Special Section: Innovative Laboratory Exercises—Focus on Laboratory Notebooks and Bioinformatics

An Inquiry-Based Biochemistry Laboratory Structure Emphasizing Competency in the Scientific Process: A Guided Approach with an Electronic Notebook Format

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Abstract

The laboratory setting is an exciting and gratifying place to teach because you can actively engage the students in the learning process through hands-on activities; it is a dynamic environment amenable to collaborative work, critical thinking, problem-solving and discovery. The guided inquiry-based approach described here guides the students through their laboratory work at a steady pace that encourages them to focus on quality observations, careful data collection and thought processes surrounding the chemistry involved. It motivates students to work in a collaborative manner with frequent opportunities for feedback, reflection, and modification of their ideas. Each laboratory activity has four stages to keep the students' efforts on track: pre-lab work, an in-lab discussion, in-lab work, and a post-lab assignment. Students are guided at each stage by an instructor created template that directs their learning while giving them the opportunity and flexibility to explore new information, ideas, and questions. These templates are easily transferred into an electronic journal (termed the E-notebook) and form the basic structural framework of the final lab reports the students submit electronically, via a learning management system. The guided-inquiry based approach presented here uses a single laboratory activity for undergraduate Introductory Biochemistry as an example. After implementation of this guided learning approach student surveys reported a higher level of course satisfaction and there was a statistically significant improvement in the quality of the student work. Therefore we firmly believe the described format to be highly effective in promoting student learning and engagement. © 2013 by The International Union of Biochemistry and Molecular Biology, 42(1):58–67, 2014.

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Overview of the Guided Inquiry-Based Approach

The teaching science laboratories continue to provide a central and distinctive environment for teaching and learning science. In order to train a new wave of skilled scientists who can keep pace with recent advances in science and technology it is crucial that the laboratory setting encourages creative thinking and problem-solving skills that mimic real life challenges. One pedagogical approach often used to foster student curiosity and increase student engagement is to give more decision-making authority to the students. However, while there is a consensus that students need to perform activities that are more student-centered, and investigative in their approach [1–9], achieving the desired learning outcome is complicated by the variable prior experiences and skills of the students. Students who have only been introduced to a traditional style of prescribed laboratory instruction are often found to follow the "recipe" in the lab manual carefully, but then do not necessarily engage in the scientific concepts or intended thought processes [6, 7]. It has been our

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experience that introducing a student-centered, inquiry based approach without a guided transition often overwhelms the student. As others have noted [7, 8], there is a recognized need to provide students with a support system that affords frequent opportunities for feedback, reflection, and modification of their ideas.

To address these concerns, we describe a transitional laboratory structure that uses instructor created templates to ensure that the desired learning outcome is achieved. Thought provoking questions are incorporated into the templates to focus the students' learning efforts and to ensure students engage in scientific inquiry throughout the process. For each laboratory activity, students develop a routine of completing four guided stages: a pre-lab assignment, an in-lab discussion, in-lab work, and a post-lab assignment (Table 1). At every stage of the week's laboratory activity, templates serve as the basis for our guided inquiry-based approach and prompt students to make entries and document their work as it progresses.

Since Introductory Biochemistry is an upper level undergraduate course it is an excellent candidate to implement this guided strategy; students have acquired foundational laboratory skills by completing courses in introductory and organic chemistry courses and are well positioned to gain competency in the entire scientific process and become engaged learners. The semester's progression of assignments (Table 2) gradually phases in a focus on student-centered learning and the expectations of a thorough lab report, so that the students are not overwhelmed. Templates are customized to reflect each lab's learning objectives and templates purposefully focus on gaining competence in one key skill in particular. For example, emphasis on the creation of effective figures with an accompanying descriptive legend is a skill that then assists in the effective interpretation of the data in a clear concise Results section. Therefore these are successive points of emphasis phased in over the course of two laboratory activities (Table 2).

Documenting Work Using the E-Notebook

The instructor created templates have yet another purpose; they establish a framework for students to easily document their lab work using an electronic format termed the "E-notebook" (Table 3). Each student journals their laboratory experience by using a distinctive font to add their own notes into the electronically provided templates. E-notebook content retains the same elements of the traditional lab report since the student's annotations transform the Pre-Lab template into an Introduction section, the In-Lab work into an Experimental section and the Post-Lab work into the Results and Discussion sections. The E-notebook is submitted twice for each weekly laboratory activity. The first submission occurs before their departure

TABLE 1 ***A guided inquiry-based approach for the biochemistry laboratory***

Format and pedagogical goals of the four-stages
Stage 1. Pre-lab Work: Students complete preparative work that enables them to • Create a basis for inquiry • Establish experimental goals and form hypotheses • Formulate questions about the upcoming lab Pedagogical Benefit: Students are afforded the opportunity to address any immediate background gaps and the stage is set for a student-led discussion at the beginning of lab.
Stage 2. In-lab Discussion: The instructor gauges the level of the students' understanding by prompting students to: • Share their hypotheses, ideas, and questions • Peer review the exercises completed in the pre-lab stage • Engage in student centered brainstorming sessions Pedagogical Benefit: Student led discussions form the basis of content coverage and ensures student engagement. The feedback received from peers and the instructor aims to level the knowledge base.
Stage 3. In-lab Work: The procedure is supplemented with thought-provoking questions and extra exercises in order to: • Promote student-centered learning and collaboration • Establish a pace that allows ideas and connections to evolve as the experiment proceeds. • Ensure the desired learning outcome is achieved. Pedagogical Benefit: Students benefit from an instructor and peer based support system and gain confidence in their ability to complete the final post-lab assignment after their departure from lab.
Stage 4. Post-lab Assignment: Students reflect on their completed work and must demonstrate an in-depth understanding of their work by: • Finalizing their data analyses • Creating publication grade figures and/or data summary tables • Assimilating information from other scientific sources • Devising explanations and arriving at well-founded conclusions Pedagogical Benefit: This stage specifically aims to develop students' metacognitive skills that raise their awareness about their own learning and thinking process.

TABLE 2 *The biochemistry laboratory sequence*

Week	*Laboratory activity*	*Skill focus*[a]
1	Properties of Buffers: A review of Weak Acid-Base Chemistry	Establishing a routine of inquiry and documentation
2	Examining Properties of Proteins using Swiss-pdb viewer, Absorbance and Fluorescence	Creation of effective figures with a descriptive legend
3	Protein stability of α-Lactalbumin: A Comparison of Apo and Holo forms using Flourimetry and Circular Dichroism	Thorough Interpretation of the collected data
4	Using Physical Properties of Proteins to Design a Protein Purification Strategy	Assimilating Information to create a sound experimental pan
5	Purification of Lysozyme from Hen Egg white using Ion Exchange Chromatography	Execution of the students' own experimental plan
6	Assessing Purity and Yield using Enzyme Assays and SDS-PAGE	Critical evaluation of the experiment and resulting data
7	Enzyme Kinetics	Spiral learning, conceptualizing lecture material by hands-on experimentation
8	A Binding Study using Isothermal Titration Calorimetry	Use of group work and discussions to form well-founded conclusions
9	An Introduction to Carbohydrate Structure: Understanding Binding site Interactions of a Glycosidase	Talking time for depth learning with extension exercises
10	MALDI-TOF Mass Spectrometry: Analysis of a Glycoprotein and its Glycan chains and Use of Peptide Mapping to Identify an Unknown Protein	Use of bioinformatic tools on the web
11	Separation of Nucleotides by High Performance Liquid Chromatography	Application of the full complement of lab skills emphasized in the earlier labs
12	Presentation Topic Researched and Prepared Complete The Notebook Assessment	Assimilating information from other sources and understanding their relevance
13	Presentations: A Metabolic Disease and the Search for a Cure. A Look at the Biochemical Methods used to Perform Leading Edge Research	Successfully conveying knowledge gained to an audience of peers

[a]As the semester progresses students are expected to apply the full complement of lab skills emphasized in the earlier labs.

from the laboratory in order to gauge the student's level of engagement and comprehension during lab. No formal grade is awarded at this point since the primary purpose is to offer individualized counseling on a noted deficiency (e.g. sparsely annotated templates are symptoms of passivity, conceptual struggles or both). The second submission of the E-notebook adds in a thorough reflection on their work and the content within their Post-lab assignment is graded (a total of 10 Post-lab assignments account for 75% of their lab grade). A lab notebook assessment (fully described in Table 3) judges the student's note-taking skills and conceptual understanding of the entire semester's work (15% of their final lab grade). A peer review of the notebook and a mock mini-assessment midway through the semester aims to instill good practices and prepare students for this cumulative assessment. As a final assignment, students are exposed to a real-world application of biochemical methods when they focus on a metabolic disease and the research strategy that aims to find a cure (the presentation constitutes10% of their final lab grade).

The weekly routine of down-loading the week's laboratory activity, submitting E-notebook content and returning

TABLE 3 ***The E-notebook guidelines provided to students***

Notebook organization:
Begin by creating a subdirectory for your E-notebook and then add a separate folder for each weekly laboratory activity (as shown in the example to the right). These folders should be chronologically ordered and contain all the documents pertaining to that week's laboratory activity. As a registrant of this course you have been added as a participant to a Sakai Lab Conference [10]. The electronically provided lab manual is located under The Resources Tab. The 12 listed laboratory activities have instructions and templates that will provide the framework for all your laboratory work. Your notations should be embedded in the provided lab templates using a distinctive font to distinguish your entries.
Your notebook is an organized record of all your work so for each week include:

- Stage 1 Pre-lab Work: a template will direct you to required reading assignments and exercises that will familiarize you with the concepts needed to complete your laboratory activity; this completed work will also serve as the Introduction/Background sections of your lab report.
- Stage 2 The In-lab Discussion: this template provides an overview of the laboratory activity and will require you to insert your own notes as the discussion proceeds during lab.
- Stage 3 The In-lab Work:
  a. The Experimental Section: this template provides the procedure that you must customize with your notes, observations, data, procedural modifications, and other specifics, and also adding in a record of collaborative work, data analyses, calculations and t relevant tables or plots.
  b. A Check on Concepts: these exercises are embedded within the procedure to gauge your level of understanding; be sure to seek support through peer discussions and instructor feedback; this work will constitute the first draft of your lab report.
- Stage 4 The Post-lab assignment: this template prompts you to complete a thorough analysis of your work and allows you to finalize the lab report begun earlier during lab; this will serve as the Results and Discussion sections of your E-notebook.

You will make two E-submissions of your compiled work using the Assignment tab within the Lab Conference:

- The first submission occurs prior to leaving the laboratory and will document your lab work through the first three stages. This submission is not formally graded but serves to demonstrate active engagement and proper note taking skills.
- The second submission consists of your completed Stage 4 Post-lab assignment; this graded assignment is due after your lab reconvenes the following week to allow time for a short discussion of the work.

Assessment of the E-notebook:
At the end of the semester you will be asked to complete a written assessment that judges your note-taking skills and conceptual understanding of your semester's work (this will constitute 15% of your lab grade).
The format involves short answer, True/False, or multiple choice questions and will require you to:

- Retrieve information stored in your E-notebook (no other sources can be consulted).
- Work within a 2-hr time-limit for completion (in order to evaluate organizational skills).
- Answer a total of 40 questions that are categorized by week and chronologically ordered (there are approximately three to four questions per weekly activity).

Examples of the type of information you may be asked to retrieve include:

- A recorded result and its interpretation
- Features of a studied biochemical system
- An observation made during an experiment
- The details of a procedural step (final concentration of a dilution, instrument setting, etc.)

You will also be asked to demonstrate higher-order learning skills practiced in the laboratory such as:

- The ability to report the importance of a procedural step (to show understanding of a method).
- The Interpretation of a graph or figure generated from your data.
- The ability to quickly repeat a calculation (showing the steps involved and units).

TABLE 4 *Example of a pre-lab template*

Title: Isothermal titration calorimetry—A study of enzyme-substrate binding

Goal and approach:

1. Define the objective of this week's laboratory:
2. Define how you will try to achieve your experimental goal
   a. Biochemical System(s) being studied:
   b. Technique(s) utilized:
   c. Underlying physical or chemical principles being applied:

Lab Specific Exercise:

1. Become familiar with the Isothermal Titration Calorimetry method at the following website: www.microcalorimetry.com/tecnology/itc.asp; click on the menu option: View animation of a typical ITC experiment [14].
2. Become familiar with lysozyme at the Proteopedia website: www.proteopedia.org/wiki/index.php/Main_Page; use the text box to search for lysozyme and explore the structures within this site [15].
3. Identify the H-bonding Residues in the Binding Site: The NAG_3 is a polysaccharide trimer, similar to the natural lysozyme substrate but with only three sugar residues. Use the Protein Data Bank website www.rcsb.org [16] to locate the pdb file 1HEW [13] ; this pdb file shows the NAG_3 co-crystallized with lysozyme at subsites A, B, and C (each subsite is a region binding with one sugar residue). Next, use SWISS-pdb viewer [17] (available from http://spdbv.vital-it.ch/) to create a figure showing the H-bonding interactions between NAG_3 and Lysozyme (directions are provided in supplemental materials). Be sure to rotate the protein to clearly view H-bonds and distances and use the centering icon on the Toolbar to maximize the figure. Finally summarize the information in the data table below:

Amino acid	Subsite location (indicate as A, B or C)	Bonding group (indicate if the H-bond is emanating from the side chain or backbone)	H-bond distance (Angstroms)
Asn 59			
Trp62			
Trp 63			
Asp 101			
Asn 103			
Ala 107			

4. Make a hypothesis: Consider typical pK_a values for amino acids, then decide which H-bond between enzyme and substrate will be affected if the pH is dropped to a pH of 3 given that lysozyme is not denatured at this pH.

graded work is all accomplished using the Sakai online learning management system [10] (Blackboard [11] is a common alternative system). This lab-specific communication platform forms a strong learning community by promoting continuous instructor-student interactions and student collaborations. In fact, all exercises are carried out in teams of three to four students in order to foster peer mentoring and ignite peer discourse that begins in the laboratory and later continues on a Discussion forum within the lab's online site. While the ease of sharing material is advantageous for the creation of a collaborative and supportive learning community, it may also place academic integrity at a greater risk. However, E-submissions can be monitored using academic integrity software (e.g. The Originality Check feature offered by Turnitin [12]) in addition to the traditional reliance on Honor Code and instructor vigilance.

The Inquiry-based Approach: A Specific Example of Implementation

To showcase our guided inquiry-based approach and describe the four stages of this strategy, an isothermal titration calorimetry (ITC) experiment examining the thermodynamics of lysozyme and tri-(*N*-acetylglucosamine) binding is used [13]. This laboratory activity is designed for a 3½-hour period and occurs in the latter half of a 13-week laboratory series after students already have completed a complete sequence of labs centered on the protein

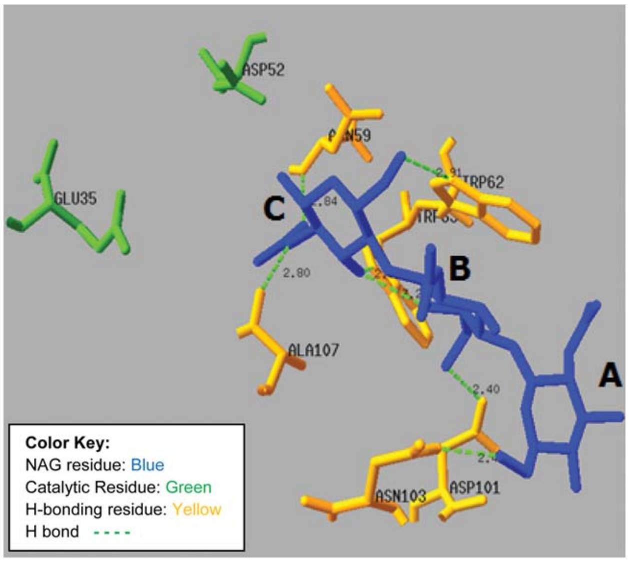

FIG 1 *Interaction of Hen Egg White Lysozyme with NAG_3. Swiss-pdb viewer was used to examine the binding site interactions of lysozyme and tri-N-acetyl-glucosamine (NAG_3). The amino acid residues that participate in the binding of the substrate are shown in yellow: Asn59, Trp62, Trp63, and Ala107 form hydrogen bonds with subsite C; while Asp101 forms an H-bond with subsite B and Asn103 forms an hydrogen bond with subsite A. The two catalytic residues of lysozyme, Glu35 and Asp52, are distant from the NAG_3 and do not cleave this competitive inhibitor.*

structure, stability, and function (see Table 2). The ITC lab activity requires students to apply knowledge gained from earlier labs, execute their own experimental plan, and fully engage in the scientific methods of investigation.

Stage 1: Pre-lab Work: Students Complete Preparative Work in Order to Create a Basis for Inquiry

This component aims to prepare students for the concepts, methods, and calculations needed to execute a successful biochemical experiment. A standard worksheet supplemented with lab specific questions is used every week to help students outline the key concepts that serve as the basis for inquiry in the laboratory. The specific pre-lab worksheet for the ITC Laboratory activity is given in Table 4. Information compiled within this preparative work is similar to the "Introduction/ Background" section of a typical lab report and therefore serves this role within the E-notebook.

From this particular pre-lab work students gain an understanding of the Isothermal Titration Calorimetry method using MicroCal's ITC website [14]. Furthermore students gain an understanding of the Enzyme–Substrate System using several online tools. Students focus on the lysozyme's binding site within the Proteopedia website [15] and become familiar with the polysaccharide trimer, NAG_3, a competitive inhibitor that resembles the substrate. Students then import the 1HEW.pdb file [16, 17] into Swiss-pdb viewer [18] and complete an exercise (described in the Supporting Information) that identifies the residues H-bonding with the three NAG residues of subsites A, B, and C (Fig. 1). Students consistently utilize SWISS-pdb viewer as a tool to model the biochemical system featured in their laboratory activity. Creation of such figures teaches the students about key structural aspects of the biochemical system they will study and also provides much needed practice in writing a descriptive legend to accompany the figure. Figure 1 is an example of student work and shows the level of competency achieved. By the latter half the semester students are capable of submitting high quality figures complete with a clear and concise figure legend.

Stage 2. In-lab Discussions: Students Establish Experimental Goals, Pose Questions, and form Hypotheses

Using their completed preparative work, the students work in small groups to establish the questions addressed by the ITC experiment and share their hypotheses formulated earlier in the pre-lab work. Students are asked to examine a typical binding isotherm provided by the instructor and their SWISS-pdb figures to formulate answers to several key bullet points stated in Table 5. Typical initial student answers are added in a bolded font while follow-up details, added after the instructor-led discussion, are included in the right-hand column. This in-lab discussion worksheet has one important goal, to guide the student through a productive student centered brainstorming session. These notes are easily transferred into a Notes section and the E-notebook gains valuable content that is useful for the comprehension and analysis of the student's own experimental work.

Stage 3 In-Lab Work: Active Engagement in the Experimental Process

Earlier student-centered steps have created a solid foundation for learning and the students are now ready to prepare reagents and enable the ITC instrument to perform their experiment using the following parameters: precise 10 μL volumes of NAG_3 (2.66 mM) are titrated into the sample cell containing 0.14 mM lysozyme at 25 °C [13]. The experiment is repeated at two different pH levels: pH 3 and pH 5 by different laboratory groups. All raw data are added to a single summary table that already contains several data entries from earlier semesters so that students have a preview of the outcome. The preview of existing data enables the students to begin collaborative data interpretation and productively use the 2-hour wait time required for the generation of their own binding isotherms. Small group work performed in rotations permits time for such student-centered learning exercises to be incorporated into all of our laboratory activities. Data collection occurs in teams of two to four students to maximize the hands-on experience

TABLE 5 *Brainstorming session summary*

Points to consider	*Follow-up details and notes from lab discussion*
Q: Is the reaction endothermic or exothermic? A: **The reaction is exothermic since negative ΔH values are indicated in the binding isotherm**	The maximal heat released during the first injections indicate the ΔH of the reaction is negative; later as the enzyme saturates ΔH of subsequent injections approach zero as saturation is reached.
Q: What is the expected binding stoichiometry? A: **Lysozyme has one binding site for the NAG_3**	One NAG_3 co-crystallizes with one lysozyme molecule in the binding site. This is in agreement with the binding curve that has a mid-point molar ratio of one.
Q: What is the molecular basis of the Enzyme-Substrate interactions? A: **Six H-bonds are formed between substrate and enzyme.**	Since catalysis and product formation is the ultimate goal, the binding interactions between enzyme and substrate are meant to be temporary. Therefore weak, non-covalent interactions like H-bonds often contribute to E-S binding.
Q. Which H-bond between enzyme and substrate will be affected if the pH is dropped to a pH of 3? A: **The H-bond of Asp 101 (pK_a of ~4) will be affected**	Only, the H-bond at subsite B will be affected. The side chain of Asp 101 (pK_a of ~4) is susceptible to protonation when the pH is lowered to 3. Such protonation could weaken or disrupt the H-bond.
Q: How will the binding curve be affected by a change in binding affinity? A: **The slope of the binding curve gives the binding affinity; a steeper slope indicates a greater affinity.**	If an H-bond is weakened or disrupted, the binding affinity will decrease; so relative to the optimal pH of 5, the pH 3 binding isotherm will rise to saturation with a less steep slope.
Q: The pH 3 condition is extreme and structural changes are a concern that will complicate our interpretation of the data. What techniques studied earlier in lab can quickly ascertain if structural changes are occurring? A. **Fluorescence was used to monitor structure and stability in an earlier lab so it can be used again here.**	The binding site has two Trp residues and fluorescence will be quite sensitive to a structural change within the binding site. Such an experiment will be designed and carried out during lab; the goal is to provide data that can rule out a conformational change as the reason for a change in binding affinity.

at an instrument, while the remaining students engage in discussions provoked by the template.

For the ITC lab activity one student-centered learning rotation is dedicated to the execution of a student-designed experiment. From earlier discussions, the students realize they must rule out the involvement of a pH-induced conformational change. Students work collaboratively in groups of two to three students and apply knowledge gained in earlier labs to design and then perform a successful fluorimetry experiment. Within an hour the students are able to generate the overlaid fluorescence spectra of lysozyme at pH 3 and pH 5 and thereby rule out a conformational change as the reason for a change in binding affinity. Pedagogically there are many benefits from this student-led group exercise. The task at hand is manageable for the students and they gain confidence in their abilities to execute an experimental plan that yields meaningful results.

With any remaining time students are assigned the task of consulting primary literature and other sources to help them incorporate substantive established scientific knowledge into their assignment. Students are given guidelines on how to conduct an effective literature search and then collaboratively create a list of key words that will yield useful journal articles. Furthermore, students create a plan that will coordinate their efforts to obtain relevant information and are directed to use a Discussion Forum within the Sakai learning management website [10] in order to share useful journal articles. This final in-lab session vastly improves the quality and focus of their literature searches and ensures that students do not waste any time on off-topic journal articles while writing up their final laboratory report.

Stage 4: The Post-lab Assignment: Students Interpret their Data and Observations and Gain Substantive Knowledge from the Primary Literature in Order to form Well-founded Conclusions

To ensure a positive learning outcome all post-lab assignments are due after the lab reconvenes the following week to allow time for a short discussion of the work. Such discussions give students the chance to recognize any deficiencies or misconceptions in their work and allow them to make the necessary adjustments. While many labs can be completed in just over a week's time, careful consideration must be given to the particular demands of a more comprehensive assignment. With a mindful appreciation for the time it takes a student to glean information from a scientific article, the culminating lab report detailed here is completed over the course of 2 weeks. For the first phase student groups must reconvene before the next lab period in order to summarize the key information from the literature. The following descriptive overview (Table 6) is the product of such a collaborative group This critical first step involved group discussions with ample feedback from the instructor; this process ensures a good understanding of the employed technique and the steps involved in the data analyses as well as the scientific significance of the experiment. At this point a solid foundation of learning has been established and a continuation of the learning process students uses a structured template to guide them through a focused reflection of the experiment. The post-lab worksheet (Table 7) outlines the expected content of their lab report, prompting students to summarize the experiment's goal, key results and key conclusions followed by a proposed future study with an emphasis on its significance. The completed worksheet is submitted to the lab's learning management site and becomes a regular entry into their

E-notebook. While the key headings provide for a consistent routine, the template is also customizable for each lab as it is supplemented with lab specific questions or prompts (italicized font). This component adds efficiency to a student's work since it replaces the traditional lengthy lab report that was often hand-written into a bound notebook and then re-typed for submission.

TABLE 6 ***Literature search summary***

An example of collaborative student work

HEW lysozyme binds to $(NAG)_3$ optimally at pH 5 and Asp101 within subsite B is one of six H-bonding residues within the active site [19, 20]. The H-bonding capabilities of Asp101 (a residue with a pK_a of 4.1) have an impact on binding affinity; this fact is supported by multiple studies:

a. Our own ITC study and earlier studies using a fluorimetric method [19, 20] both show that with the protonation of Asp101 at a low pH, HEWL binds more weakly with $(NAG)_3$; in our ITC study there is a fivefold reduction in binding affinity when the pH was lowered from the optimal pH of 5 to a pH of 3.
b. TEW lysozyme (D101G) has catalytic and thermodynamic properties similar to HEW lysozyme at pH 3; this similarity exists, because both enzymes lack hydrogen-bonding interactions of a negatively charged Asp101 side chain. HEW lysozyme at pH 3 performs like TEW lysozyme because the Asp101 side chain becomes protonated and loses its H-bond with NAG_3 at subsite B [19, 20]

Assessment and Impact of the Guided Approach

Over the years the most prominent complaint received in student surveys has been that the laboratory content was too rigorous and demanded too much time to complete. Since diluting the learning experience was not an option

TABLE 7 ***The Post lab Template for the Isothermal Titration Calorimetry Experiment***

Descriptive Title:

Goal: Define the objective of your study and the approach utilized:

Results:

a. Insert your Results: *Include one representative binding curve and then summarize the remaining data in a table. Comparisons to other studies should also appear in the data table. Accompany the figures with a descriptive legend.*
b. Interpret your results and state the key results in a bulleted format:
   1.
   2.
   3. . . .. *more as needed*
c. Compare them to an expected result (cite other studies). *As one example, be sure to compare the pH study conducted in lab to the NAG_3 binding in Turkey lysozyme because there are important similarities that assist with the interpretation of your data.*
d. Using Swisspdb viewer *show the details of the binding interactions between lysozyme and NAG_3. Add a descriptive legend to highlight the binding residues and binding interactions.*

Conclusions and Discussion:

a. State the key conclusions of the study in a bulleted format:
   1.
   2.
   3.. . .. *more as needed*
   Mention the residue(s) involved and how pH alters the binding interaction. Use other studies to support your reasoning.
b. How would you proceed to study your biochemical system further? Decide on an interesting future direction for the study. *Substantiate the significance of the proposed work by explaining the goal and anticipated outcome.*

Citations:

The notes in italics are deleted in the final submission.

from a pedagogical viewpoint, the described guided inquiry-based approach was developed to provide a more supportive in-lab experience and to encourage a more productive use of their committed time. The impact of this guided-inquiry laboratory structure was assessed both using a student survey and by comparing the quality of the students' most demanding assignment in the course (the post-lab assignment from the ITC activity described here). Data were gathered for six semesters before (n= 90 students in total) and after(n = 124 students in total) implementation. The same single laboratory instructor carried out the laboratory component of the course and the learning objectives and expectations were kept constant for all the semesters included in the data analyses. The mandatory survey tied student satisfaction to the level of support afforded by the learning environment. Since this guided learning approach has been adopted students reported a 30% increase in student satisfaction within the laboratory component of the course (87% satisfied up from 67% before implementation). Furthermore, the averaged grade on the ITC assignment increased from a mean of 83 (stdev of 1; n = 6 semesters prior) to 87 (stdev of 1; n = 6 semesters after); this is a significant increase in the students' level of performance at the 95% confidence interval using the student's t-test. Thus, the quality of their lab reports also improved and surveyed students now describe a "great lab atmosphere" and "a well-structured lab that allowed us to complete protocols fairly independently."

Summary

Undergraduate teaching laboratories have traditionally exposed students to experimentation to develop skills in specific scientific techniques and data interpretation. The current consensus favors placing the student in the center of the learning process in the laboratory [1–9] so that students develop the skills to tackle real world problems [21–25]. Our guided approach embraces this premise and incorporates thought provoking questions into every stage of the students' laboratory work. Furthermore, this structure also gives the students the opportunity to easily journal their evolving laboratory experience in an electronic format termed the "E-notebook." In the process, students discover that the best strategy for learning is to be curious about their work and to actively reflect on the meaning of their data and observations. Students are encouraged to propose multiple hypotheses, exchange ideas with their peers, and apply critical thinking skills. The peer discourse that ensues allows the instructor to gauge the level comprehension and also redirect any off-base discussions to ensure the intended learning outcome. A guided-inquiry approach has already been shown to definitively improve conceptual understanding in the laboratory setting [26–28]. Likewise we achieved a significant gain in the quality of student work and our students reported a greater level of satisfaction with the learning environment. Implementation of the described guided-inquiry approach allowed students to develop a better understanding of the scientific method and have a more positive laboratory experience.

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