

## Article

# Students' Understanding of Primary and Secondary Protein Structure: Drawing Secondary Protein Structure Reveals Student Understanding Better Than Simple Recognition of Structures

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## Abstract

The interdisciplinary nature of biochemistry courses requires students to use both chemistry and biology knowledge to understand biochemical concepts. Research that has focused on external representations in biochemistry has uncovered student difficulties in comprehending and interpreting external representations in addition to a fragmented understanding of fundamental biochemistry concepts. This project focuses on students' understanding of primary and secondary protein structure and drawings (representations) of hydrogen-bonding in alpha helices and beta sheets. Analysis demonstrated that students can recognize and identify

primary protein structure concepts when given a polypeptide. However, when asked to draw alpha helices and beta sheets and explain the role of hydrogen bonding their drawings students exhibited a fragmented understanding that lacked coherence. Faculty are encouraged to have students draw molecular level representations to make their mental models more explicit, complete, and coherent. This is in contrast to recognition and identification tasks, which do not adequately probe mental models and molecular level understanding. © 2013 by The International Union of Biochemistry and Molecular Biology, 41(6):369–376, 2013

**Keywords:** protein structure; alpha helices; beta sheets; hydrogen-bonding; undergraduate education

## Introduction

It is obvious that for some Biochemistry is a branch of Chemistry, for others it encompasses many other fields. As a discipline, Biochemistry may simply be chemical biology, but can also be physiological, medicinal, agricultural, pharmaceutical, and/or nutritional by specialty or physical, organic, and/or metabolic by interest. Thus, a Biochemistry major may be educated in a Chemistry, Biology, Life Science, BioScience, or Physiology Department. Biochemistry may exist as an independent department or, as more often the case, is linked up with Biophysics and/or Molecular

Biology and Cell Biology. Biochemistry as a child of two cultures, Biology and Chemistry, is well recognized ([1], p. 65).

Huang [1] highlights the integrative nature of the biochemistry discipline. As a “child” of both chemistry and biology, biochemistry poses interesting challenges for instructors because the content taught in biochemistry courses builds upon students' foundational chemistry and biology knowledge. Students who have a fragmented understanding of core biology and chemistry background concepts may, therefore, have difficulty learning new biochemistry concepts.

Using prior knowledge to understand new information is not a new learning theory. This follows the constructivist theory of learning, which posits that knowledge is created in the mind of the learner [2]. Through the processes of assimilation and accommodation, learners construct new knowledge [3]. During the assimilation process learners use what they already know to make sense of new incoming information. Since the new incoming information does not exactly match what the learner already knows, they will have to slightly modify their existing knowledge to

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accommodate the new knowledge. Applying the constructivist theory to biochemistry means that students' prior biology and chemistry knowledge needs to be considered in order for students to appropriately build new biochemistry knowledge.

The American Society for Biochemistry and Molecular Biology published a recommended curriculum for undergraduate biochemistry and molecular biology students that identifies the importance of teaching protein structure and function concepts [4]. Foundational topics covered during protein biochemistry are levels of protein organization and the intermolecular forces that stabilize them. Thus, students use their prior knowledge from general chemistry to consider how forces and interactions, such as hydrogen bonding, stabilize the different levels of protein structure. Previous research published in this *Journal* by Villafane and collaborators reported that students held incorrect ideas about protein alpha helix structure even after a semester of instruction [5].

This qualitative study extends Villafane's work by investigating students' recognition and identification of concepts related to primary and secondary protein structure and their representations of alpha helices and beta sheets. It is related to two studies recently reported in this *Journal* from our research group pertaining to student understanding of representations of protein structure and structure-function relationships [6, 7]. In this article, we focus on student understandings of primary and secondary protein structure.

To elucidate the difficulty students have in describing protein structure, organization, and stabilizing intermolecular forces, it is important to investigate how they are recognized, drawn, and described by students. Therefore, the guiding research questions of this study were:

- What concepts of primary structure do students recognize given a tripeptide?
- How do students represent and describe stabilizing forces such as hydrogen bonding in alpha helices and beta sheets?

## Methods

### Sampling and Participants

Maximum variation sampling methods were used to capture the central themes that cut across participants enrolled in four biochemistry courses in chemistry and biochemistry departments and one history course at a large Midwestern research institution [8]. The research participants were recruited during the fall semester from the courses by describing the study to the entire class, creating a pool of students who volunteered to be a part of the study, then randomly inviting students in the pool from each class to participate. The participants in the study

ranged from freshman to seniors including two freshmen students in the history course who were not enrolled in a biochemistry course. These students were novices in biochemistry compared to the other participants and served as a reference point in the study. The history students provided a check of the validity of the study, the degree to which the study measures what it intends to measure, student understanding of primary and secondary protein structure. Given that all the participants had some level of science instruction in high school, then one would predict that the History students, who were not enrolled in a chemistry or biochemistry course, should be able to correctly answer few of the questions in the study.

The 21 participating students are described in Table I. The Purdue University Institutional Review board approved this research project and written consent was obtained from all participants.

### Data Collection and Interview Structure

A LiveScribe Smartpen was used during each interview to collect the student's drawings and audio [9, 10]. The pen captures what is drawn and spoken during the interview by use of special paper that records the position of the pen and a microphone in the pen which records audio. The drawings and audio can be uploaded and saved into a unique computer file that can be replayed and analyzed. By recording the audio, digitizing the handwriting of the student, and automatically synching these data sources into one file researchers have the ability to capture and analyze student drawings and understanding of concepts in an innovative way. This device represents a novel data collection tool that has only been used for educational research in one prior study [10].

At the beginning of each interview, students were shown a tri-peptide (Ala-Gly-Cys) rendered as a skeleton diagram that had been previously drawn with the LiveScribe pen by the interviewer so that it would be recorded. Each student was asked several questions about the molecule.

1. What kind of molecule is this? How can you tell?
2. Can you circle the R-groups?
3. Can you name the amino acids? What are they?
4. What is the name of the bond that holds the amino acids together? Can you circle it?
5. Where is the N terminus? Where is the C terminus?

These questions helped the students to become more comfortable talking with the interviewer (MH). Simultaneously they supplied the researchers with data about concepts they could recognize and identify in the tri-peptide.

The students were then asked to describe the levels of protein organization followed by questions concerning the forces or interactions that helped stabilize alpha helices and beta sheets. Students drew alpha helices, beta strands, and beta sheets using the LiveScribe pen [9]. They also

TABLE 1

Course number and abbreviation, the number of participants, and a description of the course

Course number (abbreviation)	College: Ag = Agriculture, S = Science, LA = Liberal arts	Number of participants	Name and student population
Chemistry 333 (CHM 333)	S	2	"Principles of biochemistry" is designed for health science majors.
Chemistry 533 (CHM 533)	S	4	"Introductory Biochemistry" is designed for students majoring in chemistry.
Biochemistry 100 (BCHM100)	Ag	4	"Introduction to Biochemistry" is designed for biochemistry majors.
Biochemistry 307 (BCHM 307)	Ag	9	"Introduction to Biochemistry" is designed for students in biological science, medicine, nursing, veterinary medicine, dietetics, food sciences, animal science, botany, and nutrition,
History 151 (HIST 151)	LA	2	"American history to 1877" is designed for liberal arts majors

illustrated their understanding of the interactions that help stabilize the alpha helices and beta sheets.

## Data Analysis

Every student in the study was given a pseudonym that preserved his or her sex, but allowed for the student's identity to be confidential. All the interviews were transcribed and coded to determine what features students recognized in terms of protein structure—peptide bonds, names of amino acids, R-groups, etc.—and how students represented alpha helices and beta sheets [8]. Analysis of codes allowed us to describe concepts the students could recognize given a tripeptide and the coherence of their mental models through drawings and descriptions.

## Results and Discussion

### Recognition of Primary Protein Structure Concepts

In the first part of the interview students were asked to recognize and identify concepts associated with primary structure given a tri-peptide. Remembering factual or conceptual knowledge are low-level cognitive tasks in terms of Bloom's taxonomy [11]. Table II records student responses by course and number of students in response to each question or task. The numbers in parentheses in each column header indicates the number of students from each class. The number in each cell indicates the number of students from that particular class who answered the question or completed the task correctly. The quotes are representative responses that illustrate concepts—how students described a particular structure or phenomenon.

The history students were unable to correctly answer nearly all of the questions that required them to recognize and identify features of primary structure. Given that they were novices in the field of biochemistry and had only their instructional background from high school as resources, this result was anticipated. This outcome supports the validity of the research in that it would be expected for a novice to be unable to answer most, if not all, of the questions about protein structure.

All students in biochemistry and chemistry courses except one recognized the AGC molecular structure as a peptide. They way in which they recognized this structure involved using repeating motifs as Bill did in BCHM 100 "N, C, C, double bond O," or simply stating that they recognized amino acids as students in CHM 533 and BCHM 207 did, or by locating the N and C termini as Cara did. Seven students could correctly name all three amino acids making up the peptide. Beyond not being able to correctly identify the amino acids, the most common mistake was confusing methionine for cysteine.

Fourteen students correctly recalled that the bond between amino acids is termed a peptide bond, and within that set one student also stated that it was an amide bond. Al and Alison in BCHM 307 stated that it was an "ester linkage" confusing the structure of amide and ester functional groups where N would be replaced with O. Ken in CHM 533 identified the bond as a "glycosidic linkage" and three students did not know the name of the bond. However, 17 out of 21 students when asked to locate the peptide bonds in AGC could circle them including two students in BCHM 100, three students in BCHM 307, and one student in CHM 533 who could not correctly name the bond.



TABLE II

Student responses by course and number of students in response to questions or tasks about primary protein structure given the tripeptide AGC

Question or task	BCHM 100 (n = 4)	BCHM 307 (n = 9)	CHM 333 (n = 2)	CHM 533 (n = 4)	HIST 121 (n = 2)
Correctly recognize structure as a peptide.	4	9	1	4	0
How do you know it is a peptide?	"N, C, C, double bond O" (Bill, Bethany, and Brad)	"peptide bonds" (Abe), recognition of amino acids (7 students)	"Amino on one end, carboxyl on the other" (Cara)	Recognized amino acids (4 students)	–
Locate R groups	3	9	2	4	0
Name amino acids (AGC)	1 – AGC; 2 – AG; 1 – C	3 – AGC; 2 – AGM; 1 – AC; 1 – each M, A, and none	1 – AGC; 1 – none	2 – AGC; 1 – CG; 1 – M	2 – None
Name of bond that hold amino acids together	2 – peptide; 1 – hydrogen bond; 1 – no idea	6 – peptide; 2 – ester linkage; 1 – no idea	2 – peptide	2 – peptide; 1 – peptide; & amide; 1 – glycosidic	1 – peptide; 1 – no idea
Locate peptide bonds	2	9	2	4	0
Locate N and C termini	4	9	2	4	1

Thus, students could recognize and identify where the amino acids were bound even if they could not correctly name the type of bond. All students in biochemistry and chemistry courses could locate and identify the N and C termini.

When given a tripeptide chemistry and biochemistry students in this study were facile at recognizing features of primary structure and were able to correctly recall names of amino acids and linkages. It is important to note that recognition and identification are low-level cognitive tasks. These recall tasks do not probe the student's own mental model of proteins and protein structure.

### Student Representations and Understandings of Alpha and Beta Sheets

Table III highlights participants' responses to the interview probes concerning the interactions that stabilize secondary structures: "Can you tell me about the interactions that help the primary structure fold into the secondary structure" and "Can you draw a short piece of alpha helix or beta sheet and discuss how it is held together?" These tasks were higher-level cognitive tasks requiring students to draw their mental models of secondary structure and to describe the interrelationships between concepts that describe the structure. This student-generated representa-

tion and analysis is a very different task than recognition and identification from a given structure.

The interviewer (MH) did not suggest or use the words "hydrogen-bonding" as part of any interview prompt or follow-up probes. One student did not mention hydrogen-bonding and made no drawings at all (Hank in HIST 151, a novice). Another student in CHM 533 (Ken) did not mention hydrogen-bonding nor draw hydrogen bonds in his secondary structures. These two students will not be discussed in the analysis that follows.

Sixteen of the students used the phrase "hydrogen-bonding" to describe the interactions which would "help" the protein fold and stabilize its secondary structure. Two students included other types of interactions in their explanations. Christine in CHM 333 talked about hydrophobic and hydrophilic forces with hydrogen bonding while Hannah (HIST 151) described the same forces as Christine and also included ionic bonding. Ashlee in BCHM 307 could not recall the name of the interaction. The students' responses indicate that they do not have difficulty recalling that hydrogen bonds are important for stabilizing secondary structures. However, when asked to draw the two forms of secondary structure (alpha helix and beta sheet) it became apparent that students have a fragmented understanding of where hydrogen bonds are located and what atoms are involved.

TABLE III

*Students' responses to interview probe concerning the interactions involved stabilization of secondary structures*

<i>Course (n = number of students)</i>	<i>Number of students using the phrase "Hydrogen bonding"</i>	<i>Number of students who include another type of force with H-bonding</i>	<i>No mention of H-bonding</i>
BCHM 100 (n = 4)	4	0	0
BCHM 307	8	0	1
CHM 333	1	1	0
CHM 533	3	0	1
HIST	0	1	1

Students were asked to draw a portion of an alpha helix that included the hydrogen bonds they discussed as important stabilizing forces. Student responses were analyzed and sorted into four categories based on their representation of hydrogen-bonding as shown in Table III. Analysis of their drawings revealed that students have a fragmented understanding with difficulties ranging from where hydrogen bonds are located in an alpha helix to the atoms that are involved in hydrogen-bonding (Table IV).

Amanda-BHCM 307, Hannah-HIST 151, and Allison-BCHM 307 could all state that hydrogen bonds helped to stabilize alpha helices (as can be seen in Table III), but they were not able to draw an example of a hydrogen bond or where they were located. Two participants, Abe-CHM 307 and Kate-CHM 533, were able to draw a hydrogen bond in the alpha helix but their drawings revealed that they considered the hydrogen bonds to protrude out from the alpha helix. Eleven out of 19 participants drew hydrogen bonds running in a vertical position in an alpha helix that indicates correct placement, but the participants were not able to identify the atoms that were involved in the hydrogen bond. Three participants, Amber-BCHM 307, Ben-BHCM 100, and Kyle-CHM 533 were able to not only draw the correct location of the hydrogen bonds in an alpha helix but also indicate the atoms involved.

In addition to having a fragmented understanding of hydrogen-bonding in alpha helices, students had similar difficulty drawing hydrogen bonds in beta sheets. Table V shows the analysis of drawings for hydrogen bonds stabilizing beta sheets. Even though participants verbalized the importance of hydrogen-bonding in stabilizing beta sheets (Table III), their drawings indicate fragmentation in their understanding and incomplete mental models.

Three participants, Hannah-HIST 151, Angie-BCHM 307, and Brad-BCHM 100, were not able to draw the hydrogen bonds they spoke of earlier. They knew the terms, but could not translate their utterances into coherent, complete drawings. Their drawings reveal that they did not understand that hydrogen bonds stabilize two beta strands and not one.

However, Amber-BCHM 307, Allison-BCHM 307, Amanda-BCHM 307, and Kate-CHM 533 knew that hydrogen bonds stabilize two beta strands (as indicated by their verbal responses), but were not able to draw the hydrogen bonds that they discussed. These same participants focused their discussion of their drawings on the fact that the beta sheets could be parallel or anti-parallel, but were not able to expand on their understanding of hydrogen-bonding.

While seven of 19 participants were able to indicate hydrogen-bonding between two beta strands, they were not able to identify the atoms involved. Additionally, two students, Bethany-BCHM 100 and Abe-BCHM 307, were slightly different from the others in this category and deserve further explanation. Even though Bethany-BCHM 100 did not include two beta strands in her drawing, it was decided to include her in this category because her representation included hydrogen-bonding which differentiated her representation from those who only drew one strand and no hydrogen bonds (Hannah-HIST 151, Angie-BCHM 307, and Brad-BCHM 100). Abe-BCHM 307 on the other hand did include two beta strands in his drawing, but a closer inspection reveals that he believes that hydrogen bonds connect amino acids (indicated by "A" in his representation) between adjacent beta strands.

There were three participants, Ben-BCHM 100, Abbie-BCHM 307, and Kyle-CHM 533, who correctly drew hydrogen bonds between two beta strands and included the correct atoms involved. These students were able to verbalize and represent their understanding of the interaction of the beta strands to form sheets.

## Conclusions

Student understanding of primary and secondary protein structure is fundamental to understanding biochemistry [4]. Students in this study easily recognized and identified features of primary structure from a tripeptide. However, when students were asked to create representations of secondary protein structure and describe the interactions that stabilize this structure the lack of coherence of their mental models

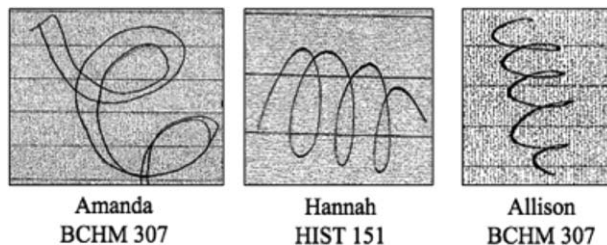
**TABLE IV**

*Students' drawings of the interactions stabilizing an alpha helix*

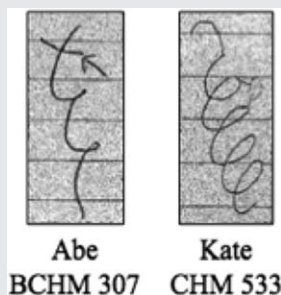
Categories and descriptions of drawings

Participants' drawing of the alpha helix

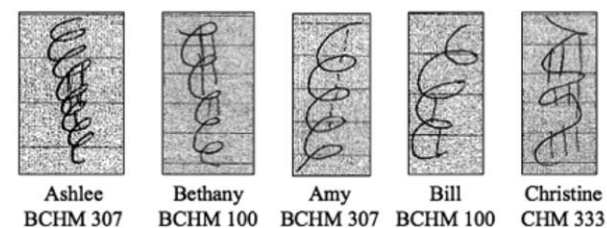
A. No hydrogen bonds shown



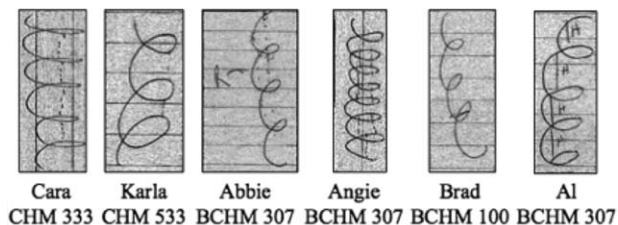
B. Hydrogen bonds are shown with incorrect placement



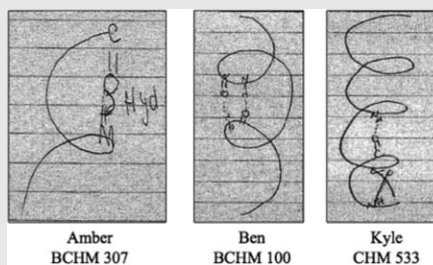
C. Hydrogen bonds shown with correct placement



D. Atoms involved in hydrogen bonding are not shown



E. Hydrogen bonds shown with correct placement and atoms involved are shown



and a fragmentation of their knowledge was revealed. These differences in response between asking students to recognize and locate versus illustrate their understanding via drawings, descriptions, and analysis has implications for facilitating student learning and assessment.

## Implications for Teaching and Research

There is an extensive research-based literature on the value and impact of student generated drawings [12–14].

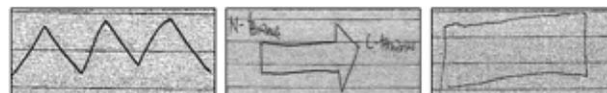
TABLE V

## Participants' drawings of the interactions stabilizing beta strands

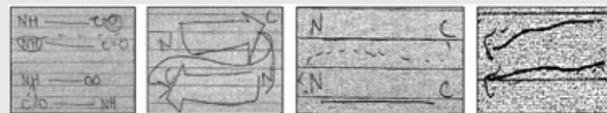
General description of drawings

Participants' drawing of beta strands

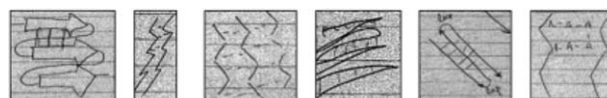
A. Drew one strand without hydrogen bonding interactions

Hannah  
HIST 151Angie  
BCHM 307Brad  
BCHM 100

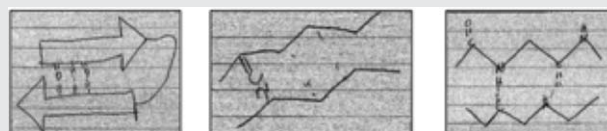
B. Drew two strands without hydrogen bonding interactions

Amber  
BCHM 307Amanda  
BCHM 307Allison  
BCHM 307Kate  
CHM 533

C. Drew two strands with hydrogen bonding interactions

Ashlee  
BCHM 307Bill  
BCHM 100Amy  
BCHM 307Christine  
CHM 333Al  
BCHM 307Abe  
BCHM 307Cara  
CHM 333Bethany  
BCHM 307Karla  
CHM 533

D. Drew two strands with hydrogen bonding interactions and atoms involved in hydrogen bonding are labeled

Ben  
BCHM 100Abbie  
BCHM 307Kyle  
CHM 533

For example, Leopold and Leutner [13] demonstrated that students who were directed to employ a drawing strategy when reading a chemistry text (specifically to draw at the molecular level), significantly outperformed students who were simply told to summarize the text on more complex tasks beyond simple recall. These findings are in accord with prior research on student drawing that found drawing promotes improved performance on higher-level cognitive assessments [15]. The conclusion across this body of research is that drawing helps students to build mental models and supports generating inferences.

Drawing molecular level representations and being specific about the types of interactions taking place both through diagrams and explanations is a strategy that can enhance knowledge acquisition and integration, help students to build flexible mental models, and help students develop a deeper understanding of the material [12–17]. Although students may be able to recognize molecular features or recall them on a multiple choice test, drawing molecular level diagrams opens up vastly different avenues for classroom discussion and assessment *We wish to emphasize that recognizing a pre-drawn molecular struc-*

*ture on a test (or other assessment) is not the same task as asking students to draw structures.*

We encourage faculty to have students draw their understanding at the molecular level when reading text, when discussing molecules in class, and when completing homework, quizzes, and examinations. For example, having students individually draw molecular interactions, compare representations with a neighbor, then share drawings and explanations across the class (thus employing a “think-pair-share” format [18]) is an approach that encourages students to make their mental models explicit and to explain concepts their drawings encode or inferences they support. While engaged in discussion or argumentation students can be prompted to supply reasoning that appropriately supports their claims and connects such claims to data. During these exchanges students may begin to realize what is missing, fragmented, or questionable in their own models, representations, and understandings.

Biochemistry education research is a relatively young field at the interface of chemistry and biology. As researchers explore student understanding at the molecular level by asking them to make explicit their mental models through



drawing we believe that the LiveScribe Smartpen is a powerful and novel tool for collecting data that synchronizes student audio with drawings [7]. We strongly encourage other researchers to consider this device in designing research and collecting data.

The results of this study point to other research questions that might be explored. It has not been established whether retention of concepts about protein structure is impacted in classrooms where students are encouraged to make their mental models explicit through drawing. Although grades in a course or on a specific exam capture student understanding of a wide range of material, future researchers might wish to compare student ability to draw their understanding of protein structure versus achievement in a course or on a specific assessment.

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