

Understanding Protein Domains: A Modular Approach

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Abstract

An understanding of protein structure, function, and interaction is central to biochemistry. One angle into this topic is to engage students in considering protein domains as modules that develop form and execute function semi-independently. Here, I describe a modular guided inquiry Lesson for students with an introductory background in molecular biology and biochemistry. The Lesson enables students to further explore how we define and investigate the structure and function of protein domains in a research context. Activities focus on bioinformatic approaches and interpretation and design of experiments to investigate protein interactions. Possible extensions into wet-lab and/or research projects are also highlighted. Students from various science majors enrolled in an intermediate-level biochemistry course reported that this Lesson strengthened their ability to analyze protein sequence and structure and to understand approaches to determining protein interactions.

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Supporting Materials: S1. Understanding Protein Domains-Module I assignment, S2. Understanding Protein Domains-Module II assignment, S3. Understanding Protein Domains-Module III assignment, and S4. Understanding Protein Domains-Module IV assignment.

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Learning Goal(s)

Students will understand that:

- protein domains have structure and function that may be both semi-independent of and contribute to overall protein structure and function.
- protein-protein interactions can be tested experimentally both *in vitro* and *in vivo*.

Learning Objective(s)

Students will be able to:

- compare protein sequences and identify conserved regions and putative domains.
- obtain, examine, and compare structural models of protein domains.
- interpret data on protein interactions (*in vitro* pull-down and *in vitro* and *in vivo* functional assays)
- propose experiments to test protein interactions.

INTRODUCTION

When introducing students to protein structure, the modular domain aspect of proteins can be fascinating in theory, but sometimes hard for students to completely grasp. How is it that segments of a protein encoded within a single gene and translated from one mRNA can have separable functions? How might various domains of a protein contribute to overall function? How can we define domains and study how they interact with other macromolecular structures within and outside of the cell? Focusing on these questions provides students with opportunities to think about structure-function relationships, to consider the impact of molecular evolution on domain structures, and to understand how scientists can demonstrate domain interactions or interactions involving whole proteins. These aims are accepted as important outcomes in the study of

biochemistry. For example, among the undergraduate learning goals identified by American Society for Biochemistry and Molecular Biology (ASBMB) are the declarations that “[s]tudents should understand that proteins are made up of domains...” and “[s]tudents should be able to discuss the interactions between a variety of biological molecules and describe how these interactions...[lead] to changes in biological function.” (1).

One route to addressing these questions is through bioinformatics. The National Institutes of Health (NIH) defines bioinformatics as “research, development, or application of computational tools and approaches for expanding the use of biological, medical, behavioral, or health data, including those to acquire, store, organize, analyze, or visualize such data.” (2). Bioinformatics is important for the study of biochemistry as it focuses on the analysis of molecular sequences (DNA, RNA, and proteins) and reveals the structure and function of macromolecules. I want to encourage students to use

bioinformatics tools to explore relationships between primary sequence, three-dimensional structure, and biological function, both in this course and in their work in other areas. Comparison of protein sequences and structures connects to learning goals articulated within ASBMB's Concept-Driven Teaching, e.g., "Use various bioinformatics approaches to analyze macromolecular primary sequence and structure." (1). Completing these assignments reinforces concepts with which biochemistry students should be comfortable, while students are simultaneously learning bioinformatics skills that they can use across disciplines. Magana *et al.* among others highlight the importance of bioinformatics education for cross-disciplinary problem solving (3). They also advocate for establishment and assessment of related learning outcomes, as in this Lesson (3).

Another set of skills that we seek to develop in undergraduate science students is their ability to propose experiments and to interpret data. This "ability to apply the process of science" is reflected as one of the Vision and Change Core Competencies for undergraduate biology education (4). The Modules described here can help build skills identified as learning goals for undergraduate biochemistry majors by the ASBMB (Students should be able to: "interpret data pertaining to tertiary and quaternary structure of molecules;" "propose a purification scheme for a particular molecule;" and "propose appropriate...chemical biology approaches to explore the... interactions of biological macromolecules.") (1).

To help students accomplish the aims described above, I developed this series of guided inquiry exercises. This approach resembles problem-based learning (PBL) in that, for at least some students, the problems introduce new content and/or require research (5). The problems may also reinforce concepts introduced earlier in the course and curriculum. This Lesson supports the aims identified by Eberlein *et al.* for PBL and related approaches: "To promote higher-order thinking skills; to help students learn to reason through problems, instead of using algorithmic approaches; to build conceptual understanding through active engagement with the material; to foster growth in teamwork and collaborative problem-solving skill" (5). This Lesson also offers an opportunity for student-centered learning advocated for by the Vision and Change report, as the projects are inquiry-driven, require active participation, and benefit from student collaboration (4). Please see the Scientific Teaching Themes section for further discussion.

The exercises I developed focus on a specific protein, the transcriptional coactivator ADA2. This protein is known to work with GCN5, a histone acetyltransferase, in various eukaryotic species. The choice of ADA2 provides a connection to a research project in my lab, which uses *Arabidopsis thaliana* as a model system. In *Arabidopsis*, gene duplication has created two versions of ADA2, referred to as ADA2a and ADA2b. ADA2b seems to have more significant biological function (6), and so I used *Arabidopsis* ADA2b as our primary starting point for some of the activities. As discussed later in this article, these Lesson activities could be adapted for any protein for which there is information about amino acid sequence, structural data, and *in vitro* or *in vivo* interactions. Alternatively, these inquiries could lay the foundation for novel research investigations, thereby building new scientific investigations on what is already known.

Intended Audience

The Lesson was used in an intermediate-level biochemistry

course at a liberal arts college. Students enrolled in the course were sophomore through senior undergraduate science majors.

Required Learning Time

The four Modules that comprise this Lesson were deployed throughout an academic semester. For each module, background information (if needed) and the assignment were provided a week in advance of a lab section during which time was allotted for students to ask questions about the Module. Following that meeting, students typically had two additional weeks to submit their work (Table 1). Scheduling of the Modules throughout the semester allowed for assessment and feedback on each assignment before the next was due. However, since the skills needed for each module are distinct, this sequential timing is unlikely to be essential. Following submission of each complete assignment, students in the Spring 2017 cohort were asked to (voluntarily) indicate how much total time they had spent preparing the assignment. In all cases, the majority of respondents indicated that they had spent either less than three hours or three to five hours engaged in these problems. That time including talking with me and/or their peers and preparing their final answers.

Pre-requisite Student Knowledge

Students should be familiar with concepts underlying the Central Dogma of molecular biology and with basic protein structure including amino acid functionality. If students are not familiar with BLAST and/or multiple sequence alignment or protein structural viewers, the instructor will need to provide background tutorial materials or other approaches to help students develop skill in using these programs. Similarly, if students have no or limited knowledge of SDS-PAGE, protein purification (GST pull down), and/or transgenic organisms, review of these approaches could be built into Modules III and IV.

Pre-requisite Teacher Knowledge

Instructors who teach molecular and cell biology at the introductory college level or above should have the prerequisite knowledge. Skill development would be similar as that outlined for students above.

SCIENTIFIC TEACHING THEMES

Active learning

As noted above, this deployment of the Modules represents guided inquiry or problem-based learning. Students have to collect and interpret data and design and explain experimental approaches to answer scientific questions. Students will need to leverage prior knowledge and acquire new knowledge and skills to prepare Module answers. Collaboration is strongly encouraged in engaging with these problems. Students in my course are accustomed to working in problem-solving groups in class and so many engage in this high impact practice [as defined by the American Association for Colleges and Universities (7)] for this project. These active learning approaches in STEM fields have been highlighted as important for enhancing student engagement, performance, and retention by Project Kaleidoscope (8), the Vision and Change report commissioned by the National Science Foundation and the American Association for the Advancement of Science (4), and by numerous individual science education studies [a sampling summarized in (9)].

Assessment

Individual written responses to short answer questions were assessed for a grade. As students are allowed to collaborate as they work through the questions, there is also a great deal of self-assessment that goes on, as student reconcile their thinking with that of their peers. As elaborated on in the Discussion, student understanding of the concepts and skills connected to the Modules was accessed through short answer questions on the final exam (e.g. interpreting a sequence alignment or data from a pull-down assay).

Inclusive teaching

These Modules represent learner-center instruction. Students are provided with background information and/or an assignment prompt to review outside of class. This gives each student time and space to connect this new work to previously developed knowledge and skills and frame questions. Individual students/small groups have opportunities to ask questions during a lab period and/or during office hours after this initial read-through. Students may also use any resources they like (with the exception of reading another student's written responses) in developing their answers. Encouraging this variety of ways to engage with the material aligns with inclusive teaching, described by Cornell's Center for Teaching Innovation as incorporating "any number of teaching approaches that address the needs of students with a variety of backgrounds, learning styles, and abilities." (10).

Aspects of this work can also prompt thought and discussion around issues of access and equity in science. For example, the bioinformatics utilities chosen are freely available and data excerpts can be chosen from research papers that are Open Access. The studies used here include authors of various nationalities working as collaborators; in general, looking at the authors of the published works chosen provides an entry point to discussing the (lack of) diversity in scientists. This latter point is reflected in a reflection prompt on inclusive teaching strategies provided by the University of Michigan's Center for Research on Learning and Teaching (11).

LESSON PLAN

Overview

These modules were used as part of a 15-week course scheduled for three hour-long lecture meetings and one three-hour lab meeting per week. For each module, an assignment sheet and in some cases, specific background reference materials were provided a week in advance of the lab meeting in which time was allotted for students to ask questions about the assignment. Following that meeting, students typically had two additional weeks to submit their work (Table 1).

The four Understanding Protein Domains (UPD) modules were worth 10% of the overall course grade, with the first two assignments being weighted slightly more due to the higher estimate of amount of work required. Based on the peer learning environment I seek to foster in my courses and the collaborative nature of science, I encouraged students to discuss use of the web-based utilities, experimental approaches, and even the UPD questions with their colleagues. However, each student was responsible for constructing their own answers prior to submission.

Module I: Comparing Sequences

The first Module (S1) involves comparing protein sequences. Students were encouraged to review written background [adapted from Young (12)] and instructor-generated screencasts reviewing BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and Clustal (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) as needed. This Module benefits from this opportunity for learner-specific instruction, as some students are experienced with these programs while others may have only been introduced to them. In addition, students were free to complete the assignment with comparable programs if they preferred. Students were directed to align eight sequences and identify domains primarily on the basis of primary sequence conservation (S1). In this assignment we studied the ADA2 transcriptional coactivator, for which paralogs exist in several species. To minimize complexity, after consultation of the literature (13), I provided students with an accession number for the *Arabidopsis thaliana* ADA2b protein as a starting point and also specified the species in which students should search for orthologs.

Module II: Comparing Structures

In this Module (S2), students use bioinformatics approaches to examine secondary, tertiary, and quaternary protein structure. Students were again encouraged to review written background information [adapted from (12)] and/or instructor-generated screencasts reviewing NCBI Structure/Cn3D (<https://www.ncbi.nlm.nih.gov/structure/>), although other structural viewers could be used as well. For ADA2, the structures of only some specific domains have been solved, which reinforced domains as protein sections that can fold and function semi-independently. This module also reinforced conservation at the structural level (S2, question 4) and offered one way to look at structural interactions (S2, question 3).

Module III: Test of Interaction

The third Module provides an opportunity for students to consider which domains of ADA2 and GCN5 might mediate chemical interactions between the two proteins. Students are asked to interpret a Glutathione S-transferase (GST) pull down experiment (14) and then propose an experiment to extend their understanding of the protein interactions involved (S3). In this example, the latter portion builds on findings from Module I, as students are asked to outline a way to test if GCN5 interacts with the plant-specific domains of ADA2b they had previously identified.

Students had been introduced to the related idea of co-immunoprecipitation through lecture and had encountered the use of epitope tags through a journal club-style review of an unrelated article from the primary literature (Table 1). However, student questions and answers revealed that more specific background about pull-down assays would be beneficial. In the Spring 2016 offering of the course, an instructor-generated screencast outlining the method was posted along with the answer key, to help increase student understanding of this approach. Please see the Teaching Discussion section for further commentary on revisions to Module III.

Module IV: Test of Function

In this last Module, I wanted to provide students with an opportunity to think about how one might test the function of a given protein domain (S4). The example here is specific to published literature on ADA2, which identifies a region of the plant-specific domain as a target for acetylation by GCN5 and then tests the requirement for a specific lysine residue through site-directed mutagenesis and an *in vivo* test (15). This example also afforded connection to a similar test of the function of the entire plant-specific domain that has been carried out in my research lab (S4; also see Extensions section below). No specific background was provided for this assignment, since students were completing this module in the last weeks of the semester and were asked to draw on prior course knowledge and problem solving skills. For example, students did not have specific experience with acetylation assays as shown in panel B in S4, but they had run SDS-PAGE in lab. Students were familiar with sequence alignment (from Module I) and histone acetylation (from lecture), which were likely helpful in interpreting panel C mentioned in S4. The experiment referenced in the second part of S4 is similar to an approach students were introduced to through a wet-lab exercise (Table 1).

TEACHING DISCUSSION

Student Outcomes

Student understanding of the concepts and skills connected to the UPD Lesson was accessed on the final exam. For example, in Spring 2016, a question that required interpretation of a sequence alignment was answered correctly by two-thirds of class (77% in Spring 2017) and a question relating to a pull down assay was answered correctly by 60% of students (64% in Spring 2017). In addition, students were asked to complete an indirect assessment of learning gains related to the goals of the UPD modules (Table 2). In general, students agreed that engaging in the UPD modules strengthened their understanding of protein domains, facilitated their ability to use sequence and structural data, and helped them interpret protein interaction. Students were least confident in their ability to design experiments to study protein interactions. This challenge was reflected in direct assessment as well. The assignment for Module 3 asks students to propose an experiment to test which domains may support protein-protein interactions. In the Spring 2016 cohort (N=35), the mean grade on this assignment was a 73%, while the combined average for the other three assignments was an 86%. This lower score may have resulted from lack of specific background on pull down assays (the focus of this assignment). I provided more explicit instructions and a review of a pull down assay via screencast in Spring 2017, and the average on the Module 3 assignment increased to 87%, although averages across the board were slightly higher for this cohort (90%).

The Modules Themselves

Data suggested that students spent the most time on Module I: 14 out of 23 respondents spent three to five hours and six students spent six or more hours working on this assignment. The higher time spent may be due in part to this being the first assignment of this type in the semester. One specific area

of challenge for students was trying to determine putative domains based on sequence conservation. In my view there is no single “right” answer here. When students discuss this challenge with me, I remind them to consider general estimates of minimum domain length (and to think about what drives this minimum). Another challenge comes from the fact that, while my students have reviewed amino acid chemistry and functionality, they are still practicing weighing identity vs. similarity when looking at sequence comparisons. Again, when asked for advice, I indicate that they should be consistent with the stringency used to choose putative domains throughout the primary sequence.

When evaluating BLAST search results, students may have difficulty recognizing multiple entries of the same protein or paralogs within a species. I encourage them to look beyond BLAST score and consider length of match, identity and similarity, etc. Module II, in which students compare structures, seems to be a little more straightforward, perhaps because there are a more limited number of structures in the databases. I encourage students to recognize this issue and consider why this might be the case.

As mentioned above, my students find proposing an experiment to test protein-protein interactions (in Module III) to be one of the more difficult items in this Lesson. I deliberately left the question fairly open, so that students could reiterate a GST pull down experiment similar to the one they had just reviewed or consider other approaches (e.g. yeast two hybrid, structural methods). In Spring 2017, I altered the instructions slightly, indicating a point value along with items that should be included in their answer: how to obtain/prepare potential interacting partners, how to assay interaction, and what controls should be used. I think this additional information helped highlight the importance of these experimental aspects. Depending on their level of expertise, students may need to research these experimental approaches before completing the assignment. Alternatively, an instructor could choose to provide more detailed background in this area prior to assigning Module III or modify his/her/their assessment.

Module IV covers both *in vitro* and *in vivo* tests of function. Necessary background will likely depend on the course and curriculum structure as well as the general student ability. For example, I use analysis of the second figure from Mao et al. (15) as a way to introduce conventional site directed mutagenesis leading to a single amino acid change (e.g. K215R). Since my students are very familiar with the amino acids by this point, many can make the leap or they can check with me or classmates. Also, my students have been introduced to the idea of transgenes and genetic rescue in another lab module; in other cases, this approach would likely require some explanation.

The Project Overall

Others have reported pedagogical strategies to help students develop bioinformatic skills including aligning sequences and investigating protein structure-function relationships, both within individual courses (e.g., 16-18) or in larger curricular structures (19,20). The Modules described here bring together these bioinformatics approaches with data analysis of wet-lab experiments designed to interrogate protein domain structure and protein-protein interactions that drive function. Because this approach can be based on existing data, it is cost effective. These Modules could be embedded within a biochemistry, molecular biology, or cell biology course.

Extensions and Adaptations

I see this course structure as adaptable in several significant ways. One could choose a protein of research or curricular interest and develop exercises using this scaffold. Focusing the exercises on a protein of interest would facilitate coupling Modules III and/or IV to wet-lab experiments, if feasible and desirable. In addition, one could adapt any of the modules to be novel investigations into the structure or function of protein domains of interest or simply rely on students' analysis of database information and published literature as I have done here. Additional extensions could involve students constructing phylogenetic trees (21) or exploring domains through other common utilities (e.g. PROSITE, <https://prosite.expasy.org/>).

From a pedagogical perspective, one may wish to adapt how the assignments are given depending on one's own teaching and/or course design preferences and based on the student population. Suggestions about places in which additional content background or skill development may be helpful are included in the reflection on "The Modules Themselves" in the Teaching Discussion. In addition, I employed a learner-center approach, which places responsibility on each student to review the assignment and any necessary background information prior to an optional class discussion. Students could be offered incentives to read the material in advance and/or be required to participate in preliminary class discussion as ways to enhance engagement and collaborative learning.

Aims

This modular approach can be used as an entire series or as individual problem-based learning experiences. It could be adapted to integrate with lab exercises or an area of research driven by the instructor or curriculum. While the Learning Goals and Learning Objectives listed earlier were developed for this specific Lesson, they connect directly to ASBMB's core concepts, specifically the idea that macromolecular structure determines function and regulation (1). The bioinformatics skills are also referenced under the Foundational Concept of Discovery (1).

SUPPORTING MATERIALS

- S1. Understanding Protein Domains-Module I assignment
- S2. Understanding Protein Domains-Module II assignment
- S3. Understanding Protein Domains-Module III assignment
- S4. Understanding Protein Domains-Module IV assignment

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Table 1. Understanding Protein Domains: Teaching Timeline. Organization of the Understanding Protein Domains Modules and related activities through a 15-week semester. **JC** indicates a journal club-style discussion and follow-up assignment that introduced students to the use of epitope tags. **Wet-Lab** indicates a lab experiment that paralleled the part of the work by Mao et al. (15) that students were asked to interpret as part of Module IV.

Week	Materials Provided	In Lab Activity	Assignment Due
1			
2			
3	Overview and Module I Background and Assignment		
4		Module I: Optional Discussion	
5			
6	Module II Background and Assignment		Module I
7	JC Overview and Paper	Module II: Optional Discussion	
8		JC Discussion (Assignment Provided After Discussion)	
9			Module II
10	Module III Assignment		JC
11	Lab Background and Protocol; Module IV Assignment	Module III: Optional Discussion	
12		Wet-Lab; Module IV: Optional Discussion	
13			Module III
14			Lab Notebook
15		Assessment	Module IV

Table 2. Understanding Protein Domains: Self reported confidence gains in understanding of and ability to study protein domains and interactions. Students in the Fall 2015 and Spring 2016 cohorts completed anonymous evaluations in the last week of class. For evaluation purposes, numerical values were assigned to confidence levels with “strongly agree” =5 and “strongly disagree”= 1.

The Understanding Protein Domains course component strengthened...	Strongly agree	Agree	Neutral	Disagree	Strongly disagree	% strongly agree/ agree	Mean
...my understanding that protein domains have separable structure & function.	10	26	6	2	0	82	4.0
...my ability to compare sequences and draw conclusions from this analysis.	10	25	6	2	0	80	4.0
...my ability to examine protein structural models and draw conclusions from this analysis.	9	24	9	2	0	75	3.9
...my ability to interpret data on protein interactions.	10	19	12	2	0	67	3.9
...my ability to propose experiments to test protein interactions.	7	18	17	2	0	57	3.7