (2014): 1) CUREs involve the use of scientific research practices; 2) they have elements of discovery; 3) the products of the course have broader relevance or importance; 4) they afford opportunities for collaboration; and 5) they emphasize the purpose of, and allow for iteration of experiments. Recently, a growing number of CURE curricula have been developed, adopted, and disseminated through various discipline-based education research (DBER) journals and open-access databases (e.g. CUREnet, n.d.). One well-known example of a CURE curriculum is the Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES; e.g. Hatfull, 2015; Jordan et al., 2014), where students collect their own soil samples to discover new viruses through microbiology, genome annotation, and bioinformatic analyses.

Table 1.2 Progression of lab formats that have been identified for chemistry teaching labs.

	Teaching Lab Formats					
Authors	**Traditional**	**Inquiry**			**CURE**	**Internship**
Domin (1999)[a]	Expository	Inquiry				
Buck et al. (2008)	Conformation	Structured	Guided	Open	Authentic inquiry	
Weaver et al. (2008)	Verification		Guided	Open	Research-based labs in class	Research apprenticeship in a lab

[a] Domin (1999) also classified two additional lab formats: discovery and problem based. These two formats do not fall under other lab headings but seem to fit well in a progression between inquiry and CUREs.

Dolan and colleagues have spearheaded several of the seminal efforts in the CURE community by defining important features of what should be incorporated into a CURE (Auchincloss et al., 2014), by developing surveys of students' perceived project ownership (Hanauer & Dolan, 2014), and by documenting students' perceived understanding about collaboration, discovery, relevance, and iteration (Corwin, Runyon, et al., 2015). However, in terms of assessing CURE curricula, they, like many others (Kinner & Lord, 2018; Kloser et al., 2013; Lefurgy & Mundorff, 2017; David Lopatto, 2007; Shaffer et al., 2014; Shanle et al., 2016) have focused on quantitative surveys rather than using qualitative instruments to get to grips with the nature of what students are actually learning during a CURE. Shortlidge & Brownell (2016), constructed a practical guide for assessing CUREs with existing tools in the literature. The majority of the resources identified were Likert or multiple-choice surveys geared toward measuring students perceptions about general skills and affective domain items (Shortlidge & Brownell, 2016). They also point out that qualitative instruments do exist to measure student experimental competence (e.g. Dasgupta, Anderson, & Pelaez, 2014, published by our research group) and other cognitive abilities, but currently, they are not as commonly used to study CUREs. As a result of the reliance on perception surveys, there is little information of what specific components, or activities, are actively affecting student learning and leading to the documented CURE outcomes that have been proposed in the literature (Corwin, Graham, et al., 2015; Table 1.1). Furthermore, as discussed earlier, these metrics are valuable but do not assess to what extent actual learning is taking place within CUREs, which is difficult to do unless the instrument is designed for a specific context and captures the ALOs for that CURE- one of the goals of the present study.

As mentioned above, there are few examples of studies that use qualitative data (e.g. Brownell et al., 2015; Mishra, Clase, Bucklin, & Daniel, 2018; Olimpo et al., 2016) to evaluate

CUREs or closely related experiences. For example, a study by Berg, Bergendahl, Lundberg, & Tibell (2003) used a combination of student surveys and interviews to evaluate student performance in different biochemistry lab styles. Students reported that more inquiry-based laboratories increased their learning, laboratory skills, and time spent on task; and the interviews revealed that students that took part in inquiry-based laboratories were more able to describe the experiment, evaluate the experimental results, and suggest new experiments or improvements to the experiment performed (Berg et al., 2003). In another qualitative study, reported by Hunter, Laursen, & Seymour (2007), research students were interviewed about the gains they made as part of their experience and how these experiences impacted their career decisions. The results suggested that in general students who participated in research continued in the scientific field. One example of a CURE, specifically, used open-ended student responses together with a Likert surveys to identify what they found enjoyable, and what they learned (Olimpo et al., 2016). Similarly, Brownell et al. (2015) used open-ended pre- and post-course surveys to study changes in student perceptions of being a scientist during their CURE of interest. They also focused on the idea of what aspects of the course helped students to 'think like a scientist.' More recently there have been efforts to move away from just documenting student's perceptions within a CURE and to begin to document actual learning. For example, a team of researchers used quantitative and qualitative analysis to document the positive effects of CUREs on the development of student competence to use external representations (ERs) and to make connections between multiple ERs (Mishra et al., 2018).

All the above-mentioned studies support the argument that authentic research and CUREs can aid the goals and development of biochemistry competencies that students should have developed upon graduation, as outlined by ASBMB (Voet et al., 2003). However, none of these studies address what specific activities of these experiences contribute to these realized outcomes, or how students integrate aspects CUREs to construct new knowledge. Though a range of outcomes of been identified by Corwin, Graham, et al. (2015) (Table 1.1), the authors also stated that additional research must be conducted on the connections between CURE activities and the proposed outcomes. Shortlidge & Brownell (2016) created an inventory of many of the assessment instruments that have been used to study CUREs, along with an outline of considerations to take into account when selecting an already developed instrument. When it comes to planning an analysis of a CURE, the first step is to identify the desired outcomes (or anticipated learning outcomes) of that particular CURE (Brownell & Kloser, 2015; Shortlidge & Brownell, 2016).

However, there is no well-documented process to identify the anticipated learning outcomes (ALOs) of a CURE. Thus, the focus of this dissertation was to first develop a process for identify research abilities (ALOs or CURAs) that are specific for the BASIL CURE, to then formulate the identified abilities into a matrix, and to use the matrix to inform the design of open-ended student assessments that target such abilities and permit measurement of the extent of learning taking place during the CURE.

1.5 CURE of Interest: The Biochemistry Authentic Scientific Inquiry Laboratory (BASIL)

This dissertation evaluates and assesses a newly developed biochemistry laboratory CURE, known as the Biochemistry Authentic Scientific Inquiry Laboratory (BASIL) (Craig, 2017; Craig et al., 2018), which is implemented by more than 15 different instructors at ten different institutions (as of 2018). This dissertation focuses on the educational research part of an NSF-funded (#1503798 and #1710051), multi-institutional, larger project entitled *Using protein function prediction to promote hypothesis-driven thinking in undergraduate biochemistry education*. The remaining institutions are *inter alia* engaged in protocol and visual material development, course instruction, and related curricular and assessment activities. The lab is based on modern biochemistry research techniques and actual research that had been done to assign putative function to proteins (McKay et al., 2015). The BASIL CURE aims to provide students with a scientific research experience through a unique combination of computational and wet-lab experiences for which a range of independent protocols have been developed for this CURE. Students that participate in a BASIL course uncover new knowledge about the function of some of the many proteins listed on the Protein Data Bank (PDB; https://www.wwpdb.org) whose structure is known, but whose function has not yet been established. In addition to developing technical skills, students get the opportunity to develop their thinking, reasoning, and visualization abilities through the application of their biochemistry knowledge to solving problems in novel situations, to generating and testing hypotheses through rigorous experimentation, and to processing and evaluating results.

Using a combination of computational programs to visualize protein structures, the students screen structural motifs for homology across related protein structures on the PDB and simulate substrate docking to discover candidates for a protein of interest (Craig, 2017; Irby, Pelaez, et al., 2018a [Chapter 3] for an outline of the course modules). Thus, the computational phase of the lab allows students to develop hypotheses for their protein's putative function, which

is then tested through experimentation. The wet-lab phase initially familiarizes students with basic biochemical techniques, but as the course progresses, lab protocols become less guided and more or authentic inquiry-based, i.e. more research-like. This includes students learning to design and perform assays to determine whether they have correctly predicted the function of their protein. Through this process, students learn how to interpret lab data in the light of computational data and information acquired from literature. There were 10 BASIL CURE lab protocols (Table 1.3) and are formatted to contain a background section that introduces either the computational programs and databases or the biochemical techniques being used as part of that particular activity. Additionally, the background sections include discussion of the method used, as well as some rationale for why a method was chosen and information about how it works. The protocols then provide an outline of either simulations to run or instructional steps that need to be completed when performing the computational modules and biochemical assays.

Table 1.3 A List of all of the BASIL CURE protocols.

BASIL CURE Protocol
Protein Expression
Protein Purification
Protein Concentration
SDS-PAGE
Enzyme Activity
BLAST
Dali
Pfam
ProMOL
PyRX

The BASIL CURE has been implemented at multiple institutions across the country, adding depth to this project by incorporating a range of demographics. For example, one of the universities is Hispanic-serving and a member of the Hispanic Association of Colleges and Universities, while another university has an above-average hearing-impaired population. Thus, the BAIL CURE, like many CURE curricula, can afford diverse student populations access to a research experience the may otherwise not have the opportunity to participate in research. In addition, none of the universities currently implementing the BASIL CURE are R1 schools. Thus,

this course may be the only experience students have doing research-like lab practices prior to graduation and entering graduate school, industry, teaching, or society in general.

1.6 Guiding Research Frameworks

The following sections provide an overview of the theoretical, conceptual, and analytical frameworks that guided this dissertation. This section is intentionally presented in a general form in order to encompass the guiding lenses and frameworks throughout the entirety of this dissertation. If additional frameworks or more specific details are needed, they will be provided for that particular dissertation part in Chapters 2-5.

1.6.1 Theoretical Frameworks

The theoretical frameworks employed by this dissertation are constructivism (1.6.1.1) and situated cognition (1.6.1.2). These frameworks are implicit in Chapters 2-4 of this dissertation, but still influence the goals and interpretations of these parts. Given its direct focus on investigating student cognition, the theoretical frameworks guiding this dissertation more explicitly inform data interpretation and study design for Chapter 5.

1.6.1.1 Constructivism

The overarching qualitative paradigm used in this project is constructivism, also referred to as interpretivism (Lincoln, Lynham, & Guba, 2011). Lincoln et al. (2011) describe the aim of this paradigm as understanding and reconstructing a phenomenon. As Bodner and Orgill (2007) outlined in their history of constructivism, before constructivism became a theoretical framework and widely-used paradigm, constructivism was a theory of learning that arose from the cognitive sciences in efforts to explain the incorporation of knowledge. One of the tenets of this learning theory is that learners construct their understanding though their experiences, striving to generate hypotheses that "fit" their observations, not necessarily "matching" with reality or coming to a right conclusion (Bodner, 1986). This idea can also be explained by a metaphor from von Glasersfeld (1989) which states that "god" is the only true knower of the *real* world because he is the one who constructed it, whereas human knowers only know the information they have constructed for themselves based on, and adapted to fit, the experience of being a part of this world.

According to Denzin and Lincoln (2011), as a theoretical framework constructivism adopts a relativist ontology, meaning that knowledge exists in relation to a given context and is not absolute, but rather co-constructed. This is in part due to the human ability to interpret, and thus construct, a reality which is shaped by a person's culture and linguistics (Patton, 2002). The goals of this dissertation project align with the tenets of the constructivist paradigm in that this project seeks to understand and communicate how students interpret and generate their own understanding in a biochemistry CURE. Students will construct their understanding of protein function by "fitting" their data with their experiences and understanding of biochemistry. Constructivism is an appropriate framework for this dissertation project because this project aims to understand how students construct an understanding of a protein's function while participating in the BASIL CURE. This project is also looked at how particular individuals or groups (a course section) experiences and learns in this particular course format, as well as how they are able to develop new knowledge of protein function for themselves, which is why this dissertation adopted a situated perspective described below.

1.6.1.2 Situated Cognition

It is also theorized that in order for a learner to obtain new knowledge there must be a perturbation of their current understanding, which is most frequently brought about by the interactions with others (von Glasersfeld, 1989). Though knowledge is held by the individual, the influence of those around them affects how they process, experience, and affirm their ideas (Solomon, 1987). Situated cognition, also referred to as "situated learning" or just "situative" (Greeno, 1997; Orgill, 2007), serves as more specific theoretical lens for this project. Situated cognition "posits that knowledge exists not as a separate entity in the mind of an individual, but that knowledge is generated as an individual interacts with his or her environment (context) to achieve a goal" (Orgill, 2007, p. 187). Thus, situated cognition views learning as a contextualized process that is adaptive and continually occurring (Bredo, 1994; Lave & Wenger, 1991). It should be noted that situated cognition does not mean that learning only occurs in a particular context, but rather learning happens when the individual engages and interacts by means of authentic activities to achieve a goal (Clancey, 1993). Because learning is intimately connected to experience, an individual's understanding of a concept is constantly under construction as they interact with the world (Brown, Collins, & Duguid, 1989).

Situated cognition has several characteristic assumptions related to the nature of reality and of learning. As part of this theory, knowledge and learning do not exist independently from the relationship of an individual and their context (Orgill, 2007). That is, an individual and the environment interact and are co-constructed by each other, to generate a reality (Bredo, 1994). It is also assumed that there is a social component to learning (Wilson & Myers, 1999). This can be through language which allows for social coordination and adaption, acting as a shared tool for signaling and coordinating activity (Bredo, 1994). Another concept to come from situated cognition is legitimate peripheral participation (LPP) (Lave & Wenger, 1991). Situated cognition assumes that learning occurs through LPP and of the agreed-upon norms, beliefs, standards, and practices set forth by a given community of practice (CoP; e.g. Macklin, 2007).

Research using situated cognition tends to focus on the learning of participants through authentic and meaningful activities (Bredo, 1994; Greeno, 1998). Orgill (2007) gives a great example of what this authentic activity may look like in terms of conducting chemistry education research:

> "Whether an activity is authentic depends on the learning environment and CoP involved in the study. If a researcher is examining how chemistry students interact with their classroom context to create knowledge and understanding, an 'authentic' activity is one that takes place in the classroom and has meaning for the community of chemistry students" (p.193).

Situated cognition fits with the analysis of student learning in the context of the BASIL CURE for developing research abilities and for interpreting novel data to understand protein function. For this study, the participants will be college students enrolled in the BASIL CURE and will participate in the CoP both as a biochemistry student and as a biochemistry researcher. Situated cognition can be used to assess the interactions of an individual (student) and their environment (a classroom, lab, lecture, and/or recitation) to generate learning (Bredo, 1994; Greeno, 1998) and to what extent features of the environment (lab activities) aid in students' learning of a desired goal (Greeno, 1998).

1.6.2 Analytical and Conceptual Frameworks

The analytical and conceptual frameworks mentioned below guided how the research was conducted, analyzed and reported for all parts of this dissertation.

1.6.2.1 Concept-Reasoning-Mode (CRM) model

The Conceptual-Reasoning-Mode (CRM) model, which has been applied mainly in biochemistry education to frame how people interpret external representations (ERs) (Schönborn & Anderson, 2009), informed all parts of this dissertation. The CRM model creates a scaffold for understanding how students may construct and use representations to rationalize experimental conclusions by incorporating conceptual knowledge (e.g. biochemical, or experimental, concepts and theories) and reasoning skills (e.g. problem solving, or data analysis) to generate their understanding of a phenomenon (Schönborn & Anderson, 2010). The CRM model informs our understanding of how students construct their knowledge of protein function because it allows organization of how students are integrating and using the components of the lab to come to their final conclusion. Additionally, the CRM model provides a convenient way of structuring statements and analyzing data for reasoning with concepts or with representations, by looking for or by making verb (reasoning)-noun (concept or mode of representation) pairs (Anderson, Schönborn, du Plessis, Gupthar, & Hull, 2013; Irby, Pelaez, et al., 2018b). More specific applications of the CRM model will be presented and discussed within relevant chapters.

1.6.2.2 The Assessment Triangle

The assessment triangle (NRC, 2001) is a framework used when conducting assessments in education research. The triad is made up of a cognition, observation, and interpterion component acting a scaffold for reasoning from evidence. In this framework, the cognition component refers to an outline of the knowledge or skills that should be sampled in order to assess students. The cognitive component is similar to Anderson's (2007) argument that the first step in assessment is to identify what cognitive abilities students should be learning and how they should be assessed. The observation component takes into consideration how an assessment will elicit certain responses and considers the type of responses that will be the most fruitful in addressing what is trying to be assessed. Lastly, the interpretation component takes into account how the observations and evidence will be analyzed, and what steps will be taken to ensure the interpretation is valid. Application of the assessment triangle will be discussed in more detail within the relevant chapters.

1.7 Roll of the Researcher

My dissertation, presented here, documents a portion of my efforts and involvement with the BASIL CURE project. This dissertation documents the research I conducted toward investigating the BASIL CURE curriculum. As a DBER researcher on this project, I designed the assessment strategy for the BASIL CURE; developed methods and data collection instruments (e.g. surveys, interviews and open-ended probes); administered and collected data sources; and carried out all of the data analysis. In addition to the research I conducted on the BASIL CURE, I was a contributor to the lab modules design and attended weekly team meetings. This gave me in-depth knowledge about running components of the lab, how to implement the lab, how to disseminate lab material to students, and how to evaluate the course and assess students learning.

My experiences and perspective towards research made me well suited to execute the research presented in this dissertation. I have over eight years of experience as a DBER researcher with projects focused in the contexts of chemistry and biochemistry. In my past research, I was a lead researcher on project investigating how students thought about chemistry problems (Irby et al., 2016); and I was a lead researcher on a project investigating student learning in a novel hybrid chemistry curriculum (Irby, Borda, & Haupt, 2018). These two projects gave me experience in assessing lab curriculums, developing methods and instruments, conducting interviews, and analyzing quantitative and qualitative data. I also have great deal of experience in biochemistry and biochemistry research; I have a B.S. in biochemistry and partook in a summer internship in a structural biology research lab. At Purdue, I have taken graduate course work in biochemistry, as well as, in qualitative and quantitative methodologies. Lastly, I have over seven years of chemistry (and biochemistry) laboratory teaching experience as TA, lab supervisor, and course coordinator. In my research, I adopt a pragmatic approach which allows me to use mixed-methods and place my focus on the research problem, rather than a particular methodology. This kind of approach is well-suited to the project described here because it allows for using multiple data sources and methods to be used to assess student learning in the BASIL CURE from different angles.

1.8 Overarching Methods and Data Sources

The different parts of this dissertation involved a combination of qualitative and quantitative methods. The specific methods, methodologies, methods, data sources, data collection, and analysis procedures are described for each part of this dissertation in their respective chapters

(Chapters 2-5). Here the methods and data sources utilized throughout this dissertation are discussed briefly.

1.8.1 Participant Recruiting

Participants of this study were recruited through purposeful sampling, based on a set of criterion (Patton, 2002; Suri, 2011). The criterion was that all participants must be either a student enrolled in an implementation of the BASIL CURE, an instructor teaching an implementation of the BASIL CURE, or project team members that worked on the creation and development of the BASIL CURE curriculum. Other than these mentioned criteria, there were no other qualifications for participation.

1.8.2 Case Study

Case study methodology is common in education research (Yin, 1993; Yin, 2006). The strength of a case study methodology is its ability to examine a particular case in-depth in relation to its real-life context (Yin, 2006). A case study is useful when trying to understand a phenomenon where the contextual conditions are relevant, and the boundaries between the context and a phenomenon are not clear (Baxter & Jack, 2008). Also, case studies can be descriptive, which is useful when specifically studying an intervention and the real-life context in which it occurred (Baxter & Jack, 2008; Yin, 2003). Case studies are compatible with the theoretical frameworks described in Section 1.6.1, which were used to inform the design, analysis, and interpretation of this dissertation.

1.8.3 Mixed Methods

This dissertation adopts a mixed methods approach (Creswell & Plano Clark, 2010; Fishman, 2013). Mixed-methods and place my focus on the research problem, rather than a particular methodology. This approach allows for flexibility to rigorously integrate different data sources in new ways that develop analytical strategies needed to improve science curriculum instruction, address novel research questions, and produce data and findings that are relevant to a diverse audience.

1.8.4 Qualitative Methods and Data Sources

The qualitative data sources collected were open-ended surveys and student probe, semi-interviews, informal observations, and collection of artifacts (i.e. BASIL protocols and lab

reports). These forms of data are the most standard in education research (Ary, Jacobs, Sorensen, & Walker, 2013). Ary et al. (2013) also goes on to explain that observations allow for insight into a setting of interest (i.e. within the BASIL lab), and interviews will allow for the students to share their own perceptions and provide further information about the lab.

1.8.4.1 Artifacts

Different BASIL CURE and student artifacts were collected for this dissertation. In Chapter 2, the BASIL protocols were collected and analyzed for the concepts (C) and representations (M) (Schönborn & Anderson, 2009, 2010) presented within them. There were ten total protocols (Table 1.3) that were collected and analyzed. The set of protocols were what outlined the BASIL CURE curriculum, and were unified documents that were created by the BASIL CURE development team to be used for all courses implementing BASIL. The BASIL CURE protocols were used to conduct a content analysis. Content analysis is a process used to interpret and better understand what is being conveyed in text or other forms of communications, resulting in exact data about the information contained within a document (Cohen, Manion, & Morrison, 2000; Hsieh & Shannon, 2005). In education research, it is common to perform a content analysis on a variety of course documents (e.g. lab protocols and student lab reports) to identify what students should be learning and to evaluate the content covered in a course.

1.8.4.2 Open-ended Surveys and Student Probes

Open-ended Surveys were used in Chapter 2 to begin to identify how instructors of the BASIL CURE to begin to understand how they, as researchers, would perform the type of activities the students were and how they would reason with the concepts and representations that the students would have been provided or would be producing within the course. Chapter 3 describes how the identified ALOs could be used to create open-ended student probes, (assessments). These probes are used in Chapter 5 as a part of the initial phase of determining if the identified CURAs are VLOs.

1.8.4.3 Semi-Structured Interviews

A semi-structured interview was conducted with the lead BASIL curriculum designer, in Chapter 2. The purpose of the interview was to follow up on the lead designers' responses to the open-ended survey and to discuss additional representations that the lead designer mentioned during the open-ended survey and provided at the interview. A semi-structured interview allows for guidance

concerning what the interviewer wants to learn from the interview but allows for flexibility to discover new, relevant, ideas being shared by the interviewee (Cohen et al., 2000).

1.8.4.4 Observations

Informal observations were used to give the researcher (S.M.I.) insight of how the BASIL CURE was conducted for a course that was used for collecting student data (Chapter 5). These observations were not used as a data source or a unit of analysis but did help to inform the researcher during the semi-structured interviews and when analyzing student responses to the open-ended, by obtaining some first-hand experience about the activities conducted within the course.

1.8.4.4.1 Qualitative Data Analysis

In general, an inductive approach (Thomas, 2006) guided by the CRM model (Schönborn & Anderson, 2009, 2010) was used to analyze the qualitative data collected. In practice, this means there were no specific concepts or representations that were predetermined; rather, the identity of the concepts and representations emerged from analysis and inductive. Interpretation and organization of the data will be aided by the use of the N'Vivo qualitative software. During the coding and theme generation process, trends in the data will begin to be interpreted by the researcher (Marshall & Rossman, 2011). As this process is being done passages and quotes that best represent the data will be selected for use as examples of the conclusions of the researcher (Marshall & Rossmanma, 2011). While doing this, there will be clear intent to seek out meaningful examples that answer the research questions of interest and adhere to the models and theories mentioned throughout. Each chapter (Chapters 2, 3 & 5) will detail the specific analysis performed.

1.8.4.5 Quantitative Methods and Data Sources

1.8.4.5.1 Likert-Surveys

In this dissertation, Likert-Surveys are used in two ways. The first (Chapters 2 and 3) used a Likert survey to determine the amount relevance an identified CURA had to the BASIL CURE based on the amount of agreement among BASIL instructors and designers for how unique a CURA was the BASIL CURE and how important having competence with an ability was to being a scientist. The second was in the form of a participant perception indicator (PPI) survey (e.g. Clase, Hein, &

Pelaez, 2008) to measure students perceptions of their knowledge, experience, and confidence (KEC) for the most relevant CURAs before and after their participation in the BASIL CURE.

1.8.4.5.2 Quantitative Analysis

A new metric called weighted-relevance (WR) (Equation 2.1; Irby, Pelaez, et al., 2018b [Chapter 2]) was used to determine which of the identified CURAs had the most agreement of importance. The PPI data were analyzed using a paired t-test to determine if there were significant changes, as well as, Cohen's *d* (Cohen, 1988) to measure effect size. In order to make comparisons between specific implementations of the BASIL CURE and to make comparisons between specific implementations and all implementations in general, normalized gain scores were also calculated (Hake, 1998)

CHAPTER 2. HOW TO IDENTIFY THE RESEARCH ABILITIES INSTRUCTORS ANTICIPATE STUDENTS WILL DEVELOP IN A BIOCHEMISTRY COURSE-BASED UNDERGRADUATE RESEARCH EXPERIENCE (CURE)

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2.1 Abstract

Course-based undergraduate research experiences (CUREs) have been described in a range of educational contexts. Although various anticipated learning outcomes (ALOs) have been proposed, processes for identifying them may not be rigorous or well documented, which can lead to inappropriate assessment and speculation about what students actually learn from CUREs. In this essay, we offer a user-friendly and rigorous approach based on evidence and an easy process to identify ALOs, namely, a five-step Process for Identifying Course-Based Undergraduate Research Abilities (PICURA), consisting of a content analysis, an open-ended survey, an interview, an alignment check, and a two-tiered Likert survey. The development of PICURA was guided by four criteria: 1) the process is iterative, 2) the overall process gives more insight than individual data sources, 3) the steps of the process allow for consensus across the data sources, and 4) the process allows for prioritization of the identified abilities. To address these criteria, we collected data from 10 participants in a multi-institutional biochemistry CURE. In this essay, we use two selected research abilities to illustrate how PICURA was used to identify and prioritize such abilities. PICURA could be applied to other CUREs in other contexts.

2.2 Introduction

In recent years, there has been a concerted effort to include more authentic research practices within science, technology, engineering, and mathematics courses across various science disciplines and educational levels (e.g. Brewer & Smith, 2011; NASEM, 2015, 2017, NRC, 2003, 2012; PCAST, 2012). In postsecondary education, the incorporation of authentic research practices

in the classroom are often referred to as course- based undergraduate research experiences (CUREs). CUREs are aimed at developing student knowledge and competence to perform more authentic research rather than the ability to follow traditional recipe-like protocols (Auchincloss et al., 2014). There have been various attempts to classify the meaning of a CURE (e.g. Brownell, Kloser, Fukami, & Shavelson, 2012; Lopatto & Tobias, 2010; Weaver, Russell, & Wink, 2008). Commonly, CUREs incorporate five components identified by Auchincloss et al. (2014): 1) they involve the use of scientific research practices; 2) they have elements of discovery; 3) their products have broader relevance or importance; 4) they afford opportunities for collaboration; and finally, 5) they emphasize the purpose of and allow for iteration of experiments. For the purpose of this essay, we adopt a broad definition of a CURE as a course wherein students engage in activities resembling those done by scientists in a particular field to conduct novel investigations about relevant phenomena that are currently unknown.

In response to the abovementioned calls for more integration of authentic scientific practices into undergraduate curricula, there has been an increase in the number of CUREs being implemented and studied across a range of disciplines, formats, and academic levels (see CUREnet [n.d.] for examples of different CURE projects). Most notably, these efforts have been primarily documented within biology and related subdisciplines (e.g. Jordan et al., 2014; Mordacq, Drane, Swarat, & Lo, 2017) in which the motivation has been to better prepare students to keep pace with ongoing research advances in the life sciences (Pelaez, Anderson, & Postlethwait, 2014). Whereas several examples of CUREs have also been cited in the chemistry education literature (e.g. Weaver et al., 2006), fewer have been published for other scientific disciplines, including biochemistry (Craig, 2017; Gray et al., 2015), the focus of this essay. Typically, when CUREs are implemented, they are either adapted from a pre-established CURE (e.g., Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science; Hatfull, 2015; Jordan et al., 2014); or they can be backward-designed to create an experience that targets a specific learning, departmental, or university goal (e.g. Shapiro et al., 2015); or, as in the present project (Craig, 2017), they can be developed and molded from a faculty member's own research.

There are few examples of published CURE studies with explicitly defined learning outcomes (Gray et al., 2015; Makarevitch et al., 2015; Olimpo et al., 2016; Staub et al., 2016). In general, when it comes to LOs in CUREs, they fall into three categories: either they are not explicitly stated, or they are adapted from the abovementioned five components of a CURE (Auchincloss et al., 2014) or another similar published source (e.g. Brownell et al., 2015; Olimpo

et al., 2016), or they are actually based on the CURE's content or technical activities (Gray et al., 2015; Kowalski et al., 2016; Makarevitch et al., 2015; Staub et al., 2016). Also, the focus tends to be on broadly applicable skills across CUREs in general. These might be good for assessing whether a course is CURE- like or not and for establishing broad goals for the students or faculty members to strive toward. But in order to more fully establish the development of research competence in students, it is essential to also identify and assess the unique and specific course-based undergraduate research abilities (or CURAs) that students might acquire during the course. In this regard, a literature review by Corwin et al. (2015) outlined various proposed benefits and outcomes of CUREs, mostly pertaining to the five components of CUREs proposed by Auchincloss et al. (2014), and commented on how well they were documented. But again, Corwin and coworkers did not focus on the range of CURAs students would be expected to develop in particular CUREs.

When CURE projects are uploaded to the CUREnet database (CUREnet, n.d.), the LOs are usually included, but not all such projects are accompanied by explanations as to what process was used to identify them. Usually, the LOs are either contrived by instructors or, at the most, identified by a group of instructors in a consensus-building session. It is often not clear whether the claimed learning outcomes are merely anticipated learning outcomes (ALOs) or objectives suggested by instructors or verified learning outcomes (VLOs) confirmed from analysis of student responses to assessments. Indeed, it seems that ALOs and LOs are often used interchangeably. Further- more, in some cases, scientists who incorporate their own research in a CURE classroom may have difficulty identifying their ALOs, let alone their VLOs. As stated by Anderson (2007), the establishment of ALOs or objectives for a course is an essential step in the educational process. ALOs will inform what and how students will be assessed, what and how instructors will teach, and what and how students will learn if they are to achieve the desired LOs. In line with this thinking, Shortlidge and Brownell (2016), Anderson (2007), and many others have pointed out that the assessment method and instrument must be selected with the specific outcomes of the CURE under study in mind. But particular problems arise when trying to evaluate a CURE with an assessment instrument that was developed for a different CURE or context. To address the above concerns, a framework has been proposed to help robustly evaluate CUREs and the learning that takes place in them (Brownell & Kloser, 2015). A key component of the framework is to first identify the outcomes of the course, but once again, these authors do not provide a clear way to do so (Brownell & Kloser, 2015; Shortlidge & Brownell, 2016). Thus, there is a need to develop a

rigorous process for identifying the CURAs that compose the ALOs associated with CUREs. In our view, this is key to more fully understanding how CUREs can benefit undergraduate education and the future of novice researchers. To our knowledge, no rigorous, data-driven process is currently available to identify such CURAs unique to a specific CURE that instructors anticipate students will develop (i.e., ALOs). In our view, this is a crucial first step before assessment design and confirmation of the actual VLOs of a CURE can be achieved.

Thus, the goal of this essay is to report on our development of a data-driven process for identifying the CURAs that instructors anticipate students will develop while experiencing the biochemistry laboratory CURE designed by Craig (2017) and coworkers. For reader convenience and clarity, an overview of our developed process is presented upfront in Figure 2.1, while the details of the process are included later in the How to Apply the Five Steps of PICURA section. To be in line with the terms CURE and CURA, we use the acronym PICURA to stand for Process for Identifying Course-Based Undergraduate Research Abilities. A list of all the acronyms used in this essay and their meanings is included in Table 2.1.

Table 2.1 Acronyms used in this chapter and their meanings.

Acronym	Meaning
ALO(s)	Anticipated Learning Outcome(s)
CURA(s)	Course-based Undergraduate Research Ability(ies)
CURE(s)	Course-based Undergraduate Research Experience(s)
LO(s)	Learning Outcome(s)
LR	An example of a "Lower-rated" CURA statement: *Recognize how proteins that are closely related by evolution can have dramatically different functions*
PICURA	Process for Identifying Course-based Undergraduate Research Abilities
TR	An example of a "Top-rated" CURA statement: *Determine using computational software whether, and where a ligand may be binding to a protein*
VLO(s)	Verified Learning Outcome(s)
WR	Weighted-relevance

The five steps of PICURA shown in Figure 2.1 are 1) a content analysis of the lab protocols, 2) an open-ended survey about how scientists conduct similar research to what the students do when performing the lab protocols, 3) a follow-up semi-structured interview, 4) an alignment check of the generated ability statements across the previous steps in the process, and 5) a Likert survey to prioritize the identified CURAs. For Steps 1–3 and Step 5, there are inputs (materials or participants used as a data source) and outputs (the resulting products from completing each step

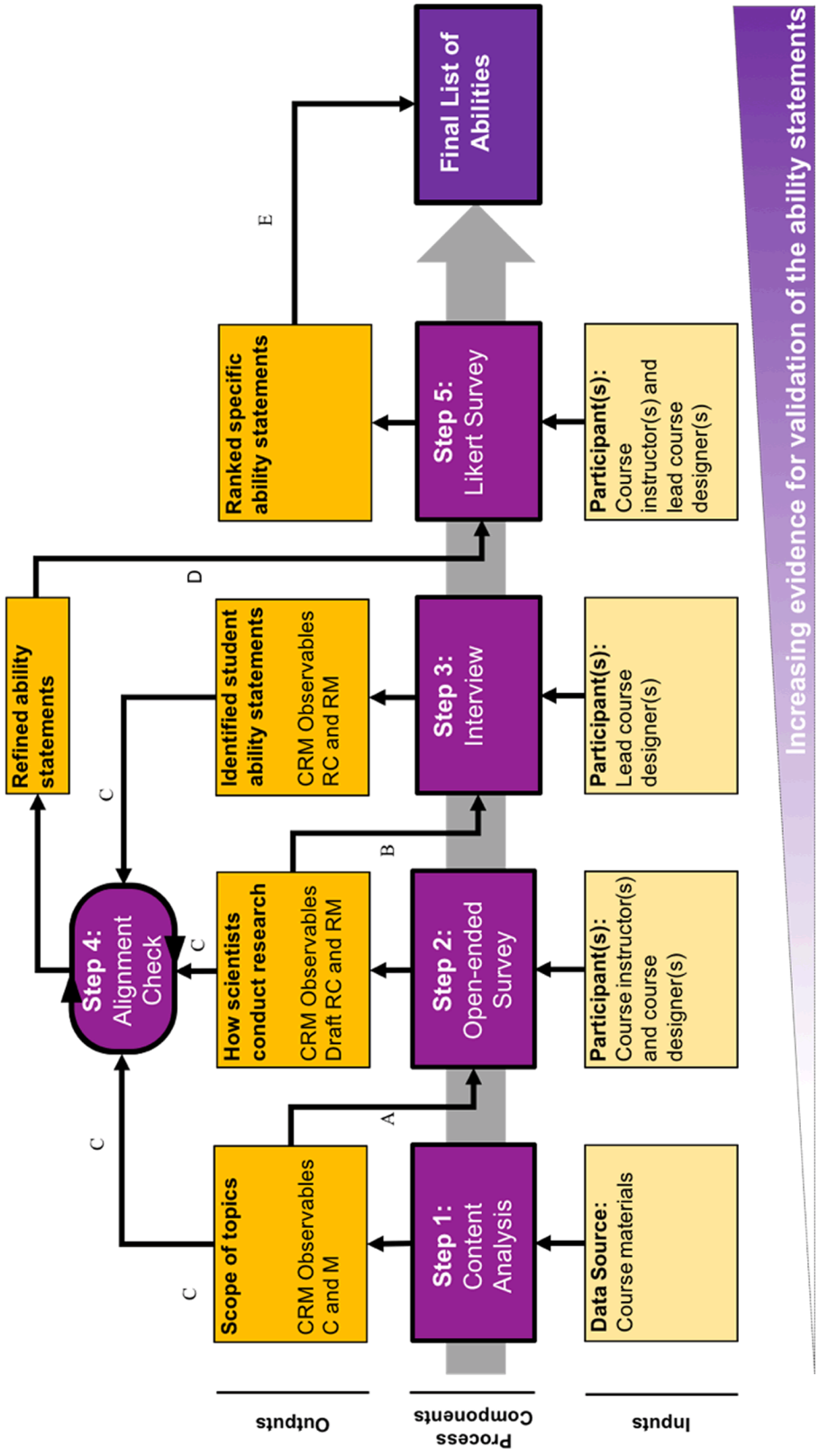
of the process). More specifically, the data sources include the CURE protocols (Step 1), nine course instructors (Steps 2 and 5), and the lead designer (Steps 2, 3, and 5). Step 4 does not have an input or output like the others. Rather, it is an alignment check to assure consensus between Steps 1–3 before proceeding to Step 5.

We identified the following criteria that we consider import- ant to ensure that the structure and design of PICURA meets our abovementioned goal of providing a rigorous process for identifying CURAs that would be well supported by the data collected at each step. For each criterion, we indicate how it relates to the various arrows and links in Figure 2.1 and refer to various supporting data, presented later in the Illustration of the Five-Step Process section, that in our view address the following four criteria:

- **Criterion 1)** Is the process iterative? That is, do the data generated by each step inform the development and design of the instrument used in the subsequent step(s)? Arrows A, B, and D in Figure 2.1 and Tables 2–5 show how data inform the process.
- **Criterion 2)** Does each part of the process give additional data so that together they give more clarity on the expected learning, in terms of CURA statements, than would be known from any one part of the process on its own? Arrows C, E, and the unlabeled arrows in Figure 2.1 and Tables 5–10 show how the final list of CURA statements are derived.
- **Criterion 3)** Do the different types of data from each step of the process combine well to achieve consensus and internal alignment about the CURAs? Arrow E in Figure 2.1 and Tables 8 and 9 (Step 4) lead to the final list of CURA statements.
- **Criterion 4)** Does the process prioritize the CURAs for instructors, researchers, or other stakeholders? That is, what is the sequence of CURA importance in terms of being relevant to the course of interest? Arrow E in Figure 2.1 and Table 2.10 demonstrate the prioritization of the final list of CURA statements.

Figure 2.1 Diagram detailing the inputs and outputs for each of the components of the five-steps of PICURA.

Arrows A, B, and D = informing the process; arrow C = alignment steps showing consensus; arrow E = prioritization. The information gained from each component is additive, meaning that each component is informed by the previous process component. Because of the nature of this process as it moves through the steps (going from a content analysis, to an open-ended survey about the course activities, to an interview, to a Likert survey), data to identify and support the abilities increased with each subsequent step. There is one feedback loop, which acts as an alignment check between the generated abilities from the interview, the scope of the topics covered identified from the content analysis, and how scientists conduct research from the open-ended survey. The final process component provided additional evidence and filtering of the abilities via a Likert survey in which participants rate their importance and uniqueness to the course.


Inputs
Process Components
Outputs
Data Source: Course materials
Step 1: Content Analysis
Scope of topics
CRM Observables C and M
Participant(s): Course instructor(s) and course designer(s)
Step 2: Open-ended Survey
How scientists conduct research
CRM Observables Draft RC and RM
Participant(s): Lead course designer(s)
Step 3: Interview
Identified student ability statements
CRM Observables RC and RM
Participant(s): Course instructor(s) and lead course designer(s)
Step 5: Likert Survey
Ranked specific ability statements
Step 4: Alignment Check
Refined ability statements
Final List of Abilities
A
B
C
C
C
C
D
E
Increasing evidence for validation of the ability statements

2.3 How to Apply the Five Steps of PICURA

2.3.1 Context: A Biochemistry Lab CURE

We used the biochemistry CURE described by Craig (2017) to develop PICURA. This CURE has only recently been developed by instructors and course designers and is currently being implemented at seven different institutions. The lab is based on modern biochemistry research techniques (McKay et al., 2015) and aims to provide students with a scientific research experience through a unique combination of computational and wet-lab experiences for which a range of independent protocols have been developed as activities for the CURE (Craig, 2017). The students uncover new knowledge about the function of some of the many proteins listed on the Protein Data Bank (PDB; www.rcsb.org) whose structure is known but whose function has not yet been established. In addition to developing technical skills, students get the opportunity to develop their thinking, reasoning, and visualization abilities through the application of their biochemistry knowledge to solving problems in novel situations, generating and testing hypotheses through rigorous experimentation, and processing and evaluating results.

Each lab protocol is formatted to contain a background section that introduces either the computational programs and databases or the biochemical techniques being used as part of that particular activity. The background sections also include discussion of the method used, as well as some rationale for why a method was chosen and information about how it works. The protocols also provide either simulations to run or instructional steps that need to be completed when performing the computational modules and biochemical assays (for a list and description of all protocols, see Craig, 2017).

2.3.2 Participants

A total of 10 project members volunteered to participate in different aspects of the development of PICURA, including acting as informants and responding to our data-gathering instruments. Of the participants, nine were instructors of our biochemistry CURE at seven different institutions. Of these volunteers, all had played a role in developing the CURE. The other participant was the "lead designer," who was also the lead principal investigator and creator of the CURE project. The authors (S.M.I., N.J.P., and T.R.A.) developed the PICURA described in this paper and are also responsible for the educational evaluation of the whole project. The work reported in this essay

was officially reviewed, and the data-collection protocols were approved by the Purdue University Institutional Review Board (IRB #1604017549).

2.3.3 The Guiding Framework for the Development of PICURA

Because the goal was to design a process for the identification of the CURAs that instructors anticipated students will develop in our biochemistry CURE, a guiding framework was needed to take inventory of the important components of the course and turn them into ability statements. Toward this end, the conceptual-reasoning-mode (CRM) model of Schönborn & Anderson (2009) was chosen to guide this work. This model has previously been successfully applied to the identification of visual competencies in biochemistry learning (Schönborn & Anderson, 2009, 2010), the structuring of verb–noun reasoning statements, and the design of assessments of student reasoning and problem-solving abilities (Anderson et al., 2013). In addition, the CRM model has been used to guide research looking at how scientists use evolutionary trees (Kong, Thawani, Anderson, & Pelaez, 2017) and how they explain molecular and cellular mechanisms (Trujillo, Anderson, & Pelaez, 2015) and, together with the assessment triangle (NRC, 2001), to guide the development of assessment instruments (Dasgupta, Anderson, & Pelaez, 2016). The CRM model offers a useful way to account for all the concepts (C), types or modes (M) of representations, and ways of reasoning (R) with those concepts (RC) and representations (RM; Schönborn & Anderson, 2009, 2010). During the development of PICURA, the CRM model was used to inform the construction of ability statements composed of verbs (reasoning skills) paired with nouns (important concepts or representations; Schönborn & Anderson, 2009). In addition, it was used to guide the data-collection and data-analysis processes at each step of PICURA and to inform the identification of the nature of the reasoning composing each CURA that is used when scientists engage with concepts and representations of phenomena.

2.3.4 Details of the Five Steps of PICURA: Instrument and Data Analysis

This section describes PICURA, the purpose of each step, and how data were collected and analyzed at each step to progressively identify the CURAs. We decided to use various qualitative research methodologies, which in our view and that of Creswell (2012), would afford an effective approach for thoroughly understanding the learning opportunities presented in our biochemistry CURE learning environment. Whereas the five steps of PICURA in Figure 2.1 were outlined in the *Introduction*, in this section, we provide readers with sufficient details to repeat the process at

their own institutions. As will become apparent, PICURA was designed to be both reflective and iterative (see Criterion 1), meaning that each data-collection step is informed by the results of the previous step (Figure 2.1, arrows A–D). The reflective nature of PICURA, in conjunction with prioritizing the course-specific ability statements in Step 5, allows for progressively gaining more insight into the nature of the CURAs.

2.3.4.1 Step 1: Content Analysis

Content analysis is a process used to interpret and better understand what is being conveyed in text or other forms of communications, resulting in exact data about the information contained within a document (Cohen, Manion, & Morrison, 2000; Hsieh & Shannon, 2005). In education research, it is common to perform a content analysis on a variety of course documents (lecture slides, student lab reports, notes, etc.) to identify what students should be learning and to evaluate the content covered in a course. The purpose of the content analysis in PICURA was to identify the concepts (C) and representations (M) of relevance to the various biochemical and computational techniques described in the lab protocols and to gain insight into how information is provided to students and how this CURE is structured.

To analyze the lab protocol data, we used an inductive approach (Thomas, 2006), guided by the CRM model (Schönborn & Anderson, 2009, 2010). In practice, this means there were no specific concepts or representations that were predetermined; rather, the identity of the concepts and representations emerged from analysis and inductive coding of the narratives in the protocols. This allowed for the development of a list of common and recurring concepts and representations that students are exposed to during the course and are accountable for knowing (see Table 2.5 for an example of this). Additionally, the content analysis gave insight into how information was presented in the protocols to the students and provided a better context of how a given course using this curriculum would be conducted. The outputs of the content analysis (Step 1) informed the development of the open-ended survey used in Step 2 (see Figure 2.1, arrow A).

2.3.4.2 Step 2: Open-Ended Survey

The next step was to survey, with open-ended questions, all instructors and designers about the important concepts and representations that scientists would use and reason with when they do research using the types of methods included in the biochemistry CURE. The open-ended survey was distributed using Qualtrics (www.qualtrics.com), an Internet-based survey and data-collection

platform. The open-ended questions provided participants with an opportunity to elaborate on the observed concepts and representations identified in the content analysis by additionally probing participants for examples of how they as scientists would use (reason with) such concepts (RC) and representations (RM) while conducting research similar to research in this biochemistry CURE (see Criterion 1). The questions were intentionally worded to have participants reflect upon how they or other scientists would approach the situation, rather than eliciting any personal beliefs about the learning objectives or ALOs of their CURE, which might vary across institutions. Two examples of the questions are provided in Table 2.2; the entire set of open-ended survey questions is provided as Supplemental Material, Section 2.8.1. Also, as part of the open-ended survey, participants were asked to list representations that were important to the course (chemical equations, visualizations of molecules, outputs from experiments, graphs, etc.). The open-ended survey results, or outputs from Step 2, were analyzed for common themes and for how the participants reported they were using or reasoning with the different concepts and representations. This led to the emergence of basal-level RC and RM statements, which were noted for further consideration in Step 3 (in line with Criteria 1 and 2). In addition, responses needing more elaboration to generate CURA statements were noted for discussion during the interview, Step 3 (see Criteria 1 and 2). All the analyzed data from Step 2 informed the development of an interview protocol for Step 3 (Figure 2.1, arrow B).

Table 2.2 Selected prompts from the course survey given to the CURE instructors and development team members.

Open-ended survey prompts about computational techniques and representations
Explain how you, or other scientists, use computational work and protein structural data to investigate protein function.
Please list and describe the types of representations you use, and how you use them, when thinking about or explaining protein function. Representations include but are not limited to items such as the following: Coomassie stained gels, graphs, computer models, activity assays, protein structures, sketches, diagrams, Bradford assays, etc.

2.3.4.3 Step 3: Interview

An interview was conducted with the lead designer to elaborate on the instructors' open-ended survey responses and to identify a clearer and more accurate range of RC and RM statements.

Additionally, the interview provided the opportunity to capture the reasoning that scientists would apply to those representations mentioned during Step 2 (Table 2.2) and provided for discussion during the interview (Table 2.3). It also afforded the opportunity to discuss how students would use the representations and the concepts related to them as part of the course as well as other difficulties the students might face (the full interview protocol is provided as Supplemental Material, Section 2.8.2). The interviewee was allowed to explain their reasoning and interpretation of these representations, the concepts related to them, and how they would expect students to use them as part of this CURE. The interviewee was also asked to discuss what students would be accountable for knowing when it comes to various representations or techniques, as well as any difficulties students have encountered with any of the CURE activities. However, the interviewer never directly asked for any opinions about the expected CURAs of this CURE. Instead, the development of the interview protocol was directly informed by the open-ended survey, Criteria 1 and 2 (Figure 2.1, arrow B).

The interview was semi-structured with a single participant, the lead designer (performed by S.M.I.). This allowed for guidance concerning what the interviewer wanted to learn from the interview, but allowed for flexibility to discover new, relevant, ideas being shared by the interviewee (Cohen et al., 2000). The lead designer was selected for the interview because the lead designer was the most intimately familiar with the CURE and so was representative of other knowledgeable faculty members designing and implementing it. This provided an opportunity to gain greater insight into the responses to the open-ended survey and into the CURE of interest.

The interview transcripts acted as the primary data source for generating and fine-tuning the relevant CURA statements. The transcript was analyzed and coded, per the CRM model (Anderson et al., 2013; Schönborn & Anderson, 2009), for reasoning with concepts (RC) and representations (RM). CURA statements were generated from the interview transcript by constructing CRM verb–noun pairs from the RC and RM codes. The initial ability statements were generated by looking at RC- or RM-coded segments and doing thought experiments about not only the direct verb–noun pair articulated by the interviewee but any others that would apply to the coded segment. In other words, taking account of the skills that the interviewee discussed led to additional possible CURA statements that could apply to the discussion of the CURE.

Table 2.3 Example of a portion of the semi-structured interview protocol informed by the open-ended survey (Figure 2.1, arrow B) question about important representations used when thinking about protein function (Table 2.2).

Interview prompts pertaining to representations
1. In the survey, there was a question asking you to list and describe the types of representations you use, and how you use them, when thinking about or explaining protein function. You provided the following representations: *A. Enzyme assays: chemical reaction drawings help us to understand how the parts of a protein catalyze a reaction.* *B. Molecular visualization: ligand binding demonstrates if a substrate binds to an active site.* i. Enzyme assays ii. Chemical reaction drawings 1. How the parts of a protein catalyze a reaction iii. Molecular visualization 1. Ligand binding Could you please talk me through: a) How you would use each representation to reason about protein function; b) What types of biochemistry representations are useful for students to be familiar with to help them in this course? c) How you would like students to use them; d) What you observed students doing with each representation? e) Whether these representations are new to the students, or did they have some previous experiences with them? If so, describe the experiences they had. f) How would you know if students were having difficulties and whether they were improving? What type of things did students do to practice and overcome these difficulties?

2.3.4.4 Alignment Check

After the initial list of CURA statements had been generated from the interview (Step 3), they were first related back and matched to the data obtained from the content analysis (Step 1) and the open-ended survey (Step 2) to ensure that there was alignment across all data sets (Criterion 3; see Tables 2.8 and 2.9 for an example). If there was a case in which a CURA statement was not aligned with all data sets (e.g., a CURA statement generated from a portion of the interview was not directly related to the CURE), it would be considered erroneous and ignored. This alignment process ensured that the identified CURA statements were supported by the data gathered about the CURE and that all the observed prominent concepts and representations had been accounted for by the CURA statements (Criterion 3). As part of this step, CURA statements were also refined and optimized by comparing statements to reduce overlap and by conducting a member check with the interview participant, which reduced the total number of CURA statements. After this step, the CURA statements were ready to be prioritized by participants using a two-tier Likert survey (Step 5; Figure 2.1, arrow D).

2.3.4.5 Likert Survey

After the specific CURA statements had been generated, a Likert-scale survey was used to prioritize them according to what the participants perceived as important to their courses, thereby addressing Criterion 4. Though the proposed utility of PICURA is that the CURAs authentically arise from data, before they can be used to assess student learning and to evaluate this CURE, they must be agreed upon in terms of their appropriateness to this CURE. To achieve this, the CURA statements were evaluated by all instructors and designers with a three-option, two-tiered, Likert survey using Qualtrics. Each CURA was rated on whether the participants expected it to be acquired or further developed by students in the course and whether it was important to the course or specific goals of each participant (Table 2.4).

Table 2.4 Example of the three-option, two-tiered, Likert scale questions for ranking the CURA statements.

Tier	Likert Question	Option 1	Option 2	Option 3
First	This ability should have been acquired:	NOT acquired in this lab course	In BOTH this lab and some other course	ONLY in this lab course
Second	How important is this to your students' functioning as a scientist?	Unimportant	Undecided	Important

For discerning which of the CURAs had the most consensus for this CURE, both Likert questions were used in tandem and a novel weighted-relevance (WR) value was calculated. WR assigns a weight to the options from the Likert scale, such that the abilities could be ranked by how relevant they were to their course. WR is a sum of the participants' scores for their responses to the Likert-scale questions times a multiple between −1 and +1 (see Equation 2.1 for an example).

Equation 2.1 Weighted-Relevance (WR) calculation.

WR = (#Important) + (#ONLY in this lab course)
+0.5(#Undecided) + 0.5(#In BOTH this lab and some other)
-(#Unimportant) - (#NOT acquired in this lab course)

A weight of +1 was given for preferred responses indicating that the CURA was unique to an individual's course and/or important (option 3 in Table 2.4). For responses that were good, but not the preferred responses, such as in cases in which the CURA was acquired in both the individual's course and another course and/or the interviewee was undecided on its importance (option 2 in Table 2.4), a multiple of +0.5 was assigned. For the negative cases, in which a participant thought that the CURA was not acquired in his or her course or was unimportant (option 1 in Table 2.4), a multiple of −1 was assigned to remove contributions to the WR scores from those abilities with these responses. Thus, the WR scale can range from −2 times the number of participants (the CURA was rated as "NOT acquired in this lab course" and "unimportant" by all participants) to positive +2 times the number of participants (the CURA was rated as "ONLY in this lab course" and "important" by all participants). Because there were 10 participants for the Likert survey, the WR score could range from −20 to +20. This score indicates the most relevant CURAs to the curriculum, which is important for prioritizing feasible outcomes for our CURE. This step put an emphasis on unique CURAs that students might not have experienced or developed fully if they had not taken a course, which was highlighted by (Shortlidge & Brownell, 2016) as a crucial step for the evaluation of CUREs.

2.4 Illustration of the Five-Step Process

In this section, we use two selected research abilities to illustrate how PICURA (Figure 2.1) was used to identify and prioritize the CURAs of relevance to the biochemistry CURE of Craig (2017). Additionally, the data will be used to demonstrate how each of our four criteria were met while designing the process.

2.4.1 Examples of How Steps 1-3 of PICURA Were Applied to Our Biochemistry CURE

2.4.1.1 Step 1: Content Analysis

The CURE protocols (see Craig, 2017) were designed in a modular manner so that they could be stand-alone protocols (i.e., they did not directly reference other protocols and could be done independently from one another) and were organized with a background section providing details on the computational program, technique, or biochemical assay that the students would be doing. This was followed by, in general, either a tutorial in the case of computational protocols or an outlined procedure in the case of biochemical protocols. The content analysis of all the lab

protocols revealed many different concepts (C) and related representations (M; see Figure 2.1). An example of how the protocols were used in Step 1 is provided in Table 2.5, with a small excerpt from the ProMOL protocol (Craig, 2017).

Table 2.5 Example of conducting Step 1 of PICURA: Content analysis of the protocols – ProMOL module.

Step 1: Content Analysis of the Protocols – ProMOL Module		
Excerpt from Protocol	**Analysis** ➔	**Output**
The first step in our function prediction process is to compare a protein of unknown function against a library of enzyme active sites from the Catalytic Site Atlas that constitute the **motif** template library of ProMOL. Each catalytic site **motif** template typically consists of 2 - 5 amino acid residues that have a fixed spatial and distance relationship. The example shown [Figure 2.2A] is an alignment for a serine protease.	Read through all lab protocols highlighting passages and coding them for whether they are pertaining to concepts or representations. Then take note of the underlying concepts or representations being portrayed. Additionally, take note of how protocols are organized, how information is presented to the reader and how protocols connect to one another.	**Concepts Detected** • Protein Homology • Protein Motifs **Representations Detected** • Protein visualization software screen shots and Stick representations of active site.

† This is a portion of a single protocol, to showcase some of the breadth of concepts and representations covered by the protocols.

In Table 2.5, the excerpt provided shows key words bolded to indicate concepts, while phrases that mention skills that students would be using pertaining to each concept are underlined. Within this example, the concepts of protein homology and protein motifs were portrayed and detected. The underlined phrases describe how to compare motifs computationally and what to look for. This protocol also contained an image of a protein of unknown function mapped onto a known protein's active site, using a protein stick representation (Figure 2.2A). The protocols outlined the key concepts and gave some examples of representations students may encounter. However, the nature of the protocols did not allow direct analysis of how the students would be connecting all the computational and biochemical techniques together or what types of explorations students might do after learning about the computational techniques or procedures to represent and link together all their collected data. Additionally, we asked the question, "Which representations other than what was presented in the protocols will the students produce or

encounter, that scientists would likely use when conducting this type of research?" In other words, we did not assume that representations presented as part of the background information in the protocols provided a complete list of all the representations students would encounter while working through the protocols. For example, students are encouraged to use additional computational simulations, while some of the biochemical protocols give an example of the results (the SDS–PAGE protocol gives an example of a stained gel), but others do not (the protein purification protocol does not provide any representations of the chromatographic results). Thus, to begin to gain greater insight into the nature of the reasoning skills necessary for performing the protocols and to document a more exhaustive list of representations, we conducted an open-ended survey as Step 2 of PICURA (see Figure 2.1, Step 2, and Table 2.6). This in turn would allow us to meet Criterion 2 of our process.

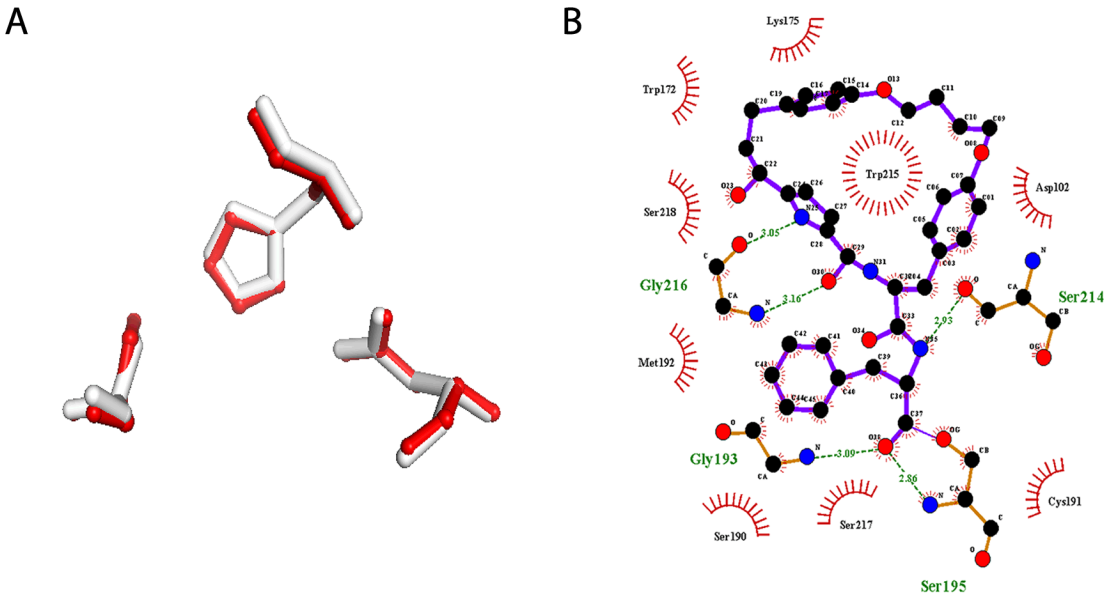


Figure 2.2 Two representations identified by PICURA.
(A) Example of an alignment with a serine protease in the ProMOL module lab protocol, as part of the content analysis. (B) LigPlot+ graph mentioned in the open-ended survey and provided for the interview by the lead designer.

2.4.1.2 Open-Ended Survey

The open-ended survey yielded data about how scientists (instructors and designers of the CURE) would perform the research described in the protocols. This, in turn, generated preliminary information about the types of reasoning the participants would expect students to use during the lab. Selected example responses from the lead designer are provided in Table 2.6. These responses were typical of the type of responses received from the other participants (unpublished data). When analyzing the lead designer's responses (Table 2.6), some of the reasoning (shown in italics) with various concepts (RC) and representations (RM; both bolded) became much clearer to the authors compared with those identified in Step 1, but the data were still not comprehensive enough to generate clear CURA statements. The nature of the responses was listed stepwise and aligned with the progression of the CURE activities and how they were presented in the protocols (Table 2.6). Though these response examples (Table 2.6) were relatively brief, some insight into exactly what scientists use each program or technique for and how they use them started to emerge. Thus, Step 2 suggested that we were starting to meet Criterion 2, in that it was yielding additional information to build on the findings from Step 1. The question about the types of representations provided an opportunity to gain greater insight regarding what other representations may be encountered or generated by students during this CURE. Some of the representations mentioned during this data-collection step had not been previously identified in the protocols (Figure 2.2B).

Though the open-ended survey provided more details about the possible set of CURAs students may be expected to develop, responses were not detailed enough to determine the extent of student proficiency that would be required for each CURA or to provide a more comprehensive range of possible CURAs for the CURE (Criteria 1 and 2; see Table 2.6). For example, the lead designer stated the need to "identify ligands that bind [substrate binding] to members of these protein families" (Table 2.6), but there are a range of CURAs that a student may need to employ to identify substrate binding. It was therefore clear to us that the responses to the open-ended surveys tended to reveal only less-than-optimal participant knowledge and that interviews would be necessary to yield a more thorough articulation of their thinking. Thus, a follow-up interview was conducted with the lead designer (whose survey responses are highlighted in Tables 2.6 and 2.7) to more deeply understand the open-ended survey responses, to discuss the role of representations mentioned in the survey, and to further probe how scientists perform this type of research, including the reasoning skills that they use (Figure 2.1, arrow B).

Table 2.6 Example of data yielded by Step 2 of PICURA: Open-ended survey.

Step 2: Open-ended Survey

Selected questions form the survey†	**Analysis** ➔	**Output:** Coded responses from the lead designer†
Q: Explain how you, or other scientists, use computational work and protein structural data to investigate protein function.	Once all the participants (instructors and designers) had completed the open-ended survey. The responses were analyzed for the concepts and representations (bolded), as well as, the reasoning (italicized) done with them*. Additionally, themes about them, how participants responded and to what level of detail was discussed was recorded.	**A:** 1. Look for structural alignments of the full **protein backbone** to *identify* **folds** or families **RM**. 2. Look for template based alignments of **small motifs** to *identify* **active sites or ligand binding sites** **RM**. 3. Look at **sequence alignments (BLAST)** to *identify* **protein families** **RM**. 4. Explore Pfam and UniProt to study the families. 5. *Identify* ligands that bind [**substrate binding**] to members of these protein families **RC**. 6. *Dock* the **ligands** to the proteins and see [*determine*] if there are favorable **binding energies** **RM**.
Q: Please list and describe the types of representations you use, and how you use them, when thinking about or explaining protein function.		**A:** 1. Enzyme assays: **chemical reaction drawings** help us *to understand* how the parts of a protein catalyze a reaction **RM**. 2. Molecular visualization: **ligand binding** *demonstrates* if a **substrate** binds to an **active site** **RC** [e.g. a LigPlot+ figure was provided and is shown in Figure 2B].
		Output: General observations
		• Participants tended to discuss items in sequential order, often similar to how they are presented to the students. • Though reasoning elements were identified, there were at a basal level.

† Full set of survey questions and responses for the lead designer is provided in the supplementary materials.
***** Underlining indicates either an RM or RC (superscript) coded segment with verbs (italics) showing reasoning associated with the noun (bold) which is either a concept or representation.

2.4.1.3 Findings from the Interview

To meet Criterion 1, we used the data for all participants from the open-ended survey to inform the design of an interview protocol (see the Supplemental Material, Section 2.8.2). During the interview, to gain greater clarity regarding the open-ended responses, we probed deeper into the nature of the concepts, representations, and related reasoning and how such knowledge is applied by students during the course (Criterion 2). In addition, the lead designer was asked how the representations were generated, what students should be able to extrapolate from them, and what meaning was attached to the different symbolism (Table 2.7). As shown by the interview quote (Table 2.7), by discussing the provided representations with the lead designer, we gained

information about how scientists and students use such representations to understand the functions of proteins.

Table 2.7 Example of conducting Step 3 of PICURA: Interview.

Step 3: Interview		
Excerpt from the interview with the lead designer†	**Analysis** ➔	**Output:** Examples of initial CURA statements from the provided quote
Interviewer: "So if you could just kind of talk to me about how these types of representations could be used when kind of forming the hypothesizes about what these proteins functions could potentially be? like how would you say look at the 3D plot or the 2-dimensional representation LigPlot+ and start to hypothesize just what these proteins are doing, or if you know you have a good substrate." **Lead Designer:** "… they do their docking studies, they get a number so let's say -8 kcal/mol for binding of a ligand to a protein and so I would like them to be able to *look* at a **LigPlot+ graph** [Figure 2.2B] like this and say ok I get a -8 for this one ligand and I get a -6 for this other one and I would like them to look at this and *count* the **hydrogen bonds** **RM**, because the more **hydrogen bonds** the more negative the **free energy** is when *binding* **RC**. but to be able to *compare* that and then the next thing that they can do is look for a '**goodness of fit**' [,or binding, between a protein and a substrate]**RC**."	The interview with the lead designer was transcribed verbatim. Then the interview transcript was coded for instances where the participant discussed reasoning with concepts and/or representations (i.e. RM or RC statements). After this, these segments were used to generate initial ability statements.	**RC and RM Abilities Detected** • Count the number of H-bonds in a molecular visualization • Determine using computational software whether, and where a ligand may be binding to a protein • Identify ligands that bind to members of a protein family • Demonstrate if a substrate binds to an active site • Estimate the relative ligand binding stability based on the number of protein ligand interactions

† Underlining indicates either an RM or RC (superscript) coded segment with verbs (italics) showing reasoning associated with the noun (bold) which is either a concept or representation.

To generate the initial ability statements, we analyzed the interview transcript for direct mention of RC and RM abilities. For example, the lead designer first mentioned how to use a "LigPlot+ graph" to "count the hydrogen bonds" between a ligand and a protein (RM segment, Table 2.7), and how difference in binding energy can come from these interactions, because "the more hydrogen bonds the more negative the free energy is when binding" (RC segment, Table 2.7). These interview quotations (Table 2.7) provided richer detail of the type of reasoning skills involved in the CURE compared with the responses from the open-ended survey (Table 2.6), but together they provided great insight into some potential CURAs (Criterion 2). For example, in

Step 2, there was a brief mention of identifying substrate binding (Table 2.6), whereas during the interview (Step 3), the lead designer gave a much more detailed explanation of how hydrogen bonds impact the favorability of protein-ligand binding interactions (Table 2.7). Thus, the interview data acted as a primary data source for the fine-tuning of specific CURA statements, but such statements were also progressively informed through the previous steps of the process in an iterative manner (Criteria 1 and 2).

Table 2.8 Alignment check (Step 4) for a top-rated (TR) CURA statement showing that the ability statement was supported by Steps 1-3 and each step added greater insight into the finalized CURA statement.

TR: Determine using computational software whether, and where a ligand may be binding to a protein	
PICURA Components	Supporting Outputs
Step 1: Content Analysis	**Concepts Detected:** • Protein Motifs • Protein Homology • Structure Function Relationships • Intermolecular forces – Protein ligand interactions **Representations Detected:** • Various computer generated protein structures (e.g. LigPlot+ and ProMOL representations)
Step 2: Open-ended Survey	"We use protein sequence alignment to find similar proteins with known function, we use domain analysis – to find proteins with similar domain composition, we use structure alignment to find similar with known functions, we use docking to simulate interactions between enzyme and possible substrate to try to choose more likely substrate. Each type of computational evidence does not generate one answer, but rather a list that can be ordered."
Step 3: Interview	"[a student] could *look at* the binding of two **ligands** to a protein that the **ligands** are almost identical, they're slightly different and see that you know let's say one ligand has a benzene ring attached to it and the other one doesn't, and the one with the benzene ring binds with 2 kilocalories per mole better than one without it and so I would hope that they would look at that and say I need to find out where that benzene ring *interacts* to cause that much better binding. and that sort of thing so by having them look at the results they obtained… **computationally** in one **program** but then *test* that in either another **program** or [run an assay in the lab]."

After the interview, quotes were coded for RC and RM components. A thought experiment was also conducted to construct the possible CURA statements that would apply to a segment (Table 2.7, outputs). An example of this is the interview quote in Table 2.7, in which one RM and two RC segments were noted. These three segments could account for at least five separate initial

CURA statements. Because the interview acted as the primary data source for the generation of these initial CURA statements, and because it was the first time that there were in-depth reasoning responses, there was a need to check for alignment with the previous two steps to make sure that the CURA statements generated were also supported by the content analysis and the open-ended survey. Thus, in combination, we were seeking consensus between the different data sources about each CURA (Criterion 3).

2.4.2 Reaching Consensus about the Generated Ability Statements (Steps 4 and 5)

In the first part of this section, data from Steps 1–3 of PICURA were highlighted to give an example of the important role of each step in clarifying the nature of the CURAs. In this section, the focus is on the alignment check (Step 4, Criterion 3) and the Likert survey (Step 5, Criterion 4), which together with all the data sources from each step provided a consensus among participants as to which CURAs they considered to be important. Results for two identified CURAs (TR and LR, Table 2.1) are provided to illustrate this alignment (Tables 2.8 and 2.9) and consensus process (Table 2.10). These two CURA statements were from the top-rated (TR) group, meaning more consensus about their importance, and the lower-rated (LR) group (less consensus) of statements identified using PICURA. The data presented here are aimed at reinforcing how each step led to greater insight into the nature of each CURA and how its relevance to the curriculum was determined.

2.4.2.1 Step 4: Ability Alignment Check across the PICURA Steps

As described in the preceding sections, the ability statements were initially generated as the output for the interview in Step 3 of PICURA (Figure 2.1 and Table 2.7). This is demonstrated by how the interview quotes informed the generation of ability statements (Table 2.7). During the interview, the participant discussed a detailed example in support of a top-rated ability statement (Table 2.8) by outlining how computational programs can be used to determine whether a ligand was binding and where it could be binding. This was also indicated in the open-ended survey response, in which it was mentioned that the alignment programs inform hypotheses about function and candidate substrates, whereas the docking simulation produces a ranked list of how likely the candidate substrates bind to a protein (Table 2.8). This CURA statement is also supported by the content analysis (Step 1), in which the concepts of motifs, homology, and ligand binding were determined to be prominent themes throughout the protocols, along with the representations from

computational software that were used to depict enzymes' active sites (Table 2.8). The amount of evidence gained in support of this CURA as we progressed from Step 1 to Steps 2 and 3 of PICURA supports why it was rated highly in Step 5 (Criteria 3 and 4; Tables 2.8 and 2.10).

Table 2.9 Alignment check (Step 4) for a low-rated (LR) CURA statement showing that the ability statement was supported by Steps 1-3 and each step added greater insight into the finalized CURA statement.

LR: Recognize how proteins that are closely related by evolution can have dramatically different functions	
PICURA Components	Supporting Outputs
Step 1: Content Analysis	**Concepts Detected:** • Protein Motifs • Protein Homology • Structure Function Relationships **Representations Detected:** • Amino acid single letter code alignment • Super imposed protein ribbon structures
Step 2: Open-ended Survey	"Some favor sequence homology to suggest function. While that is useful in some cases, I prefer a strong component of structural homology to suggest function." "I use sequence and structural data to find similar proteins with a known or hypothesized function." *No specific mention of related proteins can have different functions*
Step 3: Interview	"just because you have a **catalytic triad** that doesn't mean that an enzyme will cut proteins, maybe it will cut lipids, maybe it will cut something else. I'm hoping that they'll have *some sort of grasp* on the **physical nature of proteins** you know like the molecular weight of proteins, how they behave"

Additionally, there was evidence for the lower-rated CURA statement (Table 2.1) from the interview (Step 3) in which the participant discussed the idea that just because two enzymes have a similar active-site structure does not necessarily mean that they will have the same function, which could be quite different (Table 2.9). However, protein similarity (or homology) was discussed in a different context in the open-ended survey (Step 2) and content analysis (Step 1; Criterion 3; Table 2.9). Here, homology is used for hypothesis generation for a protein's function and not to emphasize that, although homologous, related proteins can have distinctly different functions (Table 2.9). A low WR score was measured for this item, mainly because this CURA

received very few marks for being covered in this course only and had two responses each for "not in this course" and "unimportant" (Criterion 4; Table 2.10).

It is also important to note that the alignment check for this CURE did not generate any CURA statements that were seriously misaligned. However, there were instances of CURA statements having their wording refined as part of Step 4. For example, the LR example shown in Table 2.9 was originally worded as "Realize that common protein homologies can lead to drastically different behavior" but was modified to "Recognize how proteins that are closely related by evolution can have dramatically different functions" to more deeply clarify this CURA statement. Member checking, with the lead designer, and comparing the CURA statements for overlap also led to more concise wording for each statement before the Likert survey was administered (Step 5).

2.4.2.2 Step 5: Evaluating the Importance of the CURA Statements with the Likert Survey

The two-tier Likert survey served three main roles: 1) to confirm the relevance of the generated CURA statements to the curriculum and the ability of PICURA to detect such CURAs, 2) to check for agreement among instructors about the relative importance of the CURAs, and 3) to narrow down the number of CURAs that could be used for student assessment design. Again, two selected CURA examples (Table 2.1) are used to demonstrate how Step 5 of PICURA was conducted to reach a final set of CURA statements (Figure 2.1).

Table 2.10 Example of two CURA statements from the two-tier Likert survey.
Counts represent the number of participants selecting a given response.

	Likert-Question 1			**Likert- Question 2**			
Ability*	NOT acquired in this lab course	In BOTH this lab and some other course	ONLY in this lab course	Unimportant	Undecided	Important	Weighted Relevance (WR)
TR	0	0	10	1	2	7	+17
LR	2	5	3	2	1	7	+9

* Examples of a top-rated (TR) and a lower-rated (LR) ability statements (see Table 2.1 for full description of the CURA statements).

From the Likert survey data in Table 2.10, a WR score of +17 suggested strong agreement between participants that a top-rated CURA was unique to this lab course (10/10) and was important for conducting this type of research (7/10). In contrast, a WR score of +9 for the lower-rated CURA suggested that there was less agreement among participants about how unique this ability was to this CURE, although it was generally considered to be important (7/10) to this field of research.

This final Likert step acted in two ways to confirm the importance of the selected CURAs. The first way was through the WR score (Table 2.10), which reflected the extent of agreement between each answer to the two-tier Likert survey. This permitted the selection of the most appropriate CURAs for the curriculum (Criterion 4). However, that is not to say that lower-rated CURAs are inappropriate or highly relevant to individual implementations of the CURE, rather, at this time, they are not as relevant across all institutions as the top-rated CURAs. Second, PICURA was used to identify CURAs for this CURE through unbiased data. For this implementation of PICURA, the WR scores could have ranged from −20 to +20 (see *How to Apply the Five Steps of PICURA* section for Step 5 and Equation 2.1). However, the lowest-rated CURA statement actually received a WR score of +3.5, meaning all CURA statements had a degree of agreement about uniqueness and importance. This is consistent with the data that were generated for the lower-rated statement, in which the concepts identified in Step 1 were covered by this CURA. However, excerpts from Step 2 and Step 3 show how the concepts pertaining to homology were used in differing ways (Table 2.9), so that a lower WR score was not surprising (Table 2.10). Thus, this process made it possible to find relevant candidate ability statements and to prioritize the CURA statements for future assessment development and to guide CURE evaluation (Criteria 2 and 4).

2.5 Conclusion

This essay reports on the development and application of a novel five-step process (PICURA) for the rigorous identification of the ALOs, specifically the CURAs, considered by the instructors as key to student learning in our biochemistry CURE. The scientists and educators involved with this project found that, without much effort, PICURA led them to agree on ALOs, so that they are now ready to design relevant assessments to identify the actual VLOs for their CURE. In addition, we have described a novel way to interpret two-tier Likert-scale questions by using weighted

relevance to rate the CURAs according to consensus of importance. We have demonstrated that PICURA (Figure 2.1), guided by the CRM model (Schönborn & Anderson, 2009), generates four data sources—from course materials/content analysis, an open-ended survey, an interview, and a Likert survey—that can be aligned in a consensus process with instructors to effectively identify, fine-tune, and prioritize specific CURA statements for our biochemistry CURE. Thus, we believe that PICURA meets our stated criteria. First, the process is iterative, in that the data generated by each step inform the development and design of the instrument used in the subsequent step (Criterion 1). Second, the data are additive, in that each part of the process yields more clarity on the nature of the CURAs (Criterion 2). Third, the data from each step of the process combine well to achieve consensus and internal alignment about the CURAs (Criterion 3). Finally, the process permits the prioritization of the CURAs in terms of the level of consensus about the importance and relevance of the CURAs to the course (Criterion 4). In addition, we found that the techniques of PICURA (outlined in Figure 2.1) are user-friendly and that it was an efficient way to collect and process the data from participant instructors.

It should be emphasized that using PICURA led to the identification of what the participant instructors considered the most important CURAs (ALOs) for their specific context within this specific biochemistry CURE at this point in time and, therefore, it should not be assumed that these are generalizable CURAs that will necessarily be relevant to other institutions performing the same CURE now and later. Indeed, as is the case for all courses, it is likely that the emphasis on certain ALOs will continually change, necessitating a new application of PICURA as and when required. It should also be noted that PICURA is aimed at identifying the ALOs, with the longer-term goal of using such ALOs to inform the design of assessments that will yield student responses that will allow instructors to check whether what they anticipate students will learn (ALOs) equates to verified learning outcomes (VLOs). As stated by Anderson & Rogan (2011), if this crucial alignment does not exist, instructors will need to either modify the ALOs of the course and/or the nature of the assessment to ensure that all ALOs are adequately assessed and that no assessments target the wrong ALOs.

In the context in which PICURA was developed, the CURAs identified are intended to be higher-order biochemistry research abilities, rather than technical abilities like being able to pipette or do SDS–PAGE, which, although important for researchers, can be easily assessed by checking a box that says “yes” or “no” as to whether the student can do it. As is apparent from the data presented above, the ALOs we sought to identify would reflect the scientific reasoning and

problem-solving abilities necessary for students to become competent and effective researchers in the area of biochemistry focused on by our CURE. Future use of PICURA will more fully establish to what extent the steps in this process may achieve this goal for other CUREs.

The goals of PICURA are also in agreement with the tenets of curriculum theory that advocate for the importance of ongoing course development throughout the life of a course, including periodic review of the ranking of the expected learning statements (Anderson & Rogan, 2011). This is an important component of PICURA in the CURE context, because the CURAs may change as new instructors join, instructors gain more experience, the CURE becomes more widely disseminated, and, most importantly, the research being conducted within a CURE evolves. The nature of CUREs means that, as more cohorts conduct research and novel findings are added to the collective understanding of the research being conducted, the goals of the CURE will surely change. Although the WR scores may fluctuate, as long as the protocols, techniques, and research questions remain unchanged, the CURAs will remain relevant to that CURE.

Future work will involve using PICURA to generate an entire taxonomy of CURAs for our biochemistry CURE. A taxonomy of this kind, besides informing the relevant cognition component for assessments according to the assessment triangle (NRC, 2001), could act as a framework to study additional, and deeper, reasoning about the focus of our biochemistry CURE. In this regard, we agree with Shortlidge & Brownell (2016), who identify various assessments that are available, some of which are widely used but many of which were designed for a specific context and may not meet the unique qualities of another CURE.

In conclusion, PICURA clearly permitted the participant instructors to think more deeply about the ALOs of this CURE and their particular use of the CURE in their own context than would happen if a single instructor had brainstormed the ALOs alone. This approach, in our view, is key to situations in which a particular course is run across multiple institutions, as is the case for our biochemistry CURE.

2.6 Potential Application of PICURA

Although the data presented in this essay are not intended to imply generalizability to other CUREs, we do recommend that colleagues test PICURA's usefulness in their own contexts. PICURA may be particularly useful for collaborative courses across multiple institutions and for courses in which several instructors need to come up with a set of abilities that are relevant across

settings. Additionally, the nature of the process will allow a collaborative team to optimize the ALOs and come closer to what students will really learn in order to better inform course assessment development by instructors. Though this process was developed in an upper-division biochemistry CURE, this process should be transferable across education levels, disciplines, and course formats in ways that could be useful to education researchers, instructors, and administrators. There may be a need to customize some of the steps for these other purposes. However, we suggest that any modifications to the process should primarily be to the inputs, specifically the questions given to instructors, while still applying the simple structure of the process itself (Figure 2.1). Finally, administrators could use the results of this process to inform decisions concerning implementation of educational policy and reform and to gain greater insight into the role a particular course serves within a department or institutional curriculum. This may lead to the adoption of more CURE curricula and their dissemination.

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2.8 Supplemental Material

2.8.1 The Additional Prompts from the Open-Ended Survey (Step 2) and Responses from the Lead Designer

Questions from the open-ended survey	**Responses from the lead designer**
In your own words, please describe the biochemistry lab course that you will be implementing as part of the IUSE NSF project.	In the lab, we use a CURE approach. The instructors have some ideas about the possible role of the enzymes we are studying, but the final answers are unknown and there is lots of room for discovery.
What are the necessary components that characterize a research-like laboratory course experience?	1. Develop a hypothesis 2. Propose experiments 3. Collect data 4. Interpret results 5. Repeat the process iteratively
In your own words, please describe hypothesis-driven thinking.	You encounter a problem and want to find a solution. You look at the evidence and the system. Based on that knowledge, you suggest the next thing that you think will happen. Then you design experiments to test that suggestion or hypothesis.
Please explain the process you, or other scientists, use when generating a hypothesis about protein function.	1. Collect all available data using structural bioinformatics tools. 2. Compare the results from the different tools and look for common threads. 3. Study the organism that is the source of the protein. Is there anything unique or unusual about this organism that might provide a clue about the protein's function. 4. Hypothesize a function for the protein. 5. Identify possible substrates, ligands or binding partners from the literature and commercial suppliers. 6. Buy the stuff and test your hypothesis.
Now that you have had experience learning about and teaching the computational portions of the lab course, what are some important things you have learned that you hope students will learn as well?	1. The results from the computational tools sometimes seem very clearcut, but every result contains significant uncertainty. 2. The data you input affects your results.
Explain how you, or other scientists, use enzyme assays and any other biochemical experiments to confirm protein function.	1. Enzyme assays will demonstrate if a protein will modify a substrate. 2. The assay results can indicate if a substrate might be physiological, based on the Km and Vmax values that are found. 3. It is possible to perform binding studies on a protein if you find a suitable probe.
Now that you have had experience learning about and teaching the biochemical experiments and assays used in this lab course, what are some important things you have learned that you hope students will learn as well?	1. A yellow color confirms a type of reaction. 2. That yellow color does not confirm function. It is only the beginning.

2.8.2 Full Semi-Structured Interview Protocol (Step 3)

1. In the survey, there was a question asking you to list and describe the types of representations you use, and how you use them, when thinking about or explaining protein function. You provided the following representations:

A. Enzyme assays: chemical reaction drawings help us to understand how the parts of a protein catalyze a reaction.

B. Molecular visualization: ligand binding demonstrates if a substrate binds to an active site.

   i. Enzyme assays
   ii. Chemical reaction drawings
      1. How the parts of a protein catalyze a reaction
   iii. Molecular visualization
      1. Ligand binding

   Could you please talk me through:

   g) How you would use each representation to reason about protein function;
   h) What types of biochemistry representations are useful for students to be familiar with to help them in this course?
   i) How you would like students to use them;
   j) What you observed students doing with each representation?
   k) Whether these representations are new to the students, or did they have some previous experiences with them? If so, describe the experiences they had.
   l) How would you know if students were having difficulties and whether they were improving?
   m) What type of things did students do to practice and overcome these difficulties?

2. Which biochemistry topics were most difficult for students?
   a. Were these topics new to the students, or did they have some previous experiences with them? If so, describe the experiences they had.
   b. How did you know if students were having difficulties and if they were improving?
   c. What type of things did students do to practice and overcome these difficulties?
   d. What were the biochemistry topics you hoped students would learn or improve on, as part of this course?

3. Which research skills were most difficult for students?
   a. Were these skills new to the students, or did they have some previous experiences with them? If so, describe the experiences they had.
   b. How did you know if students were having difficulties and if they were improving?
   c. What type of things did students do to practice and overcome these difficulties?
   d. What were the research skills you hoped students would learn or improve on, as part of this course?

4. How will you know if students meet the learning objectives of the course?
   a. How and what do you intend on assessing students?
   b. What things do you feel are easier or harder to monitor, observe, or assess?

Ask questions 5, 6, and 7 only if either these topics did not come up in response to above questions or you wish to explore them further:

5. What do you think about how the process detailed in the current protocols relates to the discovery process actual scientists apply to understanding protein function?

6. Please briefly explain how students learn the computational tasks in your class. What are students expected to do during this part of the lab?
    a. What do you hope students learn, and are able to do, after performing the computational portion of the lab?
    b. How do students, or scientists, use these techniques to come up with hypothesis?

7. Please briefly explain how students learn the wet lab tasks in your class. What are students expected to do during this part of the lab?
    a. What do you hope students learn, and are able to do, after performing the wet lab portion of the course?
    b. How do students use this part of the lab in assessing their hypotheses?

CHAPTER 3. ANTICIPATED LEARNING OUTCOMES FOR A BIOCHEMISTRY COURSE-BASED UNDERGRADUATE RESEARCH EXPERIENCE AIMED AT PREDICTING PROTEIN FUNCTION FROM STRUCTURE: IMPLICATIONS FOR ASSESSMENT DESIGN

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3.1 Abstract

Several course-based undergraduate research experiences (CUREs) have been published in the literature. However, only limited attempts have been made to rigorously identify the discovery-type research abilities that students actually develop during such experiences. Instead, there has been a greater focus on technical or procedural-type knowledge or general CURE skills that are too comprehensive to effectively assess. Before the extent of discovery-type learning outcomes can be established in students (termed verified learning outcomes or VLOs), it is important to rigorously identify the anticipated learning outcomes (ALOs) and to then develop student assessments that target each ALO to reveal the nature of such student learning. In this article we present a matrix of 43 ALOs, or course-based undergraduate research abilities (CURAs), that instructors anticipate students will develop during a recently-developed biochemistry CURE focusing on the prediction of protein function from structure. The CURAs were identified using the process for identifying course-based undergraduate research abilities (PICURA) and classified into seven distinct themes that enabled the characterization of the CURE and a comparison to other published inventories of research competencies and CURE aspects. These themes and the CURE protocols aligning to the CURAs were used to form the ALO matrix that was, in turn, used to inform the design of an assessment that revealed evidence that a student had developed some of the targeted CURAs. Future research will focus on further assessment development that targets other identified CURAs. This approach has potential applications to other CUREs both in biochemistry and other science disciplines.

3.2 Introduction

As the field of biochemistry continues to advance, it has become increasingly important to clarify what students should be learning from their undergraduate biochemistry coursework. As emphasized by Caldwell, Rohlman, & Benore-Parsons (2004), students will be better prepared and more competitive when applying to graduate programs or entering industry, if undergraduate programs focus on the mastering of relevant technical and problem-solving skills and key content knowledge. White, Benore, Sumter, Caldwell, & Bell (2013) extends this argument by stating that students should be given the opportunity to develop more than just the fundamentals of biochemistry; they should also be taught communication and technical skills, which can often be overlooked within a curriculum. Toward this goal it is essential for students to partake in some sort of biochemical research experience (Voet et al., 2003). Furthermore, there is a general consensus that when students have more authentic research experiences they will learn more about scientific inquiry which will, in turn, promote their attitude toward science (DeHaan, 2005). In this article we extend this thinking to focus more specifically on some of the scientific discovery skills (rather than technical skills or procedural knowledge), that are essential for the performance of sound research.

At many institutions it is not possible for students to take part in undergraduate research (DeHaan, 2005), but it is possible for them to participate in laboratory experiences that provide opportunities to experience research and to develop research and problem solving abilities (Corwin, Graham, et al., 2015; Voet et al., 2003). This is in line with recent reform efforts (Brewer & Smith, 2011; NASEM, 2015, 2017, NRC, 2003, 2012; PCAST, 2012) aimed at incorporating more authentic practices within the sciences, such as course-based Undergraduate Research Experiences (CUREs) (Auchincloss et al., 2014). CURE activities have demonstrated positive impacts on many different factors, across academic levels and scientific disciplines, ultimately leading to student persistence in science (Corwin, Graham, et al., 2015). Some recently published protein biochemistry CURE examples include: studying protein mutations in tumor and cancer-related proteins (Hekmat-Scarfe et al., 2017; Shanle et al., 2016), investigating the effect of mutating non-conserved protein regions (Ayella & Beck, 2018), and characterizing proteins of known structure but unknown function (Craig, 2017; Craig et al., 2018; Gray et al., 2015). Some CUREs have been purposely designed in a modular fashion to allow for different implementation strategies (Craig, 2017; Craig et al., 2018; McDonough et al., 2017). One such example is the

biochemistry CURE developed by the Biochemistry Authentic Scientific Inquiry Laboratory (BASIL) as a multi-institutional collaborative project, described by Craig (2017) and Craig et al. (2018). The BASIL CURE is a modular CURE focused on assigning function to proteins of known structure but unknown function. The BASIL CURE serves as the context for this study.

As advocated by various authors (e.g. Bell et al., 2017; Brownell & Kloser, 2015) the learning objectives that students are expected to achieve during a particular CURE need to be identified before a CURE can be evaluated. Such learning objectives can then be used to inform assessment design to confirm to what extent students are achieving these objectives (Anderson, 2007; Irby, Pelaez, et al., 2018b). More recently, we published an article (Irby, Pelaez, et al., 2018b) in which we suggest the use of the phrase, "Anticipated Learning Outcomes" (ALOs) instead of learning objectives. In so doing we distinguish between ALOs proposed by instructors and Verified Learning Outcomes (VLOs) based on evidence of students demonstrating that they have achieved an outcome through appropriate assessments (Irby, Pelaez, et al., 2018b). This is in line with the tenets of sound assessment design (Anderson, 2007; Anderson & Schönborn, 2008; Schönborn & Anderson, 2008) as well as those relating to the assessment triangle (NRC, 2001), which states that the three key elements of assessment are concerned with: *cognition,* identifying the set of knowledge important to measure (i.e. the Process for Identifying Course-based Undergraduate Research Abilities, used as part of this study, Irby, Pelaez, et al., 2018b); *observation*, specifying elements to be incorporated into assessment items to elicit responses about that knowledge from students (i.e. goal of this article); and *interpretation*, reasoning from evidence provided by students in response to knowledge being assessed (i.e. VLOs (Irby, Pelaez, et al., 2018b) confirmed from student responses to assessments).

Publications describing various CUREs do not always specify the related ALOs. For example, on the CURE database, CUREnet (CUREnet, n.d.), authors are required to outline what they believe are the student goals and research goals of their CURE but few discovery-type ALOs are specified. Instead, when ALOs are reported for CUREs they tend to be either too general or not clearly defined, often based on the aspects of what defines a CURE (Auchincloss et al., 2014), or are similar to the broad potential CURE outcomes reviewed by Corwin et al. (2015). In addition, some of the listed ALOs focus more on technical or procedural-type knowledge. Even when CUREs present a set of ALOs, it is often not clear how the outlined ALOs were identified or if any steps were taken to confirm if they are VLOs. Such confirmation is dependent on well aligned student assessments, which are often lacking from the literature. To address some of these issues,

Irby, Pelaez, et al. (2018b) developed a data-driven, five-step, Process for Identifying Course-based Undergraduate Research Abilities (PICURA) that instructors of our BASIL CURE anticipate students will develop during the course. We termed the identified ALOs, CURAs, after Course-based Undergraduate Research Abilities. The identification of ALOs (CURAs) by a robust process, such as PICURA (Irby, Pelaez, et al., 2018b), is an important component of course evaluation and assessment frameworks (NRC, 2001) for determining the extent of student learning and the VLOs for a course (Irby, Pelaez, et al., 2018b). The CURAs identified using PICURA and presented in this article are aimed at informing the design of assessments that will allow us to confirm to what extent students actually develop the specific research abilities that instructors anticipate students will learn during the BASIL CURE, compared with more general assessment measures that have been disseminated to assess CUREs (Shortlidge & Brownell, 2016).

In addition to identifying ALOs to determine what should be assessed for a particular CURE, ALOs can also be used as a way to characterize a CURE. For CUREs, this has previously been done by situating courses within the five aspects of CUREs (Auchincloss et al., 2014; Corwin, Runyon, et al., 2015), which are a set of features or activities that have been proposed by Auchincloss *et al*. (2014) to characterize a typical CURE. Another more general effort by the Advancing Competencies in Experimentation-Biology (ACE-Bio) network has involved the identification of a set of competencies for biological experimentation (also referred to as "ACE-Bio competencies") (Nancy Pelaez et al., 2017). Though developed in the context of the biological sciences, many of the identified experimental competencies (Nancy Pelaez et al., 2017) are skills applicable to many fields of research and should be present in courses, such as CUREs, that focus on integrating authentic scientific practices. Thus, one way to characterize the unique features of a CURE could involve identifying and aligning ALOs to more general competencies of experimental research (Nancy Pelaez et al., 2017) and the aspects that should be incorporated into a CURE (Auchincloss et al., 2014; Corwin, Runyon, et al., 2015), an approach used in the current article.

Thus, the goal of the present study was to identify the ALOs or CURAs for the BASIL CURE which, in the future, could be used to develop assessments in order to measure actual student leaning outcomes or VLOs (Irby, Pelaez, et al., 2018b). Toward achieving this goal, we addressed the following research question: "Which CURAs do the instructors of the BASIL CURE consider the most relevant learning outcomes and how do these compare to other published descriptions of CURE objectives and experimental competencies?" To address this research

question, we chose to collect data from the course instructors and lead course designer using the PICURA approach (Irby, Pelaez, et al., 2018b). In this article we present the resulting CURA statements (Table 3.1) which we then organized as an ALO matrix (Table 3.2). We also illustrate by means of one specific example how readers might use the matrix to inform the design of a student assessment to measure the development of various CURAs by a student.

3.3 Description of the BASIL CURE and Study Participants

This study focuses on a novel biochemistry CURE, developed as part of the BASIL project (Craig, 2017; Craig et al., 2018) and implemented at multiple institutions. The BASIL CURE consists of 10 protocols (listed in Table 3.2, Column 1 and described in detail in Craig (2017) and Craig et al. (2018)) which involve a combination of computational and wet lab biochemical techniques to elucidate the function of proteins whose structures have been solved but their functions have not been confirmed. The CURE was modeled after the work done by researchers in the field to elucidate the function of proteins deposited in the Protein Data Bank (e.g. McKay et al., 2015) and is described in more detail elsewhere (Craig, 2017; Craig et al., 2018; Irby, Pelaez, et al., 2018b). There were 10 participants in this study, including one lead designer and nine instructors who also contributed to the development of the BASIL CURE (Irby, Pelaez, et al., 2018b). The participants are either tenured (n = 8) or tenure-track (n = 2) faculty members at a range of US institutions who all maintain active research groups, have two or more years' experience teaching lab courses, and whose expertise is in either computational chemistry, biochemistry, or both. This study was approved by the Purdue University IRB (#1503015825 and #1604017549).

Table 3.1 Finalized list of the 43 course-based undergraduate research ability (CURA) statements grouped by weighted relevance (WR).

Identified CURA Statements	WR[†]
Top-Rated[*]	
TR1: Explain how the colorimetric enzyme assay works to allow detection of protein function	+18
TR2: Identify an enzyme active site using appropriate computational programs	+17.5
TR3: Determine the appropriate factors to consider when optimizing or interpreting an enzyme assay	+17.5
TR4: Determine using computational software whether and where a ligand may be binding to a protein	+17
TR5: Compare enzymatic results with those computationally predicted	+17
TR6: Design an enzyme assay to elucidate protein function	+16.5
TR7: Explain how the purification of tagged proteins works and ways the process can be optimized	+16.5
Middle-Rated[*]	
MR1: Assess the quality of data and how data gets altered when computationally manipulated (e.g. if going from a raw plot of data to a reciprocal plot)	+16.5
MR2: Demonstrate whether a particular substrate binds to an active site	+16
MR3: Consider how to minimize protein denaturation when planning/performing experiments	+16
MR4: Optimize the reaction parameters (e.g. substrate and enzyme concentration, pH, temperature, etc.) that are essential for the occurrence of an enzyme-catalyzed process.	+15.5
MR5: Connect the data from an enzyme assay to what the enzyme is actually doing	+15.5
MR6: Compare results of different computational methods to determine if they agree with each other	+15
MR7: Use SDS-PAGE gels for interpreting information about a protein and its expression from a plasmid	+15
MR8: Understand the effect of a residue's charge on substrate interactions with an enzyme	+14.5
MR9: Recognize parameters that will impact protein substrate interactions	+14.5
MR10: Propose modifications to ligand molecules to increase their binding affinity for a protein	+14
MR11: Determine and interpret kinetic rates in light of saturation effects	+14
MR12: Recognize the different types of atoms and/or number of atoms present in a representation of a molecule	+14
MR13: Identify which ligands bind specifically to members of a particular protein family	+13.5
MR14: Relate a graphical representation of data from an enzyme assay to what can happen biologically with that enzyme	+13.5
MR15: Grasp the limitations of research methods based on homology	+13
MR16: Determine using computational software where enzymatic activity may cause bond breaking	+12.5
MR17: Relate structurally conserved protein regions to their function	+12.5
MR18: Distinguish between the different components of an enzyme including amino acids; secondary, tertiary and quaternary structure and any non-proteinaceous components	+11.5
MR19: Recognize a bad data point on a graph of research data	+11.5
MR20: Recognize the different symbols used in a graph and their meaning	+11.5
MR21: Compare conditions to determine a binding interaction between a substrate and a protein	+11.5
MR22: Distinguish between molecules that are good at binding to an enzyme versus those that could also be a substrate for that enzyme	+10.5
MR23: Translate or map features between 2D and 3D representations of proteins	+10.5
MR24: Use kinetic data to determine important parts of a protein's structure (e.g. binding pockets and/or catalytic residues)	+10.5
Lower-Rated[*]	
LR1: Recognize the types of bonding interactions between an enzyme and its substrate	+10
LR2: Use protein and substrate electrostatic information to propose ways to improve binding	+9.5
LR3: Explain the relationship between a concept or a phenomenon and a mathematical equation representing that concept	+9.5
LR4: Identify the hydrogen bonds in a protein based on the properties of the atoms and their inter-atomic distances	+9
LR5: Recognize how proteins that are closely related by evolution can have dramatically different functions	+9
LR6: Explain the strengths and weaknesses of a Michaelis-Menten plot and a Lineweaver-Burke plot and explain when each one is easier to use	+8
LR7: Recognize that the factors that determine protein structure and function happen from interactions throughout the protein and not just from neighboring residues	+8
LR8: Explain how secondary and tertiary structure is influenced by intramolecular forces within a protein	+8
LR9: Relate a biochemical representation of a structure or process to its real life practical meaning or interpretation	+7
LR10: Determine biochemically relevant constants (such as Vmax or Km) from a Lineweaver-Burke plot	+7
LR11: Determine the presence and nature of a transition state analog	+6
LR12: Relate a data point in a Michaelis-Menten plot to one on a Lineweaver-Burke plot	+3.5

[†]Current weighted-relevance (WR) scores for the identified CURAs from the 10 participants.

[*]The weighted-relevance (WR) scores (Table 3.1), which indicate the amount of consensus among participants doing the Likert survey (Irby, Pelaez, et al., 2018b), were used to rank the CURAs into top-rated (TR) items (meaning that there was consensus about their importance to this type of research as well as their importance to different instructors' courses), middle-rated (MR), and lower-rated (LR) statements (meaning there was less consensus; see also Supporting Information (Section 3.11), Tables 3.4-3.6, for detailed rankings and how cutoffs were assigned).

3.4 Description of PICURA

The PICURA approach as described by Irby, Pelaez, et al. (2018b) was used to identify the CURAs that instructors anticipate students would develop from this CURE. PICURA consists of a five-step process that, in sequence, includes: (i) Step 1 a content analysis of course protocols, (ii) Step 2 an open-ended survey with instructors and course designers from the CURE, (iii) Step 3 an interview with the lead designer, (iv) Step 4 an alignment check to refine verb-noun CURA statements, and finally, (v) Step 5 a two-tier Likert survey to establish the final list of CURAs that participating instructors and designers consider to be the most important ALOs for the CURE. PICURA is informed by the conceptual-reasoning-mode (CRM) model (Anderson et al., 2013; Schönborn & Anderson, 2009, 2010) to guide coding of the data sources for the range of concepts (C), modes of representations (M), and reasoning skills (R) associated with them (RC and RM). The CURA statements were generated by first identifying instances where the lead designer discussed (Step 3) reasoning with concepts (RC) or representations (RM) while explaining the research associated with the CURE. A thought experiment guided by the CRM model (Anderson et al., 2013; Schönborn & Anderson, 2009, 2010) was done to identify specific RC and RM verb-noun CURA statements (Anderson et al., 2013; Irby, Pelaez, et al., 2018b). For a full description of the five steps of PICURA with respect to their inputs, analytical approach, and achieved outputs, see Irby, Pelaez, et al. (2018b).

3.5 Identification and Refinement of Technical- and Discovery-Type CURAs

Table 3.1 lists all of the final 43 CURAs that were identified as a result of the PICURA process, after generation, refinement, and alignment of 66 initial CURA statements (Steps 3–5 of PICURA). An alignment check (Step 4) was performed to check for consensus with Steps 1 and 2 (content analysis and open-ended survey). The CURA statements were subsequently member checked with the lead course designer and revised according to the lead designer's feedback and to ensure that there was no significant overlap between CURA statements.

CURA statement TR6, *Design an enzyme assay to elucidate protein function* (Table 3.1), will be used as an example of how initial CURA statements were determined and refined through the alignment check (Step 4). TR6's original wording was *Analyze kinetic data, properly, to elucidate an understanding of protein function,* which came from an interview quote when the lead

designer talked about “having students generate enzyme kinetic data and then look at the 3D structure of a protein and see what are the parts that contribute to that process” (Step 3). Here the lead designer was discussing how students generate and interpret kinetic data and make connections between a protein's structure and its function. However, when the lead designer member checked the CURA statements, the wording of TR6 got changed to its final wording (Table 3.1) because the lead designer said “it is unlikely that students will get as far as formal enzyme kinetics (Km, Vmax, turnover number, etc.), but they will be assessing activity with some screening [of] substrates.” In fact, the final wording of TR6 came directly from the lead designer, during the member check. Some examples in support of TR6 during the open-ended survey (Step 2) can be seen by the following quotes, “My goals for this project were to see if students could actually design their own experiment, analyze their results, and figure out what to do next” and “They [students] should have to consider what the possible outcomes of their experiments (computational or wet lab) are and what they should do next in the case of each outcome,” thus demonstrating the expectation for students to be learning how to design experiments. Lastly, CURA statement TR6 is supported by the BASIL CURE enzyme activity protocol (Table 3.2, Craig, 2017; Craig et al., 2018), where students design and conduct assays to evaluate the function of their protein. As the last phase of the alignment check (Step 4), overlap between CURA statements was remedied by fine-tuning the wording and/or merging statements. This procedure narrowed the list of CURA statements from 66 to 44; but after the CURA statements were rated (Step 5) one more CURA statement was removed due to its similarity to a higher-rated CURA statement, resulting in 43 final CURA statements (Table 3.1).

The final step of PICURA, Step 5, deployed a Likert scale to gauge participants’ rating of the importance of each CURA statement to this type of research and to a specific instructor's course. The Likert survey also had open-ended response boxes for the participants to comment whether any CURA statements should be added, removed, or altered. There was no consensus to add or remove any CURA statements, but the wording of TR7 was changed from “His-tagged proteins,” to just “tagged proteins” because students may encounter other tagging purification methods in this CURE (Table 3.1).

The weighted-relevance (WR) scores (Table 3.1), which indicate the amount of consensus among participants doing the Likert survey (Irby, Pelaez, et al., 2018b), were used to rank the CURAs into top-rated (TR) items (most consensus about their importance to this type of research as well as their importance to different instructors’ courses), middle-rated (MR), and lower-rated

(LR) statements (less consensus; see also Supporting Information section 3.11, Tables 3.5 and 3.6, for detailed rankings and how cutoffs were assigned). This was done to prioritize CURAs for assessment development, since the top-rated statements would be a target for assessment by different instructors implementing the BASIL CURE at various institutions. Note that the WR rankings of each CURA may fluctuate as the BASIL CURE evolves over time, because rankings vary across individuals and institutions (Irby, Pelaez, et al., 2018b). In other words, a CURA with a lower WR score does not mean that it is less important to every instructor who implements the course. In fact, in this study with 10 participants, the calculated WR scores could have ranged from −20 to +20 (Irby, Pelaez, et al., 2018b), but instead all of the identified CURA statements showed a WR score of +3.5 or greater (Table 3.1). This indicates that on average most of the instructors rated all the CURAs to be important and relevant to their BASIL CURE courses. But it is important to realize that if the BASIL CURE were implemented at other institutions, the participant instructors may rate the CURAs differently and may even suggest alternative CURAs not identified in this study.

On close scrutiny of the listed CURA statements in Table 3.1 it is clear that they require the same technical and procedural research abilities that are commonly taught in most traditional biochemistry laboratory courses, underpinned by the necessary biochemistry conceptual knowledge. For example, TR1 requires technical knowledge of the use of positive and negative controls to detect enzyme function as indicated by the presence or absence of a colored product, while MR7 requires the performance of SDS-PAGE to isolate and characterize the target protein relative to appropriate standard proteins. TR3, in contrast, is concerned with determining the important factors to consider and to optimize when designing an enzyme assay to elucidate enzyme activity. In addition to the above biochemical techniques, the computational biochemistry in the BASIL CURE requires other technical aspects such as the need to learn how to use computational software for determining ligand binding (TR4) or for identifying an enzyme active site (TR2). These CURAs that are focused on the use of computational methods are a unique feature of the BASIL CURE and are not commonly found in traditional biochemistry labs. Furthermore, most of the listed CURAs require biochemistry conceptual knowledge to master them. For example, LR10 requires students to understand the meaning of enzyme kinetics constants, Vmax and Km, and how Lineweaver-Burke plots are constructed to characterize a specific enzyme. Other examples include LR4, which requires conceptual knowledge of H-bonding and interatomic distances within

proteins, and MR11 that requires understanding of the meaning of enzyme saturation as a limiting factor in enzyme kinetics.

Table 3.2 ALO matrix organizing the CURAs into seven themes and ten BASIL CURE protocols.

CURE Component	CURE Protocol	1. Hypothesize the location and function of an enzyme active site	2. Propose a particular method based on considerations of the pros and cons of different methods	3. Interpret data to understand a biochemical meaning	4. Rationalize the design of candidate substrates	5. Visualize and determine key components of protein structure	6. Relate multiple types of data to reach a singular conclusion	7. Understand the biochemical theory behind methods
Biochemical Modules	Protein Expression							
	Protein Purification							**TR7**
	Protein Concentration							
	SDS-PAGE			MR7				
	Enzyme Activity		**TR3, TR6,** MR4	MR11, LR10	MR21		LR6, LR12	**TR1**
Computational Modules	BLAST		MR15					
	Dali		MR15			MR17		
	Pfam	**TR2**	MR15		MR13			
	ProMOL	**TR2,** MR2, MR22			MR13			
	PyRX	**TR2, TR4,** MR2, MR9, MR16, MR22			MR8, MR10, MR13, LR1			
Contained in both computational and biochemical modules or does not pertain to any singular protocol but to the CURE as a whole		LR11	MR3,	MR1, MR5, MR14, MR19, MR20, LR3, LR9	LR2, LR5	MR12, MR18, MR24, LR4, LR7, LR8	**TR5**, MR6, MR23	

In contrast to some traditional labs where students may be graded according to how well they purify a protein, determine constants, perform SDS-PAGE, or recite relevant biochemical principals or parameters, most of the CURAs in Table 3.1 do not simply address technical skills associated with the procedures of a protocol. Instead, they are also focused on the types of reasoning and problem solving needed for the discovery of new knowledge. To achieve this each of these discovery-type CURAs requires students to integrate a wide range of technical, procedural and conceptual knowledge and to apply their cognitive abilities to solving problems. For example, TR5 requires students to compare enzymatic results with those computationally predicted to

discover the function of a protein of known structure but unknown function, while MR22 requires students to discover the substrate of a protein by distinguishing between ligands that simply bind the enzyme and those that can also be processed by the enzyme. Thus, the CURAs in Table 3.1 constitute a wide range of discovery skills that researchers deploy when doing this type of research. This require the integration of technical, procedural and conceptual knowledge in ways that are not usually developed in traditional student labs and which can lead to novel research findings.

In conclusion, the data in Table 3.1 suggest that the BASIL CURE, just like most traditional biochemistry labs, covers the development of students' technical/procedural and conceptual knowledge, but in addition it also targets the integration of such knowledge and the development of discovery skills that characterize good research. Of course, no training in research should be without the essential technical skills but good research training also needs to focus on students' abilities to use such knowledge to solve novel problems and to discover new knowledge—the goal of this study and of the BASIL CURE.

3.6 An ALO Matrix of the CURAs

After the CURA statements were ranked and the wording was finalized (Table 3.1), the statements were grouped into categories according to common themes. Patterns emerged by looking across the groupings of CURA statements and identifying the common theme underlying a particular group. This was done first with the middle-rated CURAs because this group contained the most ALOs. Subsequently, the top-rated and lower-rated statements were placed into the themes already generated and new themes were added if needed. After all of the CURA statements were organized into their underlying theme or, in some cases, themes (see columns in Table 3.2), they were then further organized by aligning them with one or more BASIL protocols (rows, Table 3.2) (Craig, 2017; Craig et al., 2018). Protocols were aligned with CURAs that pertained to specific experiments and activities covered by the protocol.

The grouping of CURA statements resulted in six initial themes: (i) Hypothesizing the location and function of an enzyme active site, (ii) Proposing a particular method based on considerations of the pros and cons of different methods, (iii) Interpreting data to understand a biochemical meaning, (iv) Rationalizing the design of candidate substrates, (v) Visualizing and determining key components of protein structure, and (vi) Relating multiple types of data to reach a singular conclusion. When the top- and lower-rated CURAs were sorted into these themes, a

seventh theme, “Understanding the biochemical theory behind methods,” was needed to accommodate all the CURAs, specifically TR1, and TR7 (Table 3.2). When the new theme was checked to see if any of the middle- or lower-rated CURAs should be moved to this new theme there were no such cases. These classifications were used as the first dimension of the ALO matrix (Table 3.2, columns).

The CURA statements were then organized according to the protocols of the BASIL CURE (Table 3.2, rows). As mentioned previously, CURAs were only aligned with a specific protocol if the activities of a protocol specifically utilized a particular CURA. For example, MR7 is related to protein expression, because information from the SDS-PAGE gel will give insight into how well the expression worked. However, only during the SDS-PAGE protocols are gels and the types of information you can get from them discussed in detail. Thus, MR7 is associated only with the SDS-PAGE protocol and not the expression protocol, even though MR7 will produce information about the expression (Table 3.2). Additionally, several CURAs were not aligned with any protocol specifically. Instead, they were aligned as being *Contained in both computational and biochemical modules or does not pertain to any singular protocol but to the CURE as a whole.* Examples are TR5 because it is concerned with comparing enzymatic results with computational results; MR3 that is concerned with keeping a protein from denaturing which pertains to all biochemical protocols; and, LR9 which corresponds to the ability to make a connection between a representation and what is actually happening, as this can relate to any BASIL CURE protocol that produces a representation like a gel image or a graph of the findings.

Table 3.2 shows how all the CURA statements were organized into an ALO matrix. Note that two protocols, protein expression and protein concentration, were not specifically associated with any of the CURA statements. This is not to say that there were no CURAs related to these protocols. For example, MR3, LR3, and LR9 (Tables 3.1 and 3.2) are related to both of these two protocols and to every other biochemical protocol. Instead, rather, this finding suggests that protocols like protein expression and protein concentration were focused on procedural training that would be insufficient on their own at addressing the research goals of the BASIL CURE, which are aimed at developing students’ competence to do discovery-type research. Thus, there was a need for the row of ALOs that cover both computational and biochemical modules or the CURE as a whole (bottom row in Table 3.2). The nature of a CURE is to resemble authentic research practices, and not to merely follow or repeat steps in a protocol. Therefore, CURAs focus on the reasoning abilities scientists employ when conducting similar research activities and not

just simply the procedural steps in the protocols (Irby, Pelaez, et al., 2018b). In other words, the CURAs encompass all aspects of the CURE and focus on the CURAs most pertinent to addressing research questions about determining the function of a protein with unknown function but known structure, not just what was specifically detailed in the protocols. For this reason, the computational protocols and the enzyme activity protocols have the most CURAs specifically aligned to them because these protocols are directly related to the goals of the research being conducted in the BASIL CURE, whereas more preparatory protocols (e.g. protein concentration) did not receive any specifically aligned CURAs (Table 3.2).

The ALO matrix (Table 3.2) shows the spectrum of coverage of CURA statements, as well as their current relevance to this CURE. In addition, the ALO matrix allows for the characterization of the unique features of this CURE and how it may fit into an institution's or a program's curriculum aimed at developing students into scientists.

3.7 How the BASIL CURE Themes Compare with Experimentation Competencies and Other Aspects of CUREs

The generation of the seven themes used to construct the ALO matrix (Table 3.2), also provided the opportunity to characterize the BASIL CURE relative to other published work in the field. For example, further analysis of our BASIL CURE themes revealed that they align well with the list of ACE-Bio competencies of biological experimentation put forth by the ACE-Bio network (Nancy Pelaez et al., 2017), as well as with the aspects that define a CURE identified by Auchincloss et al. (2014) and Corwin, Runyon, et al. (2015) (Table 3.3).

These alignments were initially based on the specific CURE themes, and then checked against the CURA statements that comprise them. The association between the themes and the ACE-Bio competencies and the CURE aspects were not mutually exclusive, meaning that multiple ACE-Bio competencies and/or CURE aspects can be associated with a singular theme and vice versa. For instance, the ACE-Bio competency of “analyze” and the CURE aspect of “use of scientific practices” both have to do with processing and making decisions about data, which is a prominent theme associated with many components of the BASIL CURE (Table 3.3). For example, students propose a hypothesis for their protein's function, but they produce and analyze data to do so (Craig, 2017; Craig et al., 2018; Irby, Pelaez, et al., 2018b). Thus, the theme of *Hypothesizing the location and function of an enzyme active site* was associated with the ACE-Bio

competencies of “analyze” and “question” as well as the CURE aspect of “use of scientific practices” because it covers asking questions as well as processing data (Table 3.3). Interestingly, the BASIL CURE themes 3–5 (Tables 3.2 and 3.3) that only contain the ACE-Bio competency of “analyze” did not contain any top-rated CURAs. However, the themes that combined “analyze” with “question” (theme 1) or “conclude” (theme 6, Tables 3.2 and 3.3) contain top-rated CURAs, which implies that the participants valued those CURAs, that require students to use their analytical and questioning skills, as a key part of the course for learning how to conduct scientific research. It also suggests that competent researchers only perform data analysis with reference to a purpose such as to answer a research question or to draw conclusions. Thus, assessments should link across areas of competence and not simply focus on one area such as data analysis.

The fact that all of these efforts align is unsurprising because they have similar goals, but this alignment adds merit to the themes identified for this CURE. It was also not a surprise that no themes of this CURE aligned with the ACE-Bio competencies of conduct and communicate (Nancy Pelaez et al., 2017) (Table 3.3). This is because in this study the CURAs were generated by focusing on the reasoning abilities pertaining to this type of research (Irby, Pelaez, et al., 2018b), and did not explicitly target technical skills or research communication. Additionally, for the same reason, the CURE aspects (Auchincloss et al., 2014; Corwin, Runyon, et al., 2015) of broad relevance and collaboration were also not associated with a BASIL CURE theme (Table 3.3). This is not to say that students do not engage in such activities. On the contrary, the ability to communicate, collaborate, and conduct experiments to collect novel data are important aspects built into the course (Craig, 2017; Craig et al., 2018).

The seven ACE-Bio competency areas (Table 3.3) are big picture ideas that are too complex to easily assess in students. For this reason, the authors Pelaez et al. (2017) unpacked each competency area to identify numerous sub-competencies that are readily assessable. A similar problem exists regarding the identified CURE aspects (Auchincloss et al., 2014; Corwin, Runyon, et al., 2015) in that they too are higher level abilities, composed of numerous sub-abilities, which still need to be unpacked before effective assessments could be designed. In an analogous manner, in the present study, we have identified themes that because of their complexity will be more difficult to thoroughly assess but, as shown in the next section, the constituent CURAs can be readily assessed.

In our view, the above discussion provides an explanation for why the vast majority of published research competencies lack adequate assessments and, therefore, a strong motivation for

why assessment development should be an important target for future research in this area. Toward this end, we are actively engaged in developing and validating assessments that target our identified CURAs. An example of such an assessment task is presented in the next section.

Table 3.3 A comparison of the CURE themes for the BASIL with other documented experimental competencies and CURE aspects.

CURE Themes for the BASIL CURE Based on Identified CURA Statements	**ACE-Bio†* Competencies of Biological Experimentation** *Pelaez et al. (2017)*	**CURE Aspects†*** *Proposed by Auchincloss et al. (2014) to define what a CURE is. Definitions of the aspects came from Corwin, Runyon, et al. (2015).*
1. Hypothesize the location and function of an enzyme active site	**Analyze:** The ability to analyze and process data. **Question**: The ability to generate a research question and formulate hypotheses.	**Use of science practices:** The degree to which students engage in asking questions, building and evaluating models, proposing hypotheses, designing studies, selecting methods, gathering and analyzing data, and developing and critiquing interpretations and arguments. Students are likely to engage in several but not all scientific practices during a single CURE.
2. Propose a particular method based on considerations of the pros and cons of different methods	**Plan**: The ability to plan feasible and ethical experiments to answer research questions or test hypotheses.	**Use of science practices** **Iteration:** The degree to which students have opportunities to revise or repeat aspects of their work to fix problems, improve validity of their own and others' results, understand variation in data, or further test hypotheses.
3. Interpret data to understand a biochemical meaning	**Analyze**	**Use of science practices** **Discovery:** The degree to which students have opportunities to generate new scientific knowledge.
4. Rationalize the design of candidate substrates		
5. Visualize and determine key components of protein structure		
6. Relate multiple types of data to reach a singular conclusion	**Analyze** **Conclude**: The ability to conclude about data with inferences that are limited to the scope inherent in the experimental design.	**Use of science practices** **Discovery**
7. Understand the biochemical theory behind methods	**Identify**: The ability to identify gaps or limitations in current research knowledge through the review, filtering and synthesis of relevant literature.	**Use of science practices**
[redacted]	**Conduct**: The ability to conduct an investigation to achieve research goals.	**Use of science practices** **Iteration**
	Communicate: The ability to communicate research work in professionally appropriate modes, including visual, written, and oral formats.	**Broad relevance:** The degree to which students' work is of interest to a community beyond the classroom, which can manifest as authorship on a scientific paper or presentations or reports to stakeholders.
	Communicate	**Collaboration:** The degree to which students are encouraged to work together, help each other, build off one an- other's work, and provide and respond to feedback.

†Definitions for the ACE-Bio competencies (Nancy Pelaez et al., 2017) and CURE aspects (Corwin, Runyon, et al., 2015) were copied verbatim.

*In cases where there is more than one ACE-Bio competency or CURE aspect, the item is only defined the first time it appears.

3.8 Use of the ALO Matrix to Inform Assessment Design

In this section we demonstrate how the ALO matrix could be used by instructors in different ways to inform the design of student assessment tasks for the BASIL CURE. For example, if instructors wish to develop assessment tasks for any particular protocol, they can look along the corresponding rows in the matrix and design questions that focus on the listed CURAs. Similarly, if they wish to develop assessment tasks for any particular theme, all they need to do is look down the relevant column and focus their efforts on the listed CURAs. The ALO matrix also allows simultaneous comparison of both dimensions to guide the development of cross-protocol and/or cross-theme assessments. In all cases, the ultimate challenge is to design assessment tasks that specifically require and stimulate students to use the targeted CURAs in their answers. This can only be fully confirmed by reciprocal analysis of student answers and modification of the tasks till the task achieves its desired goals. The detail of such assessment validation is beyond the scope of this article. Instead, the assessment example provided below is intended to illustrate to what extent selected ALOs can be confirmed as VLOs through analysis of a student answer. But it is important to mention at this stage that students frequently include far less in their answers than they actually know about the problem which requires modifications to the task to facilitate more comprehensive answers. This problem is often resolved by including statements in the question like, “Include the following words or terms in your answer […].”

To illustrate the use of the matrix for assessment design we provide here an example of a student task, that was designed to incorporate CURA statements TR1, TR3, TR5, and TR6 (Figure 3.1). In so doing, the question is intended to focus on three themes of the BASIL CURE (themes 2, 6, and 7, Table 3.3) as well as the enzyme activity protocol while also requiring both computational and biochemistry knowledge (See last row of Table 3.2).

In the assessment task (Figure 3.1, students are asked to compare the results of an enzyme assay (Figure 3.1A, graph) with outputs from various computational programs and databases to predict the enzyme class (EC) of the protein of interest (Figure 3.1A, table). The data provided is similar to data that students would have generated and used as evidence when they were investigating a possible function of their assigned proteins as part of the BASIL CURE. In contrast, a discipline-based educational researcher (DBER) might have independently drafted a novel assessment task (termed a research probe) in the context of a real-life research situation that might differ from the CURE context. Appropriate background information would be provided to give all

of the conceptual subject matter knowledge required to answer the question. The research would involve validating how well the item would measure research competence (and not confounding subject matter knowledge) by conducting case study oral interviews about the novel assessment. Data could have been gathered to optimize the probe for measuring research abilities in general, and then to determine how well the assessment reveals the nature of expert knowledge and visualizations that are critical to research competence, using expert responses to the assessment for comparison to establish a measurement scale for the development of expert-like research behaviors in students (Dasgupta et al., 2016). In contrast, the case example discussed here is based on a graph that was provided by an instructor for a particular course context. Thus, the data analysis expected of students is limited to the context of the BASIL CURE, where the research goal was for students to answer a question about enzymatic activity of a protein. The students were doing research to identify if there is evidence that the protein of interest is acting like a hydrolase and to compare the assay results to computational results and not to do a formal kinetic analysis of their protein. This is also evident in the example of the CURA statement that was changed in the member check step to focus on activity and not on formal kinetics. The scope of activities that are typically done in the BASIL CURE involves assessing whether or not their protein of interest shows evidence of activity, since the students do not have enough time for a formal study of the enzyme kinetics. A few great students might respond to the item in Figure 3.1A by suggesting that the slopes could be used for a Michaelis–Menten plot, if this enzyme had been purified, by graphing these velocities on the y-axis against each substrate concentration on the x axis (LR6 in Table 3.2), As these lines might represent initial velocities. But for the BASIL CURE, the expected response from students who completed the course would be for the student to recognize that each curve could be aligned with best-fit straight lines where each slope represents a different rate of product production for each substrate concentration (Figure 3.1B, items 6 and 10).

The ALOs that this question is targeting involve the ability to identify and use evidence from an activity assay to determine the function of the protein of interest. If the student has truly developed the ALOs covered by this assessment (i.e. the ALOs qualified as VLOs), he or she would be expected to be able to discuss how the assay works (TR1), interpret important data about the protein's function (TR3), be aware of what experimental information is included or needed to make a conclusion (TR6), and relate the enzyme assay (Figure 3.1 A, graph) and computational data (Figure 3.1A, table) to see if they support each other (TR5) in predicting the function of the enzyme.

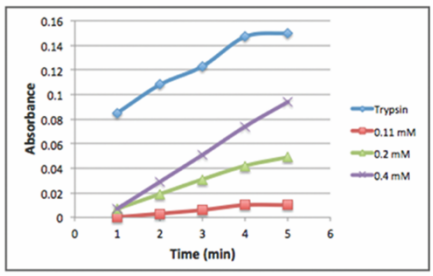
A

Compare the graph and computational outputs for a protein of interest, 3H04. The graph shows a constant concentration of 3H04 with different concentrations of p-nitrophenyl acetate (PNPA) and a positive control (Trypsin).

How does the computational prediction and the results from the assay compare? In your explanation provide justification for whether there is evidence that this protein interacts with this substrate, or not. In so doing describe:

- **the relevant biochemical concepts used when interpreting these results, and**
- **the process you used when comparing them.**

Additionally, be sure to cite the specific information you used to reach your conclusion, as well as any additional information you would have liked to have to help you reach your conclusion. Explain your reasoning in every case.



3H04	
ProMOL	**EC** 3.4.11.5, 3.7.1.8, 3.4.21.26 Hydrolase
BLAST, Dali, and Pfam common function	**EC** 3 or 3.1.1 Alpha/beta hydrolase or esterase

B

Examples of key points expected in student answers:

1. The computational results agree with one another; they all give the same EC class (EC3) which suggests the protein is a hydrolase.
2. If PNPA is hydrolyzed there will be a color change, which can be detected at a specific wavelength.
3. Trypsin is said to be a positive control, as it is a known hydrolase. However, it is necessary to know if PNPA (the substrate) was used in the trypsin assay, because the positive control should measure trypsin activity.
4. There were interactions between the protein and PNPA as can be seen by the increase in absorbance.
5. Higher concentrations of PNPA with 3H04 give corresponding higher absorbance values.
6. Increasing the concentration of PNPA increased the rate of product production.
7. Like for trypsin, for 3H04 there was an increase in absorbance over time suggesting it behaves like a hydrolase.
8. The assay appears to confirm the predictions made from the computational software results.
9. This assay does not include a negative control, which is needed to prove that these are true positives. A better study would include measures of absorbance over time (the rate of product accumulation) with the same 3 PNPA concentrations in the assay, but without any 3H04.
10. Other useful information that would help with this comparison would be: concentration of 3H04 and trypsin, optimizing the assay with constant PNPA concentration but different 3H04 and trypsin concentrations, determine why the trypsin rate of product accumulation is similar to 3H04 yet background absorbance is above zero, define the wavelength, for each curve find a best-fit straight line, etc.

C

Example of an actual student answer:

Just glancing over the data there seems to be activity occurring since there is an increase in absorbance as the reaction proceeds showing that assay was reacting with the product which would correlate to a higher absorbance when recorded.[4,7] The data is also linear as it should be because as the protein works on the substrate the concentration of the product would steadily increase.[2] There is also a correlation between the absorbance and the concentration of the protein as the concentration increases the rate of change of absorbance also changes drastically so we see three separate lines.[5,6] Since there is no crisscrossing of the lines there is nothing to worry about protein stability and the rate at which the reactions are occurring so good data is being obtained. Since all of these things are present I can say that this protein definitely interacts with this substrate.[7] Since it reacts with this substrate then the computational data was correct in assessing this as a hydrolase.[8]

Figure 3.1 Example of an assessment task developed to cover four CURA statements, TR1, TR3, TR5, and TR6.

The assessment item (panel A) is accompanied by the expected key points for students to include (panel B) and an example of a student answer (panel C). In panel C superscripts indicate where the student incorporated one of the corresponding numbered anticipated key points from panel B. The prompt (panel A) includes data from an instructor who was teaching students to identify if there is evidence that a protein of interest is acting like a hydrolase and to compare the assay results to computational results, and not to do a formal kinetic analysis of their protein of interest, which in this example is 3H04. The item includes some ambiguity that gives the opportunity to see how a student deals with real-life messy data which a scientist might gather to answer their research question.

Figure 3.1B, lists 10 key points which instructors expected students to include in their answers to demonstrate whether they have achieved the applicable ALOs. Of course, the open-ended nature of the assessment task means that students may come up with other acceptable responses not listed here. This supports the idea that there can often be multiple paths to resolving or addressing a research question, which typifies part of the nature of research abilities that we are attempting to develop in our students. The expected answer within the context of the BASIL CURE combines "analyze" with "question" (theme 1) or "conclude" (theme 6, Tables 3.2 and 3.3) as top-rated CURAs, which confirms that to do competent research in this program, students are expected to combine use of their analytical and questioning skills or their analytical and concluding skills, and not to simply "analyze" the data in terms of enzyme kinetics, which would not be a first step for characterizing proteins of known structure but unknown function.

One student response to the question (See Figure 3.1C) from a biochemistry junior who had previously completed the BASIL CURE gives some initial evidence to support the fact that the ALOs are at least being partially achieved in this student (i.e. the ALOs are being partially confirmed as VLOs). The student included six of the anticipated key points listed, indicated by the superscripts (Figure 3.1B and 3.1C). The student's response is primarily focused on the data presented about the protein of interest, primarily eliciting CURAs TR1, TR3, and TR5, and to a lesser extent TR6. For example, the student makes several connections to data trends saying things like "there is also a correlation between the absorbance and the concentration of the protein" (Figure 3.1C), but the student does not mention experimental parameters such as what wavelength the measurements were done or which controls (either present or not present) were used in the enzyme assay. In fact, there is some ambiguity in the item regarding the trypsin curve, which gives the opportunity to see how the student will deal with real-life issues. The assessment prompt identifies "trypsin" as "a positive control." Trypsin is a positive control because it is a known hydrolase, but trypsin is a protein thus it can only give "positive control" measures if p-nitrophenyl acetate (PNPA) had been included as a substrate for the trypsin curve assay. A competent research student might express concerns about the raised trypsin curve absorbance or the failure of a best-fit line to indicate zero absorbance at the zero time point for the trypsin curve. Under Figure 3.1 part B for point nine, there is mention of a negative control. An expected response would identify the need to show that PNPA on its own does not show any change in absorbance, which happens only when its hydrolysis is catalyzed by an enzyme. A response from a great student might identify the need to measure the change in absorbance over time (the rate of product accumulation) with

an assay that uses the same three different PNPA concentrations but without any 3H04 or with some protein like BSA in place of 3H04 in the solution. However, instead of identifying the need for better positive and negative controls, the following phrases in this student's response suggests some difficulties. In line two, “the assay was reacting with the product” suggests that this student does not know what “assay” means, and lines four to five “a correlation and the concentration of the protein” suggests that the student does not know that 3H04, and not PNPA, is the protein in the assay solution. Lastly, in the student response (Figure 3.1C), there is an underlined passage that raises questions about the protein's stability and the quality of the data. This was an unanticipated response but is a significant observation made by the student that demonstrates their ability to think about the data presented, showing signs of developing scientific thinking.

Although at this stage only one example of an assessment is presented with only one student response, there is clearly partial evidence that a well-designed assessment task based on data from an instructor and within the research context for a particular CURE could successfully confirm that some of the CURAs identified by instructors as ALOs could be considered as VLOs (Irby, Pelaez, et al., 2018b), whereas previously unsuspected student difficulties may need to be addressed. But in line with the tenets of sound assessment (e.g. Anderson, 2007), in the case of all assessment development for the BASIL CURE, instructors will find that several different assessment tasks will be required to cover the full range of CURAs with any one theme or protocol. Furthermore, they will likely find that a single task will seldom just assess one CURA but rather more than one related CURA of importance to a particular protocol. Thus, instructors will find that a range of assessment tasks will be required to achieve a comprehensive and more complete assessment of student learning during the BASIL CURE. In addition, it will be important to develop a range of assessment types (e.g. open-ended, scenario-type, multiple choice questions) to assess different types of knowledge and also to consider for whom the measures are valid as different students might answer different types of questions more competently (Anderson, 2007). As a result of assessment responses, more pertinent ALOs may emerge as the CURE progresses as students begin spending less time troubleshooting and assigning the type of function to focus more on specific substrates and formal kinetics. The focus currently is not to answer research questions that compare the kinetics of trypsin and 3H04 directly to determine which is a better enzyme. Rather, the current research goal is to detect the presence of hydrolase activity, as PNPA is likely not the preferred substrate for the protein of interest, in this case, 3H04. Thus, some of the CURAs that pertain to explicit kinetics were not rated highly at this time (LR6, LR10, and LR12

in Table 3.2), but this also provides a good example of the point that a different set of CURAs may become more relevant as the nature of the research advances and the focus of the research questions shifts. Given the potential for ALOs that change over time, a detailed discussion of more generalized assessment validation for CUREs is beyond the scope of this article but will be the focus of future work as novel assessment tasks are developed and validated around the identified CURAs.

3.9 Summary and Conclusions

In our view, the results of this study successfully addressed our research question: Which CURAs do the instructors of the BASIL CURE consider the most relevant learning outcomes and how do these compare to other published descriptions of CUREs and experimental competencies? We used PICURA to successfully identify 43 CURAs (Table 3.1) which were categorized by instructors as either top-, middle- or low-rated using a previously reported weighted-relevance scoring approach (Irby, Pelaez, et al., 2018b). The CURAs were then organized into a two-dimensional (2D) ALO matrix with each ALO mapped to 10 lab protocols as well as seven "big idea" themes of importance to the BASIL CURE (Table 3.2). By means of a specific example, we illustrated how the matrix might be used to inform the design of a cross-theme assessment task within one probing task that focuses on revealing student development of four top-rated CURAs. We also demonstrated with an example student answer how one might begin to validate the assessment task by checking whether the CURAs that instructors anticipated students will learn (ALOs) are actually being developed in students (i.e. VLOs), as demonstrated by their use of the targeted CURAs in the answer. We also emphasized that for optimal validation of the task, the task should reveal evidence of both sound student knowledge and a range of difficulties with the CURA, and that with a much larger sample of student responses, the information gathered might suggest a need to change a CURA or to refine the research instruction. We also illustrated how ambiguity in an assessment item might prompt deeper thinking in a student response about the research process. On the other hand, the data may reveal flaws in the probing task itself, necessitating modification of the task and its retesting until good alignment is achieved between the CURAs, the assessment task and the student answers that confirm that the targeted CURA is being thoroughly assessed in the students.

A key achievement of this project has been to identify CURAs that constitute a shift in focus from just typical procedural or technical knowledge, which is commonly found in most biochemistry lab courses, toward a greater emphasis on the discovery part of research competence, which involves alignment of data analysis to a research question and conclusions based on evidence from that data. That is not to say that technical skills and procedural knowledge are not important. On the contrary, as discussed above, none of the discovery-type CURAs listed in Table 3.1 can be mastered by students without also developing the technical competence that allow them to achieve such goals. Thus, the nature of the identified CURAs support the fact that instructors believe/anticipate that their students are developing discovery skills and research abilities that require students to reason about knowledge and to solve novel problems within the themes of the research project, much like authentic research practice. This contention is supported by the fact that our CURAs compare well (Table 3.3) to those identified in various other projects including the ACE-Bio network (Nancy Pelaez et al., 2017). By presenting preliminary evidence that our CURAs are assessable we also expose the need to unpack the various complex CURE aspects or "big idea" competencies published by Auchincloss et al. (2014) and Corwin et al. (2015) into more specific sets of connected abilities that could be assessable. Clearly, though, we believe that our work has further emphasized the urgent need to develop more validated assessments of the various research discovery abilities currently being published in the CURE literature, and how to accomplish that task. Whereas, technical and procedural-type abilities are more directly and easily assessable by simply observing students in the lab, or grading their lab notebooks, to see if they have mastered such abilities, discovery-type CURAs are far more complex and require specially designed assessment tasks to gauge whether students have developed the desired research competence. Assessments of this nature will also enable better evaluation and characterization of CUREs which is an important future step for CURE projects (Bell et al., 2017; Brownell & Kloser, 2015; Irby, Pelaez, et al., 2018b; NRC, 2001; Shortlidge & Brownell, 2016).

Although this study has successfully identified the ALOs or specific CURAs that instructors teaching the BASIL CURE consider important for students to learn, until such a time when we have developed a full range of assessments to target those CURAs and obtained student responses to them, we will not be in a position to claim with any surety that our students are actually developing such research knowledge and abilities (i.e. VLOs). This will require a longer-term study involving the meticulous development, validation, and testing of assessments that target all the CURAs and any other CURAs that may be identified by future instructors at the same or

other institutions. We do, however, believe that through this study and the research published elsewhere (see Irby, Pelaez, et al., 2018b), and the initial analysis of a response on a singular assessment item (Figure 3.1), we have taken the essential first steps toward achieving our goals.

The CURA statements generated by PICURA (Irby, Pelaez, et al., 2018b) are intended to guide researchers' investigations into student learning within this specific BASIL CURE. The ranked CURAs serve as a way to focus attention on the CURAs that in the opinion of the instructors, currently teaching this course, have the most consensus of relevance to the CURE. The AOL matrix of CURAs presented in this article constitutes the consensus opinions of 10 instructors at this point in time, following implementation of our biochemistry CURE at specific institutions for a limited number of semesters. It is likely that the list of CURAs will change as instructors gain more experience and the labs are modified or improved to stay current. Additionally, we do not claim that this list of CURAs is exhaustive as it is likely that other instructors in the future will come up with other CURAs that they consider more important. Thus, we recommend that the PICURA process should be repeated when deemed necessary (Irby, Pelaez, et al., 2018b).

Readers could benefit in multiple ways from the results of this study. Firstly, by implementing the BASIL CURE (Craig, 2017; Craig et al., 2018; Irby, Pelaez, et al., 2018b) at their own institution, their students would stand to develop the wide range of CURAs identified here that could have a significant impact on their future performance as researchers. Secondly, instructors of other CUREs, whether in biochemistry or other discipline areas, could use PICURA and the weighted relevance approach (Irby, Pelaez, et al., 2018b) we have presented in this article to identify their own CURAs. Thirdly, instructors and researchers participating in CURE projects could take our idea of focusing more on discovery-type research abilities rather than only technical and procedural type knowledge that characterizes so many of our traditional undergraduate biochemistry labs. Fourthly, in view of the dearth of good available assessments for CUREs, we strongly recommend that all participants in CURE research and instruction consider embarking upon extensive assessment development and validation projects, which target identified CURAs, on similar lines to what we advocate in this article. Finally, in so doing, future instructors will focus their assessment tasks on assessable research abilities that emerge from unpacking the big CURE ideas.

3.10 Acknowledgements

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3.11 Supporting Information

3.11.1 CURA Ratings, Cut-offs, and Final Refinements

The final step of PICURA was the two-tier Likert survey to determine the most important CURAs for the CURE as determined by weighted-relevance (WR). The 44 CURA statements were placed in three WR ranked groups: top-, middle-, and lower-rated (TR, MR, and LR, respectively) CURA statements. The TR CURA statements ranged from a WR score of +16.5 to +18 (Table 3.4). The top-rated group's lower cutoff was set to be greater than +16; CURAs receiving a WR score above +16 are greater than 80% (16/20) of the possible positive WR scores. This yielded a total of nine TR CURA statements. These statements were examined and reduced to a set of 7 top-rated CURAs. First, ability B9 was removed because of its overlap with TR1 (B3), as both covered colorimetric enzyme assays and would likely be covered by identical assessments, resulting in now 43 CURA statements (Table 3.4). Additionally, C3 was removed from the top-rated set of CURAs because it had one participant rating it as not part of their course (tier 1). C3 also received a disproportional fraction of its WR score from tier 2 compared to other CURAs in the top-rated group (Tables 3.1 and 3.4). The cut-off between the middle-rated (Table 3.5) and lower-rated (Table 3.6) CURA statements was set to a WR score of +10 (half of the positive range). It is important to note that the highest-rated CURA (TR1, Tables 3.1 and 3.4) had a WR of +18 and the lowest-rated CURA (Table 3.6) had a WR of +3.5, which, since the WR scale for this study ranges

from -20 to +20, meant PICURA was able to reliably identify relevant CURAs as all scores were positive.

Table 3.4 Top-rated CURAs based on weighted-relevance (WR) from course instructors' and designers' responses to the two-tier Likert abilities survey. The wording of the CURA statements is the wording used for the survey and is not necessarily the final wording (see Table 3.1 for final wording of CURAs).

CURA Statements†	**Likert Survey Question Tier 1**			**Likert Survey Question Tier 2**			**WR**
	NOT acquired in this lab course	In BOTH this lab and some other course	ONLY in this lab course	Unimportant	Undecided	Important	
TR1 (B3): Explain how the colorimetric enzyme assay works to allow detection of protein function	0	2	8	0	2	8	**+18**
TR2 (C9): Identify an enzyme active site using appropriate computational programs	0	0	10	1	1	8	**+17.5**
TR3 (B6): Determine the appropriate factors to consider when optimizing or interpreting an enzyme assay	0	2	8	0	3	7	**+17.5**
TR4 (C4): Determine using computational software whether, and where a ligand may be binding to a protein	0	0	10	1	2	7	**+17**
TR5 (C8): Compare enzymatic results with those computationally predicted	0	0	10	1	2	7	**+17**
TR6 (B1): Design an enzyme assay to elucidate protein function	0	4	6	0	3	7	**+16.5**
TR7 (B4): Explain how His-tag purification works and ways the process can be optimized	0	4	6	0	3	7	**+16.5**
CURA Statements Removed from the Set of Top-Rated Abilities							
(B9): Use an enzyme assay and absorbance data to establish the presence of catalytic activity	0	4	6	0	2	8	**+17**
(C3): Assess the quality of data and how data gets altered when computationally manipulated	1	3	6	0	0	10	**+16.5**

† The TR indicator is based on WR rank and the letters in parenthesis stands for the ability category the CURA statement was grouped in: biochemistry research protocols (B), computational methods (C), enzyme functions and properties (E), molecular models (M), and plotting or interpreting data (P).

3.11.2 Middle and Low Scoring CURA Statements Weighted-Relevance Scores

Table 3.5 Middle-rated (MR) CURAs based on WR from course instructors' and designers' responses to the Likert survey (PICURA Step 5).

CURA Statement	**Likert Survey Question Tier 1**			**Likert Survey Question Tier 2**			WR
	NOT acquired in this lab course	In BOTH this lab and some other course	ONLY in this lab course	Unimportant	Undecided	Important	
MR1	1	3	6	0	0	10	**+16.5**
MR2	0	2	8	1	2	7	**+16**
MR3	0	6	4	0	2	8	**+16**
MR4	0	4	6	1	1	8	**+15.5**
MR5	0	4	6	1	1	8	**+15.5**
MR6	1	2	7	1	0	9	**+15**
MR7	0	8	2	0	2	8	**+15**
MR8	0	5	5	1	2	7	**+14.5**
MR9	0	6	4	1	1	8	**+14.5**
MR10	0	1	9	2	3	5	**+14**
MR11	1	6	3	0	2	8	**+14**
MR12	1	7	2	0	1	9	**+14**
MR13	1	2	7	1	3	6	**+13.5**
MR14	0	6	4	1	3	6	**+13.5**
MR15	1	2	7	2	0	8	**+13**
MR16	1	0	9	2	3	5	**+12.5**
MR17	1	4	5	1	3	6	**+12.5**
MR18	1	8	1	1	1	8	**+11.5**
MR19	1	9	0	1	0	9	**+11.5**
MR20	2	8	0	0	1	9	**+11.5**
MR21	1	2	7	2	3	5	**+11.5**
MR22	2	1	7	2	2	6	**+10.5**
MR23	1	5	4	2	2	6	**+10.5**
MR24	2	2	6	2	1	7	**+10.5**

Table 3.6 Lower-rated (LR) CURAs based on WR from course instructors' and designers' responses to the Likert survey (PICURA Step 5).

CURA Statement	**Likert Survey Question Tier 1**			**Likert Survey Question Tier 2**			WR
	NOT acquired in this lab course	In BOTH this lab and some other course	ONLY in this lab course	Unimportant	Undecided	Important	
LR1	2	6	2	1	2	7	**+10**
LR2	2	2	6	2	3	5	**+9.5**
LR3	2	8	0	1	1	8	**+9.5**
LR4	2	4	4	2	2	6	**+9**
LR5	2	5	3	2	1	7	**+9**
LR6	2	5	3	2	3	5	**+8**
LR7	3	4	3	1	4	5	**+8**
LR8	3	6	1	1	2	7	**+8**
LR9	2	5	3	2	5	3	**+7**
LR10	2	7	1	2	3	5	**+7**
LR11	3	3	4	3	1	6	**+6**
LR12	2	6	2	4	3	3	**+3.5**

3.11.3 CURAs Organized by BASIL CURE Themes

CURA statements organized by CURE theme identified for the BASIL CURE. The CURA statements are listed in order of weighted-relevance score (numbers in parenthesis is the weighted-relevance score).

1. Hypothesize the location and function of an enzyme active site

- **TR2**: Identify an enzyme active site using appropriate computational programs. (+17.5)
- **TR4**: Determine using computational software whether, and where, a ligand may be binding to a protein. (+17)
- **MR2**: Demonstrate whether a particular substrate binds to an active site (+16)
- **MR9**: Recognize parameters that will impact protein substrate interactions (+14.5)
- **MR16**: Determine using computational software where enzymatic activity may cause bond breaking. (+12.5)
- **MR22**: Distinguish between molecules that are good at binding to an enzyme versus those that could also be a substrate for that enzyme (+10.5)
- **LR11**: Determine the presence and nature of a transition state analog (+6)

2. Propose a particular method based on considerations of the pros and cons of different methods

- **TR3**: Determine the appropriate factors to consider when optimizing or interpreting an enzyme assay. (+17.5)
- **TR6**: Design an enzyme assay to elucidate protein function. (+16.5)
- **MR3**: Consider how to minimize protein denaturation when planning/performing experiments (+16)
- **MR4**: Optimize the reaction parameters (e.g. substrate and enzyme concentration, pH, temperature, etc.) that are essential for the occurrence of an enzyme-catalyzed process. (+15.5)
- **MR15**: Grasp the limitations of research methods based on homology (+13)

3. Interpret data to understand a biochemical meaning

- **MR1**: Assess the quality of data and how data gets altered when computationally manipulated (e.g. if going from a raw plot of data to a reciprocal plot) (+16.5)
- **MR5**: Connect the data from an enzyme assay to what the enzyme is actually doing. (+15.5)
- **MR7**: Use SDS-PAGE gels for interpreting information about a protein and its expression from a plasmid. (+15)
- **MR11**: Determine and interpret kinetic rates in light of saturation effects (+14)
- **MR14**: Relate a graphical representation of data from an enzyme assay to what can happen biologically with that enzyme. (+13.5)
- **MR19**: Recognize a bad data point on a graph of research data (+11.5)
- **MR20**: Recognize the different symbols used in a graph and their meaning (+11.5)
- **LR3**: Explain the relationship between a concept or a phenomenon and a mathematical equation representing that concept. (+9.5)
- **LR9**: Relate a biochemical representation of a structure or process to its real life practical meaning or interpretation (+7)
- **LR10**: Determine biochemically relevant constants (such as Vmax or Km) from a Lineweaver-Burke plot (+7)

4. Rationalize the design of candidate substrates

- **MR8**: Understand the effect of a residue's charge on substrate interactions with an enzyme. (+14.5)
- **MR10**: Propose modifications to ligand molecules to increase their binding affinity for a protein. (+14)
- **MR13**: Identify which ligands bind specifically to members of a particular protein family. (+13.5)
- **MR21**: Compare conditions to determine a binding interaction between a substrate and a protein (+11.5)
- **LR1**: Recognize the types of bonding interactions between an enzyme and its substrate (+10)
- **LR2**: Use protein and substrate electrostatic information to propose ways to improve binding (+9.5)
- **LR5**: Recognize how proteins that are closely related by evolution can have dramatically different functions (+9)

5. Visualize and determine key components of protein structure

- **MR12**: Recognize the different types of atoms and/or number of atoms present in a representation of a molecule (+14)
- **MR17**: Relate structurally conserved protein regions to their function (+12.5)
- **MR18**: Distinguish between the different components of an enzyme including amino acids; secondary, tertiary and quaternary structure and any non-proteinaceous components (+11.5)
- **MR24**: Use kinetic data to determine important parts of a protein's structure (e.g. binding pockets and/or catalytic residues) (+10.5)
- **LR4**: Identify the hydrogen bonds in a protein based on the properties of the atoms and their inter-atomic distances (+9)
- **LR7**: Recognize that the factors that determine protein structure and function happen from interactions throughout the protein and not just from neighboring residues (+8)
- **LR8**: Explain how secondary and tertiary structure is influenced by intramolecular forces within a protein (+8)

6. Relate multiple types of data to reach a singular conclusion

- **TR5**: Compare enzymatic results with those computationally predicted. (+17)
- **MR6**: Compare results of different computational methods to determine if they agree with each other (+15)
- **MR23**: Translate or map features between 2D and 3D representations of proteins (+10.5)
- **LR6**: Explain the strengths and weaknesses of a Michaelis-Menten plot and a Lineweaver-Burke plot and explain when each one is easier to use (+8)
- **LR12**: Relate a data point in a Michaelis-Menten plot to one on a Lineweaver-Burke plot (+3.5)

7. Understand the biochemical theory behind methods

- **TR1**: Explain how the colorimetric enzyme assay works to allow detection of protein function. (+18)
- **TR7**: Explain how the purification of tagged proteins work and ways the process can be optimized. (+16.5)

CHAPTER 4. STUDENT PERCEPTIONS OF THEIR GAINS IN COURSE-BASED UNDERGRADUATE RESEARCH ABILITIES IDENTIFIED AS THE ANTICIPATED LEARNING OUTCOMES FOR A BIOCHEMISTRY CURE

A version of this chapter is being prepared for submission as a stand-alone manuscript: **Irby, S. M.**, Pelaez, N. J., & Anderson, T. R. Student Perceptions of their Gains in Course-based Undergraduate Research Abilities Identified as the Anticipated Learning Outcomes (ALOs) for a Biochemistry CURE.

4.1 Abstract

Course-based Undergraduate Research Experiences (CUREs) are innovative teaching strategies that afford students the opportunity to conduct research as part of their coursework. The Biochemistry Authentic Scientific Inquiry Laboratory (BASIL) CURE, is an example of one of these courses where students use computational and biochemical techniques to conduct novel research to evaluate the function of proteins with known structure, but unknown function. The goal of this study was to investigate changes in students' perceived knowledge, experience, and confidence (KEC) regarding previously identified research abilities or anticipated learning outcomes (ALOs) for the BASIL CURE. Towards this goal we addressed the following research questions: **RQ1**) How do students' perceived knowledge, experience, and confidence (KEC) regarding the specific ALOs change across all implementations during the BASIL CURE? and **RQ2**) How do student perceptions of their KEC regarding specific ALOs from the BASIL CURE vary across different course structures and institutional contexts? To answer these RQs, we used a Participant Perception Indicator (PPI) survey to measure the change in students' KEC for the identified BAIL CURE ALOs. Participants in this study were students in one of ten courses implementing the BASIL CURE at seven different academic institutions. In general, there were significant gains across the BASIL ALOs investigated by this study, with large effect sizes, in students' perceptions of their KEC for the BASIL specific ALOs, as well as, the computational and biochemical techniques they were exposed to. Additionally, differences in responses were associated with the components of the BASIL CURE students participated in. This study is an important initial step toward assessing student learning in the BASIL CURE, because the way students perceived and experienced the BASIL CURE as well as what and how components of the BASIL CURE were taught will, in turn, impact actual learning and will inform how best to assess

actual student learning. Future work will focus on whether or not students can actually perform these research abilities (ALOs), to determine if they are verified learning outcomes (VLOs).

4.2 Introduction

Course-based Undergraduate Research Experiences (CUREs) are increasingly being incorporated into undergraduate programs to afford students more opportunities to develop their knowledge of how research is practiced (Auchincloss et al., 2014). CUREs have commonly been defined by the features (Auchincloss et al., 2014) or activities incorporated into them (Corwin, Graham, et al., 2015). For example, Auchincloss et al. (2014) describes CUREs as courses with the following features: collaboration, discovery, broad relevance, iteration, and use of science practices. These five features are reflected in the description of CUREs by others in the field (Brownell, Kloser, Fukami, & Shavelson, 2012; Irby, Pelaez, et al., 2018b; Lopatto & Tobias, 2010b; Weaver et al., 2008). In a recent study by our group (Irby, Pelaez, et al., 2018b) we have extended the definition as "as a course wherein students engage in activities resembling those done by scientists in a particular field to conduct novel investigations about relevant phenomena that are currently unknown." CUREs have been documented having a wide range of benefits to student learning; however, not all of these benefits are well established (Corwin, Graham, et al., 2015).

CURE curricula have been studied primarily by using Likert-scale self-reported survey data as a measure of student learning in and success of CUREs. For example, Shanle et al. (2016) used Likert surveys to determine whether students self-identified as a scientist and if students were confident with laboratory techniques as one method to evaluate student performance in a CURE. Lefurgy & Mundorff (2017) assessed student learning gains by having students rate their satisfaction and perceived benefit items ranging from "lab reports" to "knowledge of basic modern biochemistry laboratory techniques." Surveys have been used to measure the differences between CURE and non-CURE lab course students perceptions on collaboration, discover and relevance, and iteration (Corwin, Runyon, et al., 2015); as well as, the difference in the perceived degree of project ownership (Hanauer & Dolan, 2014). Lastly, others have used more general instruments such as the Survey of Undergraduate Research Experiences (SURE; David Lopatto, 2004, 2007) or the Undergraduate Research Student Self-Assessment (URSSA; Weston & Laursen, 2015) to assess students perceived competencies with general scientific traits research skills (Shaffer et al., 2014). Perception survey data, such as the examples discussed above, are useful as part of an

assessment and evaluation strategy and provide valuable information about how students perceive CUREs. They also can offer a convenient way to compare curricula and can help to identify areas that should be assessed further in specific courses. However, many of these studies are not paired with measures of actual student learning or are created based around specific course-based undergraduate research abilities (CURAs) for a CURE of interest (Irby, Pelaez, et al., 2018a, 2018b).

Though not a measure of actual student learning, self-reported perception surveys can give insight into what was experienced by a student within a course and can be used to investigate students' perceptions of their abilities. One method to do so is by using a multi-scale approach, such as a Participant Perception Indicator (PPI) survey (e.g. Berger & Carlson, 1988; Clase, Gundlach, & Pelaez, 2010; Glazer, 2015; Hensiek et al., 2016). A PPI instrument asks students to rate their Knowledge, Experience, and Confidence (KEC) regarding each of the items on the survey. PPI surveys and the type of data they collect emerged from Self-efficacy theory (Bandura, 1995; Lunenburg, 2011). A PPI survey is an important metric to use as a part of an assessment strategy because it measures a sense of ability which will impact a student's actual learning. An example of this is, if a student perceives that their KEC has increased, they may then also have a higher self-efficacy surrounding the abilities and techniques they learned from a CURE, which could make a student more likely to be willing and able to attempt to apply them. Gains in a student's self-efficacy could, in turn, positively impact their actual ability (Ross, 2006). PPI surveys on their own may provide better indicators of students' procedural knowledge (e.g. Hensiek et al., 2016), as students may be able to better gauge their KEC for technical skill, such as using a volumetric pipet, than their discovery-type knowledge. This is not to say that procedural knowledge is not an important part of research knowledge, but it is important for students to also study the more applied skills, such as the experimental or research abilities listed in Table 4.1 which we identified in a previous study (Irby, Pelaez, et al., 2018a). A PPI survey, or other self-reported forms of data, should be used in parallel with other measures of student learning, but can still give valuable insight into what students experienced within a course *(see model guiding this study,* Section 4.3). This is because there may be a greater degree of the Dunning-Kruger effect (Kruger & Dunning, 1999), due to students needing to understand the item fully and have introspective abilities, which is less of a problem for items that are more procedural and concrete (Dunning, 2011), and can lead to overconfidence (Rozenblit & Keil, 2002).

As mentioned above, self-reported surveys used to study CUREs tend to focus on general skills and do not use any processes to identify specific anticipated learning outcomes (ALOs) that are expected for students to develop during a CURE, such as the Process for Identifying Course-based Undergraduate Research Abilities (PICURA; Irby, Pelaez, et al., 2018b, 2018a). Using a method like PICURA to identify the ALOs, specifically Course-based Undergraduate Research abilities (CURAs) allows for understanding the experience of students in a specific CURE and what they perceived they learned. This is an important initial component for evaluating a CURE but should not be the only evaluation measure of student learning, since the ALOs used for the PPI are very involved, applied, abilities. PICURA has been performed previously for our CURE of interest, the Biochemistry Authentic Science Inquiry Lab (BASIL) CURE, which in turn permitted the characterization of the CURE and informed the assessment of student learning (see Table 4.1; Irby, Pelaez, et al., 2018b, 2018a).

Table 4.1 ALOs from the PPI survey

ALOs	Description	BASIL CURE Components	BASIL CURE Protocol(s)
ALO1	Explain how the colorimetric enzyme assay works to allow detection of protein function	Biochem (B)	Enzyme Activity
ALO2	Identify an enzyme active site using appropriate computational programs	Comp (C)	Pfam, ProMOL, PyRx
ALO3	Determine the appropriate factors to consider when optimizing or interpreting an enzyme assay	Biochem (B)	Enzyme Activity
ALO4	Determine using computational software whether, and where, a ligand may be binding to a protein	Comp (C)	PyRx
ALO5	Compare enzymatic results with those computationally predicted	Both (B/C)	Not pertained within any single protocol
ALO6	Design an enzyme assay to elucidate protein function	Biochem (B)	Enzyme Activity
ALO7	Explain how the purification of tagged proteins work and ways the process can be optimized	Biochem (B)	Protein Purification

4.3 Model Guiding This Study

According to Anderson (2007), there are four key components of the educational process: course objectives (or ALOs), teaching, learning, and assessment. Here we have adapted and expanded his model to help to explain how ALOs direct an “educational cycle” which can lead to verified learning outcomes (VLOs; Figure 4.1). This educational cycle, like the original model, put forth by Anderson (2007), describes the interplay between what and how, course content is taught,

learned, and assessed. Sound curriculum design should have these three activities aligned and based on well-defined learning outcomes for a particular course (e.g. Irby, Pelaez, et al., 2018a, 2018b).

Assessment is an essential component of both chemistry education research and practice (e.g. Anderson, 2007; NRC, 2001). What and how we assess learning should be informed by what and how students learn (Figure 4.1). For a specific course, it is crucial to also account for what and how course content was taught (Figure 4.1). In our previous work (Irby, Pelaez, et al., 2018a, 2018b), we have focused on rigorously identifying ALOs of the BASIL CURE to be used to develop measures to assess student learning for these ALOs (Table 4.1). In this study, we used a PPI survey to gain insight into the student experience from participating in different BASIL CURE implementations. The PPI data can reveal how the way the BASIL CURE was taught and what activities students were exposed to in a course implementing the BASIL CURE may impact how students perceived their learning. The focus of this study was on the highlighted dashed-arrows in the model (Figure 4.1), the PPI survey will reveal what students perceived they experienced in a variety of BASIL CURE courses (Tables 4.2 and 4.3) and helped to determine what will be appropriate to assess.

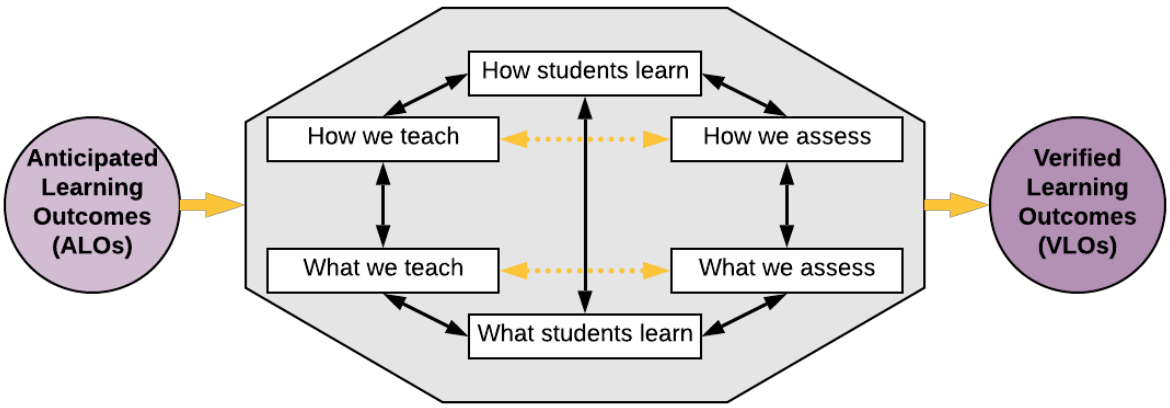

Figure 4.1 An adapted model from Anderson (2007) outlining how anticipated leaning outcomes (ALOs) should inform teaching, learning, and assessment.

The double-headed arrows indicate that there should be an interplay between what and how we teach, students learn, and course assessments. Sound curriculum design places focus on these three aspects and align them with each other. Aligning this teaching, learning, and assessment cycle with specific ALOs will allow for determining whether or not they are verified learning outcomes (VLOs).

4.4 Research Question

The goal of this study was to collect data about students' perceived knowledge, experience, and confidence for the identified ALOs (Table 4.1) and for the related techniques and methods (Irby, Pelaez, et al., 2018b, 2018a) that students were exposed to in their course implementing the BASIL CURE (Craig, 2017; Craig et al., 2018; Irby, Pelaez, et al., 2018b, 2018a). (Tables 4.2 and 4.3). Which, can be used to identify the appropriate features of the BASIL CURE for specific implementations (Figure 4.1, highlighted dashed-arrows). Towards achieving the above goal the following research questions were addressed:

- **RQ1)** How do students' perceived knowledge, experience, and confidence (KEC) regarding the specific ALOs change across all implementations during the BASIL CURE?
- **RQ2)** How do student perceptions of their KEC regarding specific ALOs from the BASIL CURE vary across different course structures and institutional contexts?

4.5 Study Context

The focus of this study was on the BASIL CURE (Craig, 2017; Craig et al., 2018; Irby, Pelaez, et al., 2018b, 2018a). Students in this CURE seek to determine the function of proteins of known structure but unknown function, using computational and biochemical (wet-lab) techniques. This CURE was molded after research conducted by scientists in this field (McKay et al., 2015). More detailed information about the BASIL CURE has been previously published (Craig, 2017; Craig et al., 2018; Irby, Pelaez, et al., 2018b, 2018a). The BASIL CURE consisted of 10 lab protocols and was designed in a modular fashion so it could be implemented as computational techniques only (comp only), wet-lab biochemical techniques only (biochem only), or in full (both). An example of how the BASIL CURE was implemented in four different courses is shown in Figure 4.2. Figure 4.2 shows the components of the BASIL CURE, the constituent activities, as well as other meetings and presentations, students participated in. Two courses MW2-2 and SW1-9 (Tables 4.2 and 4.3) employed all portions of the BASIL CURE (Table 4.2). In comparison, course NE3-7 only implemented the computational portions of the BASIL CURE, and NE3-8 only implemented the biochemical portions of the BASIL CURE (Figure 4.2, Tables 4.2 and 4.3).

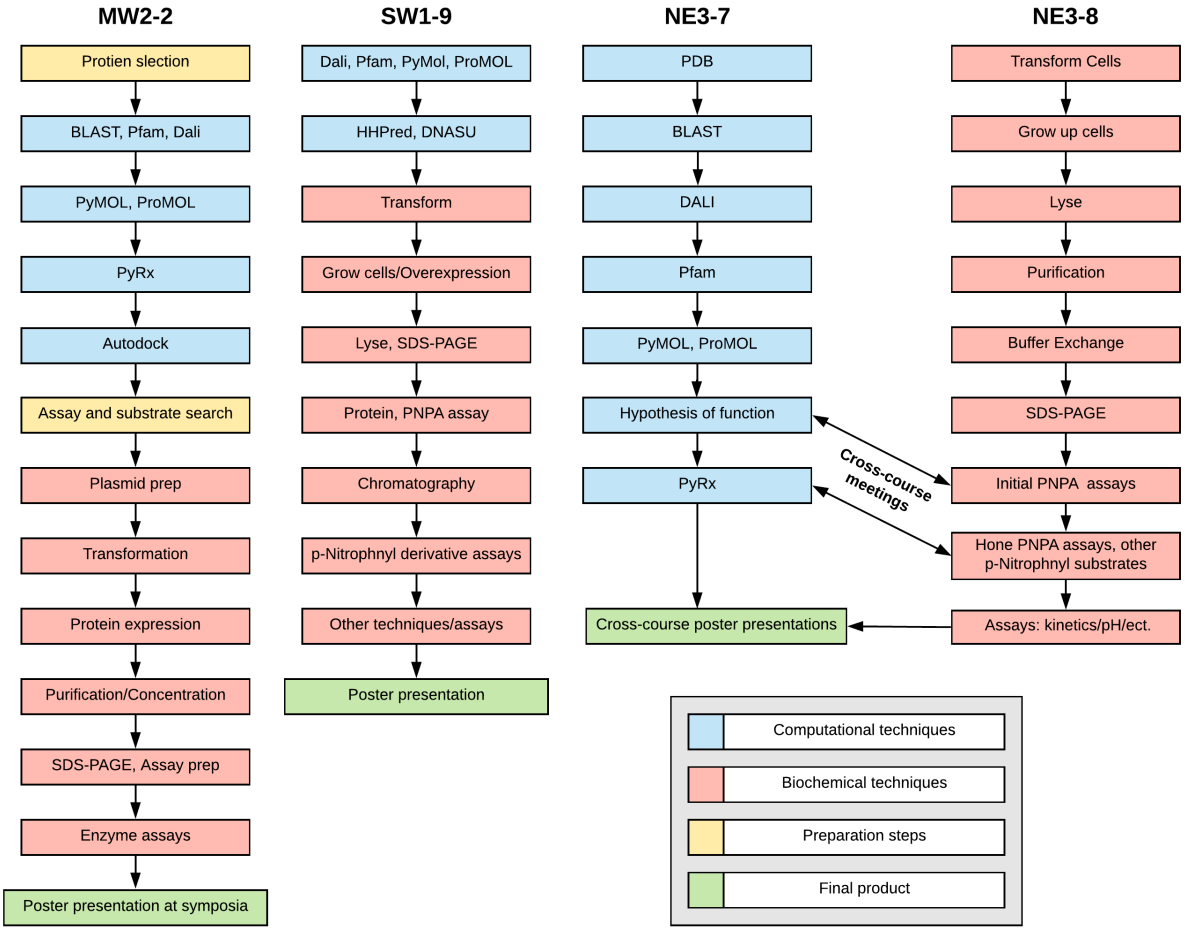

Figure 4.2 Outline of four different BASIL CURE courses and how they each implemented the BASIL curriculum.

The courses either implemented both the computational and biochemical components (MW2-2 and SW1-9), only the computational components (NE3-7) or only the biochemical components (NE3-8) of the BASIL curriculum. The two courses (NE3-7 and NE3-8) that only implemented half of the BASIL curriculum were at the same institution, during the same semester (Tables 4.1 and 4.3). Courses NE3-7 and NE3-8 had cross-course meetings during the semester to share what they have learned about the proteins of interest and to put together and give a joint poster presentation.

Data for this study was collected from ten courses from seven different institutions across the United States, representing a diverse range of institution types (Tables 4.2 and 4.3). All of the students were at least Sophomores and 84.4% of the students were Juniors or Seniors (Table 4.4). Students self-identified as being a part of 10 different majors, with students identifying as being a

biochemistry, chemistry, or biology major, or a closely related major (e.g. biotechnology, Table 4.4). The specific demographics for courses with more than 10 students participating in both the pre- and post-PPI survey are reported in Table 4.6.

Table 4.2 Institution Information

Institution ID	**Region**	**Carnegie Classification** ("The Carnegie Classification of Institutions of Higher Education," n.d.)
MW1	Midwestern	Baccalaureate College: Arts & Sciences Focus
MW2	Midwestern	Special Focus Four-Year: Other Health Professions School
NE1	Northeastern	Doctoral University: Moderate Research Activity
NE2	Northeastern	Master's College & University: Larger Programs
NE3	Northeastern	Baccalaureate College: Arts & Sciences Focus
SW1	Southwestern	Master's College & University: Larger Programs
W1	Western	Master's College & University: Larger Programs

4.6 Participant Perception Indicator (PPI) Survey

4.6.1 Developing the PPI Survey

This PPI survey contained three categories for participants to rate their perceived knowledge, experience, and confidence. The first category was the seven anticipated learning outcomes (ALOs) that were previously selected from forty-three ALOs for the BASIL CURE (Irby, Pelaez, et al., 2018b, 2018a). These ALOs were also referred to as CURAs in Irby, Pelaez, et al. (2018b, 2018a) and were identified by the PICURA (Irby, Pelaez, et al., 2018b). For the present study, we selected the seven ALOs (Table 4.1) that were rated the highest by the BASIL instructors based on how unique they considered the ALOs to be to the BASIL CURE and how important they were to the functioning of a scientist in this field of research (Irby, Pelaez, et al., 2018a, 2018b). Also, in Table 4.1, the seven ALOs are aligned with the BASIL CURE components and protocols that directly correspond to them (Table 4.1). The other two categories were the computational and biochemical (wet-lab) techniques identified by the instructors as being present in their version of the BASIL CURE. All the items in the PPI survey are provided in the Supplemental Material (Section 4.11.1).

4.6.2 Conducting the PPI Survey

Data collection took place during the first half of 2018, after being piloted during the previous year. To match student responses, students generated their own unique participant ID number, keeping their true identity anonymous. Instructors asked students to take the online PPI survey at the beginning and end of their course implementing the BASIL CURE. The only inclusion criteria were that students had to be a part of a BASIL CURE course, complete both the pre- and post-PPI survey and enter the same participant ID number that they generated so that their responses could be paired together. Table 4.3 shows when each participating course was taught, which BASIL CURE components were part of the course, and how many students participated.

Table 4.3 Course and PPI survey participation data.

Institution	Instructor	Term[a]	Components	Number of students		
				Pre	Post	Pre/Post Paired[b,c]
MW1						
	MW1-1	H '18	Both	6	5	5 (12)
MW2						
	MW2-2	S '18	Both	13	12	10 (12)
NE1						
	NE1-3	S '18	Both	9	7	7 (9)
NE2						
	NE2-4	S '18	Both	8	2	1 (28)
	NE2-5	S '18	Both	1	6	1 (11)
	NE2-6	S '18	Both	11	4	4 (20)
NE3						
	NE3-7	S '18	Comp Only	12	11	11 (14)
	NE3-8	S '18	Biochem Only	15	14	11 (16)
SW1						
	SW1-9	S '18	Both	19	15	11 (24)
W1						
	W1-10	W '18	Biochem Only	8	5	3 (16)
			Total	**102**	**81**	**64**

[a]When the course occurred: H = first half of spring semester, S = spring semester, F = fall semester, and W= winter quarter

[b]Paired refers to students who completed both the pre and the post PPI survey in 2018 so their individual change in KEC can be tracked.

[c]Number in parenthesis is total course enrollment based on instructor post-course PPI survey, the pre/post student numbers may be different than the reported course enrollment based on the students adding/dropping the course, incorrectly inputting their identification number, or choosing not to participate in either the pre/post survey.

In the Spring semester and Winter quarter of 2018, there was a total of 64 students that participated in both the pre- and post-PPI survey (Table 4.3). These students participated in the BASIL CURE as a part of 10 different courses at seven different institutions (Table 4.3).

Instructors also took the PPI survey and had to self-identify in order to link student responses to instructor responses. The instructors were able to indicate the specific components and techniques their course implemented. The student averages for their KEC on the computational (Comp Avg) and biochemical (Biochem Avg) techniques were based on only the techniques instructors reported that the students conducted as part of their course. In the case of NE3-7 (comp only) and NE3-8 (biochem only), since they had cross-course interaction the techniques that counted were based for the students average for Comp Avg and Biochem Avg KEC was based on their instructor's and the companion course instructor's (for NE3-7 and NE3-8) PPI survey of that the students should have been exposed to.

When taking the PPI survey participants rated their knowledge, experience, and confidence regarding each item on the following scale: **1** = "none," **2** = "a little," **3** = "some," **4** = "much," and **5** = "a great deal." Participation in the survey was voluntary, and no compensation was given, nor were the instructors able to access any of their results until after final grades were submitted. This was in accordance with Purdue University's Institutional Review Board (IRB# 1604017549).

4.6.3 Data Analysis

Responses for knowledge (K), experience (E), and confidence (C) for each PPI item were averaged together to generate a "KEC" score for each item (Clase et al., 2010). Initially, all of the 2018 paired data were analyzed together (Figure 4.1, Table 4.7), and then by individual courses with 10 or more paired pre-/post-PPI responses (Table 4.3). Significance between pre- and post-PPI responses was determined by performing a paired t-test, with an alpha level of 0.05. Since the pooled data included students from various courses (Table 4.3), tests for normality were conducted, and no major violations of this assumption were detected (see Figure 4.4 for Q-Q plots). Lastly, since population sizes differ (from all pooled, large n, to an individual course, small n) normalized gain of averages (gain scores; Hake, 1998) was calculated, as well as, the effect size was determined using Cohen's *d* (with a value > 0.80 considered a large effect; Cohen, 1988).

Table 4.4 Student demographics for all paired data from 2018.

Major[a]	Academic Rank			Total
	Sophomore	Junior	Senior	
Biochemistry	1	5	9	15
Biochemistry and Molecular Biology	3	8	3	14
Biology	-	10	2	12
Biology and Chemistry	1	-	-	1
Biomedical Sciences	-	1	-	1
Biotechnology	-	-	2	2
Chemistry	-	2	-	2
Health Sciences	1	3	6	10
Neuroscience	1	-	-	1
Zoology	3	3	-	6
Total	10	32	22	**64**

[a]Demographics for each individual course with 10 or more paired responses is provided in the supplemental material

4.7 Results and Discussion

4.7.1 Change in KEC Across Institutions

When analyzing all the PPI paired responses together (n=64), students reported that their pre-course KEC for the seven ALOs was between "none" and "a little" (Figure 4.3, Table 4.7). On the post-course PPI, students reported that their KEC increased to be between "some" and "much" for all seven of the ALOs (Figure 4.3, Table 4.7). All of these increases were found to be significant ($p < 0.001$) and had large effect sizes ($d \geq 1.24$) for all seven of the ALOs, with gain scores between 41-56% (Table 4.3). The ALO with the highest pre- (2.06) and post-course (3.69) KEC score was for ALO7 (Figure 4.3) with a post-course KEC approaching our "much" rating, (Figure 4.3). This could be because protein purification and protein tagging is a common component of biochemistry courses, and was indicated by instructors of the BASIL CURE that it is an ability that students may experience in other courses, besides the BASIL CURE (Irby, Pelaez, et al., 2018a). Thus it is unsurprising that students entered the BASIL CURE with some KEC for ALO7, and that they would be able to build upon this ability by participating in the course. Additionally, ALO6 (Table 4.2) received the largest change in KEC score of 1.69, followed by ALO2 with an increase of 1.65 (Figure 4.3, Table 4.2 and 4.7). These two ALOs are integral to the BASIL CURE because they encompass two of the main activities: determining the active site (ALO2, Table 4.3) and identifying candidate ligands, followed by enzyme assays to assess the predicted protein function (ALO6, Table 4.3). The BASIL CURE as a whole was effective at increasing students' perceptions

of their KEC for these seven ALOs (RQ1), across the BASIL courses (Figure 4.3, Tables 4.1, 4.3, and 4.7). However, due to the modular nature of the BASIL CURE (Craig, 2017; Craig et al., 2018) analysis of how specific implementations responded to the PPI survey is needed to understand the true effects the CURE has on students' perceptions of their KEC for the identified items.

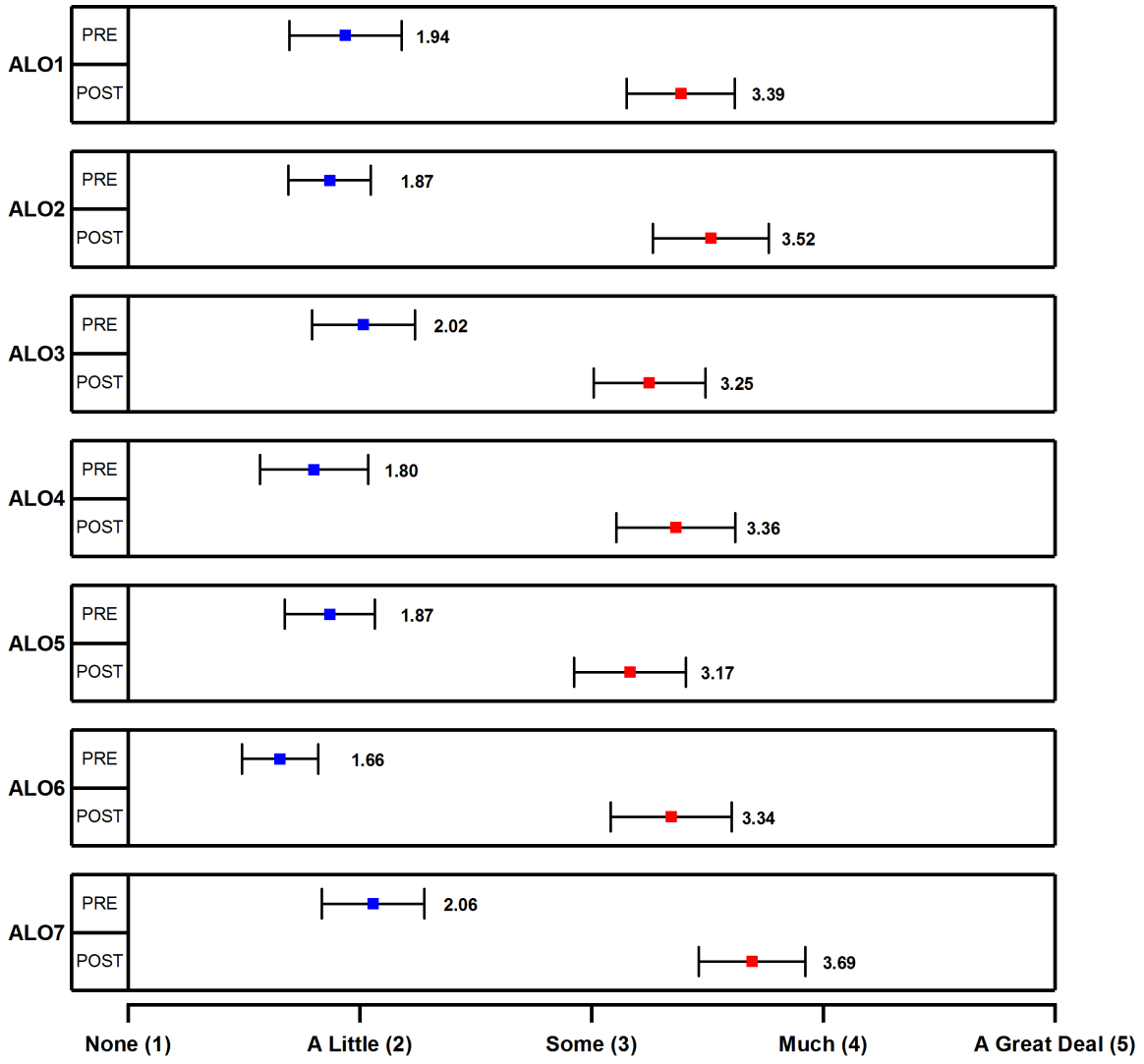

Figure 4.3 Plots of the pre-PPI (blue) and post-PPI (red) KEC ratings for the seven ALOs showing all of the paired PPI data for all courses from Spring 2018 (n=64).

In all of the plots the square dots indicate the average and the whickers represent the 95% confidence interval

4.7.2 Change in Students' KEC Metrics across Courses and Institutions

In this section, we focus on the implementation of the BASIL CURE as part of four different courses at three different institutions, with 10 or more student responses from each institution. Two of the institutions incorporated both the computational and biochemical components of the BASIL CURE (MW2-2 and SW1-9), while one course only included the computational components (NE3-7), and one only included the biochemical components (NE3-8). In addition to the instructor responses to the PPI survey, course syllabi were collected better understand the structure of these four courses, which is outlined in Figure 4.2.

The same general trends were observed for the two courses, from two different institutions, that included both computational and biochemical BASIL CURE components, as when all courses were pooled and analyzed together. Significant changes with large effect sizes were found for all seven of the ALOs with only one exception; the change in KEC score for ALO3 (Table 4.3) was not significant ($p = 0.15$) and had a low effect size ($d = 0.56$) for SW1-9 (Table 4.5). ALO3 pertains to determining which factors are important for interpreting and optimizing enzyme assays. Findings show that in contrast to the other biochemical containing implementations (MW2-2 and NE3-7) and the pooled data, the students in SW1-9 seemed to have a relatively higher amount of experience with ALO3 (pre KEC = 2.76, Figure 4.3, Table 4.5) resulting in a much lower gain score (22%). For the BASIL CURE this ALO is typically associated with conducting a p-Nitrophenyl Acetate (PNPA) assay to detect protein hydrolase activity, which was the case for NE3-8 and SW1-9 (Figure 4.2). Students in MW2-2 did not conduct PNPA assays at all; instead students developed their own enzyme assays based on the predicted function of their protein. When looking at the courses that contained biochemical techniques (Figure 4.2), students in SW1-9 performed multiple types of assays in addition to the PNPA assays: other p-Nitrophenyl derivatives, agarose electrophoresis, and thin-layer chromatography (TLC) to assess the type of hydrolase a protein may be. In contrast, students in NE3-8 focused their attention on just the PNPA assay, running it multiple times while varying the conditions of the assay; while MW2-2 has students select and design their own enzyme assays (Figure 4.2). Thus, since the students in SW1-9 had the opportunity to perform more types of assays, they may have had less time to spend on identifying important factors for optimizing or interpreting a given assay, resulting in a smaller change (0.48, Table 4.5) to the KEC score for ALO3 (RQ2).

Table 4.5 Comparison of changes in students' KEC scores from pre to post on the PPI survey for four different individual implementations of the BASIL CURE.

Metric	ALO1 (B)	ALO2 (C)	ALO3 (B)	ALO4 (C)	ALO5 (B/C)	ALO6 (B)	ALO7 (B)	Comp Avg	Biochem Avg
MW2-2 (Both, n=10)									
Pre	1.13	2.70	1.37	3.07	2.37	1.33	1.17	1.72	1.47
Post	3.00	3.97	3.33	3.90	2.97	3.53	3.50	3.13	2.82
Change	1.87	1.27	1.97	0.83	0.60	2.20	2.33	1.41	1.36
Gain Score	48%	55%	54%	43%	23%	60%	61%	43%	38%
Cohen's *d*[a]	2.47	2.16	3.66	1.12	0.99	3.54	4.33	4.11	3.17
t-value[a]	5.59[e]	5.46[e]	9.21[e]	2.75[c]	2.21[c]	9.19[e]	9.04[e]	10.43[e]	8.84[e]
SW1-9 (Both, n=11)									
Pre	2.30	1.91	2.76	1.88	2.06	2.03	3.00	1.89	2.59
Post	3.45	3.42	3.24	3.06	3.21	3.42	3.91	2.85	3.62
Change	1.15	1.52	0.48	1.18	1.15	1.39	0.91	0.96	1.03
Gain Score	43%	49%	22%	38%	39%	47%	45%	31%	43%
Cohen's *d*[a]	1.19	1.97	0.56	1.13	1.26	1.86	0.97	1.64	1.43
t-value[a]	4.57[d]	5.84[e]	1.55	3.95[d]	5.30[e]	4.53[d]	5.21[e]	4.69[e]	7.23[e]
NE3-7 (Comp Only, n=11)									
Pre	1.85	1.39	1.88	1.18	1.94	1.55	1.70	1.22	1.97
Post	2.27	4.00	1.91	3.88	3.12	1.94	2.76	3.50	2.47
Change	0.42	2.61	0.03	2.70	1.18	0.39	1.06	2.28	0.49
Gain Score	13%	72%	1%	71%	39%	11%	32%	60%	16%
Cohen's *d*[a]	0.41	3.61	0.04	3.92	0.97	0.48	1.30	4.39	0.68
t-value[a]	1.57	8.13[e]	0.12	9.94[e]	3.82[d]	2.08	5.05[e]	12.02[e]	2.03
NE3-8 (Biochem Only, n=11)									
Pre	2.94	1.82	2.70	1.91	1.94	2.09	2.79	1.58	2.69
Post	4.09	2.76	3.61	2.12	2.82	3.79	4.21	1.94	3.62
Change	1.15	0.94	0.91	0.21	0.88	1.70	1.42	0.35	0.92
Gain Score	56%	30%	39%	7%	29%	58%	64%	10%	40%
Cohen's *d*[a]	1.44	0.88	0.88	0.17	0.88	2.06	1.52	0.54	1.84
t-value[a]	3.98[d]	5.10[e]	2.98[c]	0.82	4.24[d]	5.42[e]	3.76[d]	4.59[d]	5.94[e]

[a]Numbers are shaded green if they are considered to be a large effect size (Cohen's $d > 0.8$) or were found to be significant ($p \leq 0.05$)
[b]Indicates that an ALO pertains to B=biochemical (wet-lab) techniques or C=computational techniques
[c]$p \leq 0.05$
[d]$p < 0.01$
[e]$p < 0.001$

Two other courses that were further analyzed, NE3-7 and NE3-8 (See Figure 4.2), were from the same institution (Tables 4.1 and 4.3). NE3-7 only covered the computational portions of the BASIL CURE, and NE3-8 only covered the biochemical wet-lab portions (Figure 4.2). However, the two courses participated in cross-course meetings to share their results and experiences from the portions of the BASIL CURE they participated in (Figure 4.2). During these meetings students presented their findings from the parts of the BASIL CURE they conducted to each other at two different time points during the semester and they worked together to synthesize

their results, to create a poster presentation on all of the components of the BASIL CURE at the end of the semester (Figure 4.2).

Unlike the courses that included all of the BASIL CURE components (MW2-2 and SW1-9), course NE3-7 (computational only) only showed significant increases in KEC scores, with large effect sizes, for the ALOs that pertained to the computational components of the BASIL CURE (ALO2, ALO4, ALO5) and the computational techniques (Table 4.5). This is unsurprising since these students did not directly participate in the biochemical components of the BASIL CURE. However, the NE3-7 students did perceive a significant gain in KEC for one biochemical related ALO, ALO7 (Tables 4.2 and 4.5). The biochemistry only course, NE3-8, did have significant increases across all items except for ALO4, a computational related ALO (Tables 4.2 and 4.5), but had lower effect sizes and gain scores for the ALOs that related computational abilities (ALO2 and ALO5, Table 4.2) than for the biochemical related items (Table 4.5).

The results from these two courses (NE3-7 and NE3-8) could be attributed to several factors. When the students from the two courses interacted, those in the computational course (NE3-7) may have provided more details about how they used the computational programs, whereas the students from biochemical course (NE3-8) may have focused only on their results and not on optimization, troubleshooting, and design of assays and protocols (RQ2). Therefore, conversations about the results from biochemical techniques did not impact the KEC of the biochemical related ALOs (Tables 4.2 and 4.5). The one exception of this was ALO7 which, as discussed earlier, students may have already been familiar with this ALO prior to the course and could have made the biochemical students (NE3-8) more comfortable discussing this ALO with their computational peers (Figure 4.2, Table 4.5). The biochemical students (NE3-8) showed significant increases in KEC for ALO2 and for the computational techniques, but with a low effect size (Table 4.5). Thus, suggesting from having exposer to the computational components of the BASIL CURE through the cross-course meetings students were afforded the opportunity to learn about computational techniques, an area of chemistry undergraduate students are often not exposed to (RQ2).

4.8 Conclusion

This work successfully addressed the guiding research questions: Towards this goal we addressed the following research questions: **RQ1**) How do students' perceived knowledge, experience, and

confidence (KEC) regarding the specific ALOs across all implementations change during the BASIL CURE? and **RQ2**) How do student perceptions of their KEC regarding specific ALOs from the BASIL CURE vary across different course structures and institutional contexts? There was an increase in students' perception of their KEC for all of the seven BASIL CURE ALOs (Figure 4.3, Table 4.7) that were previously identified (Irby, Pelaez, et al., 2018a, 2018b) and used for this PPI survey when looking across all implementations (RQ1). In general, these gains were significant and showed large effect sizes (Figure 4.2, Table 4.7) across all implementations of the BASIL CURE (RQ1). When focusing on the course sections with 10 or more paired responses (Table 4.5), the same general trend was observed for the courses implementing all components of the BASIL CURE (MW2-2 and SW1-9, Figure 4.2, Tables 4.2, 4.5, and 4.7-4.11). However, SW1-9 did not show a significant increase in student perceived KEC for ALO3. This could have been due to the students being exposed to more types of assays and with less time available for optimizing a particular assay (RQ2). There were also differences in students change in their perceived KEC based on if they only participated directly in the computational (NE3-7) or the biochemical (NE3-8) components of the BASIL CURE (RQ2). These differences are also an indicator that the students were reliably responding to the PPI (e.g. the computational only course, NE3-7, only reported a perceived increase on the computational related items, Table 4.5 and 4.10). Additionally, there is some initial evidence that students that participated in a course that only implements the biochemical components (e.g. NE3-8, Tables 4.5 and 4.11) also show an increase in their perceived KEC for computational related ALOs (Table 4.2) when given opportunities to collaborate with students that are participating in a course that is only implementing the computational components (RQ2, Figure 3). However, this data suggests that this trend does not seem as impactful in increasing the KEC for computational only students (NE3-7) for biochemical ALOs or techniques (Table 4.5) from cross course meetings and joint presentations (RQ2, Tables 4.5, 4.10 and 4.11, Figure 4.3).

The results from the PPI survey about student's increase in their perceived KEC are promising and consistent with other metrics used to study CUREs (Corwin, Runyon, et al., 2015; Lefurgy & Mundorff, 2017; Shaffer et al., 2014; Shanle et al., 2016). Though students' perceptions of their learning in the BASIL CURE is important, it becomes more meaningful when this type of data can be compared to data that measures actual student learning. One way this data can be used is, as illustrated in Figure 4.1, now that there is data on what students perceived they learned in courses implementing the BASIL CURE, sound assessment strategies (Anderson, 2007; NRC,

2001) can be designed to measure actual student learning for identified ALOs (Irby, Pelaez, et al., 2018b, 2018a) to determine if they are VLOs. By measuring actual learning by eliciting student responses to student probes for ALOs (Irby, Pelaez, et al., 2018a), or by performing in-depth studies of courses implementing the BASIL CURE in unique ways, such as NE3-7 and NE3-8, to better understand how unique implementation styles of a CURE can impact the student learning and experiences. Future research will, therefore, focus on individual case studies of BASIL CURE implementations to using a combination of open-ended assessments, interview, and student-generated artifacts to assess actual student learning compared to and informed by the PPI survey results.

4.9 Limitations

As discussed in the introduction, self-reported data such as a PPI survey used here only reflects a student's perceived learning and what they experienced from participating in the BASIL CURE. This information is important but does not necessarily reflect the actual amount of learning for a student participating in the BASIL CURE which will need to be established using a different assessment instrument.

4.10 Acknowledgments

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Supplemental Material

4.10.1 PPI Survey Items

Category 1: Research abilities you may have gained or experienced as part of the course
Indicate your feelings of knowledge, experience, and confidence about the following:

ALO1: Explain how the colorimetric enzyme assay works to allow detection of protein function
ALO2: Identify an enzyme active site using appropriate computational programs
ALO3: Determine the appropriate factors to consider when optimizing or interpreting an enzyme assay
ALO4: Determine using computational software whether, and where, a ligand may be binding to a protein
ALO5: Compare enzymatic results with those computationally predicted
ALO6: Design an enzyme assay to elucidate protein function
ALO7: Explain how the purification of tagged proteins work and ways the process can be optimized

ALOQ: Please take a minute to explain how you would use ONE of the above research abilities that you feel the most competent in doing for your own research. Share your thoughts here in as much detail as possible and please include the ability number for the research ability you choose to discuss (i.e. ALO1-ALO7).

Category 2: Computational programs and databases you may have used as part of the course
Indicate your feelings of knowledge, experience, and confidence about the following:

C1: Autodock
C2: BLAST
C3: CHARMM
C4: Chemspider
C5: Chimera
C6: ConSCRIPT
C7: Dali
C8: DNASU
C9: Entrez
C10: LigPlot+
C11: MarkUS
C12: PDB
C13: Pfam
C14: Plasmid maps
C15: ProMOL
C16: PSI4 program through the WebMO interface
C17: PubChem
C18: PyMOL
C19: PyRx
C20: SBKB

CQ: Please take a minute to consider how you would use the ONE computational technique above that you would feel most competent using in your own research. Share your thoughts here in as much detail as possible.

Category 3: Biochemical assays, methods, or tools you may have used as part of the course
Indicate your feelings of knowledge, experience, and confidence about the following:

B1: Protein expression
B2: Cell culture and growth
B3: Metal ion affinity chromatography
B4: Bradford assay
B5: BCA assay
B6: SDS-PAGE
B7: Activity assay with p-nitrophenyl acetate
B8: Western blot
B9: ImageJ

BQ: Please take a minute to consider how you would use the ONE biochemical technique above that you would feel the most competent using in your own research. Share your thoughts here in as much detail as possible.

4.10.2 2018 Specific Course Student Demographics

Table 4.6 Student demographics for all paired data from 2018 with 10 or more students

Major	Academic Rank			Total
	Sophomore	Junior	Senior	
MW2-2 (Both)				
Health Sciences	1	3	6	10
SW1-9 (Both)				
Biochemistry	1	2	-	3
Biology	-	7	1	8
NE3-7 (Comp Only)				
Biochemistry	-	1	-	1
Biochemistry and Molecular Biology	3	1	2	6
Biology	-	1	1	2
Biology and Chemistry	1	-	-	1
Neuroscience	1	-	-	1
NE3-8 (Biochem Only)				
Biochemistry	1	-	2	3
Biochemistry and Molecular Biology	-	6	-	6
Biology	-	2	-	2

4.10.3 Detailed Statistics

Table 4.7 Results and statistics of the all of the 2018 student paired PPI data (n=64)

	ALO1 (B)	ALO2 (C)	ALO3 (B)	ALO4 (C)	ALO5 (B/C)	ALO6 (B)	ALO7 (B)
Pre	1.94	1.87	2.02	1.80	1.87	1.66	2.06
Pre STD	1.12	0.82	1.03	1.08	0.90	0.76	1.02
C.I.	0.27	0.20	0.25	0.27	0.22	0.19	0.25
Post	3.39	3.52	3.25	3.36	3.17	3.34	3.69
Post STD	0.94	1.00	0.96	1.03	0.96	1.04	0.92
C.I.	0.23	0.24	0.24	0.25	0.24	0.26	0.22
Change	1.45	1.65	1.23	1.56	1.30	1.69	1.64
Gain Score	47%	53%	41%	49%	41%	50%	56%
Cohen's d	1.40	1.80	1.24	1.48	1.39	1.85	1.69
p-Value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

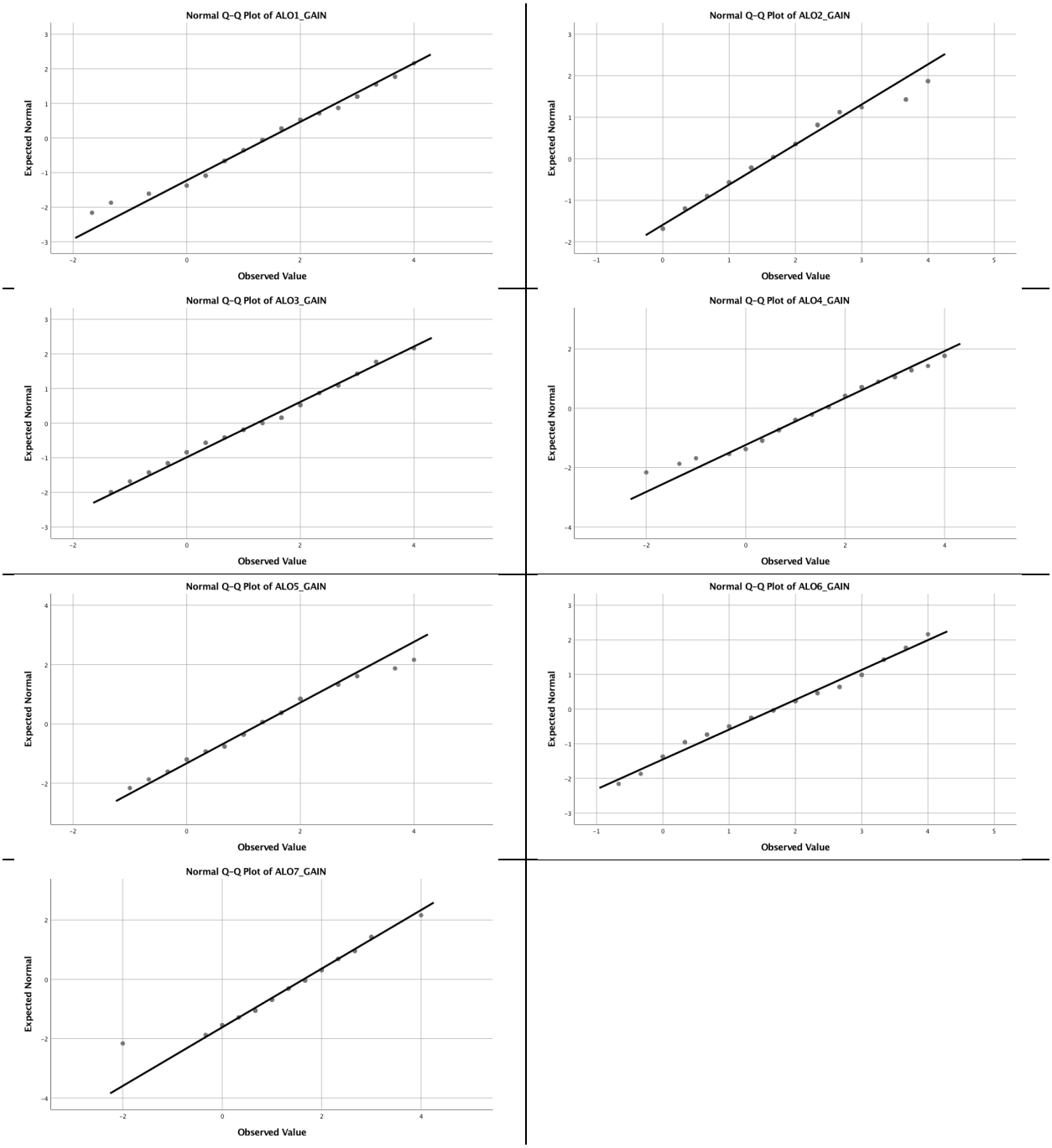

Figure 4.4 Q-Q plots to assess the normality of the student pooled paired responses

Table 4.8 Results and statistics for MW2-2 (Both, n=10) student paired PPI data.

	ALO1 (B)	ALO2 (C)	ALO3 (B)	ALO4 (C)	ALO5 (B/C)	ALO6 (B)	ALO7 (B)	Avg C	Avg B
Pre	1.13	2.70	1.37	3.07	2.37	1.33	1.17	1.72	1.47
Pre STD	0.32	0.71	0.46	0.89	0.74	0.38	0.32	0.20	0.30
C.I.	0.20	0.44	0.28	0.55	0.46	0.24	0.20	0.13	0.19
Post	3.00	3.97	3.33	3.90	2.97	3.53	3.50	3.13	2.82
Post STD	1.02	0.43	0.61	0.57	0.43	0.79	0.69	0.44	0.52
C.I.	0.63	0.27	0.38	0.35	0.27	0.49	0.43	0.27	0.32
Change	1.87	1.27	1.97	0.83	0.60	2.20	2.33	1.41	1.36
Gain Score	48%	55%	54%	43%	23%	60%	61%	43%	38%
Cohen's d	2.47	2.16	3.66	1.12	0.99	3.54	4.33	4.11	3.17
p-value	<0.001	<0.001	<0.001	<0.05	0.05	<0.001	<0.001	<0.001	<0.001

Table 4.9 Results and statistics for SW1-9 (Both, n=11) student paired PPI data.

	ALO1 (B)	ALO2 (C)	ALO3 (B)	ALO4 (C)	ALO5 (B/C)	ALO6 (B)	ALO7 (B)	Avg C	Avg B
Pre	2.30	1.91	2.76	1.88	2.06	2.03	3.00	1.89	2.58
Pre STD	1.01	0.72	0.99	1.24	1.08	0.84	0.92	0.63	0.61
C.I.	0.59	0.42	0.58	0.73	0.64	0.49	0.54	0.37	0.36
Post	3.45	3.42	3.24	3.06	3.21	3.42	3.91	2.85	3.59
Post STD	0.93	0.82	0.72	0.81	0.70	0.65	0.96	0.54	0.73
C.I.	0.55	0.48	0.42	0.48	0.42	0.38	0.56	0.32	0.43
Change	1.15	1.52	0.48	1.18	1.15	1.39	0.91	0.96	1.01
Gain Score	43%	49%	22%	38%	39%	47%	45%	31%	42%
Cohen's d	1.19	1.97	0.56	1.13	1.26	1.86	0.97	1.64	1.50
p-value	<0.01	<0.001	0.15	<0.01	<0.001	<0.01	<0.001	<0.001	<0.001

Table 4.10 Results and statistics for NE3-7 (Comp Only, n=11) student paired PPI data.

	ALO1 (B)	ALO2 (C)	ALO3 (B)	ALO4 (C)	ALO5 (B/C)	ALO6 (B)	ALO7 (B)	Avg C	Avg B
Pre	1.85	1.39	1.88	1.18	1.94	1.55	1.70	1.22	1.97
Pre STD	1.34	0.44	0.86	0.43	1.00	0.92	0.80	0.14	0.78
C.I.	0.79	0.26	0.51	0.25	0.59	0.54	0.47	0.08	0.46
Post	2.27	4.00	1.91	3.88	3.12	1.94	2.76	3.50	2.47
Post STD	0.63	0.92	0.63	0.87	1.41	0.71	0.83	0.72	0.68
C.I.	0.37	0.54	0.37	0.52	0.83	0.42	0.49	0.43	0.40
Change	0.42	2.61	0.03	2.70	1.18	0.39	1.06	2.28	0.49
Gain Score	13%	72%	1%	71%	39%	11%	32%	60%	16%
Cohen's d	0.41	3.61	0.04	3.92	0.97	0.48	1.30	4.39	0.68
p-value	0.15	<0.001	0.91	<0.001	<0.01	0.07	<0.001	<0.001	0.07

Table 4.11 Results and statistics for NE3-8 (Biochem Only, n=11) student paired PPI data.

	ALO1 (B)	ALO2 (C)	ALO3 (B)	ALO4 (C)	ALO5 (B/C)	ALO6 (B)	ALO7 (B)	Avg C	Avg B
Pre	2.94	1.82	2.70	1.91	1.94	2.09	2.79	1.58	2.69
Pre STD	1.04	0.96	1.10	1.34	1.04	0.82	0.98	0.63	0.55
C.I.	0.62	0.57	0.65	0.79	0.62	0.48	0.58	0.37	0.32
Post	4.09	2.76	3.61	2.12	2.82	3.79	4.21	1.94	3.62
Post STD	0.45	1.17	0.96	1.10	0.95	0.83	0.89	0.68	0.45
C.I.	0.27	0.69	0.57	0.65	0.56	0.49	0.52	0.40	0.27
Change	1.15	0.94	0.91	0.21	0.88	1.70	1.42	0.35	0.92
Gain Score	56%	30%	39%	7%	29%	58%	64%	10%	40%
Cohen's d	1.44	0.88	0.88	0.17	0.88	2.06	1.52	0.54	1.84
p-value	<0.01	<0.001	<0.05	0.43	<0.01	<0.001	<0.01	<0.01	<0.001

CHAPTER 5. VLOS REVEALED BY ASSESSMENT OF TOP-RATED CURAS

5.1 Introduction and motivation

There is a dearth of literature focusing on the development of students' research abilities, especially in biochemistry CUREs. This is despite numerous calls to incorporate the teaching of research abilities into undergraduate curricula, by various policy documents (Brewer & Smith, 2011; NASEM, 2015, 2017, NRC, 2003, 2012; PCAST, 2012), the American Society of Biochemistry and Molecular Biology's benchmarks (ASBMB, n.d.), and a network of scientists and discipline-based education researchers that worked to categorize research abilities (Nancy Pelaez et al., 2017). Studies suggest there may be a close relationship between the development of conceptual understanding and the experimental process (e.g. Bernhard, 2018; Chandrasekharan & Nersessian, 2015; Jeffery, Pelaez, & Anderson, 2018, n.d.). Though much is unknown about the extent of student experimental and research abilities, there has been documented previously that students struggle with experimental design and interpretation (Dasgupta et al., 2014), as well as with integrating concepts and theories from lecture or other contexts into the laboratory setting (Dasgupta et al., 2014; Nakhleh, 1994).

Recently, researchers have been focusing on studying expert practice and student difficulties to inform the design of teaching strategies to foster the opportunity for students to develop research abilities. For example, in biochemistry and biology, there have been efforts to study and characterize the way experts explain and conduct research (Jeffery et al., 2018; Nancy Pelaez et al., 2017; Trujillo et al., 2015) in order to scaffold student learning and development with experimental and research abilities. This is similar to how Irby, Pelaez, et al. (2018b [Chapter 2]) had instructors and designers discuss how they would execute research projects like those conducted by the students participating in the BASIL CURE, in order to identify CURAs as ALOs. There have also been efforts toward creating new teaching strategies, such as CUREs (e.g. Auchincloss et al., 2014; Corwin, Graham, et al., 2015; Craig et al., 2018), for exposing students to research abilities.

There has been little work using in-depth qualitative studies investigating the learning taking place within CURE settings. As discussed in Chapters 1 and 4, though some CUREs assess students' experimental abilities as a result of participating in a CURE or other advanced biochemistry lab courses, their abilities were primarily determined by Likert-scale perception

studies (e.g. Roberts, 2001; Wang, Schembri, Ramakrishna, Sagulenko, & Fuerst, 2012; Witherow & Carson, 2011). There has, however, been some examples of studies looking and connecting qualitative data to student survey responses (e.g. Brownell et al., 2015; Olimpo et al., 2016), but more in-depth qualitative CURE studies are needed to determine students actual development of research abilities, the focus of this study. Lastly, a synthesis of proposed CURE outcomes indicated that research abilities, such as collecting novel data and analyze results, are critical to achieving all of the proposed medium- and long-term outcomes previously identified (Corwin, Graham, et al., 2015; Table 1.1).

There is currently a gap in the literature about how students interpret and model phenomena from novel self-generated data, and the reasoning, visual and problem-solving abilities that are engaged when performing such tasks. Studies, where student understanding of data interpretation was examined, have reported that students often neglect to consider scientific theory used to inform the data analysis and focus instead on the quantity of data collected (Ryder & Leach, 2000). One report on a research-like course suggested that the ability of students to interpret data increased as the course progressed (Brownell et al., 2015). In biochemistry, little is known about how students perceive biochemistry data and relate it to the actual function of biological molecules, i.e. proteins. In fact, there are only a few examples of studies that have specifically looked at biochemistry students protein problem-solving abilities. Halmo et al. (2018), for example, investigated the domain-specific and domain-general problem-solving abilities regarding protein structure-function problem during a think-aloud interview. It was identified that students had difficulties with: 1) what components of an amino acid drive tertiary structure, 2) how amino acid categories can aid in predicting protein structure, function and dynamics, 3) how noncovalent interactions are a part of the causal mechanism of attraction, and 4) contradicting themselves as while solving the problem (Halmo et al., 2018). It is suggested that more conceptual curriculums such as Project Oriented Guided Inquiry Learning (POGIL; https://www.pogil.org) or CUREs could help to improve biochemistry students' problem-solving abilities.

This chapter focuses on investigating to what extent students in the BASIL CURE demonstrate knowledge for the top-rated Course-based Undergraduate Research Abilities (CURAs) as the Anticipated Learning Outcomes (ALOs) identified for this CURE, to determine if there is support for the CURAs to be considered Verified Learning Outcomes (VLOs) previously described in Chapters 2-4 as the Anticipated Learning Outcomes (ALOs) for the BASIL CURE. Chapter 3 (Irby, Pelaez, et al., 2018a) gave an example of a student response to an open-ended

assessment (probe; Figure 3.1) created based on the top-rated CURAs. This and other open-ended probe questions have been developed for the BASIL CURE are presented here as the primary data source for gathering evidence of students demonstrating their ability to be able to perform the seven top-rated CURAs. This chapter presents the initial phase of data analysis from a case study conducted for a single BASIL CURE course (NE1-3). This was done by analyzing student responses to open-ended probes about how to hypothesize and evaluate the function of proteins of interest. This study is the initial phase of a more extensive study to determine if the identified ALOs for the BASIL CURE (Craig, 2017; Craig et al., 2018; Irby, Pelaez, et al., 2018a, 2018b) are in fact VLOs, as well as, contribute to the CURE, experimental ability, and biochemistry problem-solving literature about student learning and understanding of how to apply biochemistry concepts to computational and biochemical methods to study protein function. In addition to open-ended probes, semi-structured interviews were conducted, and student artifacts were collected. Though these sources of data are not currently presented and analyzed, they will be critical to establish whether or not the identified ALOs have evidence to be considered as VLOs (see future work Section 5.8).

5.2 Goals and Guiding Research Questions for this Study

The goal of this study was to determine to what extent students are able to develop the top-rated CURAs while experiencing the BASIL CURE. To this end, this study was guided by the following research questions (RQs):

- **RQ1)** What evidence is there that the identified top-rated CURAs (ALOs) are VLOs supported by student data?
- **RQ2)** What is the range of student competencies with each CURA (ALO) detected in the responses to the open-ended assessments (probes)?
- **RQ3)** What is the nature of student difficulties relating to the competencies?

5.3 Methods

5.3.1 Methodology – Case Study

A case study methodology (Yin, 1993; Yin, 2006) was used to address the above mentioned RQs (see also Section 1.8.2). The case was bounded by a single implementation of the BASIL CURE with its own specific context that the learning will be taking place within (The context is described in more detail in Section 5.3.1.1.1). The type of case study here would be considered an embedded

case study (Baxter & Jack, 2008; Yin, 2006) because each student will be treated as a unit of analysis, to determine the extent of learning each student demonstrated during their participation in the same BASIL CURE implementation (the context).

5.3.1.1 Context

The BASIL curriculum has been implemented at more than 10 institutions by 15 different instructors (as of 2018). As stated previously (Irby, Pelaez, et al., 2018a, 2018b), in order to fully understand how students are performing in this CURE course, it is important to do an in-depth study of the specific ALOs that characterize the BASIL curriculum. In addition, to complement the student and instructor perception study across multiple implementations (Chapter 4), a case study of a single implementation is needed to gain an in-depth understanding of student development with the top-rated CURAs identified as ALOs of the BASIL CURE (Irby, Pelaez, et al., 2018a, 2018b). The characteristics of this specific implementation of the BASIL curriculum, as well as why this implementation was selected, are described below.

5.3.1.1.1 Details for the Specific Implementation of the Course

The course chosen to be the context of this case study was NE1-3 (Chapter 4; Tables 3.2 and 3.3). NE1 is a Northeastern University with a Carnegie Classification ("The Carnegie Classification of Institutions of Higher Education," n.d.) of "Doctoral Universities: Moderate Research Activity." NE1 was chosen because this is the institution where the "lead designer" (Irby, Pelaez, et al., 2018b) of the BASIL project resides, as well as other faculty members that are involved in the implementation and design of the BASIL curriculum and have experience doing aspects of the BASIL CURE as part of their personal research. In other words, implementations of the BASIL CURE at NE1 served as a flag ship course.

Though the overall flow of the course is similar to a typical BASIL CURE, as described previously (Craig, 2017; Craig et al., 2018; Irby, Pelaez, et al., 2018b, 2018a [Chapters 2 and 3]) and in Chapter 4 (Figure 4.3), there are some distinct differences. The exact topics covered by NE1-3 are presented in Table 5.1, and the structure of the course activities is presented in Tables 5.1 and 5.2. However, some aspects are unique to this course implementation. The first is that instead of a having a range of proteins with different putative functions, all of the proteins provided for students to study have the putative function and classification of being a nucleoside diphosphate X (NUDIX) hydrolase (see Table 5.3 for a list of the proteins studied by the students)

which cleave between the two phosphates (Figure 5.1; Bessman, Frick, O'Handley, & Csanády, 1996; Sheikh, O'Handley, & Bessman, 1998; Srouji, Xu, Park, Kirsch, & Brenner, 2017; Xu, Shen, Dunn, & Bessman, 2003). The NUDIX hydrolase family of proteins active sites contain a highly conserved amino acid sequence known as the NUDIX box: GX_5EX_7REU-XEEXGU, where "X" represents any amino acid and "U" represents one of the bulky, hydrophobic amino acids (e.g. isoleucine, leucine, or valine; Bessman et al., 1996; Sheikh et al., 1998; Xu et al., 2003). Because of this, students in NE3-1 did not use ProMOL as part of their bioinformatics work because though NUDIX hydrolases have a well-defined active site motif, they go through a major conformational change that makes ProMOL results less reliable. Also, because there was strong evidence to support that the proteins (Table 5.3) students were studying were related to the NUDIX hydrolase family, students conducted enzyme assays as outlined by Sheikh et al. (1998) instead of the standard PNPA assays that are the typically performed in the BASIL CURE to detect the presence of hydrolase function. These assays included the putative NUDIX hydrolase of interest (Table 5.3) candidate substrates (e.g. FAD, CoA, GDP glucose, ADP, CDP choline, AD4A, UDP glucose, NAD+, and NADH) and metal cofactors (e.g. Mn^{2+} or Mg^{2+}), in the presence of different buffering conditions. The reaction is dependent on the presence of a metal cation cofactor, and to stop the reaction, the assay is quenched with EDTA. To detect whether or not the protein of interest behaved as a NUDIX hydrolase, Calf Intestinal Alkaline Phosphatase (CIAP) was added to cleave and produce inorganic phosphates (Figure 5.1) which can then be identified by adding molybdate and ascorbic acid (Ames & Dubin, 1960; Sheikh et al., 1998).

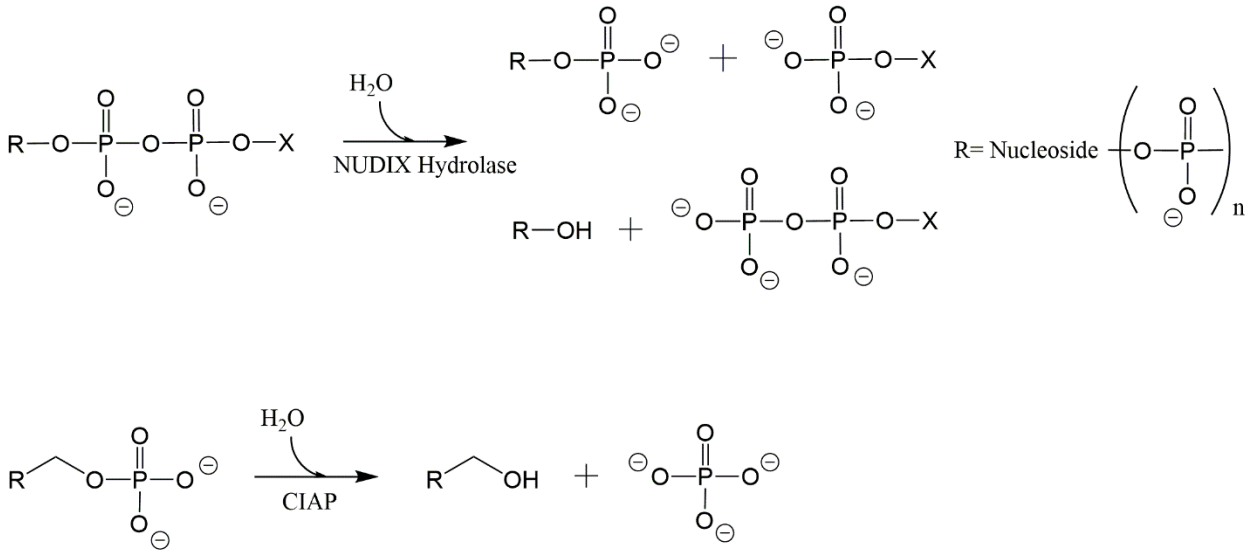

Figure 5.1 Enzymatic reactions taking place in enzyme assays. First, the NUDIX Hydrolase cleaves the diphosphate bond, followed by Calf Intestinal Alkaline Phosphatase (CIAP) generating free phosphates.

Table 5.1 Specific topics covered by NE1-3 and an outline of which of the general BASIL CURE techniques were performed.

Topics Listed in the Couse Syllabus	**Computational Techniques Indicated by the Instructor on the PPI Survey (Section 4.11.1)**	**Biochemical Techniques Indicated by the Instructor on the PPI Survey (Section 4.11.1)**
• Bioinformatics (PDB, PyMOL, ProMOL, BLAST, UniPROT, Pfam, & DALI) • Plasmid purification & Agarose gel analysis • Transformation • Cell growth, protein overexpression, & Cell harvesting • Cell lysis • Analysis of cell lysate (Bradford assay & SDS-PAGE) • Affinity purification of target protein • Analysis of purified proteins (Bradford assay & SDS-PAGE) • Desalt and concentration of fractions • Bioinformatics (PyRX & ligand docking) • Enzyme assays • Kinetics	• PyRx • Dali • Pfam • PyMOL • Chemspider • PubChem • Entrez • BLAST • PDB • DNASU • Plasmid Maps • Chimera	• Protein expression • Metal ion affinity chromatography • Bradford assay • SDS-PAGE • Cell culture and growth

Table 5.2 An outline of the course activities, assignment due dates, and instructor notes provided for NE1-3, as well as, alignment of when different data sources were collected.

Course Schedule		Instructor Notes and Course Due Dates	Data Collection
Weeks 1-3	Bioinformatics protocols	→ Creation of a figure and caption due (Week 3)	Pre-Course PPI (Week 1)
Week 4	Miniprep and plasmid isolation	→ Experimental section for bioinformatics due	
Week 5	Transformation and plating		
Week 6	Large growths and overexpression	→ Write a results/discussion section for the bioinformatics	
Week 7	Purification	→ Annotated bibliography for the introduction with at least 5 references due	
Week 8	SDS-PAGE		
Week 9	Spring break		
Week 10	Practice assays: learn all of the above stuff and determine how much enzyme is needed to get a blue solution with the right absorbance	→ Figure and caption for plasmid prep and overexpression/purification due (Week 10) → Experimental section for protein production due (Week 11) **Notes from Instructor:** • If the students get positive results, is it because they made a mistake or because the assay actually worked? • If the students get negative results, is it because they made a mistake or because the assay did not work? • Our reaction volumes are only 50 uL so proper pipetting technique is a must • Related to above, proper mixing of solutions is essential as well • Organization of workspace/workflow – adding one component and moving the tube to a different position so you know it's done • Getting the timing down – we typically run 10-20 experiments for one assay and they are time-staggered so the students have to get used to doing things at a quick pace when timing is crucial	
Week 11	Practice assays: "master" all of the above stuff and see the effects of pH		
Week 12	Real assays with their enzymes; determine how much enzyme to add		Open-ended Probes
Week 13	Real assays; test various substrates	→ Lab reports due	
Week 14	Real assays; test various metals	→ Last ePortfolio due	Interviews
Week 15	Real assays; test various buffers and pH	→ Final project summary due	Post-Course PPI

Table 5.3 Proteins studied by students participating in course NE1-3 that implemented the BASIL CURE.

PDB ID	Description	Lab Group	Number of Group Members
3FCM	NUDIX hydrolase from Clostridium perfringens	1	2
3GRN	MUTT PROTEIN FROM Methanosarcina mazei Go1	2	3
3GWY	Putative CTP pyrophosphohydrolase from Bacteroides fragilis	3	2
3R03	NUDIX hydrolase from Rhodospirillum rubrum	4	2

5.3.1.2 Participants

Participants for this study were recruited through purposeful sampling based on a set of criteria (Patton, 2002; Suri, 2011). Here, the criteria was that all participants must be enrolled in the biochemistry lab course (NE1-3) that was implementing the BASIL CURE and complete all components of the study. There were no other screening measures.

Six students (Table 5.4) volunteered to participate and completed all parts of this study (open-ended probes, semi-structured interview, PPI survey, and final lab report). All of the students (all names are pseudonyms) that participated in this study self-identified as either a college junior and senior (Table 5.4) and as either biochemistry majors (3/6) or a closely related major: Biotechnology (2/6) or Biomedical Science (1/6). Five of the students had previously participated in undergraduate research outside of their course work (Table 5.4). Of these students, only Alex participated in research that was similar to the research conducted in the BASIL CURE.

Table 5.4 Students that participated in this study.

Student	Lab Group	Protein	Major	Class rank	Previous Research
Ryan	1	3FCM	Biotechnology	Senior	**Yes** - nucleic acid structural biochemistry
Alex	1	3FCM	Biotechnology	Junior	**Yes** – conducted research with the instructor of NE1-3 and lead course designer on research similar to the BASIL CURE on different NUDIX Hydrolase
Jean	2	3GRN	Biochemistry	Senior	**No**
Sage	2	3GRN	Biochemistry	Junior	**Yes** – bacterial signaling
Pat	3	3GWY	Biomedical Science	Junior	**Yes** – studied *C. elegans* and biofilms
Kris	4	3R03	Biochemistry	Senior	**Yes** - total synthesis organic chemistry

5.3.2 Data Sources Collected and Developed

5.3.2.1 Design and Implementation of Open-ended Student Probes for Collecting Data About Students Understanding of the Identified Top-Rated CURAs for the BASIL CURE

The probes were designed to align with the seven top-rated CURAs previously identified (Irby, Pelaez, et al., 2018b, 2018a [Chapters 2 and 3]) and are presented in Table 5.5. The questions were designed to invoke student reasoning skills, problem-solving, and visual competencies, as well as explanation skills about the goals of the BASIL CURE and the identified CURAs. The questions were formatted as either general questions pertaining to the top-rated CURA statements modeled after the activities and experimental outputs from the BASIL CURE, or as a "scenario" with provided data. An example of one of these probes is shown in Figure 3.1 (see Appendix C for all of the probes). The probes were aimed at collecting student data about how they applied the top-rated CURAs (Table 5.5) as they worked through problems pertaining to the computational and biochemical portions of the BASIL CURE. The computational questions were written as a "scenario," because the computational programs are intended to be used together to triangulate a hypothesis (Appendix C). The computational scenario questions were modeled from the two main "steps" from this portion of the lab, identifying the enzyme class and determining a suitable substrate. However, the biochemical related questions were stand-alone question because some of the CURAs related to techniques used as preparatory steps to be able to conduct an enzyme assay. There were also questions that focused on comparing both computational data and enzyme assay data (Figure 3.1 and Appendix C).

The open-ended probes were designed to be administered towards the end of the semester. This instrument was administered as a Qualtrics survey (Qualtrics, 2017) that students completed online. After the Purdue research team reviewed the draft probes, the probes were sent to the lead designer of the CURE (Fall 2017). They were probes were modified for content and clarity based on the lead designer's comments. Then the probes were piloted by students currently taking the CURE course, on a volunteer basis (Fall 2017). The results of the pilot informed additional changes, to assure the probes are eliciting the types of responses they were designed for. Final revisions were made and were administered to students who were enrolled in NE1-3 during the 2018 Spring semester.

5.3.2.2 Semi-Structured Interviews

Semi-structured interviews (DiCicco-Bloom & Crabtree, 2006) were also performed with the students; the reason being that the interviews can better reveal the extent of a student's knowledge towards the top-rated CURAs. Semi-structured interviews allowed for the flexibility to document and elicit a range of student explanations of their experiences, while providing a guide for the conversation to maintain a focus related to the research questions and goals of the project. In particular, the interviews were centered around student describing the research they conducted on their proteins of interest and their ability to describe their knowledge and use of the top-rated CURAs identified for the BASIL CURE. The semi-structured interview protocol is provided in Appendix E. Student interviews have been conducted but have not yet been fully analyzed and are, therefore, not part of this current study but will be used in later phases of this study (see Section 5.8).

5.3.2.3 Research frameworks Guiding Instrument Development

The CRM model (Anderson & Schönborn, 2008; Anderson et al., 2013; Schönborn & Anderson, 2009, 2010) as described earlier (Section 1.6.2.1) played a role in both the development and analysis of student data. First, the CRM model was used in the design of any student probes or interviews to assess students competencies in the identified CURAs (Irby, Pelaez, et al., 2018b, 2018a) because the CRM model greatly influenced them. Secondly, the nature of these student assessments will be to explore how students reason (R) with the various course-related concepts (C) and various modes of representations (M) that they produce, are given, or encounter during the CURE. Anderson et al. (2013) have provided examples of reasoning abilities within the CRM model with concepts and representations.

5.3.3 Data Collection

The open-ended probes were administered online through Qualtrics after the students had completed all of the computational components and had run all of the biochemical assays at least once (Table 5.2). Semi-structured interviews were conducted during week 14 of the course at NE1. This was the second to last week in the course (Table 5.2), so students had an opportunity to complete all aspects of the course and were primarily focused on data analysis and re-doing some experiments if needed. The students who volunteered and are listed in Table 5.4, also provided copies of their final lab reports and links to their ePortfolios that were required for their course.

Similarly, to the semi-structured interviews that were conducted, but will be analyzed in later phases of this study (see Section 5.8).

5.3.4 Analysis of Open-Ended Probes

Student responses to the open-ended probes (See Appendix B) were coded for which of the top-rated CURAs they corresponded to (See Appendix C). They were then analyzed for examples of "complete" or "incomplete" knowledge for a given CURA (Table 5.6). Complete refers to a student answer that was able to explain how they would apply an ability and why they would do so, as well as, using accurate content knowledge. Segments that were coded as incomplete represented a student misapplying an ability or using incorrect content knowledge (i.e. histidine residues are attracted to the negative charge of metal affinity column) as part of their explanation of a CURA.

5.4 Results and Discussion

The open-ended probes revealed a range of student abilities from relatively complete to incomplete knowledge for each CURA (or ALO), suggesting that there is evidence that the identified CURAs are VLOs. This was first observed in Chapter 3 (Figure 3.1) where Sage's (Table 5.4) response for the open-ended probe question Q2.11 (Appendix C and D, Figure 3.1, Table 5.5) was used to demonstrate that there was initial evidence that students are achieving an ability to articulate the identified CURAs for the BASIL CURE (Table 5.5). Continuing with this initial phase of analysis is a discussion of each of the biochemical, computational, and combined top-rated CURAs (Table 5.5) with examples of student responses to the open-ended probes (Table 5.6) that showcase complete and incomplete knowledge students portrayed on the open-ended probes for the top-rated CURAs. Table 5.5 aligns each of the seven top-rated CURAs with the associated BASIL protocols and open-ended probe questions. Additionally, Table 5.5 aligns the top-rated CURAs with the components of the MA(t)CH model (methods, analogy, theory, context, and how to mechanisms) for analyzing scientific explanations (Jeffery et al., 2018; Trujillo et al., 2015). The MA(t)CH model has been able to document scientists' experimental explanations and will be applied in the future analysis of the collected data to help assess the full range of competencies demonstrated by the student participants (See section 5.7).

Table 5.5 Top-rated CURAs aligned with the BASIL protocols, student probe questions, and MA(t)CH components.

Top-rated (TR) CURA Statements	BASIL Protocols	Open-ended Probe Question	MA(t)CH Components
TR1. Explain how the colorimetric enzyme assay works to allow detection of protein function	Enzymatic Activity	Q2.1, 2.4, 2.5, 2.7, 2.11, & 2.12	M/A/H
TR2. Identify an enzyme active site using appropriate computational programs	PyRX Molecular Docking, ProMOL, and Pfam	Q1.4, 1.5, 1.6, 1.7, 1.8 & 1.10	M/A
TR3. Determine the appropriate factors to consider when optimizing or interpreting an enzyme assay	Enzyme Activity	Q2,1, 2.2, 2.4 2.11, & 2.12	M/H – A/H
TR4. Determine using computational software whether, and where, a ligand may be binding to a protein	PyRX Molecular Docking	Q1.5, 1.6, 1.7, 1.8 & 21	A/H
TR5. Compare enzymatic results with those computationally predicted	Enzyme Activity and refer back to all computational protocol results	Q2.1, 2.5, 2.11, 21	M/A/H
TR6. Design an enzyme assay to elucidate protein function	Enzyme Activity	Q2.1, 2.2, 2.7, 2.11, & 2.12	M/H
TR7. Explain how the purification of tagged proteins work and ways the process can be optimized	Protein Purification	Q2.5, 2.6	M/H

In general, the six students (Table 5.4) that participated in the open-ended probes (Appendix C, Table 5.5) had more instances of applying correct knowledge than incorrect knowledge (Table 5.6) for each of the top-rated CURAs. Many of the students, as can be seen by the examples of "complete knowledge" in Table 5.6 were able to articulate and describe how they would apply CURAs when answering the open-ended probes. The 'incomplete knowledge" ranged from students either not entirely being able to describe or apply a particular CURA or described a misunderstanding in their knowledge for that ability. An example of this is for Q1.6:

> Based on the database (Pfam, BLAST, and DALI) outputs above, what is the predicted function of B51L? Explain your answer. Be sure to explain how Pfam, BLAST, and DALI work, and how you integrate the data from each program to inform your prediction (Appendix C).

For this question, Alex responded with "The data would suggest that B51L is a Uracil Phosphoribosyl transferase because that kind of protein has the most hits after alignment," which does answer the question at a very basal level. However, Alex fails to describe how the results from the database searches compared or discussed any evaluation of the 'hits' and relied on the number of results for a particular putative function is returned by the computational programs (Appendix D, Table 5.6). Thus, this response was identified as incomplete for CURA TR2: *Identify an enzyme active site using appropriate computational programs,* which is concerned with identifying potential active sites to propose the putative function of a protein of interest. This is because though Alex's answer was correct, he did not fully articulate his reasoning, which may be supplemented in further analysis from the other collected data sources (see section 5.7). This is in

contrast to Sage's response to the same question (Q1.6, Appendix C and D), which was identified as a complete response (TR2, Table 5.6). Sage described what different computational programs are used for and sited specific metrics such as 'RMSD' and 'Z-score' to determine which type of enzymes were most closely aligned with the protein of interest (Table 5.6). The level of detail and articulation of how the programs were used and how Sage came to a conclusion illustrated that Sage had complete knowledge, based on this initial phase of analysis.

Of the six students who participated in this study, all demonstrated at least one instance of complete knowledge (Table 5.6). Alex and Sage had the most instances of their responses being classified as incomplete (Table 5.6), whereas Jean and Ryan had fewer examples of excerpts being identified as incomplete. Pat and Kris did not have any instances of their responses being identified as incomplete. This is promising and may suggest that the open-ended probes are capturing a range of student competencies with the CURAs. However, this needs to be investigated further with the other collected data sources and compared to other collected metrics (i.e. PPI data) to see if there is further evidence of this.

Below will briefly outline the results for the other six top-rated CURAs (Table 5.5), to provide some examples of the complete and incomplete knowledge exhibited in student responses to the open-ended probes. These examples will be presented based on BASIL CURE component, computational (5.4.1) and biochemical (5.4.2).

5.4.1 Computational CURAs

Of the seven top-rated CURAs (or ALOs) for the BASIL CURE, two of them specifically pertained to the computational components of the CURE, TR2 and TR4 (Table 5.5). These two CURAs were directly related to the PyRx Molecular docking (TR2 and TR4), ProMOL (TR2), and Pfam (TR2) protocols (Chapter 3, Irby, Pelaez, et al., 2018a), and had to do with identifying an enzyme active (TR2) and determining whether and where a potential ligand may be binding to a protein. TR2 was discussed as a detailed example above.

5.4.1.1 TR4: Determine using computational software whether, and where, a ligand may be binding to a protein

Ryan, Pat, and Sage all determined the proper "best" ligand based on the data presented in Q1.8, and cited pieces of evidence such as more interactions with the protein and/or lower but similar free energy values as another potential ligand but bound near the "binding pocket" [active site]

was a better candidate ligand (TR4, Table 5.6). This can be seen by Pat's response for Q1.8 Appendix C-E:

> "I think CTP is a better ligand choice. CTP appears to interact with B51L all along its own structure while 5GP only interacts on one end and with fewer residues. I would use the other programs to see if CTP is a ligand used by other uracil phosphoribosyl transferases, which would make it a more likely candidate for B51L." (Pat, Q1.8)

Here Pat illustrates how the visual representation provided in Q1.8 (Appendix C) were used determine the number of interactions between candidate ligands and the protein of interest, B51L. Pat also goes on to discuss that she would also use additional sources of information to see if the identified candidate ligand (CTP) has been documented as a ligand with any other related proteins. However, Alex demonstrated incomplete knowledge by only stating that you can use the PDB ligand finder for ligands that bind to similar proteins, failing to explain how this information would be used to determine the ligand for a specific protein of interest (Table 5.6).

5.4.2 Biochemical CURAs

Four of the seven top-rated CURAs (ALOs) were associated with the biochemical portions of the BASIL CURE, TR1, TR3, TR6, and TR7 (Table 5.5). Majority of these CURAs pertained to the enzyme activity protocol (TR1, TR3, and TR6), whereas TR7 related to protein purification protocol (Chapter 3, Irby, Pelaez, et al., 2018a).

5.4.2.1 TR1: Explain how the colorimetric enzyme assay works to allow detection of protein function

When responding to question Q2.4: *How does the colorimetric enzyme assay work in general and specifically for the assay you conducted in this course? Additionally, what information can be gained about a protein's function? What limitations may there be?* There were some very articulate examples describing how colorimetric enzyme assays work. Kris discussed in detail how the enzyme assays that were used and developed for the proteins investigated in NE1-3 worked (Table 5.6). Kris's articulation of the details of the NUDIX was correct (see Section 5.3.1.1.1) and represented correct knowledge. Alex, on the other hand, did not provide as much detail on the assay, but did discuss the importance of co-factors for NUDIX hydrolases and discussed what the interpretation of the conditions that produced the largest absorbance would mean "The substrate + co-factor + enzyme combination that produces the highest OD should give a good suggestion as

to what the protein's function is". Sage presented some confusion between the Bradford assay conducted to determine the concentration of protein and the enzyme assay. In Sage's answer, the Bradford assay was discussed as if it was the enzyme assay and that you would need to add calf intestinal phosphatase to detect color and that a Bradford assay detects activity (Table 5.6). Sage's answer was classified as incomplete and will need to be investigated further to determine if Sage has a true misunderstanding between the two types of assays and their purposes, or if Sage misinterpreted the question.

5.4.2.2 TR3: Determine the appropriate factors to consider when optimizing or interpreting an enzyme assay

When discussing the appropriate factors to consider when optimizing or interpreting an enzyme assay (Q2.2, Appendix C), Sage explained the need to run an initial enzyme reaction progress curve to determine proper reaction conditions for enzyme stability and that the reaction does not occur too slowly or rapidly (Table 5.6). Similarly, Pat explained the need to test specific reaction conditions, such as temperature, pH, cofactors, coenzymes, and concentration, to determine what combination produces the most activity, so that you can achieve the strongest signal (Table 5.6). Sage's and Pat's responses are examples of complete knowledge because they both were able to articulate various parameters that are important to take into account when designing and running enzyme assays. One student, Jean, discussed that the calibration curve used to interpret the enzyme assays is key to optimizing results, stating that it is important to assure that the "calibration curve and its range or the correct use of enzyme concentration in the different enzyme-ligand samples" (Q2.2, Table 5.6). This is correct, having an appropriate calibration curve is critical to properly interpreting the results of a colorimetric enzyme assay, however making a proper calibration curve itself, does not optimize an enzyme assay. Jean's response would be considered incomplete, and similar to Sage's response for Q2.4 (TR1, Table 5.6, Section 5.4.2.1) will need to be explored further to see if Jean has a misunderstanding in what it is meant to optimize an enzyme reaction or if Jean may have misinterpreted the question.

5.4.2.3 TR6: Design an enzyme assay to elucidate protein function

When asked on the open-ended probes about how a student would propose biochemically confirming a protein's function (Q2.1, Appendix C), there were examples of complete and incomplete knowledge towards this ability (TR6, Tables 5.5 and 5.6). A good example of this is came from Kris's answer to Q2.1, where Kris discussed the need to include positive and negative

controls, that the assay should agree with physiological conditions of the enzyme, and that "the only thing that should be able to act with or on the enzyme is the ligand itself, otherwise results won't carry as much weight. Positive and negative results should be easy to discern." All of the considerations Kris discussed are things that should, and need, to be considered when designing assays. Kris also outlined why the conditions were mentioned and gave examples, rather than just simply listing a set of conditions without any explanation as to why. Sage, on the other hand, once again confused a Bradford assay and using a nano-drop to determine protein concentration as methods for detecting and determining enzyme activity (Table 5.6). Though these methods and considering relative concentrations of a protein in an assay is important, these methods themselves are not an enzyme assay and do not provide information of an enzymes activity. Sage's difficulty with the purpose of these methods continues to persist. This may be due with Sage's understanding of an assay. We documented previously (Irby, Pelaez, et al., 2018a [Chapter 3, Section 3.8, Figure 3.1]) that Sage misused the word 'assay' when reasoning through the question and data presented in question Q2.11 (Appendix C, Figure 3.1) and could be one reason why Sage is having difficulty answering questions specifically about enzyme assays (Table 5.6, Section 5.4.2.1).

5.4.2.4 TR7: Explain how the purification of tagged proteins work and ways the process can be optimized

There was one open-ended probe question (Q2.6, Appendix C) designed to reveal students' competence with tagged protein purification (TR5, Table 5.5). Q2.6 specifically asked students *How can purification by protein tags be optimized?* Several students were able to discuss ways to optimize tagged purification. For example, Pat explained that it is important to take into account the residues that other proteins in the solution may contain because "[…] if there are a lot of proteins with a high concentration of histidine, a His tag might not be appropriate because extraneous proteins may bind to the matrix and cause impurities in the final result" (Table 5.6). Kris discussed the need to design simple tags that can be engineered into plasmids and do not impact the overall structure and function of the protein. Thus "the properties bestowed by the tag whether that be molecular weight or chemical properties, should allow the protein to be differentiated and separated predictably and reliably from other proteins" (Kris, Q2.6, Table 5.6).

Though several students indicated a complete knowledge of using protein tags for purification, several students showed incomplete or no knowledge about how tagged proteins worked or how to optimize them. Two students, Alex and Sage, stated that they either did not know much about how protein tags work or how to optimize protein purification (Table 5.6). Ryan

attempted to explain how His-tag purification works, but misidentified histidine residues as having positive charges which then interact with a negatively charged metal-based resin (Table 5.6). Lastly, Jean, merely stated that they would choose "the most suitable tag compound" without any explanation about how to do so.

5.4.3 Combined CURA TR5: Compare enzymatic results with those computationally predicted

Students were able to make connections between the computational outputs and the biochemical data presented. This was shown previously for Sage (Irby, Pelaez, et al., 2018a [Chapter 3, Section 3.8, Figure 3.1]). Additionally, Ryan and Jean also demonstrated evidence of having complete knowledge for this CURA (TR5, Tables 5.5 and 5.6). Jean demonstrated this by his response to Q2.1 (Appendix C), where he stated that using computational programs to narrow down "the 4 or 5 most suitable ligands can be purchased and together with the protein in analysis, used to create an assay describing the minimum enzyme concentration to produce enzyme activity with each substrate. In order to determine the activity of the enzyme […] a colored complex can be formed by adding a suitable reactant and absorbance can be used to determine the enzyme's preferred substrate" (Table 5.6). Another example of complete knowledge for linking together computational and enzymatic results is Ryan's response to Q2.11 (Appendix C, Figure 3.1A), "[…] The computational outputs point to 3H04 being a hydrolase, and the graph shows that PNPA and the protein interact. They are both pointing to the protein being a hydrolase protein. This is able to be hypothesized based on the structure of PNPA and where the molecule could be hydrolyzed." Alex, for example, did not have any examples of connecting computational and enzymatic results together. In responding to Q2.11, Alex only commented on the enzymatic data presented in the question by saying "the concentration of PNPA increases the level of absorbance increases. Which would suggest that activity increases respectively to PNPA concentration." However, to truly explore the full range of students' competencies with CURA TR5 (Table 5.5) the other data sources will need to be analyzed. This is because the nature of the open-ended probes (Appendix C) tends to segregate the data types or may over scaffold the students to integrate them together. Whereas the way the explain their research and results in their final lab reports and during the semi-structured interview may better represent how they integrated using both types of data to come to a conclude their protein's function.

Table 5.6 Examples of students demonstrating complete and incomplete knowledge for the top-rated CURAs when responding to the open-ended probes.

More examples of excerpts from the open-ended probes (Appendix C and D) can be found in Appendix E.

CURAs		Examples of complete and incomplete knowledge of the top-rated CURAs for the BASIL CURE
TR1: Explain how the colorimetric enzyme assay works to allow detection of protein function	**Complete**	• […] a color change indicates a positive result. Dyes or other molecular indicators will bind and allow color change in the presence of bi-products resulting from a successful reaction between your enzyme and the ligand. […] Although NUDIX hydrolase enzymes cleave diphosphate bonds, the AMES reagent will not be able to detect a successful reaction since the nucleotides are still bound. Calf intestinal phosphatase then cleaves the phosphates from these bi-products to release free phosphates which can be detected. The AMES reagent (molybdate and ascorbic acid) is incredibly sensitive to free phosphate and will turn blue. The intensity of the blue can be detected using UV visible spectrometers. (Kris, Q2.4) • A colorimetric enzyme assay works by producing a measurable molecule after the reaction, one that most likely produces a color shift significant enough that it can be measured using OD. The substrate + co-factor + enzyme combination that produces the highest OD should give a good suggestion as to what the protein's function is. (Alex, Q2.4)
	Incomplete	• In our course, Bradford assays were primarily used where there was a color change to blue when there were high amounts of activity otherwise there was a fractional change in the color and the activity could only be recorded via a spectrophotometer. The assay functions by the dye binding with protein and then changing color. In our case, we added some calf intestinal protein that binds with our reaction products so whenever there is a reaction that occurs there is a colorimetric change that can be recorded allowing us to see if reactions are occurring or not. (Sage, Q2.4)
TR2: Identify an enzyme active site using appropriate computational programs	**Complete**	• When it comes to utilizing BLAST, Pfam, and DALI I personally found that BLAST was by far the worst option when it came to actually finding useful results. Since all it provided was a sequential similarity and nothing else, so I would not know much of the possibilities when it came to actual protein function. That is where Pfam came in handy since it utilizes motif similarities to obtain a set of proteins that share functionality to the protein in question. So, Pfam was a lot more helpful since it gave me a wider array of results because it looked at motifs and then built a family based on that motif. Then, DALI was just overall the most helpful since it did a comparison of every single side chain between the two or more proteins given and then creates overlays of it and gives a lot of useful information like RMSD, Z-score and more which can really help to find extremely similar proteins that give us clues to the function of the protein in question. So, BLAST just gave a lot of similar sequences that could be useful or could be a waste of time, Pfam gave a solid family to work with and DALI allowed to work out the bad seeds of the family to really narrow down the possible function of the protein. Looking at all the data, I would say that it is a phosphoribosyltransferase and potentially a Uracil phosphoribosyltransferase. The reason why I say this is because the results from DALI show a very significant z-score for all other phosphoribosyltransferases, which means that there is a high similarity in the significant folds of the proteins. Alongside that, the results from Pfam indicate the same when comparing the sequence and the HMM. In the Blast results, %ident with higher than 50 all have something to do with phosphoribosyltransferases. (Sage, Q1.6)
	Incomplete	• The data would suggest that B51L is a Uracil Phosphoribosyltransferase because that kind of protein has the most hits after alignment. (Alex, Q1.6) • I thought it was a EC 3: Hydrolase but its probably a EC 2: Transferase after the database results (Alex, Q1.7)

Table 5.6 Continued

CURAs	Examples of complete and incomplete knowledge of the top-rated CURAs for the BASIL CURE	
TR3: Determine the appropriate factors to consider when optimizing or interpreting an enzyme assay	**Complete**	• One of the optimization setups that should be run is enzyme reaction progress curve which allows us to find the best concentrations to use for the assays. Since we want the enzyme to remain stable during the whole experiment, an enzyme reaction progress curve can help us a lot since it will show if the substrate is running out too quickly or if there are too little protein and things of that nature. Once results from the curve are obtained it allows us to ensure that the reactions are occurring at an appropriate speed during the assays. Alongside that, the most important thing to worry about in an assay is the enzyme stability. Once, a general idea of how the reaction is going to progress is obtained, we can compare results to it to see if there is something wrong with our stability. In the data, we should normally see 3 lines based on concentration that do not converge and plateau out after a steady increase almost like a bacterium's growth phase diagram. If that is not seen and there are issues with specific concentrations then those concentrations would have to be re-run and stability would have to be checked. Since an enzyme reaction progress curve would rule out a high concentration problem. (Sage, Q2.2) • Enzyme assays are interpreted based on optical density readings. To optimize assays, I would test many different conditions (temperature, pH, concentrations) to determine which produces the most activity. I might need to add various cofactors or coenzymes based on the enzyme. If all my assays produce small OD readings, I would continue to change variables until a greater activity is noted (Pat Q2.2)
	Incomplete	• Factors that are important when optimizing or interpreting an enzyme assay can be things like the reliability of the calibration curve and its range or the correct use of enzyme concentration in the different enzyme-ligand samples (Jean, Q2.2)
TR4: Determine using computational software whether, and where, a ligand may be binding to a protein	**Complete**	• I think CTP is a better ligand choice. CTP appears to interact with B51L all along its own structure while 5GP only interacts on one end and with fewer residues. I would use the other programs to see if CTP is a ligand used by other uracil phosphoribosyltransferases, which would make it a more likely candidate for B51L. (Pat, Q1.8) • CTP looks to have more interactions with the protein even though the free energy value is similar between CTP and 5GP. CTP could be a more appropriate ligand due to the extra interaction as it looks almost like a binding pocket. (Ryan, Q1.8) • […] I would say that CTP (Citrine Triphosphate) is the appropriate candidate substrate for B51L due to it creating a better binding with B51L and also having the lower kcal/mol value. Since the value is a bit lower that means that it has a more spontaneous reaction than 5GP and it has more binding sites with B51L meaning that it will be more stable in the active site so the reaction is more likely to occur. (Sage, Q1.8)
	Incomplete	• Using the PDB's ligand finder look for ligands associated with Uracil Phosphoribosyltransferase (Alex, Q21)

Table 5.6 Continued

CURAs	Examples of complete and incomplete knowledge of the top-rated CURAs for the BASIL CURE	
TR5: Compare enzymatic results with those computationally predicted	**Complete**	• Since it reacts with this substrate then the computational data was correct in assessing this as a hydrolase. (Sage, Q2.11) • Firstly, the pdb structure of the protein analyzed and of the possible ligands for it have to be downloaded from PDB and then the affinity between the protein and each possible ligand can be estimated with the docking function in PyRx. Secondly, the plasmid coding for the protein in analysis can be purchased and induced in E. Coli to transcribe our protein. The protein can then be extracted from E. Coli cells and purified. The 4 or 5 most suitable ligands can be purchased and, together with the protein in analysis, used to create an assay describing the minimum enzyme concentration to produce enzyme activity with each substrate. In order to determine the activity of the enzyme, the product coming from the ligand needs to be quantified. To do that, a colored complex can be formed by adding a suitable reactant and absorbance can be used to determine the enzyme's preferred substrate. (Jean, Q2.1) • 3H04 interacts with PNAP and does so almost linearly based on the graph. The slope depends on the concentration of the ligand present, the more ligand, the more PNPA is hydrolyzed. This graph was likely produced from data from a color assay. The computational outputs point to 3H04 being a hydrolase, and the graph shows that PNPA and the protein interact. They are both pointing to the protein being a hydrolase protein. This is able to be hypothesized based on the structure of PNPA and where the molecule could be hydrolized. (Ryan, Q2.11)
	Incomplete	• Alex did not respond to any of the probes that demonstrated any knowledge of this ability
TR6: Design an enzyme assay to elucidate protein function	**Complete**	• […] After choosing which ligands are likely a good fit to test and pure concentrated protein is collected, the protein can be screened against these ligands in various assays. The assay should include a positive or negative result if there is evidence of a reaction between the protein and substrate. For example, a molecule or dye may change color or fluoresce in the presence of any biproducts resulting in the chemical reaction between the enzyme and ligand. The assay chosen is going to have to agree, physiologically, with the enzyme. This means that the enzyme must tolerate the conditions the assay requires and that the pH and reagents in the assay do not alter the protein's function. The only thing that should be able to act with or on the enzyme is the ligand itself, otherwise results won't carry as much weight. Positive and negative results should be easy to discern. (Kris, Q2.1)
	Incomplete	• I would personally run several assays to confirm the function of the protein and utilize Bradford assay and nanodrop to obtain data on the assays to identify functionality. The reason why I chose Bradford assay and nanodrop is due to more experience with those techniques and understanding the results of those techniques a bit better. I would have several graphs with calibration curves to obtain the protein activity. Since I would run a decreasing amount of protein in each sample I would expect to see a more or less decreasing linear model where activity is occurring since activity is decreasing due to a drop in the number of available proteins. Since the models are based on activity occurring whenever activity does occur I would be able to find functionality based on activity. (Sage, Q2.1)

Table 5.6 Continued

CURAs	Examples of complete and incomplete knowledge of the top-rated CURAs for the BASIL CURE	
TR7: Explain how the purification of tagged proteins work and ways the process can be optimized	**Complete**	• Protein tags should be **optimized** based on the other proteins that will be in solution. If there are a lot of proteins with a high concentration of histidine, a His tag might not be appropriate because extraneous proteins may bind to the matrix and cause impurities in the final result. (Pat, Q2.6) • The purification tag may not interfere with the overall structure and function of the protein in question and should operate the same had the tag not been there. The purification tag should be relative easy to engineer into the plasmid, easy for the cell to transcribe and translate and allow the protein to be separated away from others. In other words, the properties bestowed by the tag whether that be molecular weight or chemical properties, should allow the protein to be differentiated and separated predictably and reliably from other proteins. (Kris, Q2.6)
	Incomplete	• I'm not sure, I don't know much about how protein tags work to be honest. I suppose you'd want a high affinity between two molecules and a wash that allows you to elute everything in the supernatant besides the protein (Alex, Q2.6) • I am unsure about the **optimization** of the protein purification step so I cannot accurately describe this situation. (Sage, Q2.6) • Histidines have a positive charge and the His-tag is usually between six and eight histidines at either the N or C terminus of a protein. They can attract a negative charge in a column and are useful for purification. A column is usually filled with a resin or beads that can be saturated with a solution that doesn't out-compete the tagged protein. (Ryan, Q2.5) • By choosing the most suitable tag compound (Jean, Q2.6)

5.5 Conclusions

The goal of this study was to determine if there was any initial evidence for the ALOs, in terms of CURAs, to be considered VLOs. Towards this goal, thee research questions were addressed: RQ1) What evidence is there that the identified top-rated CURAs (ALOs) are VLOs supported by student data? RQ2) What is the range of student competencies with each CURA (ALO) detected in the responses to the open-ended assessments (probes)? and RQ3) What is the nature of student difficulties relating to the competencies?

Overall the open-ended probes were able to reveal evidence of a range of student abilities to discuss and implement the different top-rated CURAs (RQs 1 and 2). Though this is a preliminary analysis of this data, there does appear to be evidence to support that the open-ended probes were successful at capturing a range of student knowledge and abilities for the assessed CURAs (Section 5.4, Table 5.6). All of the students were able to articulate some examples of "complete knowledge" for one or more of the CURAs. Of the six students, two of them (Pat and Kris) had no instances of responses being identified as incomplete, two students (Jean and Ryan) had a few cases of responses being classified as incomplete, and two students (Alex and Sage) had many instances of responses being classified as incomplete (Table 5.6). This suggests that the open-ended probes may be able to identify a range of competencies with the identified top-rated CURAs (Tables 5.5 and 5.6) for the BASIL CURE (RQ2). However, more analysis of all the data will be needed to determine whether the instances of incomplete knowledge, is truly incomplete, or just an artifact of students interpreting and responding to the open-ended probes.

Based on the open-ended probes, students seemed to have the most difficulties discussing how to optimize tagged proteins (TR7, Tables 5.5 and 5.6). Only Pat and Kris demonstrated complete knowledge, the four other student participants all had their responses for Q2.6 (Appendix C) classified as incomplete knowledge (Table 5.6). It is unclear at this point what the root of the difficulty is. One limitation is that there was only one question on the open-ended probe given to the students that dealt with tagged protein purification, where most other CURAs were aligned to multiple questions (Table 5.5). Thus, given the opportunity to discuss tagged purification during an interview may remedy some of the incomplete knowledge that was documented, this may be true for all instances of incomplete knowledge. One student, Sage, showed repeated difficulty discussing assays and differentiating between assays used to determine protein concentration (i.e. Bradford assay) and enzymatic assays (Table 5.6). Jean also showed some evidence of having the same difficulty (Table 5.6). This will be a focus of future analysis to see if other students reveal

similar difficulties when discussing their research during interviews and final lab reports, as well as seeing if Sage and Jean continue to conflate different types of assays. Though more analysis needs to be conducted, it appears that the open-ended probes were able to reveal the nature of student difficulties with the top-rated CURAs (RQ3).

Additionally, it appears that there is some initial evidence to support that the students are developing competencies with the ALOs (CURAs) that instructors expect students to develop during the BASIL CURE - i.e. that the ALOs can be confirmed as VLOs. This is based on the number of well-articulated excerpts that were considered to be complete knowledge (Table 5.6) and because all six of the student participants at least one instance documented complete knowledge for a CURA. Though this will need to be evaluated further (see Section 5.8), the data analyzed so far supports the validity of the PICURA method (Chapter 2; Irby, Pelaez, et al., 2018b) and the process in which the open-ended probes were created (Chapter 3: Irby, Pelaez, et al., 2018a).

5.6 Considerations and Limitations

The data and analysis presented in this chapter is only for the initial phase of analysis of one of the data sources (open-ended probes). Because of this, it is uncertain whether or not a student's complete or incomplete knowledge demonstrated thus far, is a true reflection of their understanding or just their performance on the open-ended probes. Since many of the open-ended probes (Appendix C) asked students to answer questions based on provided data and not directly about the top-rated CURAs, it is possible that some knowledge currently regarded as incomplete may be resolved when analyzing the interview data where students are asked to explain their research and answered some targeted questions about the top-rated CURAs. Thus, though some basal evidence about students understanding of these abilities and whether or not they are VLOs has been documented, more research is necessary to reach any final conclusions.

5.7 Future Work

Future work will further analyze the open-ended probes, as well as the student interviews and student work. Following a case study approach, the analysis of all data sources will be converged and analyzed holistically and not as separate analyses (Baxter & Jack, 2008; Creswell, 2013). Codes from all the data sources will be grouped together in themes, or categories, to begin to note

commonalities within and between codes (Creswell, 2013). This organization will allow a connection between the raw data and how it addresses/serves as evidence for the posed research questions. During the coding and theme generation process, trends in the data will begin to be interpreted by the researcher (Marshall & Rossman, 2011). As this process is conducted, passages and quotes that best represent the data will be selected for use as examples of the researcher's conclusions (Marshall & Rossmanma, 2011). While doing this, there will be a clear intent to seek out meaningful examples that answer the research questions of interest and adhere to the models and theories mentioned throughout this dissertation.

One way to assess how students came to their conclusions and applied the identified CURAs is to examine their explanations of the research they conducted. To understand this, a model of how scientists explained their research will be applied (Jeffery et al., 2018; Trujillo et al., 2015). This model is referred to as the MA(t)CH model, which serves as a tool to characterize the components of scientific explanations in terms of the Methods, Analogies, Context, and "How" mechanisms (Trujillo et al., 2015), and has been revised to include theory and its role in mediating between the method, analogy, and "how" components (Jeffery et al., 2018). This will provide a way to characterize a student's explanation, and how they support the conclusions they came to, based on their work in the lab. Emergent codes will be developed through the lens of the CRM and MA(t)CH models (Jeffery et al., 2018; Trujillo et al., 2015) for BASIL CURE students' explanations of their protein's function and for their knowledge of the top-rated CURAs (Table 5.5).

CHAPTER 6. GENERAL DISCUSSION

This dissertation addressed the following four overarching research questions:

- **RQ1)** How can ALOs be rigorously identified for the BASIL CURE? (Chapter 2)
- **RQ2)** How can the identified anticipated learning outcomes (ALOs) be used to develop a matrix that characterizes the BASIL CURE? (Chapters 3)
- **RQ3)** What are students' perceptions of their knowledge, experience, and confidence regarding their abilities to perform the top-rated ALOs for this CURE (Chapters 4)
- **RQ4)** What are appropriate assessments for student achievement of the identified ALOs and what is the nature of student learning, and related difficulties, developed by students during the BASIL CURE? (Chapters 3 and 5)

All four of the above research questions were addressed and led to several outputs that we expect will make an important impact on both the CURE and assessment fields. By addressing RQ1, we successfully developed a novel data-driven Process for Identifying Course-based Undergraduate Research Abilities (PICURA). We then addressed RQ2 by using PICURA to identify a range of CURAs for the BASIL CURE which was then formulated into a matrix of CURAs (Figure 2.1) (Irby, Pelaez, et al., 2018b). To our knowledge, PICURA is the first documented process that uses data from instructors to identify ALOs of relevance to a CURE curriculum. Although PICURA was developed in the context of an upper-division biochemistry CURE, the data inputs (artifact content analysis, open-ended survey, semi-structured interview, and a Likert survey) are not unique to the BASIL CURE and could benefit any CURE or other curriculum development effort being taught by one or instructors, including large-scale collaborative efforts in which the same course is taught across multiple institutions.

In addressing RQ 2, PICURA was applied to the BASIL CURE to identify and rank 43 ALOs (Table 3.1). The ALOs were then organized into an ALO matrix (Table 3.2), which permitted the characterization of the BASIL CURE based on its specific activities and components, and informed the design of student assessments that target the CURAs (RQ2). The development of our taxonomy-like matrix adds to the list of other educational taxonomies developed by education researchers and instructors, including those published by Adams (2015), Couch, Brown, Schelpat, Graham, & Knight (2015), Kidwell, Fisher, Braun, & Swanson (2012), Krathwohl (2002), Marzano (2001), Marzano & Kendall (2007), and Towns, Raker, Becker, Harle, & Sutcliffe (2012) to structure and characterize learning within a course or curriculum. However, the ALO matrix created as a part of this dissertation (Irby, Pelaez, et al., 2018a [Chapter 3]) is the first

of its kind to align course components, with specific ALOs, and learning themes. The ALO matrix also allows for aligning the BASIL CURE ALOs with other efforts in the CURE literature and experimental competency literature (Table 3.3), which could bring both communities together and add support to the merit of the identified CURAs and PICURA. The ALO matrix could also serve as an assessment creation tool (see Section 3.8, Figure 3.1, Appendix C) to generate open-ended assessments that measure actual student learning within the BASIL CURE (RQ4, See also Chapter 5).

As discussed throughout this dissertation, and documented by Shortlidge & Brownell (2016), student perception surveys have been used as a primary means of measuring the success of CURE curricula. When addressing RQ3 (Chapter 4**)** we described a Participant Perception Indicator (PPI) survey (e.g. Clase et al., 2008; Hensiek et al., 2016) used to identify to what extent students perceived a change in their knowledge, experience, and confidence (KEC) for the identified BASIL CURE ALOs and techniques they conducted as a part of their specific implementation of BASIL CURE. It was found across all implementations that there were significant gains in KEC, with large effect sizes for the top-rated CURAs (RQ3, Figure 4.3, Table 4.7). This study also documented that the perceived learning by the students was dependent on the specific activity they participate in (RQ3, Figure 4.2, Table 4.5). Though this study, like many other CURE studies, used a perception survey, several notable differences that will add to the literature. This study is, to our knowledge, the first in the CURE literature to use a multiple-tier metric (i.e. having students rate themselves on their knowledge, experience, and confidence) for items that were rigorously identified for a specific CURE (i.e. using PICURA), instead of asking students to rate the degree to which they agree with more general statements. Additionally, rather than being used as the only proxy for student learning the PPI survey was used in conjunction with our assessments of actual learning (Chapter 5) to help inform what should be assessed for each BASIL implementation and what areas of difficulty to focus remedial efforts. Thus together the results of the Chapters 4 and 5, (especially once more assessment data has been collected) will allow for the first time in the CURE field a comparison of students' perceived and actual learning and provide instructors with insights into how students are developing the identified CURAs and their self-efficacy (e.g. Bandura, 1977, 1995; Lunenburg, 2011).

The final research question of this dissertation, RQ4, was addressed in part by Chapters 3 and 5. Chapter 5 described a case study that began to document the range of complete and incomplete student knowledge of the top-rated CURAs. This was done by conducting an initial

analysis of student responses to open-ended assessments that targeted selected CURAs (Figure 3.1 and Appendix C). This initial analysis yielded preliminary evidence that the top-rated ALOs identified by PICURA for the BASIL CURE may be VLOs. However, further analysis of the responses to the open-ended assessments, as well as, analysis of the student interview data and final lab reports are needed to confirm these findings. Additionally, the open-ended assessment data revealed preliminary evidence of various student difficulties especially with understanding the differences between protein and enzymatic assays. This needs to be explored further but could also provide valuable insight to the biochemistry and molecular biology education communities about this important area of research.

This dissertation is positioned to make important contributions to both the assessment and CURE literature. As discussed earlier, there have been many different documented benefits of CURE curricula (Table 1.1, Corwin, Graham, et al., 2015). However, many CUREs have only been studied by quantitative, self-reported, Likert-scale surveys, many of which have been aggregated by Shortlidge & Brownell (2016). This dissertation documents ways to move beyond these types of surveys through the use of other methods (see Chapter 3 [Irby, Pelaez, et al., 2018a] and Chapter 5), as well as how to use Likert-scale surveys (Chapter 4) to capture students' experiences and guide in-depth qualitative analysis. This dissertation, specifically Chapter 2 (Irby, Pelaez, et al., 2018b) and Chapter 3 (Irby, Pelaez, et al., 2018a), is the first documented case of using and providing a process (PICURA) and other tools (such as an ALO Matrix) to document ALOs and use them to guide assessment of a CURE. This is an essential first step for assessing CUREs (Brownell & Kloser, 2015); as until now, there has been no documented data-driven process of how to do so. Lastly, this work contributes to the assessment methods literature by demonstrating new approaches for identifying ALOs and how to determine if there is appropriate evidence for them to be confirmed as VLOs.

This dissertation will have direct implications for advancing the goals of the BASIL CURE. Instructors will now be able to use PICURA to continually update the identified ALOs and matrix for their own courses, to design their own assessments and where relevant use student response data to inform the modification of lab activities and protocols to improve student performance. This could include determining which CURAs should be top-, middle- or lower-rated and confirming which of these are VLOs, or whether some assessments need modifying to enable such verification. Additionally, one mission of the BASIL CURE team is for the dissemination of the BASIL curriculum. Towards this goal, in my view, it would be of great benefit to the teaching of

biochemistry if more institutions would consider implementing the BASIL CURE. This dissertation will also inform other potential adopters of BASIL and provide them with materials for monitoring student learning about research abilities.

Additionally, the publications produced from this dissertation will in my view make important contributions to the literature regarding the assessment of student learning from CUREs. As discussed earlier, there are numerous published CUREs but until the present study none have fully come to grips with what such CUREs are actually developing in students in terms of research abilities and how best to specifically assess such abilities. This is because this dissertation is the first, to our knowledge, to develop, document, and align ALOs with course-specific data sources from instructors allowing for the assessment of student learning of consensus ability statements. This is a key achievement of this dissertation, by identifying ALOs, in terms of CURAs, shifts the focus from typical procedural or technical knowledge, commonly found in most undergraduate teaching lab courses, places a greater emphasis on discovery-type research abilities, which involves alignment of data analysis to a research question and conclusions based on evidence from that data (Chapters 2 and 3). These ALOs then can be used for embarking on assessment development and validation projects (e.g. the work described in Chapters 3-5). In so doing, instructors and discipline-based education researchers can focus their assessment tasks on assessable research abilities that emerge from unpacking the big CURE ideas and will supplement general assessment measures commonly used to study CUREs (Shortlidge & Brownell, 2016). This culminates in providing discipline-based education researchers and instructors tools and a model for how to rigorously assess student learning in their courses, whether they are interested in CUREs or other course formats, because this dissertation was grounded in assessment frameworks (Anderson, 2007; Brownell & Kloser, 2015; NRC, 2001) - allowing for the determination of what students actually learn from participating in a CURE. This dissertation does not only outline how to identify ALOs, but how to determine VLOs. This is a significant outcome of this work, because too often people report their learning outcomes, or objectives, with no distinction between if they are anticipated or verified; let alone, detail the process for how they identified learning outcomes. The work of this dissertation outlined detailed steps for identifying ALOs and determining if they are also VLOs, which is a valuable contribution to the assessment field. Lastly, the outcomes of this dissertation give insight on how to assess innovative course formats that are being implemented at multiple institutions by many different instructors.

APPENDIX A. CHAPTER 2 PERMISSION

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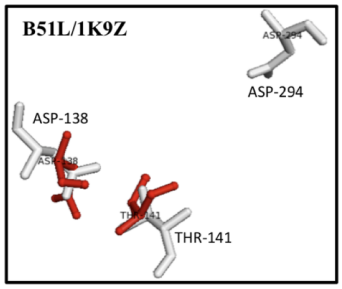
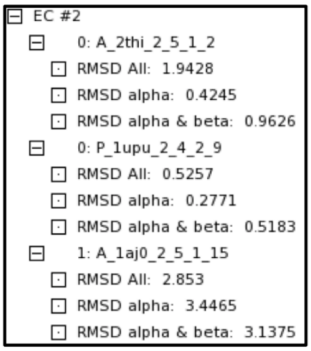
APPENDIX C. OPEN-ENDED STUDENT PROBES

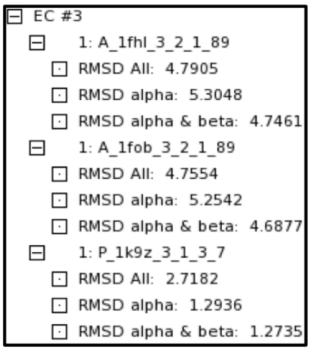
Computational

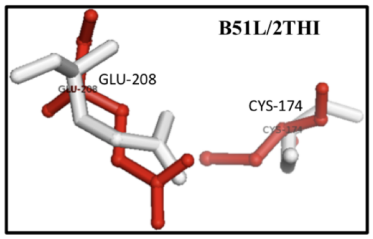
Q16.

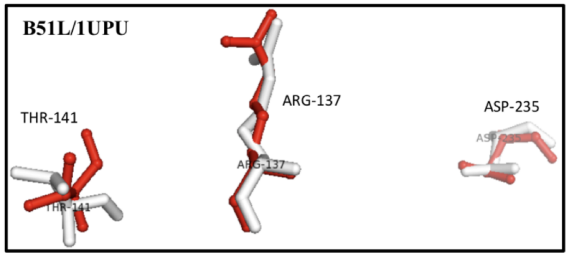
The following questions all pertain to the same uncharacterized protein (PDB# B51L) that has had its structure determined and deposited in the protein data bank. Your help is needed to elucidate the function of B51L.











EC 2: Transferase Nomenclature	EC 3: Hydrolases

• EC 2.4.2: Pentosyltransferases
  ○ EC 2.4.2.9: Uracil phosphoribosyltransferase
• EC 2.5.1: Transferring Alkyl or Aryl Groups, Other than Methyl Groups
  ○ EC 2.5.1.2: Thiamine pyridinylase
	• EC 3.1.3: Phosphoric Monoester Hydrolases
	  ○ EC 3.1.3.7: 3'(2'),5'-bisphosphate nucleotidase

Q1.5.

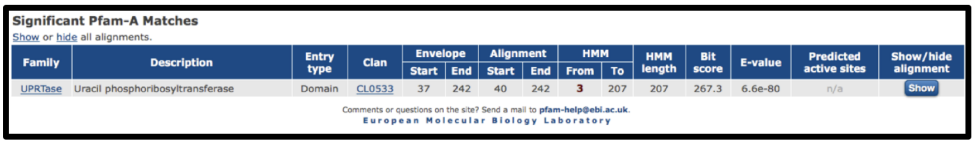
Presented above are the outputs from ProMOL's Motif Finder.

Use the provided outputs to explain:

- **Why or why not each of the presented motif alignments may characterize the motif of B51L's active site.**
- **How you used the ProMOL results to determine a suitable motif for B51L and what this tells us about the possible function and class of the enzyme.**

Additionally, be sure to explain what the data outputs represent, and how you would integrate the different information in the ProMOL outputs depicted above to plan your next steps.

Q9. **Below are three outputs from the Pfam, BLAST, and DALI databases for B51L.**



Significant Pfam-A Matches

Show or hide all alignments.

Family	Description	Entry type	Clan	Envelope Start	Envelope End	Alignment Start	Alignment End	HMM From	HMM To	HMM length	Bit score	E-value	Predicted active sites	Show/hide alignment
UPRTase	Uracil phosphoribosyltransferase	Domain	CL0533	37	242	40	242	3	207	207	267.3	6.6e-80	n/a	Show

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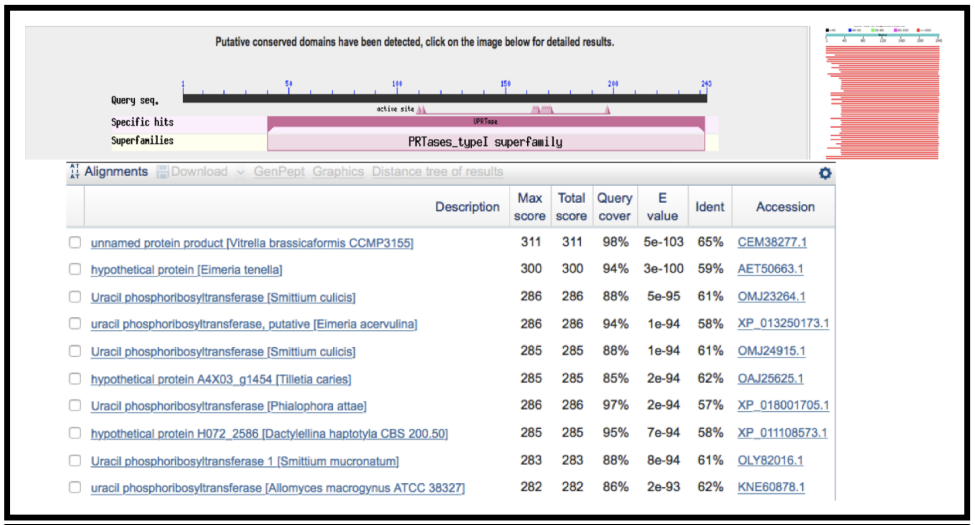


Alignments Download GenPept Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
unnamed protein product [Vitrella brassicaformis CCMP3155]	311	311	98%	5e-103	65%	CEM38277.1
hypothetical protein [Eimeria tenella]	300	300	94%	3e-100	59%	AET50663.1
Uracil phosphoribosyltransferase [Smittium culicis]	286	286	88%	5e-95	61%	OMJ23264.1
uracil phosphoribosyltransferase, putative [Eimeria acervulina]	286	286	94%	1e-94	58%	XP_013250173.1
Uracil phosphoribosyltransferase [Smittium culicis]	285	285	88%	1e-94	61%	OMJ24915.1
hypothetical protein A4X03_g1454 [Tilletia caries]	285	285	85%	2e-94	62%	OAJ25625.1
Uracil phosphoribosyltransferase [Phialophora attae]	286	286	97%	2e-94	57%	XP_018001705.1
hypothetical protein H072_2586 [Dactylellina haptotyla CBS 200.50]	285	285	95%	7e-94	58%	XP_011108573.1
Uracil phosphoribosyltransferase 1 [Smittium mucronatum]	283	283	88%	8e-94	61%	OLY82016.1
uracil phosphoribosyltransferase [Allomyces macrogynus ATCC 38327]	282	282	86%	2e-93	62%	KNE60878.1

No:	Chain	Z	rmsd	lali	nres	%id	PDB	Description
1:	1jlr-A	37.6	0.4	224	235	100	PDB	MOLECULE: URACIL PHOSPHORIBOSYLTRANSFERASE;
2:	4p82-A	14.3	2.8	152	180	16	PDB	MOLECULE: BIFUNCTIONAL PROTEIN PYRR;
3:	5vn4-A	13.3	2.9	159	237	16	PDB	MOLECULE: ADENINE PHOSPHORIBOSYLTRANSFERASE, PUTATIVE;
4:	2xbu-B	12.8	2.7	139	202	14	PDB	MOLECULE: HYPOXANTHINE-GUANINE PHOSPHORIBOSYLTRANSFERASE;
5:	3qw4-C	12.7	3.5	147	447	10	PDB	MOLECULE: UMP SYNTHASE;
6:	4rv4-B	12.7	2.9	141	211	11	PDB	MOLECULE: OROTATE PHOSPHORIBOSYLTRANSFERASE;
7:	1y0b-D	12.3	3.1	150	194	12	PDB	MOLECULE: XANTHINE PHOSPHORIBOSYLTRANSFERASE;
8:	1wd5-A	12.2	2.8	142	208	18	PDB	MOLECULE: HYPOTHETICAL PROTEIN TT1426;
9:	4trb-A	11.9	2.5	139	209	17	PDB	MOLECULE: PURINE PHOSPHORIBOSYLTRANSFERASE (GPT-1);
10:	4paw-A	11.5	3.1	142	197	13	PDB	MOLECULE: OROTATE PHOSPHORIBOSYLTRANSFERASE;

Q1.6.

Based on the database (Pfam, BLAST, and DALI) outputs above, what is the predicted function of B51L? Explain your answer.

Be sure to explain how Pfam, BLAST, and DALI work, and how you integrate the data from each program to inform your prediction.

Q1.7.

How do these database results compare to the ProMOL results? To which class of enzyme do you now think B51L belongs?

Be sure to cite specific pieces of evidence to explain how you came to your conclusion, including how you used the data outputs to predict the specific type and function of the B51L enzyme.

Q21.

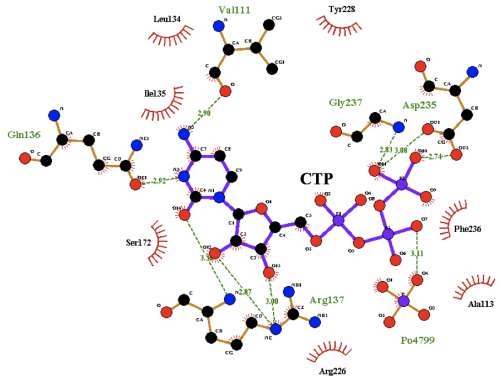
Based on your prediction of function and enzyme class, how would you

choose potential candidate ligands that may interact with the protein of interest? Use the insights from all of the outputs provided so far to identify a potential candidate ligand and explain how you would start screening to identify a ligand for the B51L protein.

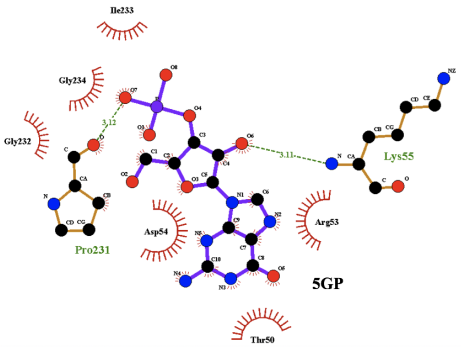
Be sure to name any programs you might use, how you would use the outputs and representations they produce, and how you would integrate evidence from the different programs to inform your thinking. Additionally, be sure to cite specific pieces of information available to you that you would use to inform your decisions.

Q11.

The representations below show how two substrates (purple backbones) may be interacting with B51L by using the calculated interaction from PyRx. The energies provided were also calculated from PyRx.



CTP bound to B51L
-7.5 kcal/mol



5GP bound to B51L
-7.4 kcal/mol

Q1.8.

Which of the above ligands would be an appropriate candidate substrate for B51L? How did you come to this conclusion? Be sure to explain how you used the above representations and the evidence needed to inform your ideas about the best ligand substrate.

Additionally, be sure to cite specific pieces of information available to you that you would use to inform your decisions and how you integrate information from other computational programs and the data outputs from them (above) to inform your conclusion.

Biochemical

Q2.1.

How would you propose biochemically confirming a protein's function? Please outline:

- **the specific steps you would take and your reasoning behind choosing them,**
- **the expected types of data you would gather, and representations they would produce, and**
- **how you would use them in order to make conclusions about a protein's function.**

Q2.2.

What are the appropriate factors to consider when optimizing or interpreting an enzyme assay?

Be sure to include your reasoning into how these factors will impact the assay results. Additionally, mention what types of outputs or representations from an enzyme assay would inform you that you would need to consider making changes to your methods.

Q2.4.

How does the colorimetric enzyme assay work in general and specifically for the assay you conducted in this course? Additionally, what information can be gained about a protein's function? What limitations may there be?

Be sure to discuss in detail the concepts and theories behind the methods and of this type of assay and how the outputs and representations, from the assay, would be interpreted to uncover insight about a protein's function.

Q2.5.

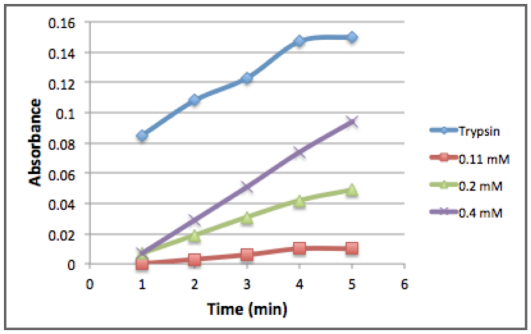
Explain how the purification of tagged proteins works. What biochemical concepts govern how this method functions? How would you know if purification of a protein of interest was a success?

Be sure to discuss the data that would be gathered and how it would be presented and interpreted to determine if a protein of interest had been purified.

Q2.6.

How can purification by protein tags be optimized?

Be sure to explain how the factors you mention will contribute to a better purification. Additionally, mention what types of outputs or representations would suggest the need to make changes to your methods and what specific changes you would make.



3H04	
ProMOL	**EC** 3.4.11.5, 3.7.1.8, 3.4.21.26 Hydrolase
BLAST, Dali, and Pfam common function	**EC** 3 or 3.1.1 Alpha/beta hydrolase or esterase

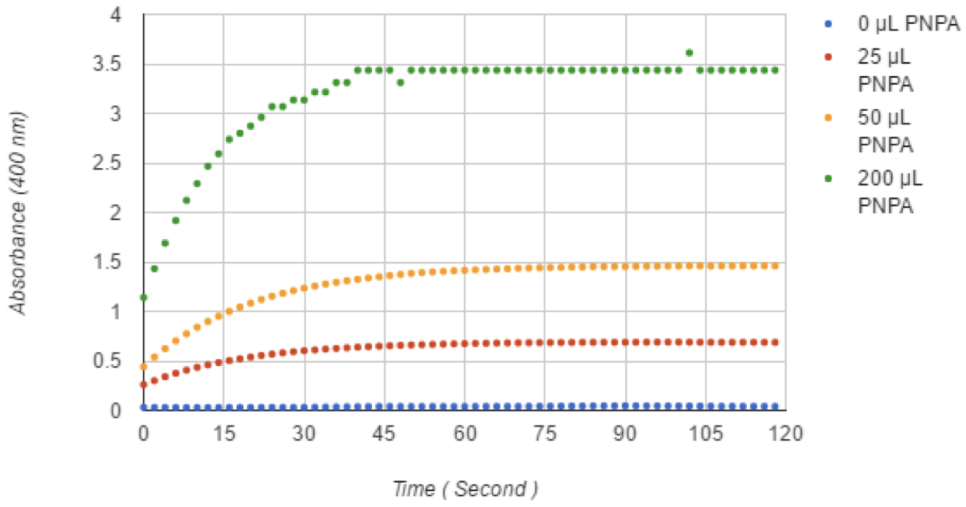
Q2.11.

Compare the above graph and computational outputs for a protein of interest. The graph shows a constant concentration of 3H04 with different concentrations of p-nitrophenyl acetate (PNPA) and a positive control (Trypsin).

How does the computational prediction and the results from the assay compare? In your explanation provide justification for whether there is evidence that this protein interacts with this substrate, or not. In so doing describe:

- **the relevant biochemical concepts used when interpreting these results, and**
- **the process you used when comparing them.**

Additionally, be sure to cite the specific information you used to reach your conclusion, as well as any additional information you would have liked to have to help you reach your conclusion. Explain your reasoning in every case.



Q2.12.

The data in this plot was generated by a series of activity assays done on a constant concentration of protein from a purified fraction, at four different concentrations of p-nitrophenyl acetate (PNPA).

What can you conclude about the function of the protein of interest? As part of your analysis of this graph please discuss:

- **any relevant biochemical knowledge you used when interpreting the graph,**
- **how this graph was generated,**
- **what else could be done with this data, and**
- **any other experiments you would do to enhance your results and data presented above.**

Also, be sure to discuss the specific information you can infer from this graph and if there is any additional information that would be necessary to generate a conclusion about the protein's function.

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APPENDIX D. STUDENT ANSWERS TO THE OPEN-ENDED PROBES

Q1.5: Presented above are the outputs from ProMOL's Motif Finder. Use the provided outputs to explain: Why or why not each of the presented motif alignments may characterize the motif of B51L's active site. How you used the ProMOL results to determine a suitable motif for B51L and what this tells us about the possible function and class of the enzyme. Additionally, be sure to explain what the data outputs represent, and how you would integrate the different information in the ProMOL outputs depicted above to plan your next steps.

Student	Answer
Pat	• The motif of B51L appears to align best with 1UPU as shown in the lower right image. 2THI and 1K9Z don't align as well. For this reason, I would guess that B51L has a similar active site as 1UPU, which has an EC number of 2.4.2.9. Enzymes in this group are uracil phosphoribosyltransferases. • For my next step, I would look at different programs that can match sequences and try to find similar structures. If these results support my hypothesis here, I can be more assured this is accurate.
Kris	• Motif alignments are a good first step in determining the relative active site and function of the enzyme. With the available structure, you can screen substrates and similar enzymes with likely active site residues and observe and calculate the goodness-of-fit. • Based on these results, EC3 or phosphoric monoester hydrolases appears to be the best fit. Based on the RMSD and visual ques, the best fit and likely residues involved in the active site are ARG137 and ASP235 when compared against 1UPU. • Motif alignments don't always paint the full picture in regards to overall function. Just because two pieces fit together, this does not mean any interesting chemical reactions will take place at this site in regards to the enzyme's function. The results can tell you that if the structures match at a certain point and these local residues are involved in the active site of the protein with known function, you can start to make assumptions of the location and behavior of the active site of the protein of unknown function.
Jean	• According to the data reported above, the active site for the B51L protein is most likely to be around THR-141 since the total RMSD in the B51L/1UPU motif allignment is 0.5257. Moreover, THR-141 appears as a docking site also for the B51l/1K9Z with a total RMSD of 2.7182. All the other motif allingnments have to high RMSDs to be considered possible true motif of B51L's activation site. The function of the B51L protein is probably the one of pentosyltransferase since the RMSD between B51L and uracil phosphoribosyltransferase is 0.5257.
Ryan	• I am unfamiliar with ProMOL's Motif finder and its outputs. Though I can hypothesize that the presented motif alignments are done through sequence alignment and then structures are compared. The structures of similarity are representative of active motifs in B51L. To determine suitable motifs the RMSD value should be low, the lower the number the closer the compared structures are in similarity in space. The motifs that point to a possible function of B51L are Uracil phosphoribosyltransferase, thiamine pyridinylase, and phosphoric monoester hydrolase. In short, B51L may be a transferase or a hydrolase.
Alex	• B51L/1K9Z - poor alignment, 2 out of 3 residues are present, 1 of the 3 residues aren't present in B51L and supports that the comparison is not a suitable motif • B51L/2THI - poor alignment, 2 out of 2 residues are present, though both residues are present the alignment produced by this motif is poor. • B51L/1UPU - good alignment, 3 out of 3 residues are present, this comparison presents the most suitable motif to be used to determine function and class of the enzyme.
Sage	• When it comes to the motif alignments telling us something about B51L's active site, it is important for the motifs to match as well as they possibly can since a deviation of similarity can cause deviation of the active site. Although there are motifs that match quite well there is still a difference between them thus there is a possibility that the active site is not completely conserved. So, even though they are extremely similar if there is a problem at a crucial location then the active site might not function and the similarities would not matter. • In ProMOL results, to me, the most important information is the RMSD since it tells me the deviation between each of the motifs. So, a lower RMSD or the lowest RMSD would tell me the best match for the motifs and most likely to conserve the active site of B51L. Out of all of the motifs, the lowest RMSD was given by 1UPU which has an overall RMSD of 0.5257. Once I have this protein that has a very high chance of having similar active site activity to B51L then I would look at its known substrate. Since the EC for 1UPU is 2.4.2.9 then it is a Uracil phosphoribosyltransferase. So, most likely B51L is some sort of pentosyltransferase and maybe even a Uracil transferase so I would personally add the substrate to a list of substrates to be tested with the protein. • Alongside that, I would utilize the transferase EC to obtain all possible transferases recorded in the PDB database and run PyRx Molecule-Ligand application to obtain the affinities for the substrates and then run them in PyMOL to see if the bindings are occurring near the active site location.

Q1.6: Based on the database (Pfam, BLAST, and DALI) outputs above, what is the predicted function of B51L? Explain your answer. Be sure to explain how Pfam, BLAST, and DALI work, and how you integrate the data from each program to inform your prediction.

Student	Answer
Pat	• Pfam shows that B51L is from a family made of uracil phosphoribosyltransferases. The BLAST results compared my protein's sequence to sequences in a database. Some of the top results returned are different uracil phosphoribosyltransferases. DALI compared the 3D structure of B51L to structures in a database. I'm looking for high z-scores (shows a good quality of fit), a low RMSD (to show that atoms in the 3D structure are close together between two structures), and %ID (to see how many residues are the same between structures). The best result here is a uracil phosphoribosyltransferase. • Since all three programs returned uracil phosphoribosyltransferases, I am more confident in saying that B51L will have a similar function.
Kris	• Based on the data inquiry above, the function of B51L is likely a phosphoribosyltransferase enzyme that transforms some nucleobase into a nucleobase monophasphate molecule. For example, the closest related protein based on BLAST and DALI is the uracil phosphoribosyltransferase protein that transforms uracil into UMP. • Pfam works by compiling active site, structure, sequence and known functions of enzymes with similar function. For example, hydrolases is an overarching family where a NUDIX hydrolase is a subfamily. Superoxide dismutases are another family with similar enzymatic function.The phosphoribosyltransferase proteins likely all perform similar functions using similar chemical mechanisms on different substrates and different parts of the body. • DALI and BLAST searches only take the sequence of your desired protein and compares them against all other known sequences to look for the closest fit. The premise is that proteins with similar sequences are more likely to have similar function. If the function of the matched protein is known and well characterized, it can help elucidate the unknown function of a desired protein.
Jean	• According to the data above, B51L is a pentosyl transferase since, when compared to uracyl phosphoribosyltransferase, the e-value (from pfam) is 6.6e-80, the z-score (from BLAST) is 37.6 and the RMSD is 0.4.
Ryan	• The predicted function for B51L is a uracil phosphoribosyltransferase. DALI used structural similarities to grade the protein of choice against others from the PDB. It had a 100%id and low root mean squared deviation meaning the distances of marked atoms between the unknown B51L protein and the uracil phosphoribosyltransferase was very little. BLAST, on the other hand, uses sequence alignments to find similarities, instead of structure. several hits were for sequence similarity between uracil phosphoribosyltransferase, all of them having high %id and very low E values. this means the hits were very good, and there is a lot of confidence in their similarity. Pfam specifically uses sequence alignments of known domains. The unknown protein has a uracil phosphoribosyltransferase domain, again pointing to that being its potential function.
Alex	• The data would suggest that B51L is a Uracil Phosphoribosyltransferase because that kind of protein has the most hits after alignment.
Sage	• When it comes to utilizing BLAST, Pfam, and DALI I personally found that BLAST was by far the worst option when it came to actually finding useful results. Since all it provided was a sequential similarity and nothing else, so I would not know much of the possibilities when it came to actual protein function. That is where Pfam came in handy since it utilizes motif similarities to obtain a set of proteins that share functionality to the protein in question. So, Pfam was a lot more helpful since it gave me a wider array of results because it looked at motifs and then built a family based on that motif. Then, DALI was just overall the most helpful since it did a comparison of every single side chain between the two or more proteins given and then creates overlays of it and gives a lot of useful information like RMSD, Z-score and more which can really help to find extremely similar proteins that give us clues to the function of the protein in question. So, BLAST just gave a lot of similar sequences that could be useful or could be a waste of time, Pfam gave a solid family to work with and DALI allowed to work out the bad seeds of the family to really narrow down the possible function of the protein. • Looking at all the data, I would say that it is a phosphoribosyltransferase and potentially a Uracil phosphoribosyltransferase. The reason why I say this is because the results from DALI show a very significant z-score for all other phosphoribosyltransferases, which means that there is a high similarity in the significant folds of the proteins. Alongside that, the results from Pfam indicate the same when comparing the sequence and the HMM. In the Blast results, %ident with higher than 50 all have something to do with phosphoribosyltransferases.

Q1.7: How do these database results compare to the ProMOL results? To which class of enzyme do you now think B51L belongs? Be sure to cite specific pieces of evidence to explain how you came to your conclusion, including how you used the data outputs to predict the specific type and function of the B51L enzyme.

Student	Answer
Pat	• I thought that the ProMOL results showed that the motif for B51L was most similar to another enzyme that is a uracil phosphoribosyltransferase. Pfam showed that B51L came from a family of uracil phosphoribosyltransferases. BLAST determined that B51L has a similar primary structure to several other uracil phosphoribosyltransferases. DALI shows that the 3D structure is similar to a uracil phosphoribosyltransferase and other types of phosphosyltransferases.
Kris	• Based on the name of the most closely related protein on the sequence level and the best fit and alignment provided by ProMOL, the enzymatic class of the B51L protein is likely phosphoric monoester hydrolase. We know that the best fit in alignment is the EC 3.1.3 protein 1UPU, a phosphoric monoester hydrolase, and the most closely related protein according to the BLAST and DALI searches is the uracil phosphoribosyltransferase protein. Since this protein uses phosphoribosylpyrophosphate to transform uracil into UMP, a phosphoric monoester hydrolase function, it's likely B51L performs a similar reaction on some kind of nucleobase.
Jean	• They have the same outcome: B51L belongs to EC 2.4.2: Pentosyltransferases
Ryan	• Again, I am unfamiliar with ProMOL and how it finds it motifs but the databases use known information to find similarities and elicit information about B51L. From the databases, B51L seems to function as a uracil phosphoribosyltransferase, especially since all three databases shown had very good hits for it as well.
Alex	• I thought it was a EC 3: Hydrolase but its probably a EC 2: Transferase after the database results
Sage	• These results heavily support the ProMOL results, within ProMOL it was shown that 1UPU has the best motif similarity to B51L with an RMSD all of 0.5257 which Uracil phosphoribosyltransferase. As described in the earlier question, all the data obtained from the different databases supports a function of phosphoribosyltransferase so B51L is a trasnferase enzyme.

Q21: Based on your prediction of function and enzyme class, how would you choose potential candidate ligands that may interact with the protein of interest? Use the insights from all of the outputs provided so far to identify a potential candidate ligand and explain how you would start screening to identify a ligand for the B51L protein. Be sure to name any programs you might use, how you would use the outputs and representations they produce, and how you would integrate evidence from the different programs to inform your thinking. Additionally, be sure to cite specific pieces of information available to you that you would use to inform your decisions.

Student	Answer
Pat	• I would start by gathering a list of ligands used by other uracil phosphoribosyltransferases in the same family as B51L. To do this, I would use the PDB to search for them. Then I would use PyRx to test these ligands and how well they fit with B51L. Of the ligands that show a good fit, I would look at the structure in PyMOL and make sure it is in a potential active site. Any ligands I think are a good fit at this point are candidates for enzyme assays.
Kris	• I would use PyRx to find a closely fit binding event between a number of ligands B51L may work with. I would look up literature of the most closely related proteins to B51L according to DALI and BLAST and discover which ligands bind to their active sites and which residues are involved. Based on the information I have thus far, I would probably test all of the nucleotides as well as GTP, CTP, ATP, TTP and UTP or any variant of phosphate(s) attacked to the nucleotides.
Jean	• Pfam can be used to obtain a list of possible ligands. Then the structure for those ligands can be downloaded from PDB and the ligand docking function from PyRx can be used to determine the suitability of each ligand as a substrate for B51L.
Ryan	• A good start would be to use ligands that interact with known uracil phosphoribosyltransferase. Then finding similar ligands through the PDB ligand search could make a more robust list of possible ligands to text in silico though PyRx. From the good hits off of PyRx, the next step is to get a visual of the protein and ligands interaction using PyMol, CN3D, UCSF Chimera, or another program that is similar. PyRx can sometimes have hits that are false and it is easy to tell by getting a visual if the ligand is in an odd place.
Alex	• Using the PDB's ligand finder look for ligands associated with Uracil Phosphoribosyltransferase
Sage	• I would choose potential ligands by going to PDB.org search all phosphoribosyltransferases via the EC number and then record and download it as a CSV file. Then, I would utilize PyRx to upload all of the ligands that are usable from the list, basically remove all ligands that were used to identify the structure of the proteins like selenomethionine, and then run them through the program. Once I have received the output of the program I would separate the ligands via high binding affinities vs. low binding affinities in kcal. Then, I would use PyMOL to upload the files from PyRx, highlighting the active site motif of B51L and see if the ligands are located in or around the active site. If they are then, I would separate out those ligands and run an assay on them to find activities.

Q1.8: Which of the above ligands would be an appropriate candidate substrate for B51L? How did you come to this conclusion? Be sure to explain how you used the above representations and the evidence needed to inform your ideas about the best ligand substrate. Additionally, be sure to cite specific pieces of information available to you that you would use to inform your decisions and how you integrate information from other computational programs and the data outputs from them (above) to inform your conclusion.

Student	Answer
Pat	• I think CTP is a better ligand choice. CTP appears to interact with B51L all along its own structure while 5GP only interacts on one end and with fewer residues. • I would use the other programs to see if CTP is a ligand used by other uracil phosphoribosyltransferases, which would make it a more likely candidate for B51L.
Kris	• Visually, both substrates seem to fit pretty well into the predicted active site of B51L, however, the binding energies are more telling pieces of information. Any binding energy lower than -6 or -7, typically, is a decent candidate for substrate testing in assay. While both CTP and 5GP are good fits (lower than -7) the CTP binding is -.1 kcal/mol lower than that of 5GP, suggesting an even stronger relationship between this substrate and B51L. The substrate also fits nicely between ARG137 and ASP235, residues that were likely involved in the active site as per the ProMOL results while the 5GP does not bind at these residues.
Jean	• CTP wold be a better ligand for B51L then 5GP. Firstly, the binding affinity between CTP and B51L is lower (therefore better) then the one between 5GP and B51L. Secondly, B51L has more possible docking sites in CTP then in 5GP
Ryan	• CTP looks to have more interactions with the protein even though the free energy value is similar between CTP and 5GP. CTP could be a more appropriate ligand due to the extra interaction as it looks almost like a binding pocket.
Alex	• CTP's affinity is 0.1 kcal/mol less than that of 5GP and the substrate (purple backbones) appear more exposed than 5GP, also theres more of them
Sage	• Since I am having a hard time properly reading the image, I will try my best based on what I understand from it and the given binding affinities. I would say that CTP (Citrine Triphosphate) is the appropriate candidate substrate for B51L due to it creating a better binding with B51L and also having the lower kcal/mol value. Since the value is a bit lower that means that it has a more spontaneous reaction than 5GP and it has more binding sites with B51L meaning that it will be more stable in the active site so the reaction is more likely to occur.

Q2.1: How would you propose biochemically confirming a protein's function? Please outline: the specific steps you would take and your reasoning behind choosing them, the expected types of data you would gather, and representations they would produce, and how you would use them in order to make conclusions about a protein's function.

Student	Answer
Pat	• After purifying a protein, I would perform enzyme assays using many different ligands and at different conditions (temperature, pH, varying concentrations of enzyme and substrate). I would expect to gather data through optical density measurements. This will show me if a reaction took place and allow me to quantify the activity level. Reactions that produce the highest OD reading would be assumed to have the most function. The ligand used here will help determine function along with bioinformatics research data.
Kris	• There are a number of ways one could biochemically confirm a protein's function, but the overall premise looks at chemical assay analysis. After choosing which ligands are likely a good fit to test and pure concentrated protein is collected, the protein can be screened against these ligands in various assays. The assay should include a positive or negative result if there is evidence of a reaction between the protein and substrate. For example, a molecule or dye may change color or fluoresce in the presence of any biproducts resulting in the chemical reaction between the enzyme and ligand. The assay chosen is going to have to agree, physiologically, with the enzyme. This means that the enzyme must tolerate the conditions the assay requires and that the pH and reagents in the assay do not alter the protein's function. The only thing that should be able to act with or on the enzyme is the ligand itself, otherwise results won't carry as much weight. Positive and negative results should be easy to discern.
Jean	• Firstly, the pdb structure of the protein analyzed and of the possible ligands for it have to be downloaded from PDB and then the affinity between the protein and each possible ligand can be estimated with the docking function in PyRx. • Secondly, the plasmid coding for the protein in analysis can be purchased and induced in E. Coli to transcribe our protein. The protein can then be extracted from E. Coli cells and purified. The 4 or 5 most suitable ligands can be purchased and, together with the protein in analysis, used to create an assay describing the minimum enzyme concentration to produce enzyme activity with each substrate. In order to determine the activity of the enzyme, the product coming from the ligand needs to be quntified. To do that, a colored complex can be formed by adding a suitable reactant and absorbance can be used to determine the enzyme's preferred substrate.
Ryan	• After finding potentially ligands in silico and after producing and purifying the B51L protein, the next step would be to run assays with each ligand. This would also involve finding ions that the enzyme needs to function as well as optimizing temperature and pH for activity. Seeing the quantitative color change in some assays over others would be positive qualitative results and further comparison to a standard with a spectrophotometer quantitative results as to which ligands and conditions best suit the enzyme. Quick conclusions can be made from assays like this as to which ligands are interacting with the enzyme.
Alex	• Performing a substrate assay that produces a measurable molecule after catabolism, one that most likely produces a color shift significant enough that it can be measured using OD. The substrate + co-factor + enzyme combination that produces the highest OD should give a good suggestion as to what the protein's function is • From there you could manipulate pH, co-factors, temp, substrates, and protein concentration. Establish extinction coefficient and create a standard curve.
Sage	• I would personally run several assays to confirm the function of the protein and utilize Bradford assay and nanodrop to obtain data on the assays to identify functionality. The reason why I chose Bradford assay and nanodrop is due to more experience with those techniques and understanding the results of those techniques a bit better. I would have several graphs with calibration curves to obtain the protein activity. Since I would run a decreasing amount of protein in each sample I would expect to see a more or less decreasing linear model where activity is occurring since activity is decreasing due to a drop in the number of available proteins. Since the models are based on activity occurring whenever activity does occur I would be able to find functionality based on activity.

Q2.2: What are the appropriate factors to consider when optimizing or interpreting an enzyme assay? Be sure to include your reasoning into how these factors will impact the assay results. Additionally, mention what types of outputs or representations from an enzyme assay would inform you that you would need to consider making changes to your methods.

Student	Answer
Pat	• Enzyme assays are interpreted based on optical density readings. To optimize assays, I would test many different conditions (temperature, pH, concentrations) to determine which produces the most activity. I might need to add various cofactors or coenzymes based on the enzyme. If all my assays produce small OD readings, I would continue to change variables until a greater activity is noted.
Kris	• Assays are very time sensitive, reagent concentration sensitive and must be treated with great quantitative care. Otherwise, a positive or negative result may not be telling. Factors to consider in assays are the pH, salinity, buffers used, indicator molecule and ligands tested. All of these factors must be held constant so that the only reagent affecting the protein is the ligand itself and they must not affect or alter the function of the protein. Understanding what the likely biproducts of a successful reaction between the protein and ligand is crucial as the indicator molecule or dye must be able to detect the presence of this successful event in a relatively sensitive way. The efficiency of the protein is not known, so being able to detect minute reaction success may be critical.
Jean	• Factors that are important when optimizing or interpreting an enzyme assay can be things like the reliability of the calibration curve and its range or the correct use of enzyme concentration in the different enzyme-ligand samples
Ryan	• Enzymes need cofactors to function, like metal ions. This is one thing that can be optimized. Another factor is pH, not all enzymes function at the same pH, especially depending on where in a cell the enzyme acts and what it does, this can also be optimized. The concentration of ligand present can affect an assay and can change how the results are interpreted.
Alex	• protein concentration, co-factors, pH, and substrate are all good factors to establish when performing an assay
Sage	• One of the optimization setups that should be run is enzyme reaction progress curve which allows us to find the best concentrations to use for the assays. Since we want the enzyme to remain stable during the whole experiment, an enzyme reaction progress curve can help us a lot since it will show if the substrate is running out too quickly or if there are too little protein and things of that nature. Once results from the curve are obtained it allows us to ensure that the reactions are occurring at an appropriate speed during the assays. • Alongside that, the most important thing to worry about in an assay is the enzyme stability. Once, a general idea of how the reaction is going to progress is obtained, we can compare results to it to see if there is something wrong with our stability. In the data, we should normally see 3 lines based on concentration that do not converge and plateau out after a steady increase almost like a bacterium's growth phase diagram. If that is not seen and there are issues with specific concentrations then those concentrations would have to be re-run and stability would have to be checked. Since an enzyme reaction progress curve would rule out a high concentration problem.

Q2.4: How does the colorimetric enzyme assay work in general and specifically for the assay you conducted in this course? Additionally, what information can be gained about a protein's function? What limitations may there be? Be sure to discuss in detail the concepts and theories behind the methods and of this type of assay and how the outputs and representations, from the assay, would be interpreted to uncover insight about a protein's function.

Student	Answer
Pat	• My protein was a NUDIX enzyme which cleave diphosphate bonds. In order to detect the phosphate groups, we needed calf intestinal alkaline phosphatase to free the terminal phosphate groups. This phosphate will bind to MoO4 2- and produce a blue solution. To stop the reaction, EDTA was used to remove the catalytic metal ion. How blue the solution gets will help determine how active the enzyme was. This assay is very sensitive so concentrations must be kept very small to produce a notable difference in the level of blue color.
Kris	• Colorimetric enzyme assays work by which a color change indicates a positive result. Dyes or other molecular indicators will bind and allow color change in the presence of bi-products resulting from a successful reaction between your enzyme and the ligand. For example, the assay we ran in lab was based on the ability to detect free phosphate. Although NUDIX hydrolase enzymes cleave diphosphate bonds, the AMES reagent will not be able to detect a successful reaction since the nucleotides are still bound. Calf intestinal phosphatase then cleaves the phosphates from these bi-products to release free phosphates which can be detected. The AMES reagent (molybdate and ascorbic acid) is incredibly sensitive to free phosphate and will turn blue. The intensity of the blue can be detected using UV visible spectrometers. You are looking to see that a solution with enzyme and ligand be more intensely blue than one without (the control group).
Jean	• A colored complex can be formed with the products from each enzyme ligand reaction by adding a suitable reactant and absorbance can be used to determine the enzyme's preferred substrate.
Ryan	• For this course as phosphates are freed, they are able to interact with the reaction solution and a color change happens. In our case, the enzyme produced a blue produced. This works since the enzyme under investigation is a diphosphate hydrolase. So far we have gained information on what ion to use with the enzyme as well as ligands that the enzyme interacts with. A problem with results from assays like this is they are qualitative over quantitative. there are also limits of time and how much can be tested in a single assay.
Alex	• A colorimetric enzyme assay works by producing a measurable molecule after the reaction, one that most likely produces a color shift significant enough that it can be measured using OD. The substrate + co-factor + enzyme combination that produces the highest OD should give a good suggestion as to what the protein's function is. • The color shift sometimes won't be large enough to measure activity. Some substrates may have exposed portions of the measured product that could result in false positives - so using good controls for these colorimetric assays are important
Sage	• In general, the way that colorimetric enzyme function is that as the assay is run there is a quantitative and qualitative change in the solution that can or cannot be visible to the eye. Once the solution is placed in a spectrophotometer then actual values can be obtained and activity can be recorded based on the color change that occurred during the assay. In our course, Bradford assays were primarily used where there was a color change to blue when there were high amounts of activity otherwise there was a fractional change in the color and the activity could only be recorded via a spectrophotometer. The assay functions by the dye binding with protein and then changing color. In our case, we added some calf intestinal protein that binds with our reaction products so whenever there is a reaction that occurs there is a colorimetric change that can be recorded allowing us to see if reactions are occurring or not. The main limitation of this method is that too much buffer can interfere with the dye and gives us improper reading and if there is detergent in the mix the same would occur.

Q2.5: Explain how the purification of tagged proteins works. What biochemical concepts govern how this method functions? How would you know if purification of a protein of interest was a success? Be sure to discuss the data that would be gathered and how it would be presented and interpreted to determine if a protein of interest had been purified.

Student	Answer
Pat	• Tagged proteins will bind to a matrix during affinity column chromatography while other proteins wash through. In my case, the enzyme had a His6 tag, which binds to Ni2+ in the Ni2+:NTA resin. After washing the other proteins, an elution buffer is added to remove the target protein from the matrix. I added imidazole, which competes with the His tag for binding to Ni2+. • To determine if I have purified the protein, I would run a SDS-PAGE gel of fractions gathered during the wash and elution phases. The wash should show a large number of bands with different molecular weights. The elution fractions should show one large band that decreases over the time of collection.
Kris	• Purification tags are built into the protein of interest as blueprinted from the engineered plasmid. The plasmid will contain the information needed to place extra amino acid 'tags' at the C or N terminal region of the protein. The purpose of these tags is to be able to separate these proteins from all other proteins lysed from a bacterial cell. Cells naturally do not have these tags and can be easily teased from the protein solution. The purification tag we used in class was the His tag which contains six histidine residues on the N-terminal region of our NUDIX proteins. The residue, histidine, contains the molecule chemically equivalent to imidazole which can be targeted on separation column chromatography. Metal ions bound to the column substrate chelate the nitrogen atoms of the histidine tag, allowing other proteins to flow off the column. High concentrations of imidazole solution then swaps out for the protein and the purified protein can be washed and collected. You are able to collect samples of this flow through and try and detect a single band corresponding to your protein of interest on gel electrophoresis. No matter the tag used, the chemical properties they possess differ from natural proteins which allows biochemists to isolate the protein they need.
Jean	• A protein can be purified using different kind of columns (affinity, size exclusion, etc.), acidity variations and formation of temporary complexes by tagging the protein with another compound and them elute it by taking it off. A Bradford assay can be used to qualitatively determine the presence of the protein and a gel can be run to determine the purity of it
Ryan	• Histidines have a positive charge and the His-tag is usually between six and eight histidines at either the N or C terminus of a protein. They can attract a negative charge in a column and are useful for purification. A column is usually filled with a resin or beads that can be saturated with a solution that doesn't out-compete the tagged protein. Another solution is then used to wash away proteins that are not of interest. a final solution is used to elute the protein of interest by out competing and pushing off the tagged protein from the resin. Purification is then tested by running SDS-PAGE with different samples from the washes and elutions. the bands will be very telling of purity and when the protein eluted.
Alex	• Histidine tags are utilized during purification to attach proteins to a column. Using gels loaded with fractions of washes containing protein are run using electrophoresis to identify bands
Sage	• In the case of the protein for my group, the protein contained His-tags and kanR tags. The KanR tags allow us to grow the protein without a problem since adding kanamycin to the growth media allows us to ensure that only the bacterium with our protein survives. After that, the cells were lysed via sonication and an SDS-Page was run on small fractions of the materials to see if the protein was present in our solution. Then, His-tags were used to purify our protein via metal-ion chromatography. The way this works is that since His can be bound with Nickel quite well, we set up a metal-ion chromatography with Nickel resin. As the protein ran through the material the His-tags wrapped around the nickel and were stuck there. Afterwords imidazole was used to release the protein from the resin since imidazole can also bind to the resin and as imidazole replaced the His-tags our proteins were slowly removed from the resin and fell into a solution completely separate from other proteins or molecular debris that was previously present in the solution. Then a Bradford assay can be run alongside an SDS-Page to find the concentration and purity of protein samples.

Q2.6: How can purification by protein tags be optimized? Be sure to explain how the factors you mention will contribute to a better purification. Additionally, mention what types of outputs or representations would suggest the need to make changes to your methods and what specific changes you would make.

Student	Answer
Pat	• Protein tags should be optimized based on the other proteins that will be in solution. If there are a lot of proteins with a high concentration of histidine, a His tag might not be appropriate because extraneous proteins may bind to the matrix and cause impurities in the final result.
Kris	• The purification tag may not interfere with the overall structure and function of the protein in question and should operate the same had the tag not been there. The purification tag should be relative easy to engineer into the plasmid, easy for the cell to transcribe and translate and allow the protein to be separated away from others. In other words, the properties bestowed by the tag whether that be molecular weight or chemical properties, should allow the protein to be differentiated and separated predictably and reliably from other proteins.
Jean	• By choosing the most suitable tag compound
Ryan	• Finding the right solutions for binding, washing, and elution is crucial. If a solution outcompetes the protein of interest for binding then the protein will be washed out with the rest of the proteins. Also solutions should not interact with the protein or tags themselves. Another thing to keep in mind is the resin itself, it should be chosen to interact with the tag used. Taking the time to add binding buffer slowly and across enough column volumes allows for the solution the resin was stored in to be washed out and diluted down.
Alex	• I'm not sure, I don't know much about how protein tags work to be honest. • I suppose you'd want a high affinity between two molecules and a wash that allows you to elute everything in the supernatant besides the protein
Sage	• I am unsure about the optimization of the protein purification step so I cannot accurately describe this situation.

Q2.11: Compare the above graph and computational outputs for a protein of interest. The graph shows a constant concentration of 3H04 with different concentrations of p-nitrophenyl acetate (PNPA) and a positive control (Trypsin). How does the computational prediction and the results from the assay compare? In your explanation provide justification for whether there is evidence that this protein interacts with this substrate, or not. In so doing describe: the relevant biochemical concepts used when interpreting these results, and the process you used when comparing them. Additionally, be sure to cite the specific information you used to reach your conclusion, as well as any additional information you would have liked to have to help you reach your conclusion. Explain your reasoning in every case.

Student	Answer
Pat	• The computational prediction suggests that the enzyme is a hydrolase. The assay results show that activity was faster with a higher concentration of the enzyme, as the 0.4 mM line has a greater slope than the 0.11 mM line. I think this increase in absorbance with a higher concentration of enzyme suggests that the protein interacts with the substrate. Otherwise, the lines should have had no slope if the protein didn't interact.
Kris	• The positive control of trypsin under the same conditions allows to see how the absorbance changes with the concentration of the substrate over time. Since slopes of absorbance are similar between the control and the concentration at .2mM, it can be inferred that the rate of reaction at that concentration is similar for the protein of interest at that concentration as the control trypsin. The enzyme is even more responsive to PNPA at higher concentrations like .4mM, indicating it is an efficient enzyme working on the substrate PNPA. If there was no interaction, there would be no appreciable slope despite the change in concentration of PNPA. Little to no absorbance would be observed.
Jean	• The absorbance grows relatively to the concentration PNPA so product is formed and the protein interacts with the substrate
Ryan	• 3H04 interacts with PNAP and does so almost linearly based on the graph. The slope depends on the concentration of the ligand present, the more ligand, the more PNPA is hydrolyzed. This graph was likely produced from data from a color assay. The computational outputs point to 3H04 being a hydrolase, and the graph shows that PNPA and the protein interact. They are both pointing to the protein being a hydrolase protein. This is able to be hypothesized based on the structure of PNPA and where the molecule could be hydrolized.
Alex	• As the concentration of PNPA increases the level of absorbance increases. Which would suggest that activity increases respectively to PNPA concentration.
Sage	• Just glancing over the data there seems to be activity occurring since there is an increase in absorbance as the reaction proceeds showing that assay was reacting with the product which would correlate to a higher absorbance when recorded. The data is also linear as it should be because as the protein works on the substrate the concentration of the product would steadily increase. There is also a correlation between the absorbance and the concentration of the protein as the concentration increases the rate of change of absorbance also changes drastically so we see three separate lines. Since there is no crisscrossing of the lines there is nothing to worry about protein stability and the rate at which the reactions are occurring so good data is being obtained. Since all of these things are present I can say that this protein definitely interacts with this substrate. Since it reacts with this substrate then the computational data was correct in assessing this as a hydrolase.

Q2.12: The data in this plot was generated by a series of activity assays done on a constant concentration of protein from a purified fraction, at four different concentrations of p-nitrophenyl acetate (PNPA). What can you conclude about the function of the protein of interest? As part of your analysis of this graph please discuss: any relevant biochemical knowledge you used when interpreting the graph, how this graph was generated, what else could be done with this data, and any other experiments you would do to enhance your results and data presented above. Also, be sure to discuss the specific information you can infer from this graph and if there is any additional information that would be necessary to generate a conclusion about the protein's function.

Student	Answer
Pat	• At about 45 seconds, the increase in absorbance generally stops which indicates that PNPA was used up. Since even 200 uL was used up in the same time as 25 uL of PNPA, I would like to see higher concentrations used until the graph shows that it takes longer than 45 seconds to exhaust the supply of PNPA.
Kris	• The protein of interest appears to be sensitive to PNPA and will react and produce a positive result at any small concentration of the substrate. It appears the protein will continue to react with the substrate until it is used up, hence the flat-lining after 45 seconds. What is interesting is that this "flat-lined' absorbance seems to be around 45 seconds regardless of the concentration of PNPA which could be telling of the kinetics of the reaction. The data for this graph appears to have been collected by visible spectroscopy of the color blue/purple given the wavelength set. Other experiments may be to see what is the highest concentration of PNPA you'd need to reach before the enzyme was not able to keep up with demand and react all the substrate in 45 seconds. Another would be to test similar substrates or retest PNPA under different conditions like temperature, pH or the presence of specific cofactors.
Jean	• All the substrate is consumed in the first 45 seconds
Ryan	• The protein is able to interact efficiently with PNPA as a substrate. The more substrate present the better, that way there is an increased likelihood that the protein and substrate will bump into one another and interact. This graph was likely generated by a protein assay looking at the concentration where the protein was allowed to react set amounts of time. The optimal reaction time can also be gathered from the graph. The next experiment could be to keep the PNPA concentration constant and changing protein substrate. Knowing more about experimental conditions would help with data analysis.
Alex	• B51L metabolizes PNPA, using different levels of protein concentration and over a longer period of time could possibly enhance the results
Sage	• Since there is no activity at 0 uL of PNPA that tells us that our control is functioning properly and there is no issue with contamination or any other funny business. The graph also shows a good progression curve with a steady increase into a curve into a plateau which should be present since the max amount of product has been produced so there should be no further change in the curve. I would say that the 25 and 50 uL PNPA concentrations were perfect while the 200 uL PNPA is a bit erratic due to the high concentration of substrate so I would say that this graph also tells us a good concentration to work with.

APPENDIX E. STUDENT OPEN-ENDED RESPONCES ORGANIZED BY TOP-RATED CURAS

Below are the student responses to the open-ended probes (Appendices C and D) organized by the top-rated CURA or CURAs a response (or portion of a response) pertained to.

TR1: Explain how the colorimetric enzyme assay works to allow detection of protein function

Student	Open-ended Assessment Answers
Kris	• […] The assay should include a positive or negative result if there is evidence of a reaction between the protein and substrate. For example, a molecule or dye may change color or fluoresce in the presence of any biproducts resulting in the chemical reaction between the enzyme and ligand. The assay chosen is going to have to agree, physiologically, with the enzyme. This means that the enzyme must tolerate the conditions the assay requires and that the pH and reagents in the assay do not alter the protein's function. The only thing that should be able to act with or on the enzyme is the ligand itself, otherwise results won't carry as much weight. Positive and negative results should be easy to discern. (Q2.1) • […] Understanding what the likely biproducts of a successful reaction between the protein and ligand is crucial as the indicator molecule or dye must be able to detect the presence of this successful event in a relatively sensitive way. […] (KRIS, Q2.2) • Colorimetric enzyme assays work by which a color change indicates a positive result. Dyes or other molecular indicators will bind and allow color change in the presence of bi-products resulting from a successful reaction between your enzyme and the ligand. For example, the assay we ran in lab was based on the ability to detect free phosphate. Although NUDIX hydrolase enzymes cleave diphosphate bonds, the AMES reagent will not be able to detect a successful reaction since the nucleotides are still bound. Calf intestinal phosphatase then cleaves the phosphates from these bi-products to release free phosphates which can be detected. The AMES reagent (molybdate and ascorbic acid) is incredibly sensitive to free phosphate and will turn blue. The intensity of the blue can be detected using UV visible spectrometers. You are looking to see that a solution with enzyme and ligand be more intensely blue than one without (the control group). (KRIS, Q2.4) • The positive control of trypsin under the same conditions allows to see how the absorbance changes with the concentration of the substrate over time. […] The enzyme is even more responsive to PNPA at higher concentrations like .4mM, indicating it is an efficient enzyme working on the substrate PNPA. If there was no interaction, there would be no appreciable slope despite the change in concentration of PNPA. Little to no absorbance would be observed. (Q2.11)
Pat	• […] I would expect to gather data through optical density measurements. This will show me if a reaction took place and allow me to quantify the activity level. Reactions that produce the highest OD reading would be assumed to have the most function. (Q2.1) • My protein was a NUDIX enzyme which cleave diphosphate bonds. In order to detect the phosphate groups, we needed calf intestinal alkaline phosphatase to free the terminal phosphate groups. Thisphosphate will bind to MoO4 2- and produce a blue solution. To stop the reaction, EDTA was used to remove the catalytic metal ion. (Q2.4) • […] I think this increase in absorbance with a higher concentration of enzyme suggests that the protein interacts with the substrate. Otherwise, the lines should have had no slope if the protein didn't interact. (Q2.11)
Alex	• Performing a substrate assay that produces a measurable molecule after catabolism, one that most likely produces a color shift significant enough that it can be measured using OD. The substrate + co-factor + enzyme combination that produces the highest OD should give a good suggestion as to what the protein's function is From there you could manipulate pH, co-factors, temp, substrates, and protein concentration. Establish extinction coefficient and create a standard curve. (Q2.1) • A colorimetric enzyme assay works by producing a measurable molecule after the reaction, one that most likely produces a color shift significant enough that it can be measured using OD. The substrate + co-factor + enzyme combination that produces the highest OD should give a good suggestion as to what the protein's function is. (Q2.4)
Sage	• I would personally run several assays to confirm the function of the protein and utilize Bradford assay and nanodrop to obtain data on the assays to identify functionality. The reason why I chose Bradford assay and nanodrop is due to more experience with those techniques and understanding the results of those techniques a bit better. I would have several graphs with calibration curves to obtain the protein activity. Since I would run a decreasing amount of protein in each sample I would expect to see a more or less decreasing linear model where activity is occurring since activity is decreasing due to a drop in the number of available proteins. Since the models are based on activity occurring whenever activity does occur I would be able to find functionality based on activity. (Q2.1) • […] the way that colorimetric enzyme function is that as the assay is run there is a quantitative and qualitative change in the solution that can or cannot be visible to the eye. Once the solution is placed in a spectrophotometer then actual values can be obtained and activity can be recorded based on the color change that occurred during the assay. In our course, Bradford assays were primarily used where there was a color change to blue when there were high amounts of activity otherwise there was a fractional change in the color and the activity could only be recorded via a spectrophotometer. The assay functions by the dye binding with protein and then changing color. In our case, we added some calf intestinal protein that binds with our reaction products so whenever there is a reaction that occurs there is a colorimetric change that can be recorded allowing us to see if reactions are occurring or not. (Q2.4) • […] there is an increase in absorbance as the reaction proceeds showing that assay was reacting with the product which would correlate to a higher absorbance when recorded. The data is also linear as it should be because as the protein works on the substrate the concentration of the product would steadily increase. There is also a correlation between the absorbance and the concentration of the protein as the concentration increases the rate of change of absorbance also changes drastically so we see three separate lines. Since there is no crisscrossing of the lines there is nothing to worry about protein stability and the rate at which the reactions are occurring so good data is being obtained. (Q2.11) (Irby, Pelaez, et al., 2018a)
Ryan	• Seeing the quantitative color change in some assays over others would be positive qualitative results and further comparison to a standard with a spectrophotometer quantitative results as to which ligands and conditions best suit the enzyme. Quick conclusions can be made from assays like this as to which ligands are interacting with the enzyme. (Ryan, Q2.1) • For this course as phosphates are freed, they are able to interact with the reaction solution and a color change happens. In our case, the enzyme produced a blue produced. This works since the enzyme under investigation is a diphosphate hydrolase. (Ryan, Q2.4) • The absorbance grows relatively to the concentration PNPA so product is formed and the protein interacts with the substrate (Ryan, Q2.11)
Jean	• A colored complex can be formed with the products from each enzyme ligand reaction by adding a suitable reactant and absorbance can be used to determine the enzyme's preferred substrate. (Jean, Q2.4) • The slope depends on the concentration of the ligand present, the more ligand, the more PNPA is hydrolyzed. This graph was likely produced from data from a color assay. (Jean, Q2.11)

TR2: Identify an enzyme active site using appropriate computational programs

Student	Open-ended Assessment Answers
Kris	• Based on the data inquiry above, the function of B51L is likely a phosphoribosyltransferase enzyme that transforms some nucleobase into a nucleobase monophasphate molecule. For example, the closest related protein based on BLAST and DALI is the uracil phosphoribosyltransferase protein that transforms uracil into UMP. Pfam works by compiling active site, structure, sequence and known functions of enzymes with similar function. For example, hydrolases is an overarching family where a NUDIX hydrolase is a subfamily. Superoxide dismutases are another family with similar enzymatic function. The phosphoribosyltransferase proteins likely all perform similar functions using similar chemical mechanisms on different substrates and different parts of the body. DALI and BLAST searches only take the sequence of your desired protein and compares them against all other known sequences to look for the closest fit. The premise is that proteins with similar sequences are more likely to have similar function. If the function of the matched protein is known and well characterized, it can help elucidate the unknown function of a desired protein. (Q1.6) • Based on the name of the most closely related protein on the sequence level and the best fit and alignment provided by ProMOL, the enzymatic class of the B51L protein is likely phosphoric monoester hydrolase. We know that the best fit in alignment is the EC 3.1.3 protein 1UPU, a phosphoric monoester hydrolase, and the most closely related protein according to the BLAST and DALI searches is the uracil phosphoribosyltransferase protein. Since this protein uses phosphoribosylpyrophosphate to transform uracil into UMP, a phosphoric monoester hydrolase function, it's likely B51L performs a similar reaction on some kind of nucleobase. (Q1.7) • […] I would look up literature of the most closely related proteins to B51L according to DALI and BLAST and discover which ligands bind to their active sites and which residues are involved. […] (Q21) • […] The substrate also fits nicely between ARG137 and ASP235, residues that were likely involved in the active site as per the ProMOL results while the 5GP does not bind at these residues. (Q1.8)
Pat	• Pfam shows that B51L is from a family made of uracil phosphoribosyltransferases. The BLAST results compared my protein's sequence to sequences in a database. Some of the top results returned are different uracil phosphoribosyltransferases. DALI compared the 3D structure of B51L to structures in a database. I'm looking for high z-scores (shows a good quality of fit), a low RMSD (to show that atoms in the 3D structure are close together between two structures), and %ID (to see how many residues are the same between structures). The best result here is a uracil phosphoribosyltransferase. Since all three programs returned uracil phosphoribosyltransferases, I am more confident in saying that B51L will have a similar function. (Q1.6) • I thought that the ProMOL results showed that the motif for B51L was most similar to another enzyme that is a uracil phosphoribosyltransferase. Pfam showed that B51L came from a family of uracil phosphoribosyltransferases. BLAST determined that B51L has a similar primary structure to several other uracil phosphoribosyltransferases. DALI shows that the 3D structure is similar to a uracil phosphoribosyltransferase and other types of phosphosyltransferases. (Q1.7) • […] Then I would use PyRx to test these ligands and how well they fit with B51L. Of the ligands that show a good fit, I would look at the structure in PyMOL and make sure it is in a potential active site. […] (Q21)
Alex	• The data would suggest that B51L is a Uracil Phosphoribosyltransferase because that kind of protein has the most hits after alignment. (Q1.6) • I thought it was a EC 3: Hydrolase but its probably a EC 2: Transferase after the database results (Q1.7)
Sage	• When it comes to utilizing BLAST, Pfam, and DALI I personally found that BLAST was by far the worst option when it came to actually finding useful results. Since all it provided was a sequential similarity and nothing else, so I would not know much of the possibilities when it came to actual protein function. That is where Pfam came in handy since it utilizes motif similarities to obtain a set of proteins that share functionality to the protein in question. So, Pfam was a lot more helpful since it gave me a wider array of results because it looked at motifs and then built a family based on that motif. Then, DALI was just overall the most helpful since it did a comparison of every single side chain between the two or more proteins given and then creates overlays of it and gives a lot of useful information like RMSD, Z-score and more which can really help to find extremely similar proteins that give us clues to the function of the protein in question. So, BLAST just gave a lot of similar sequences that could be useful or could be a waste of time, Pfam gave a solid family to work with and DALI allowed to work out the bad seeds of the family to really narrow down the possible function of the protein. Looking at all the data, I would say that it is a phosphoribosyltransferase and potentially a Uracil phosphoribosyltransferase. The reason why I say this is because the results from DALI show a very significant z-score for all other phosphoribosyltransferases, which means that there is a high similarity in the significant folds of the proteins. Alongside that, the results from Pfam indicate the same when comparing the sequence and the HMM. In the Blast results, %ident with higher than 50 all have something to do with phosphoribosyltransferases. (Sage, Q1.6) • These results heavily support the ProMOL results, within ProMOL it was shown that 1UPU has the best motif similarity to B51L with an RMSD all of 0.5257 which Uracil phosphoribosyltransferase. As described in the earlier question, all the data obtained from the different databases supports a function of phosphoribosyltransferase so B51L is a trasnferase enzyme. (Q1.7) • […] Then, I would use PyMOL to upload the files from PyRx, highlighting the active site motif of B51L and see if the ligands are located in or around the active site. If they are then, I would separate out those ligands and run an assay on them to find activities. (Q21) • […] Since the value is a bit lower that means that it has a more spontaneous reaction than 5GP and it has more binding sites with B51L meaning that it will be more stable in the active site so the reaction is more likely to occur. (Q1.8)

Ryan	• The predicted function for B51L is a uracil phosphoribosyltransferase. DALI used structural similarities to grade the protein of choice against others from the PDB. It had a 100%id and low root mean squared deviation meaning the distances of marked atoms between the unknown B51L protein and the uracil phosphoribosyltransferase was very little. BLAST, on the other hand, uses sequence alignments to find similarities, instead of structure. several hits were for sequence similarity between uracil phosphoribosyltransferase, all of them having high %id and very low E values. this means the hits were very good, and there is a lot of confidence in their similarity. Pfam specifically uses sequence alignments of known domains. The unknown protein has a uracil phosphoribosyltransferase domain, again pointing to that being its potential function. (Ryan, Q1.6) • Again, I am unfamiliar with ProMOL and how it finds it motifs but the databases use known information to find similarities and elicit information about B51L. From the databases, B51L seems to function as a uracil phosphoribosyltransferase, especially since all three databases shown had very good hits for it as well. (Ryan, Q1.7) • From the good hits off of PyRx, the next step is to get a visual of the protein and ligands interaction using PyMol, CN3D, UCSF Chimera, or another program that is similar.(Ryan, Q21) • CTP could be a more appropriate ligand due to the extra interaction as it looks almost like a binding pocket. (Ryan, Q1.8)
Jean	• According to the data above, B51L is a pentosyl transferase since, when compared to uracyl phosphoribosyltransferase, the e-value (from pfam) is 6.6e-80, the z-score (from BLAST) is 37.6 and the RMSD is 0.4. (Jean, Q1.6) • Secondly, B51L has more possible docking sites in CTP then in 5GP (Jean, Q1.8)

TR3: Determine the appropriate factors to consider when optimizing or interpreting an enzyme assay

Student	Open-ended Assessment Answers
Kris	• […] After choosing which ligands are likely a good fit to test and pure concentrated protein is collected, the protein can be screened against these ligands in various assays. The assay should include a positive or negative result if there is evidence of a reaction between the protein and substrate. For example, a molecule or dye may change color or fluoresce in the presence of any biproducts resulting in the chemical reaction between the enzyme and ligand. The assay chosen is going to have to agree, physiologically, with the enzyme. This means that the enzyme must tolerate the conditions the assay requires and that the pH and reagents in the assay do not alter the protein's function. The only thing that should be able to act with or on the enzyme is the ligand itself, otherwise results won't carry as much weight. Positive and negative results should be easy to discern. (Q2.1) • Assays are very time sensitive, reagent concentration sensitive and must be treated with great quantitative care. Otherwise, a positive or negative result may not be telling. Factors to consider in assays are the pH, salinity, buffers used, indicator molecule and ligands tested. All of these factors must be held constant so that the only reagent affecting the protein is the ligand itself and they must not affect or alter the function of the protein. Understanding what the likely biproducts of a successful reaction between the protein and ligand is crucial as the indicator molecule or dye must be able to detect the presence of this successful event in a relatively sensitive way. The efficiency of the protein is not known, so being able to detect minute reaction success may be critical. (Q2.2) • […] The AMES reagent (molybdate and ascorbic acid) is incredibly sensitive to free phosphate and will turn blue. The intensity of the blue can be detected using UV visible spectrometers. You are looking to see that a solution with enzyme and ligand be more intensely blue than one without (the control group). (Q2.4) • The positive control of trypsin under the same conditions allows to see how the absorbance changes with the concentration of the substrate over time. Since slopes of absorbance are similar between the control and the concentration at .2mM, it can be inferred that the rate of reaction at that concentration is similar for the protein of interest at that concentration as the control trypsin. The enzyme is even more responsive to PNPA at higher concentrations like .4mM, indicating it is an efficient enzyme working on the substrate PNPA. If there was no interaction, there would be no appreciable slope despite the change in concentration of PNPA. Little to no absorbance would be observed. (Q2.11)
Pat	• After purifying a protein, I would perform enzyme assays using many different ligands and at different conditions (temperature, pH, varying concentrations of enzyme and substrate). (Q2.1) • Enzyme assays are interpreted based on optical density readings. To optimize assays, I would test many different conditions(temperature, pH, concentrations) to determine which produces the most activity. I might need to add various cofactors or coenzymes based on the enzyme. If all my assays produce small OD readings, I would continue to change variables until a greater activity is noted (Pat Q2.2) • […] How blue the solution gets will help determine how active the enzyme was. This assay is very sensitive so concentrations must be kept very small to produce a notable difference in the level of blue color. (Q2.4) • The assay results show that activity was faster with a higher concentration of the enzyme, as the 0.4 mM line has a greater slope than the 0.11 mM line. I think this increase in absorbance with a higher concentration of enzyme suggests that the protein interacts with the substrate. Otherwise, the lines should have had no slope if the protein didn't interact. (Q2.11)
Alex	• […] The substrate + co-factor + enzyme combination that produces the highest OD should give a good suggestion as to what the protein's function is From there you could manipulate pH, co-factors, temp, substrates, and protein concentration. […] (Q2.1) • protein concentration, co-factors, pH, and substrate are all good factors to establish when performing an assay (Q2.2) • The color shift sometimes won't be large enough to measure activity. Some substrates may have exposed portions of the measured product that could result in false positives - so using good controls for these colorimetric assays are important (Q2.4) • As the concentration of PNPA increases the level of absorbance increases. Which would suggest that activity increases respectively to PNPA concentration. (Q2.11)
Sage	• One of the optimization setups that should be run is enzyme reaction progress curve which allows us to find the best concentrations to use for the assays. Since we want the enzyme to remain stable during the whole experiment, an enzyme reaction progress curve can help us a lot since it will show if the substrate is running out too quickly or if there are too little protein and things of that nature. Once results from the curve are obtained it allows us to ensure that the reactions are occurring at an appropriate speed during the assays. Alongside that, the most important thing to worry about in an assay is the enzyme stability. Once, a general idea of how the reaction is going to progress is obtained, we can compare results to it to see if there is something wrong with our stability. In the data, we should normally see 3 lines based on concentration that do not converge and plateau out after a steady increase almost like a bacterium's growth phase diagram. If that is not seen and there are issues with specific concentrations then those concentrations would have to be re-run and stability would have to be checked. Since an enzyme reaction progress curve would rule out a high concentration problem. (Q2.2) • […] The main limitation of this method is that too much buffer can interfere with the dye and gives us improper reading and if there is detergent in the mix the same would occur. (Q2.4) • there is an increase in absorbance as the reaction proceeds showing that assay was reacting with the product which would correlate to a higher absorbance when recorded. The data is also linear as it should be because as the protein works on the substrate the concentration of the product would steadily increase. There is also a correlation between the absorbance and the concentration of the protein as the concentration increases the rate of change of absorbance also changes drastically so we see three separate lines. Since there is no crisscrossing of the lines there is nothing to worry about protein stability and the rate at which the reactions are occurring so good data is being obtained. (Q2.11)

Ryan	• After finding potentially ligands in silico and after producing and purifying the B51L protein, the next step would be to run assays with each ligand. This would also involve finding ions that the enzyme needs to function as well as optimizing temperature and pH for activity. (Ryan, Q2.1) • Enzymes need cofactors to function, like metal ions. This is one thing that can be optimized. Another factor is pH, not all enzymes function at the same pH, especially depending on where in a cell the enzyme acts and what it does, this can also be optimized. The concentration of ligand present can affect an assay and can change how the results are interpreted. (Ryan, Q2.2) • So far we have gained information on what ion to use with the enzyme as well as ligands that the enzyme interacts with. A problem with results from assays like this is they are qualitative over quantitative. there are also limits of time and how much can be tested in a single assay. (Ryan, Q2.4)
Jean	• Factors that are important when optimizing or interpreting an enzyme assay can be things like the reliability of the calibration curve and its range or the correct use of enzyme concentration in the different enzyme-ligand samples (Jean, Q2.2)

TR4: Determine using computational software whether, and where, a ligand may be binding to a protein

Student	Open-ended Assessment Answers
Kris	• I would use PyRx to find a closely fit binding event between a number of ligands B51L may work with. I would look up literature of the most closely related proteins to B51L according to DALI and BLAST and discover which ligands bind to their active sites and which residues are involved. Based on the information I have thus far, I would probably test all of the nucleotides as well as GTP, CTP, ATP, TTP and UTP or any variant of phosphate(s) attacked to the nucleotides. (Q21) • Visually, both substrates seem to fit pretty well into the predicted active site of B51L, however, the binding energies are more telling pieces of information. Any binding energy lower than -6 or -7, typically, is a decent candidate for substrate testing in assay. While both CTP and 5GP are good fits (lower than -7) the CTP binding is -.1 kcal/mol lower than that of 5GP, suggesting an even stronger relationship between this substrate and B51L. The substrate also fits nicely between ARG137 and ASP235, residues that were likely involved in the active site as per the ProMOL results while the 5GP does not bind at these residues. (Q1.8) • […] After choosing which ligands are likely a good fit to test and pure concentrated protein is collected, the protein can be screened against these ligands in various assays. […] (Q2.1)
Pat	• I would start by gathering a list of ligands used by other uracil phosphoribosyltransferases in the same family as B51L. To do this, I would use the PDB to search for them. Then I would use PyRx to test these ligands and how well they fit with B51L. Of the ligands that show a good fit, I would look at the structure in PyMOL and make sure it is in a potential active site. Any ligands I think are a good fit at this point are candidates for enzyme assays. (Q21) • I think CTP is a better ligand choice. CTP appears to interact with B51L all along its own structure while 5GP only interacts on one end and with fewer residues. I would use the other programs to see if CTP is a ligand used by other uracil phosphoribosyltransferases, which would make it a more likely candidate for B51L. (Pat, Q1.8) • […] The ligand used here will help determine function along with bioinformatics research data. (Q2.1)
Alex	• Using the PDB's ligand finder look for ligands associated with Uracil Phosphoribosyltransferase (Alex, Q21) • CTP's affinity is 0.1 kcal/mol less than that of 5GP and the substrate (purple backbones) appear more exposed than 5GP, also there is more of them (Q1.8)
Sage	• I would choose potential ligands by going to PDB.org search all phosphoribosyltransferases via the EC number and then record and download it as a CSV file. Then, I would utilize PyRx to upload all of the ligands that are usable from the list, basically remove all ligands that were used to identify the structure of the proteins like selenomethionine, and then run them through the program. Once I have received the output of the program I would separate the ligands via high binding affinities vs. low binding affinities in kcal. Then, I would use PyMOL to upload the files from PyRx, highlighting the active site motif of B51L and see if the ligands are located in or around the active site. If they are then, I would separate out those ligands and run an assay on them to find activities. (Q21) • […] I would say that CTP (Citrine Triphosphate) is the appropriate candidate substrate for B51L due to it creating a better binding with B51L and also having the lower kcal/mol value. Since the value is a bit lower that means that it has a more spontaneous reaction than 5GP and it has more binding sites with B51L meaning that it will be more stable in the active site so the reaction is more likely to occur. (Sage, Q1.8)
Ryan	• A good start would be to use ligands that interact with known uracil phosphoribosyltransferase. Then finding similar ligands through the PDB ligand search could make a more robust list of possible ligands to text in silico though PyRx. From the good hits off of PyRx, the next step is to get a visual of the protein and ligands interaction using PyMol, CN3D, UCSF Chimera, or another program that is similar. PyRx can sometimes have hits that are false and it is easy to tell by getting a visual if the ligand is in an odd place. (Ryan, Q21) • CTP looks to have more interactions with the protein even though the free energy value is similar between CTP and 5GP. CTP could be a more appropriate ligand due to the extra interaction as it looks almost like a binding pocket. (Ryan, Q1.8)
Jean	• Pfam can be used to obtain a list of possible ligands. Then the structure for those ligands can be downloaded from PDB and the ligand docking function from PyRx can be used to determine the suitability of each ligand as a substrate for B51L. (Jean, Q21) • CTP would be a better ligand for B51L then 5GP. Firstly, the binding affinity between CTP and B51L is lower (therefore better) then the one between 5GP and B51L. Secondly, B51L has more possible docking sites in CTP then in 5GP (Jean, Q1.8) • Firstly, the pdb structure of the protein analyzed and of the possible ligands for it have to be downloaded from PDB and then the affinity between the protein and each possible ligand can be estimated with the docking function in PyRx. (Jean, Q2.1)

TR5: Compare enzymatic results with those computationally predicted

Student	Open-ended Assessment Answers
Kris	• […] I would probably test all of the nucleotides as well as GTP, CTP, ATP, TTP and UTP or any variant of phosphate(s) attacked to the nucleotides. (Q21) • […] After choosing which ligands are likely a good fit to test and pure concentrated protein is collected, the protein can be screened against these ligands in various assays. […] (2.1)
Pat	• […] Any ligands I think are a good fit at this point are candidates for enzyme assays. (Q21) • […] The ligand used here will help determine function along with bioinformatics research data. The ligand used here will help determine function along with bioinformatics research data. (Q2.1) • The computational prediction suggests that the enzyme is a hydrolase. (Q2.11)
Alex	• N/A
Sage	• […] If they are then, I would separate out those ligands and run an assay on them to find activities. (Q21) • Since it reacts with this substrate then the computational data was correct in assessing this as a hydrolase. (SAGE, Q2.11)
Ryan	• After finding potentially ligands in silico and after producing and purifying the B51L protein, the next step would be to run assays with each ligand. This would also involve finding ions that the enzyme needs to function as well as optimizing temperature and pH for activity. Seeing the quantitative color change in some assays over others would be positive qualitative results and further comparison to a standard with a spectrophotometer quantitative results as to which ligands and conditions best suit the enzyme. Quick conclusions can be made from assays like this as to which ligands are interacting with the enzyme. (Ryan, Q2.1) • 3H04 interacts with PNAP and does so almost linearly based on the graph. The slope depends on the concentration of the ligand present, the more ligand, the more PNPA is hydrolyzed. This graph was likely produced from data from a color assay. The computational outputs point to 3H04 being a hydrolase, and the graph shows that PNPA and the protein interact. They are both pointing to the protein being a hydrolase protein. This is able to be hypothesized based on the structure of PNPA and where the molecule could be hydrolized. (Jean, Q2.11)
Jean	• Firstly, the pdb structure of the protein analyzed and of the possible ligands for it have to be downloaded from PDB and then the affinity between the protein and each possible ligand can be estimated with the docking function in PyRx. Secondly, the plasmid coding for the protein in analysis can be purchased and induced in E. Coli to transcribe our protein. The protein can then be extracted from E. Coli cells and purified. The 4 or 5 most suitable ligands can be purchased and, together with the protein in analysis, used to create an assay describing the minimum enzyme concentration to produce enzyme activity with each substrate. In order to determine the activity of the enzyme, the product coming from the ligand needs to be quntified. To do that, a colored complex can be formed by adding a suitable reactant and absorbance can be used to determine the enzyme's preferred substrate. (Jean, Q2.1)

TR6: Design an enzyme assay to elucidate protein function

Student	Open-ended Assessment Answers
Kris	• […] After choosing which ligands are likely a good fit to test and pure concentrated protein is collected, the protein can be screened against these ligands in various assays. The assay should include a positive or negative result if there is evidence of a reaction between the protein and substrate. For example, a molecule or dye may change color or fluoresce in the presence of any biproducts resulting in the chemical reaction between the enzyme and ligand. The assay chosen is going to have to agree, physiologically, with the enzyme. This means that the enzyme must tolerate the conditions the assay requires and that the pH and reagents in the assay do not alter the protein's function. The only thing that should be able to act with or on the enzyme is the ligand itself, otherwise results won't carry as much weight. Positive and negative results should be easy to discern. (Kris, Q2.1 • The positive control of trypsin under the same conditions allows to see how the absorbance changes with the concentration of the substrate over time. Since slopes of absorbance are similar between the control and the concentration at .2mM, it can be inferred that the rate of reaction at that concentration is similar for the protein of interest at that concentration as the control trypsin. […] (Q2.11)
Pat	• […] I would perform enzyme assays using many different ligands and at different conditions (temperature, pH, varying concentrations of enzyme and substrate). I would expect to gather data through optical density measurements. This will show me if a reaction took place and allow me to quantify the activity level. Reactions that produce the highest OD reading would be assumed to have the most function. The ligand used here will help determine function along with bioinformatics research data. (Q2.1)
Alex	• Performing a substrate assay that produces a measurable molecule after catabolism, one that most likely produces a color shift significant enough that it can be measured using OD. The substrate + co-factor + enzyme combination that produces the highest OD should give a good suggestion as to what the protein's function is From there you could manipulate pH, co-factors, temp, substrates, and protein concentration. Establish extinction coefficient and create a standard curve. (Q2.1)
Sage	• I would personally run several assays to confirm the function of the protein and utilize Bradford assay and nanodrop to obtain data on the assays to identify functionality. The reason why I chose Bradford assay and nanodrop is due to more experience with those techniques and understanding the results of those techniques a bit better. I would have several graphs with calibration curves to obtain the protein activity. Since I would run a decreasing amount of protein in each sample I would expect to see a more or less decreasing linear model where activity is occurring since activity is decreasing due to a drop in the number of available proteins. Since the models are based on activity occurring whenever activity does occur I would be able to find functionality based on activity. (Sage, Q2.1)
Ryan	• After finding potentially ligands in silico and after producing and purifying the B51L protein, the next step would be to run assays with each ligand. This would also involve finding ions that the enzyme needs to function as well as optimizing temperature and pH for activity. Seeing the quantitative color change in some assays over others would be positive qualitative results and further comparison to a standard with a spectrophotometer quantitative results as to which ligands and conditions best suit the enzyme. Quick conclusions can be made from assays like this as to which ligands are interacting with the enzyme. (Ryan, Q2.1)
Jean	• In order to determine the activity of the enzyme, the product coming from the ligand needs to be quantified. To do that, a colored complex can be formed by adding a suitable reactant and absorbance can be used to determine the enzyme's preferred substrate. (Jean, Q2.1)

TR7: Explain how the purification of tagged proteins work and ways the process can be optimized

Student	Open-ended Assessment Answers
Kris	• Purification tags are built into the protein of interest as blueprinted from the engineered plasmid. The plasmid will contain the information needed to place extra amino acid 'tags' at the C or N terminal region of the protein. The purpose of these tags is to be able to separate these proteins from all other proteins lysed from a bacterial cell. Cells naturally do not have these tags and can be easily teased from the protein solution. The purification tag we used in class was the His tag which contains six histidine residues on the N-terminal region of our NUDIX proteins. The residue, histidine, contains the molecule chemically equivalent to imidazole which can be targeted on separation column chromatography. Metal ions bound to the column substrate chelate the nitrogen atoms of the histidine tag, allowing other proteins to flow off the column. High concentrations of imidazole solution then swaps out for the protein and the purified protein can be washed and collected. You are able to collect samples of this flow through and try and detect a single band corresponding to your protein of interest on gel electrophoresis. No matter the tag used, the chemical properties they possess differ from natural proteins which allows biochemists to isolate the protein they need. (Q2.5) • The purification tag may not interfere with the overall structure and function of the protein in question and should operate the same had the tag not been there. The purification tag should be relative easy to engineer into the plasmid, easy for the cell to transcribe and translate and allow the protein to be separated away from others. In other words, the properties bestowed by the tag whether that be molecular weight or chemical properties, should allow the protein to be differentiated and separated predictably and reliably from other proteins. (Kris, Q2.6)
Pat	• Tagged proteins will bind to a matrix during affinity column chromatography while other proteins wash through. In my case, the enzyme had a His6 tag, which binds to Ni2+ in the Ni2+:NTA resin. After washing the other proteins, an elution buffer is added to remove the target protein from the matrix. I added imidazole, which competes with the Histag for binding to Ni2+. […] (Q2.5) • Protein tags should be optimized based on the other proteins that will be in solution. If there are a lot of proteins with a high concentration of histidine, a His tag might not be appropriate because extraneous proteins may bind to the matrix and cause impurities in the final result. (PAT, Q2.6)
Alex	• Histidine tags are utilized during purification to attach proteins to a column. […] (Q2.5) • I'm not sure, I don't know much about how protein tags work to be honest. I suppose you'd want a high affinity between two molecules and a wash that allows you to elute everything in the supernatant besides the protein (ALEX, Q2.6)
Sage	• […] the protein for my group, the protein contained His-tags and kanR tags. The KanR tags allow us to grow the protein without a problem since adding kanamycin to the growth media allows us to ensure that only the bacterium with our protein survives. After that, the cells were lysed via sonication and an SDS-Page was run on small fractions of the materials to see if the protein was present in our solution. […] (Q2.5) • […] His-tags were used to purify our protein via metal-ion chromatography. The way this works is that since His can be bound with Nickel quite well, we set up a metal-ion chromatography with Nickel resin. As the protein ran through the material the His-tags wrapped around the nickel and were stuck there. Afterwords imidazole was used to release the protein from the resin since imidazole can also bind to the resin and as imidazole replaced the His-tags our proteins were slowly removed from the resin and fell into a solution completely separate from other proteins or molecular debris that was previously present in the solution. […] (Q2.5) • I am unsure about the optimization of the protein purification step so I cannot accurately describe this situation. (Sage, Q2.6)
Ryan	• Histidines have a positive charge and the His-tag is usually between six and eight histidines at either the N or C terminus of a protein. They can attract a negative charge in a column and are useful for purification. A column is usually filled with a resin or beads that can be saturated with a solution that doesn't out-compete the tagged protein. Another solution is then used to wash away proteins that are not of interest. a final solution is used to elute the protein of interest by out competing and pushing off the tagged protein from the resin. (Ryan, Q2.5) • Finding the right solutions for binding, washing, and elution is crucial. If a solution outcompetes the protein of interest for binding then the protein will be washed out with the rest of the proteins. Also solutions should not interact with the protein or tags themselves. Another thing to keep in mind is the resin itself, it should be chosen to interact with the tag used. Taking the time to add binding buffer slowly and across enough column volumes allows for the solution the resin was stored in to be washed out and diluted down. (Ryan, Q2.6)
Jean	• A protein can be purified using different kind of columns (affinity, size exclusion, etc.), acidity variations and formation of temporary complexes by tagging the protein with another compound and them elute it by taking it off. (Jean, Q2.5) • By choosing the most suitable tag compound (Jean, Q2.6)

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VITA

Stefan Mark Irby

EDUCATION

Ph.D. Chemistry – Chemical Education)	Purdue University	2019
B.S. Biochemistry	Western Washington University	2014
A.A. Business	Green River Community College	2010
High School Diploma	Tahoma Senior High School	2010

RESEARCH

Doctoral Researcher – Biochemistry Education	Purdue University	August '14 – May '19
Undergraduate Researcher – Chemistry Education	Western Washington University	March '11 – June '14
Research Intern	Fred Hutchinson Cancer Research Center	June '13 – August '13

TEACHING

General Chemistry Course Coordinator	Purdue University	June '16 – August '16
General Chemistry Lab Supervisor	Purdue University	August '15 – May '19
Graduate Chemistry Teaching Assistant	Purdue University	August '14 – May '15
Chemistry Lab Teaching Assistant	Western Washington University	January '11 – June '14

PROFESSIONAL DEVELOPMENT

Certificate of Foundations in College Teaching	**Purdue University**
2018	West Lafayette, Indiana
Success Strategies Workshop for New Faculty Members	**United States Air Force Academy**
2018	Colorado Springs, Colorado
Inclusion in the Classroom Workshop	**United States Air Force Academy**
2018	Colorado Springs, Colorado
Graduate Student and Postdoc Career-Development Event	**ASBMB**
2017	Chicago, Illinois
Safe Zone Certified	**LGBTQ Center, Purdue University**
2017	West Lafayette, Indiana

DEPARTMENTAL AND UNIVERSITY VOLUNTEERING AND OUTREACH

Purdue University

2018	General Chemistry Administrative Assistant Selection Committee Member
2018	Graduate Teaching Assistant Advisor and Panel Member
2018	Phi Lambda Upsilon Spring Fest Science Outreach
2017 - 2018	Disability Resource Center Mentoring Program - Mentor
2017	Science in Schools Outreach Program
2017	Chemistry Proposal Writing Help Session - Panel Member
2016	National Chemistry Week Outreach Program
2015 - 2018 (annually)	Chemistry Department Recruitment
2015 - Present	Chemistry Graduate Student Mentoring Program - Mentor
2015 - 2017 (annually)	Chemistry Graduate Student Orientation

Western Washington University

2012	Compass 2 Campus Mentoring Program - Mentor

AWARDS, HONORS, FELLOWSHIPS AND SCHOLARSHIPS

2018	**For Mentoring New Graduate Teaching Assistants and Improving Chemistry Education and Instruction** Purdue University, Department of Chemistry
2018	**United States Air Force Academy Faculty Development Fellowship** United States Air Force Academy
2018	**Phi Lambda Upsilon Travel Grant** Chemistry Honor society, Nu Chapter - Purdue University
2017	**ASBMB Graduate Student Travel Award** American Society for Biochemistry and Molecular Biology
2016	**Graduate Research Fellowship Program Honorable Mention** National Science Foundation
2015	**Initiated into Phi Lambda Upsilon** Chemistry Honor society, Nu Chapter - Purdue University
2013	**Summer Undergraduate Research Program** Fred Hutchinson Cancer Research Center
2013	**Barbara French Duzan Scholarship** Western Washington University
2013	**Hach Land Grant Undergraduate Scholarship** American Chemical Society
2012	**Julia Ann Rutherford Memorial Scholarship** American Chemical Society - Puget Sound Chapter
2012	**Hach Land Grant Undergraduate Scholarship** American Chemical Society
2012	**Chemistry Department Tuition Waiver Scholarship** Western Washington University
2012	**Brilliant Scientist of Tomorrow Book Scholarship** Brilliant Scientist of Tomorrow
2010	**Future Woodring Scholar** Woodring College of Education - Western Washington University
2010	**Future Educator of Tomorrow Scholarship** Tahoma Education Association
2009	**Initiated into Phi Theta Kappa** International Honor Society of the Two-Year College, Alpha Chi Beta Chapter

Publications

Irby, S. M., Pelaez, N. J., & Anderson, T. R. (**2018**). Anticipated Learning Outcomes for a Biochemistry Course-based Undergraduate Research Experience Aimed at Predicting Protein Function from Structure: Implications for Assessment Design. *Biochemistry and Molecular Biology Education*, *46*(5), 478–492.

Irby, S. M., Pelaez, N. J., & Anderson, T. R. (**2018**). How to Identify the Research Abilities Instructors Anticipate Students will Develop in a Biochemistry Course-Based Undergraduate Research Experience (CURE). *CBE—Life Sciences Education*, *17*(2), es4, 1–14.

Craig, P. A., Anderson, T., Bernstein, H. J., Daubner, C., Goodman, A., **Irby, S. M.**, … Stewart, R. (**2018**). Using protein function prediction to promote hypothesis-driven thinking in undergraduate biochemistry education. *The Chemist*, *91*(1), 1–8.

Irby, S. M., Borda, E. J., & Haupt, J. (**2018**). The Effects of Implementing a Hybrid Wet-Lab and Online Module-Lab Curriculum into a General Chemistry Course: Impacts on Student Performance and Engagement with the Chemistry Triplet. *Journal of Chemical Education*, *95*(2), 224-232.

Irby, S. M., Phu, A. L., Borda, E. J., Haskell, T. R., Steed, N., & Meyer, Z. (**2016**). Use of a card sort task to assess students' ability to coordinate three levels of representation in chemistry. *Chemistry Education Research and Practice*, *17*(2), 337-352.

Invited Presentations *underlined author denotes presenter(s)*

Irby, S. M., Pelaez, N. J., & Anderson, T. R. (2017, May). A Process for Defining and Validating Learning Competencies for Course-Based Undergraduate Research Experiences in a Biochemistry Laboratory Curriculum. Presented at Fourth NSF RCN-UBE funded ACE-Bio Network Retreat, Highlands, NC.

Oral Presentations *underlined author denotes presenter(s)*

Irby, S. M., Pelaez, N. J., & Anderson, T. R. (**2018**, July). A data-driven process for identifying the Anticipated Learning Outcomes (ALOs) of a biochemistry Course-based Undergraduate Research Experience (CURE). Presented at Biennial Conference on Chemical Education, Notre Dame, IN.

Irby, S. M. & Borda, E. J. (**2018**, July). Effects of implementing a hybrid wet lab and online module lab curriculum into a general chemistry course: Impacts on student performance and engagement with the chemistry triplet. Presented at Biennial Conference on Chemical Education, Notre Dame, IN.

Irby, S., Phu, A., Borda, E., and Haskell, T. (2014, August). Scratching the surface of chemistry: A progression for categorizing chemistry problems. Presented at Biennial Conference on Chemical Education, Allendale, MI.

Poster Presentations

Irby, S. M., Pelaez, N. J., & Anderson, T. R. (2017, April). A Process for Defining and Validating Learning Competencies for Course-Based Undergraduate Research Experiences in a Biochemistry Laboratory Curriculum. Poster session presented at Experimental Biology, Chicago, IL.

Irby, S. M., Pelaez, N. J., & Anderson, T. R. (2016, August). Towards an understanding of reasoning about proteins of unknown function in biochemistry course-based undergraduate research experiences. Poster session presented at Biennial Conference of Chemical Education, Greeley, CO.

Irby, S., Phu, A., Borda, E., and Haskell, T. (2014, May). Scratching the surface of chemistry: A progression for categorizing chemistry problems. Poster session presented at Western Washington University Scholars Week, Bellingham, WA.

Irby, S. and Stoddard, B. (2013, August). Characterization of the Homing Endonuclease I-Cth. Fred Hutchinson Cancer Research Center's Summer Undergraduate Research Program competitive poster session. Seattle, WA.

Irby, S. and Borda, E. (2012, June). Analysis of module learning and student outcomes: Using technology to increase gains in chemical education. Poster session presented at ACS North West Regional Meeting, Boise, ID.

Irby, S. and Borda, E. (2012, May). Analysis of module learning and student outcomes: Using technology to increase gains in chemical education. Poster session presented at Western Washington University Scholars Week, Bellingham, WA.

Acknowledged Research and Intellectual Contributions

Craig, P. A. (2018). Lessons from my undegraduate research students. *Journal of Biological Chemistry*, *293*(27), 10447-10452.

Craig, P. A. (2017) A survey on faculty perspectives on the transition to a biochemistry course-based undergraduate research experience laboratory. *Biochemistry and Molecular Biology Education*, *45*(5), 426-436.

PUBLICATIONS

Irby, S. M., Pelaez, N. J., & Anderson, T. R. (2018a). Anticipated Learning Outcomes for a Biochemistry Course-based Undergraduate Research Experience Aimed at Predicting Protein Function from Structure: Implications for Assessment Design. *Biochemistry and Molecular Biology Education*, 46(5), 478–492. https://doi.org/10.1002/bmb.21173

Irby, S. M., Pelaez, N. J., & Anderson, T. R. (2018b). How to Identify the Research Abilities Instructors Anticipate Students will Develop in a Biochemistry Course-Based Undergraduate Research Experience (CURE). *CBE—Life Sciences Education*, 17(2), es4, 1–14. https://doi.org/10.1187/cbe.17-12-0250