EXPERT EXPLANATIONS OF PROTEIN-FOLDING AND DYNAMICS RESEARCH: IMPLICATIONS FOR BIOCHEMISTRY INSTRUCTION

by

Kathleen A. Jeffery

A Dissertation

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Doctor of Philosophy

Department of Chemistry

West Lafayette, Indiana

May 2019

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Trevor Anderson, Chair

Department of Chemistry

Dr. Chittaranjan Das

Department of Chemistry

Dr. Minjung Ryu

Department of Curriculum and Instruction, Department of Chemistry

Dr. David Eichinger

Department of Curriculum and Instruction, Department of Biology

Approved by:

Dr. Christine Hrycyna

Head of the Graduate Program

This work is dedicated to my past, current, and future teachers and students.

Thank you for enriching my life through your knowledge and vision.

ACKNOWLEDGMENTS

This dissertation is the result of the efforts of many people. First, I would like to thank my immediate advisors, Drs. Trevor Anderson and Nancy Pelaez, who have challenged me to become a better researcher and individual, and mentored me by sharing their experiences as professionals and individuals. I would also like to thank my committee members, Drs. Chittaranjan Das, Minjung Ryu, and David Eichinger, for their questions and feedback. Second, I want to thank Dr. Ryan Sweeder who invited me to take part in education research as an undergraduate, and so helped me find one of my life's passions. Without his initial and continual support, I would not be where I am today. Third, I would like to thank past and present members of the Visualization in Biochemistry Education (VIBE) research group, as well as the many members of the Division of Chemical Education at Purdue, for their research support, feedback, and most of all, friendship. I would especially like to thank my lab mate, Stefan Irby, who has suffered my presence for the past five years with good humor, navigating coursework and teaching with me, as well as helping me refine and improve my research ideas. I would also like to thank Elijah Roth – whom I taught with more semesters than he probably wanted – for partnering with me to teach physical chemistry (somehow making great memories along the way) and challenging me to become a better individual. Additionally, I must thank Stephanie Santos-Díaz for her friendship as well as for inspiring me with her passion, strength, and vulnerability. Additional thanks goes out to Drs. Christopher Randles, Franziska Lang, Ashton Hjerstedt, and Hannah Sturtevant, who supported me in many different ways, as well as everyone I managed to strong-arm into ridiculously long work days over the years. I would also like to especially thank my parents and family, as well as friends near and far, for putting up with me and cheering me on throughout my graduate school journey. I would not be as mentally or physically healthy without your love and support over the years. Finally, I would like to thank my research participants, including both research scientists and students, who made this research possible and taught me so much.

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ABSTRACT

Author: Jeffery, Kathleen, A. PhD
Institution: Purdue University
Degree Received: May 2019
Title: Expert Explanations of Protein-Folding and Dynamics Research: Implications for Biochemistry Instruction
Committee Chair: Trevor R. Anderson

Recent calls in education have emphasized the critical need for curricula in the sciences to support student development of the general and disciplinary-specific practices that are relevant to modern scientific research and careers, as well as foundational scientific knowledge that reflects recent advances. In this regard, the life sciences, including biochemistry, have been under pressure to develop curricula that reflect current research knowledge and practices, and that develop student competence in areas such as experimentation and visualization. In contrast to these calls, biochemistry textbooks, and instruction based on them, seldom discuss how disciplinary knowledge is combined with experimental work or other disciplinary resources to investigate and communicate about biochemical phenomena. This is of great concern given that graduates entering life science careers must be able to reason with relevant disciplinary knowledge, utilize experimental research methods, and navigate data representations in order to solve research problems. It is therefore crucial for biochemistry instruction to expose students to the ways in which expert scientists navigate and reason with disciplinary resources in cutting-edge scientific research on topics such as protein folding and dynamics, the focus of this project. Thus, this dissertation aims to fill a gap in our understanding of how expert research scientists explain protein-folding and dynamics research, and how that research knowledge can be used to inform the development of instructional materials in this crucially important area of biochemistry. To address this goal, we explore three overarching research questions: How can we model experts' explanations of their research related to protein folding and dynamics? (RQ1); How do experts use representations to explain their protein-folding and dynamics research? (RQ2); and How can we use expert research to inform the design and implementation of instructional materials aimed at developing biochemistry students' understanding of protein-folding and dynamics? (RQ3). To address these research questions, we first collected and analyzed interview data from four experts to explore the nature of their research explanations. This data was used to develop a model (i.e.

the MAtCH model) of how experts integrate theoretical knowledge with their research context, methods, and analogies when explaining how phenomena operate (RQ1). In doing so, we also established how the experts use and combine explanatory models depending on the phenomena discussed and their explanatory aims, as well as how they explain thermodynamic and kinetic concepts relevant to protein folding in ways that align with their experimental research methods. We then examined selected representations from the expert interviews to explore how experts use language and representations to create meaning when explaining their research (RQ2). In comparing these to representations from biochemistry textbooks, analysis of the data indicated that textbooks generally explain 'what is known' but seldom explain 'how it is known,' whereas the experts use a combination of language, multiple representations, and gestures to explain how experimental research methods can provide evidence for phenomena. From this analysis, suggestions were made regarding the design of instructional materials to support discussion of experimental research methods and student interpretation of representations in classroom activities. In the final study, these suggestions were used in combination with additional analysis of expert research to inform the development anticipated learning outcomes (ALOs) and the design of instructional materials aimed at developing biochemistry students' understanding of protein folding and dynamics (RQ3). The materials focus on the use of hydrogen-deuterium exchange mass spectrometry (HDX-MS) to study changes in protein structure due to denaturation and interactions with other molecules. The instructional materials were piloted in an undergraduate biochemistry course for the health sciences, and the nature of students' understandings were explored. Our findings suggest that research practice – including research context, experimental methods, and representations – influences reasoning and explanation, providing additional evidence of the importance of developing discursive literacy in science students. To that end, a major implication of this work is that student knowledge of experimentation and representation may be a critical component of developing functional scientific understanding. Each of the studies contained in this dissertation therefore suggests ways in which practitioners may use our findings to modify instruction and instructional materials so that they are more aligned with expert practices. In order to teach students how scientific research underpins factual knowledge in biochemistry, future research should continue to explore experts' use of disciplinary resources and ways of thinking in order to inform teaching and learning strategies and materials that can support the development of students' disciplinary literacy.

CHAPTER 1. INTRODUCTION & RATIONALE

1.1 Calls for Curricular Reform

Recent calls for science education reform at the university level have repeatedly encouraged a greater focus on the development of knowledge and skills relevant to modern scientific research and careers in medicine and industry. Many of these calls advocate for competencies that reflect recent advances in foundational scientific knowledge as well as general and disciplinary-specific practices in which scientists engage (e.g. Brewer & Smith, 2011; Tansey, Baird, et al., 2013; White, Benore, et al., 2013). Life science faculty, in particular, are under constant pressure to develop curricula that reflect a rapidly changing knowledge base (Brewe, Pelaez & Cooke, 2013). For biochemistry and the molecular life sciences, important competencies include knowledge and use of advanced experimental techniques, modeling and analysis of complex systems, application of quantitative and qualitative reasoning in problem-solving, and evaluation of evidence and claims (AAMC-HHMI, 2009; Brewer & Smith, 2011). Furthermore, due to the abstract and invisible nature of the processes at the center of most life science research, practitioners use an extensive variety of experimental tools and techniques, external representations, and activities to conduct and communicate their work. For example, practitioners engage in spoken and written communication, they construct models and analogies to reason during problem solving, and they create and interpret data sets, images, graphs, and outputs of simulative environments (e.g. Kozma & Russell, 1997; Ochs, Gonzales & Jacoby, 1996; Nersessian, 2009). Given the importance of these processes to scientific investigation and understanding, supporting students' visual and discursive literacy is a critical objective of curricula for science majors (Airey & Linder, 2009; Krajcik & Sutherland, 2010; Metros & Woolsey, 2006).

Recent calls in educational reform also emphasize the foundational nature and increasingly close relationship of the physical and mathematical sciences with life science research and practice (AAMC-HHMI, 2009; NRC, 2003; Wright, Provost, et al., 2013). This is echoed at the primary and secondary levels where mathematics has been identified as a core scientific practice (NGSS, 2013). The Bio2010 report argues that, as "…the ways in which we think about and pursue research in biology are changing rapidly… modern biology is becoming more dependent on the physical sciences…," concluding "…that the best preparation for the biomedical research of the future is a

broadly based education with a strong foundation in the physical sciences and mathematics" (NRC, 2003; p. 10-11, 24). Brewe et al. (2013) highlight that many complex topics, such as random motion and microstate thermodynamics, are often taught in graduate-level courses despite their significance to introductory-level life science topics like bioenergetics and cellular activities. Consequently, significant opportunities for educational transformation exist at the intersection of the life and physical sciences, particularly in upper-level courses where the knowledge and skills that students are expected to demonstrate become increasingly sophisticated (Brewe et al., 2013).

1.2 Selection of Context: Student Difficulties with Thermodynamics and Kinetics in Biochemistry

In response to the above-mentioned calls, this dissertation focuses on explanations of protein-folding and dynamics research involving the application of thermodynamic and kinetic principles. This combination of context and content was chosen because the physical basis of interactions underlying structure-function relationships, the thermodynamics of macromolecular structure formation, and free energy, have all been identified as threshold concepts for biochemistry, without which a learner cannot progress to a deeper level of understanding (Loertscher et al., 2014). Moreover, numerous cutting-edge research projects pursued in the industrial, pharmaceutical, and medical fields require the application of biochemical knowledge and experimental methods involving physical processes governed by thermodynamics and kinetics. Research into neurodegenerative diseases and the development of protein drugs, for example, require an understanding of the fundamental physical processes that underlie protein folding and dynamics and the methods used to study them. Without sound knowledge of these principles and their application, students are not fully equipped to navigate the representations, experimental tools, and activities employed in the life sciences to investigate, model, and communicate about an abstract and invisible world (e.g. Airey & Linder, 2009).

1.2.1 Student Difficulties with Thermodynamics and Kinetics

The overarching conclusion suggested by research on student difficulties with thermodynamics and kinetics, is that students at all levels have difficulty understanding and applying foundational physical principles, with a concerning consistency in alternative conceptions and an inability to

transfer ideas to new contexts like biochemistry (e.g. Sears, Thompson, & Saxon, 2007; Wolfson, Rowland, Lawrie, & Wright, 2014).

Research has characterized difficulties with thermodynamics and kinetics for secondary students to upper-level undergraduates across engineering, the life sciences, and the physical sciences. As Table 1.1 demonstrates, the majority of such studies have taken place in the physical sciences, particular in chemistry (also see reviews by Bain, Moon, Mack & Towns, 2014; Bain & Towns, 2016). These studies have primarily investigated alternative conceptions (e.g. Nilsson & Niedderer, 2014; Thomas & Schwenz, 1998), focusing on general thermodynamic concepts like spontaneity, or specific laws and associated concepts like state versus path functions. Research suggests that some of these difficulties appear to result from conflating the meaning of everyday language with scientific terminology (e.g. 'spontaneity') or the confusing, and sometimes incompatible, ways energy is described in different disciplines (e.g. Cooper & Klymkowsky, 2013; Dreyfus et al., 2012). Still other difficulties appear to stem from students' inability to relate or interpret the physical meaning of mathematical representations, leading instead to procedural use of thermodynamic and kinetic equations (e.g. Bektasli & Çakmakci, 2011; Çakmakci, Donnelly, & Leach, 2005; Kermen & Méheut, 2011; Hadfield & Wieman, 2010). Research also indicates that students have difficulty differentiating between thermodynamic, kinetic, and equilibrium ideas, often conflating them (e.g. Çakmakci, 2010; Çakmakci & Aydogdu, 2011; Sözbilir, 2002; Sözbilir & Bennett, 2006; Turányi & Tóth, 2013). Common examples of this confusion include students' use of 'spontaneity' to describe the rate of a reaction (Sözbilir, 2002), relating rate to extent of reaction (Banerjee, 1995), and that exothermic reactions occur faster (Sözbilir, Tacettin, & Canpolat, 2010).

Table 1.1 Examples of research studies on student difficulties in thermodynamics and kinetics across disciplines.

Discipline	References	Examples of Topics Addressed
Engineering	Haglund, Andersson, & Elmgren (2015)	Entropy
Physics	Meltzer (2007)	Work, heat, first law, second law
	Pollock, Thompson & Mountcastle (2007)	Work, path/state functions
	Dreyfus, Redish & Watkins (2012)	Energy (across different disciplines)
Chemistry	Gabriela, Ribeiro, Costa Pereira & Maskill (1990)	Reactions, spontaneity
	van Roon, van Sprang & Verdonk (1994)	Work, heat
	Thomas & Schwenz (1998)	Equilibrium, spontaneity, free energy, first law, second law
	Boo & Watson (2001)	Reactions, energy
	Çakmakci, Donnelly & Leach (2005)	Relationship between concentration and reaction rate
	Sözbilir & Bennett (2006; 2007)	Enthalpy, spontaneity; Entropy
	Hadfield & Wieman (2010)	First law
	Kermen & Méheut (2011)	Predicting direction of chemical change
	Sreenivasulu & Subramaniam (2013)	Third law
Life Sciences	Sears, Thompson, & Saxon (2007)	Equilibrium
	Turányi & Tóth (2013)	Reaction rate, equilibrium, enthalpy, heat, catalysts
	Wolfson, Rowland, Lawrie, & Wright (2014)	Free energy, equilibrium, reaction rate

1.2.2 Biochemistry Students Difficulties with Concepts Involving Thermodynamics and Kinetics

Student difficulties with fundamental principles persist into upper-division biochemistry courses. The persistent misconception that breaking bonds releases energy, for instance, continues amongst biochemistry majors and biochemistry and physiology students (Galley, 2004; Villafañe, Loertscher, et al., 2011). Wolfson et al. (Wolfson, Rowland, Lawrie, & Wright, 2014) specifically pursued students' conceptions of energy in biochemical phenomena and found that students

struggle to understand the relationship between free energy change/equilibrium and enzymes or reaction rates, and that many students appear to think no reaction can be reversed by changing concentrations. Moreover, Sears and colleagues found that only a quarter of the upper-level undergraduates in their biochemistry course could determine the correct equilibrium constant for acetic acid, which they argue results from a poor understanding of association and dissociation concepts in general and a lack of meaningful mathematical equations (Sears, Thompson, & Saxon, 2007). Of particular concern, given the importance of structure-function relationships and the energy considerations that make folding and interactions favorable or unfavorable, some biochemistry students believe the interior of an alpha helix contains the side chains of amino acids, even after instruction (Villafañe et al., 2011). Robic (2010) describes ten common misconceptions about protein structure, folding, and stability, including ideas like 'unfolded proteins are simply stretched out polypeptide chains.' Investigation of student ability to solve a protein structure-function problem has also revealed that students struggle with understanding what components of amino acids drive tertiary structure formation; how amino acids can be used to predict protein structure, function, and dynamics; and how attraction due to charge is the underlying causal mechanism for noncovalent interactions (Halmo et al., 2018). Without a strong foundation in thermodynamics and kinetics, it is not surprising that students have difficulty understanding more complex biochemical structures and processes that rely on such fundamental principles.

1.2.3 Current Trends in Biochemistry Instructional Materials

Current biochemistry textbooks offer little support for developing an integrated understanding of thermodynamics and kinetics and their application to complex biochemical processes or experimental methods. The study of dynamic processes, like protein folding, usually involves significant amounts of mathematical description, symbolism, and information-rich representations (see, for example, Liu et al., 2016), but textbooks often fail to provide representations that reflect how scientific work is documented in primary literature (Rybarczyk, 2011). Instructional materials, including textbooks, may also contain potentially inaccurate multimedia resources (e.g. Goodsell & Johnson, 2007) as well as representations that have been decontextualized, modified by publishers, and/or separated from other representations that support meaning-making (Bowen & Roth, 2002; Roth & Bowen, 1999; Roth, Bowen, & McGinn, 1999). This likely compounds student difficulties with complex subject matter, given that previous work has established the importance

of representations in developing student understanding of complex scientific concepts (e.g. Ainsworth, 2008; Kozma & Russell, 2005), in addition to characterizing the many difficulties science students face when interpreting representations (e.g. Pinto & Ametller, 2002; Schönborn, Anderson, & Grayson, 2002; Schönborn & Anderson, 2006). Moreover, despite the necessity of experimental methods to create biochemical knowledge, very few textbooks or educational research studies focus on the role of experimental work in developing understanding. Experimental work and tools are often treated as merely part of providing concrete experiences to students in lab (e.g. Trumper, 2003), but experimental work may shape the nature of an individual's conceptual understanding (Bernhard, 2007; 2010; 2018; Nersessian & Chandrasekharan, 2009). With the way that subject matter related to thermodynamics, kinetics, and protein folding is currently organized in most textbooks (and thus most courses), life science students often receive a disjointed description of protein folding and how it is studied.

Recently, several educational activities related to protein folding, protein dynamics, and computer-aided modeling of proteins or protein drugs have been published (e.g. Helgren & Hagen, 2017; Lipchock, Ginther, et al., 2017; McLaughlin, 2017; Pickard IV, Miller, et al., 2014; Prigozhin, Scott, & Denos, 2014; White, 2006). However, none of these activities consider how thermodynamic and kinetic principles are applied to the biochemical system or experimental methods involved, nor are they written with careful consideration for how to support students in constructing explanations, analyzing experimental data to develop models, or interpreting representations. Practitioners of the life sciences must be equipped with the knowledge and skills that will help them navigate their discipline on their own.

As a first step, this dissertation explores how several experts explain protein-folding and dynamics research, with the goal of using the findings to inform biochemistry instruction and to generate instructional materials that foster student understanding of biochemical research methods, the application of physical principles in the life sciences, and the ability to integrate theoretical and experimental knowledge to explain the study of biochemical phenomena.

1.3 Using Expert Scientific Research and Practices to Inform Curriculum Design

As the primary source of scientific knowledge, studying experts provides education researchers with insight into disciplinary questions at the ever-expanding forefront of science, as well as insight into how experts use representations, experimental tools, and activities to create and

communicate disciplinary knowledge (Lemke, 1998; Airey & Linder, 2009). Experts can also provide insight into practical applications of knowledge and ways of thinking, which can be translated and structured for use in educational contexts. However, in the past few decades, more education research has focused on the characterization of student conceptions and reasoning difficulties instead of studying expert practice. This is in part due to a belief that the expert-novice research approach highlights discontinuities and differences between expert and novice understanding, and ignores the value and relevance of student knowledge resources in educational contexts (Smith, diSessa & Roschelle, 1994). It is given that students' knowledge will fall short when judged by scientific standards. I argue that the purpose of studying experts is to use expert practice as a target of what knowledge and skills instructors should aim to develop in their students. That is, for example, if a biochemist is skilled at interpreting multiple complex graphs to draw conclusions, then biochemistry instructors should support the development of visualization and data interpretation skills in their students. To develop strategies and materials to support student development, it is critical to have a deep and explicit understanding of expert knowledge and ways of knowing in order to know where learning is heading (Lajoie, 2003; Nersessian, 1995; Smith et al., 1994).

In the life sciences – and especially those that borrow heavily from the physical sciences – developing expert ways of thinking and knowing means understanding the knowledge and skills experts use to investigate, model, and manipulate an abstract and invisible world. It means making sense of the complex intellectual endeavor that is science by investigating authentic scientific practice with an eye for how it can be productively translated, structured, and enacted in an educational context (Passmore, Gouvea, & Giere, 2014). Naturally, there are significant differences between scientists and students so student experiences with disciplinary resources and practices must be carefully designed to support intended learning goals (Kozma et al., 2000). However, ultimately, students must be sufficiently equipped with the knowledge and skills necessary to navigate their discipline on their own. In light of these considerations, this dissertation adopts a backwards design approach. Backwards design is based on the principle that curricular design begins with identifying the desired or anticipated learning outcomes (ALOs; see Irby, Pelaez & Anderson, 2018a), that is, what students should know, understand, and be able to do, as well as what is acceptable evidence that learning has occurred (Brewer & Smith, 2011; Wiggins

& McTighe, 2005). Learning experiences and instruction are shaped by a clear vision of these two things.

1.4 Overarching Research Questions

My dissertation aims to demonstrate how expert data from cutting-edge researchers might be used to inform undergraduate teaching of biochemistry. More specifically, I aim to fill a gap in our knowledge by investigating how experts integrate theoretical knowledge of thermodynamics and kinetics with experimental methods and representations in the context of protein-folding and dynamics research, and how such expert knowledge can be used to inform the development of instructional materials in this crucially important area of biochemistry. Towards achieving these aims, this dissertation project is divided into three parts (Chapters 4-6; Figure 1.1) which respectively address the following three overarching research questions:

1. How can we model experts' explanations of their research related to protein folding and dynamics? (Chapter 4);
2. How do experts use representations to explain their protein-folding and dynamics research? (Chapter 5); and
3. How can we use expert research to inform the design and implementation of instructional materials aimed at developing biochemistry students' understanding of protein folding and dynamics? (Chapter 6)

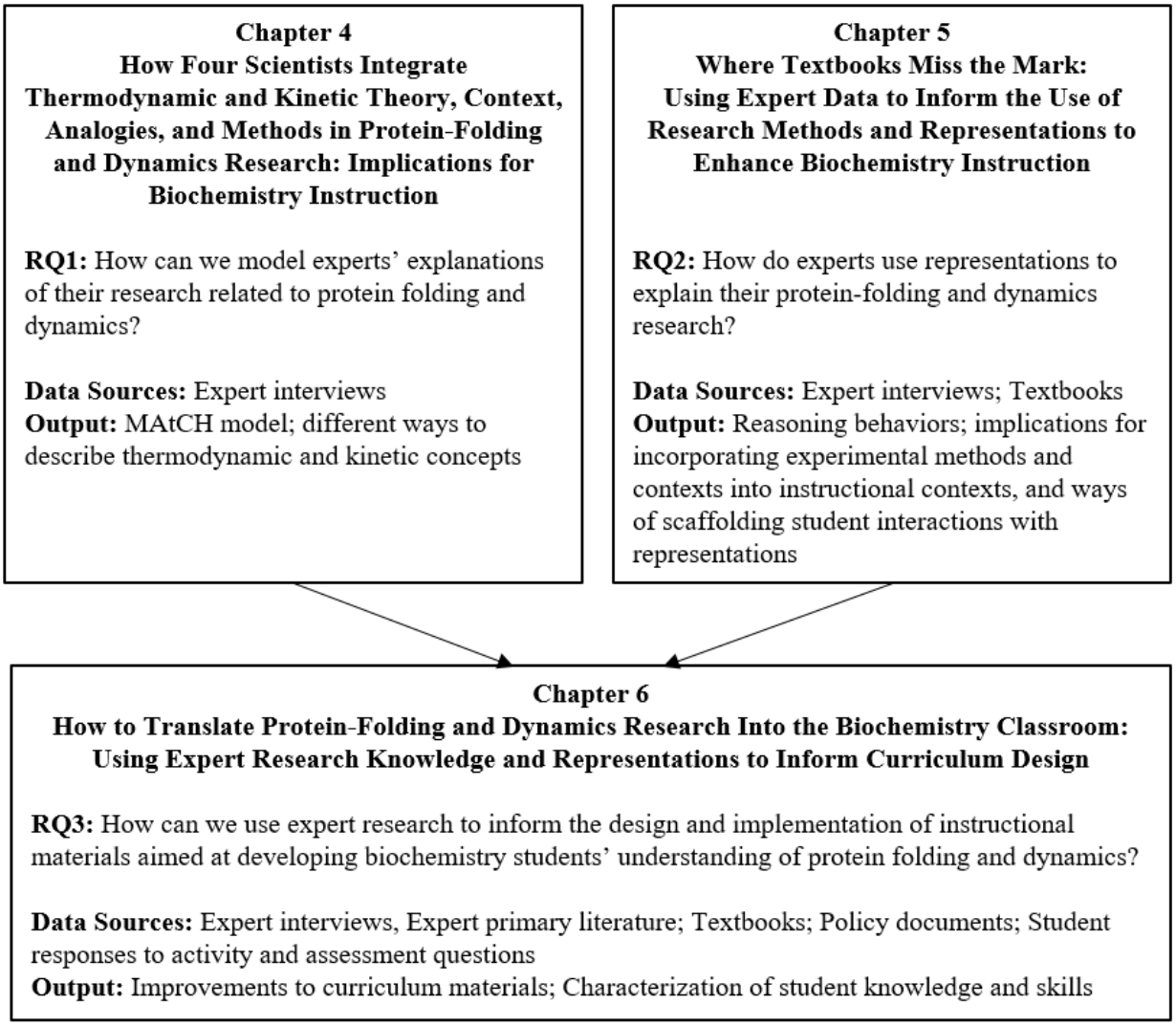


Figure 1.1 Flow chart of the three chapters of this dissertation project with brief descriptions of their purpose, data sources, and outputs.

As illustrated in Figure 1.1, the various parts of this dissertation build upon each other. The study described in Chapter 4 was conducted to describe and model the components and structure of experts' research explanations. The study in Chapter 5 extended this characterization by looking at the interaction of spoken language, external representations, and gestures in these explanations. The findings in Chapters 4 and 5 suggest several implications for teaching and learning, particularly ways in which instructors and instructional materials might incorporate research contexts and experimental methods into the classroom, support student integration of theoretical

and experimental knowledge, and scaffold student interpretation of representations. Chapter 6, therefore, uses the findings from the expert data to inform the design of instructional materials about protein-folding and dynamics research. These resources aim to foster student understanding of biochemical research methods, the application of physical principles in the life sciences, and reasoning with data representations.

The main focus of this dissertation project is to explore expert knowledge and practice and then use selected aspects of such knowledge to inform undergraduate biochemistry instruction. Thus, Chapter 2 provides an overview of literature of general relevance to expert knowledge and practice, while the Results Chapters 4-6 contain discussions of literature of more direct relevance to the findings of this dissertation. Chapter 3 offers an overview of the theoretical, methodological, and analytical frameworks that influenced this research. Chapter 7 offers a general synthesizing discussion to discuss the findings of each study in terms of the overarching research questions, how the findings relate and contribute to the literature, as well as implications for future research and teaching practice.

CHAPTER 2. AN OVERVIEW OF RELEVANT LITERATURE

2.1 Scientific Explanation

Explanation is a central goal of science and thus science education (Salmon, 1989). In the life sciences, historical reconstructions and examinations of scientific discourse have enhanced understanding of scientific explanation, especially explanations of mechanistic processes which seek to establish causal links between agents and events (Bechtel & Richardson, 1993; Darden, 2008; Machamer, Darden, & Craver, 2000). A growing number of problems reported in the life sciences address emergent phenomena – like protein folding and dynamics – where the overall behavior of the system emerges from underlying random processes rather than a regular sequential mechanistic process (Chi, Roscoe, et al., 2012). Although many important biological and chemical processes are emergent (e.g. diffusion, molecular binding), thinking from an emergent perspective is quite different from the linear narrative that dominates most human interactions, making emergent explanations difficult for many students to understand (Chi et al., 2012; Talanquer, 2014). I argue, and aim to show through this dissertation, that constructing rich accounts of how experts explain their research provides insight into how instructors can support students in constructing these difficult but essential types of explanations.

There are five major philosophical models of scientific explanations relevant to research and practice in science education (Braaten & Windschitl, 2011). Like others have suggested, I contend that the model of scientific explanation which is most appropriate depends on the purpose(s) of an investigation and its explanatory aims (Brigandt, 2010, 2013; Craver, 2006; Van Fraassen, 1980). For example, a researcher may offer an explanation that takes the form of a statistical-probabilistic pattern that relates the occurrence of a disease to trends in environmental factors in order to make health recommendations. Although the researcher does not provide an underlying cause for the disease, their aim is a predictive tool and thus a mathematical account ‘suffices’ as an explanation. Thus, in this dissertation I consider ‘explanation’ to include descriptions of observable phenomena, theoretical accounts of how phenomena progress according to any of the philosophical models, and/or the process of clarifying ideas, reasoning, and findings regarding a phenomenon (Achinstein, 1983; Knorr-Cetina, 1999; Salmon, 1989).

In the process of developing explanations, scientists manipulate many tools and engage in a wide variety of physical and mental activities. This literature review is organized around relevant meaning-making resources that science experts use to construct and communicate knowledge and ways of knowing.

2.2 Disciplinary Discourse

Scientific disciplines rely on a range of meaning-making resources to develop, represent, and share disciplinary knowledge. This collection of discursive resources includes spoken and written language, gestures, mathematics, external representations (ERs), experimental tools, and activities (Airey & Linder, 2009; Lemke, 1998). ERs include pictures, diagrams, graphs, physical models, or simulated computer models; experimental tools include equipment to run experiments or take measurements; and activities are the ways in which science is done, such as experimental routines or modeling processes. No single resource itself is capable of fully communicating a disciplinary way of knowing and one resource cannot be reduced to another in a one-to-one manner (Givry & Roth, 2006; Kress and van Leeuwen, 2001). Instead, each resource helps characterize a different facet of disciplinary knowledge and therefore in combination with other resources affords holistic understanding (Airey & Linder, 2009; Kress & van Leeuwen, 2001). To gain expertise in any discipline requires knowing the system of concepts and theories created to explain phenomena, understanding how they are represented, and being able to productively coordinate and translate between multiple discursive resources (Airey & Linder, 2009; Lemke, 1998, 2002; 2004; Offerdahl, Arneson, & Byrne, 2017). The following sections therefore contain an overview of research related to scientific thought and reasoning in practice, which informs what disciplinary resources are important to consider when characterizing expert explanations (Chapter 4 and 5), how experts may employ those resources (Chapters 4 and 5), and, ultimately, what resources science students must become proficient at using (Chapter 6). I focus particularly on the roles of two key scientific activities (Section 2.4), external representations (Section 2.5.1), and gestures (Section 2.5.2), as these topics are directly relevant to the expert case studies presented later in Chapters 4 and 5 of this dissertation.

2.3 Expert Cognition

Past research has investigated expert cognition in a variety of ways including analyses of historical cases, contrived problem-solving tasks, naturalistic studies of (scientific) work, and combinations of these settings (e.g. Chi, 2006; Dunbar & Blanchette, 2001; Nersessian, 1992a; Trickett & Trafton, 2007). In the context of this project, this literature suggests features of knowledge and reasoning skills, which can be explored in scientific contexts to inform learning goals, strategies, and materials to support student development. By definition, expertise refers to the "…manifestation of skills and understanding resulting from the accumulation of a large body of knowledge" (Chi, 2006, p. 167). Much of the research related to expertise, therefore, has focused on understanding the knowledge structures and problem-solving strategies that distinguish experts, often in comparison to novices. Research on the structure of expert knowledge suggests that experts, through their broad experiences, possess a substantial body of well-connected, detailed knowledge organized around certain core concepts or principles (Chi, Feltovich, & Glaser, 1981; Irby, Phu, et al., 2016; Larkin, McDermott, Simon, & Simon, 1980; NRC, 2000). Studies of experts in chess, circuitry, physics, radiologists, and even architecture suggest that experts are more sensitive to critical features, perceive more detailed features and more relations between them, and are therefore more likely to chunk information into large, meaningful patterns (e.g. de Groot, 1965; Glaser & Chi, 1988; Sowden, Davies, & Roling, 2000). The recognition of core concepts and patterns is what guides experts in accessing relevant knowledge, and interpreting, representing, and solving both familiar and novel problems (Larkin et al., 1980; NRC, 2000). The extent of their knowledge allows experts to flexibly adapt to new situations and fluently correct mistakes (Smith et al, 1994).

In addition to their extensive knowledge, experts are also characterized by a broad range of other cognitive processes including the ability to synthesize, analyze, and evaluate information, to detect causal relational patterns (Goldwater & Gentner, 2015), to reason analogically (Dunbar, 1997; Dunbar & Blanchette, 2001; Genter & Gentner, 1983; Nersessian & Chandrasekharan, 2009), to reason both locally and globally (Kitano, 2002), to use mental models and conceptual simulation (Clement, 2009; Gorman & Carlson, 1990; Nersessian, 1992a, 1999, 2002, 2009; Trickett & Trafton, 2002; 2007), and to manipulate, coordinate, and connect representations to solve problems (Kozma, 2003; Kozma & Russell, 1997; Trickett, Trafton, & Schunn, 2009). It is possible that some of these cognitive processes are domain-independent, however evidence suggests that

activities are highly contextualized (e.g. Bowen, Roth & McGinn, 1999; Roth & Bowen, 1999). Experts, for instance, enact different practices to interpret representations as a result of different knowledge resources when they engage in tasks that require knowledge outside their domain (e.g. Roth & Bowen, 2001), and demonstrate differences in knowledge structure and reasoning based on their occupation (Medin, Lynch, Coley, & Atran, 1997). This suggests that any inferences about expert knowledge structures or strategies ought to be anchored in specific domains (Chi, 2006) through the study of authentic, domain-specific tasks (Dunbar & Blanchette, 2001; Smith et al., 1994). That is one reason this dissertation explores experts' explanations of their own research. At this time, there are still very few studies of expert scientists completing authentic tasks due to the complex nature of scientific work and only recent adoption of more naturalistic methodologies in cognitive science (Nersessian, 2002a). Studying scientific knowledge and ways of knowing means exploring a wide variety of discursive activities such as: designing, performing, and troubleshooting real-world and thought experiments; using and developing mathematical tools, modelling tools, methods, and/or instruments; and, constructing arguments and devising means of communicating abstract and complex data and models.

As this project investigates how experts explain their research, I provide an overview of literature regarding two key activities that underlie scientific explanation and representation: modeling (Section 2.4.1) and analogical reasoning (Section 2.4.2).

2.4 Key Activities in Science

Because it is concerned with the study of an abstract, molecular world, biochemistry relies heavily on a variety of activities and tools to investigate, manipulate, and understand complex biochemical processes like protein folding and dynamics. Scientific activities refer to the ways in which science is done, such as experimental routines or discussion around experimental results. While engaging in activities, scientists use tools and representations to create disciplinary ways of knowing; for example, taking measurements with an apparatus (Airey & Linder, 2009). Perhaps two of the most critical activities underlying scientific thought and communication are modeling and analogical reasoning. In this dissertation, these activities are explicitly featured or alluded to in each of the experts' explanations of their research.

2.4.1 Modeling

Previous studies of scientific cognition in practice have provided great insight into the processes underlying discovery and problem solving. Contrary to the customary, positivistic view of scientific cognition as being based on 'formal' hypothetico-deductive logic, in practice scientists employ many non-formal ways of reasoning (Clement, 1988; 2008; Magnani, Nersessian, & Thagard, 1999). Many historical studies of revolutionary changes in scientific understanding indicate that non-formal reasoning happened first and was later followed by quantitative formalisms of the theory (Nersessian, 1992a; 1995; 1999; 2002a; 2002b). Non-formal reasoning often involves the use of models as cognitive tools and thus the use of models in cognition is now considered a special kind of 'model-based reasoning', which itself involves dynamic integration of multiple types of reasoning activities in order to define and manipulate a problem (Clement, 2008; Magnani et al., 1999; Nersessian, 2002a). Studies of scientific practice and cognition indicate that modeling is a standard practice of science in both theoretical and experimental work (Cartwright, 1983; Chandrasekharan & Nersessian, 2014; Hesse, 1970; Magnani et al., 1999; Morgan & Morrison, 1999). Due to their centrality in scientific practice and ties to a range of cognitive phenomena, any review of cognition in science is incomplete without a basic understanding of modeling.

Models can be broadly thought of as structural or behavioral representations of the components and/or relations of situations, events, processes, or entities (e.g. Harrison & Treagust, 2000; Johnson-Laird, 1983; Rouse & Morris, 1986). In practice, scientists create both external and internal models to represent salient aspects of a phenomenon, and manipulate them in order to make inferences and increase their understanding (e.g. Boon & Knuuttila, 2008). External models include material or computational representations like organisms, computer simulations, or engineered devices, which may be explored in experimental contexts to learn about phenomena (e.g. Nersessian, 2009; 2012), as well as other representations, like pictures, graphs, mathematical equations, and gestures, which are similarly manipulated or used to convey information (e.g. Bailer-Jones, 2002; Ochs et al., 1996; Roth, Bowen & McGinn, 1999). The idea of an internal, mental model is typically attributed to Craik (1943), who proposed that individuals carry "small-scale model[s] of external reality" in their head which allow them to envision alternatives, and use prior knowledge to make decisions and react to situations (as quoted in Johnson-Laird, 2013). Cognitive science research supports the idea that mental models are based on perception and are

imagistic or iconic in nature, representing reality through similarity (e.g. Nersessian 2008; Hegarty, 1992; Shephard & Metzler, 1971). Because mental models are restricted only by the limitations imposed on them by their user, they can supply information that goes beyond what is derivable from either data or theory and can therefore underlie not only deductive, but also inductive and creative processes, which can lead to new ways of understanding phenomena (Johnson-Laird, 2004). Thus, inferences are not necessarily – or even typically – made by following formal logic, but instead are based on various models, with each model representing a distinct possibility that is possibly inconsistent with others, but nonetheless useful in different situations (Johnson-Laird, 2013; Morrison & Morgan, 1999). For instance, narrative explanations of possible mechanisms for biochemical phenomena, as explored in this dissertation as well as elsewhere (Trujillo, Anderson, & Pelaez, 2015), can be considered as a type of polished internal model, creatively constructed from theory and/or experimental data (e.g. Nersessian, 1992b).

Cognitive-historical and ethnographic studies of science demonstrate how scientists manipulate external and internal models simultaneously to organize information and facilitate reasoning during problem-solving (e.g. Chandrasekharan & Nersessian, 2014; Nersessian, 2009). Nersessian (2009), for example, describes how scientists combine biological and engineered models of *in vivo* phenomena taken from primary literature with bio-engineered *in vitro* models and mental models of both *in vivo* and *in vitro* phenomena, of devices as *in vitro* models, and of devices as engineered models. Because both internal and external models work together, a scientist's model of a situation is composed of not only theory and/or data, but also includes any metaphors, analogies, mathematical concepts, diagrams, techniques, etc. they brought to bear on the problem at hand (Boumans, 1999). Recent studies even suggest close relationship between the development of conceptual understanding and the experimental process (e.g. Nersessian & Chandrasekharan, 2009). Reasoning in scientific practice, therefore, cannot be meaningfully understood without considering both external and internal models (e.g. Hegarty, 2004a; Larkin & Simon, 1987). This dissertation explores both, as presented in the experts' explanations. The process of creating and manipulating internal and external models involves a range of reasoning strategies, including the use of analogical (e.g. Dunbar, 1997, Gentner & Markman, 1997; Nersessian & Chandrasekharan, 2009), simulative (e.g. Clement, 2009; Hegarty, 2004b; Nersessian, 2009; Trickett & Trafton, 2007), and distributed reasoning (e.g. Dunbar, 2000; Nersessian, Kurz-Milcke, et al., 2003).

Chapters 4 and 5 of this dissertation attend to how experts use various models – including mathematical models, external representations, and *in vitro* and *in silico* experiments – in explanations of their research, as well as actions or language that suggest the aforementioned kinds of reasoning strategies.

2.4.2 Analogical Reasoning

Analogical reasoning and modeling are fundamentally related. All models, internal or external, are analogies in that they represent something which they are not. Accordingly, the process of constructing models requires analogical reasoning. Analogy and analogical reasoning are abundant in science and play an important role in many problem-solving and theorizing activities, including explanation and representation (Bailer-Jones, 2002; Meheus, 2000). Many phenomena and methods in science are too abstract, complex, unobservable, or impossible to explain without the use of analogy. Analogical reasoning is therefore an essential skill in biochemistry and the molecular life sciences because they depend heavily on understanding the abstract world of molecular structures and processes (Anderson & Schönborn, 2008; Schönborn & Anderson, 2008). It is for this reason that analogical reasoning is explored in this dissertation.

Although different approaches to analogical reasoning have been proposed, they share the idea of mapping information between a known 'source' domain to an unfamiliar 'target' domain in order to make inferences about the 'target' (Gentner, 1989; Holyoak & Thagard, 1989). Put simply: scientists use the familiar to make sense of the new (Hesse, 1970). In scientific practice, analogy serves two main functions. First, and most significantly, analogy stands to relate a phenomenon and a model of a phenomenon (Bailer-Jones, 2002). That is, models are not literal descriptions of nature, but rather analogues of nature (Hesse, 1953). The practice of scientific modeling shares a structure with analogical reasoning in that scientists attempt to understand the 'target' phenomenon by mapping and comparing it to a known 'source' model of the phenomenon. I include both internal and external models in this function on the basis of the following example: a three-dimensional computer simulation of immunoglobulin IgM is not a molecule of IgM, nor is one's mental model. A second function of analogy is to formally relate theoretical treatments of phenomena (Bailer-Jones, 2002) in order to better understand what the 'target' domain may or may not be like, and thus enrich theory related to the phenomenon (Hesse, 1970; Dunbar, 1999; Psillos, 1995). This may be done by employing the same equations in different scientific domains

or explaining one phenomenon by comparison to another, such as how Maxwell used water pressure to describe Faraday's electric lines (Maxwell, 1855; Nersessian, 1995; 2002b) or Boyle imagined gas particles as moving coiled springs (e.g. Harrison & Treagust, 2006; Clement; 1988).

Scientists are flexible analogists, engaging in different kinds of analogical reasoning depending on their goal; whether that be hypothesis generation, experimental design and troubleshooting, or explanation (Dunbar, 1997; Dunbar & Blanchette, 2001; Holyoak, Holyoak & Thagard, 1996). During hypothesis generation, for example, a scientist considers structural relations as they search prior knowledge for similar cases or generalize from previous experimental results (Dunbar & Blanchette, 2001; Klahr & Dunbar, 1988). Analogies allow scientists to perform many steps all at once, instead of proceeding in a step-wise manner (Dunbar, 2000). Scientists frequently employ analogy to reason through experimental data and laboratory experiments, using local analogies (e.g. from a prior experiment to a newer, similar experiment) to reason about singular unexpected findings or using distant analogies as explanatory devices (Dunbar, 1995; 2000). In the life sciences, research has demonstrated that scientists constructing descriptions of biological mechanisms will take known mechanisms from other contexts or fields, abstract the general structure, and then fill in the functional roles with the particulars of their target phenomenon (Darden & Craver, 2002; Darden, 2002). Another analogical reasoning strategy cobbles together groups of common components from elsewhere in the field to create an organized mechanism (Darden, 2002).

As perhaps the most prominent meaning-making resource, it is not unsurprising that scientific language is heavy with analogy. Language, scientific or otherwise, is possibly the most information-heavy and pervasive meaning-making resource (Lemke, 1998; Norris, 2004). Scientists employ easily recognizable formal analogies such as "ATP is the energy currency of the cell," but the implicit use of familiar, embodied experiences is also a part of scientific knowledge and reasoning (e.g. Amin, 2009; Brookes & Etkina, 2007). Spatial analogies and the use of spatial words and grammar, for example, are especially ubiquitous in science because physical space is a familiar foundation on which to build and communicate understandings of abstract ideas, like energy (e.g. Lancor, 2012). The idea that our bodily experiences in the world influences cognition is known as embodied cognition (Johnson, 1987; Nunez, 1999; Varela, Thompson, & Rosch, 1991). In language, the conceptualization of an abstract concept by referencing a more familiar and concrete concept or domain is known as conceptual metaphor (Lakoff & Johnson, 1980; 1999).

Conceptual metaphors are based on concrete embodied experiences (Amin, 2009; 2015; Amin, Jeppsson & Haglund, 2015; Lakoff & Johnson, 1999; Treagust & Duit, 2015). Recurring patterns in everyday sensorimotor experiences involving interaction with physical objects are abstracted into image schemata (Lakoff & Johnson, 1980; 1999). Image schemata allow an individual to use concrete, bodily experiences to think about abstract domains (Johnson, 1987). Many of the source domains used by scientists consist of image schemata drawn from sensorimotor experiences. For example, the discussion of energy uses image schemata like possession, containment, and the movement of possession; e.g. "the molecule has kinetic energy" (Amin, 2009; Lancor, 2012). In thermodynamics, a common conceptual metaphor known as the Location Event Structure metaphor, relates abstract concepts like the state functions of energy or entropy to substances or locations, with changes in state involving some sort of flow or movement (Amin, 2009; Amin, Jeppsson, et al., 2012; Brookes & Etkina, 2007; Close & Scherr, 2015; Jeppsson, Haglund, et al., 2013). Conceptual metaphors are believed to support creative insight during scientific reasoning (Clement, 2009) and assist the coordination of qualitative and quantitative reasoning (Jeppsson et al., 2013). It has also been suggested that multiple conceptual metaphors may be required to fully understand an abstract concept (Amin, 2015).

Because analogy and analogical reasoning enable scientists to understand and communicate abstract concepts, they function as pivotal meaning-making resources in science, and are therefore a critical component of scientific explanations. Past characterizations of the role of analogy in science indicate the importance of attending to experts' language, use of representations, and how they relate and/or compare representations or processes. It is for these reasons that this dissertation specifically attends to experts' use of analogies and analogical reasoning, including how they reason with experimental methods to explain phenomena, and their use of certain kinds of language (see Chapters 4 and 5).

2.5 Representation

Any attempt to understand a world that cannot be directly observed must rely on representations to mediate between the phenomenon and imperceptible entities or processes, as well as to negotiate meaning with other members of the scientific community (e.g. Airey and Linder, 2009; Kozma, Chin, Russell & Marx, 2000; Offerdahl, Arneson, & Byrne, 2017; Schönborn & Anderson, 2006). Each type of representation has its own set of affordances, making it a valuable meaning-making

resource in the community. Lemke (1998) captures the significance of representation in science when he states that “Science does not speak of the world in the language of words alone, and in many cases it simply cannot do so” (p. 6). Spoken and written language, for example, are terrible at expressing precise, intermediate degrees of change – that is, at describing quantitative variation – whereas graphs, mathematics, and gestures excel at presenting information in a smooth and continuous manner (Lemke, 2002). Because it aims to understand the abstract and invisible world of molecular and cellular processes, biochemistry relies heavily on the use of representations to bridge experience and thought. In this section, I briefly introduce two types of representations that are explored in this research: external representations (ERs) and gestures. Expert use of ERs and gestures is the main focus of Chapter 5, while expert and student use of ERs to design and modify instructional materials is the main focus of Chapter 6.

2.5.1 External Representations

External representations convey spatial information and include visualizations such as diagrams, graphs, physical models, equations, photographs, animations, or data read-outs generated from experimental tools. In the act of interpreting ERs, scientists are guided by the concerns of their domain, drawing on a large variety of experience-based, domain-specific resources and practices (e.g. Bowen et al., 1999). Effective use of ERs therefore requires a combination of conceptual knowledge, knowledge of modes, and the ability to apply cognitive skills to perceive, process, and express ERs (Anderson, Schönborn, du Plessis, Gupthar, & Hull, 2013; Mnguni, Schönborn, & Anderson, 2009). This is especially true in fields like biochemistry, where ERs often employ discipline-specific symbolic conventions and may stretch across multiple levels of complexity or abstraction (Schönborn & Anderson, 2006; 2010).

Historical and ethnographic studies of scientists demonstrate that ERs are an essential component of scientific investigation and the creation of scientific knowledge (e.g. Dunbar, 1997; Kozma et al., 2000; Lynch & Woolgar, 1990; Magnani, 2002; 2013). Scientists employ ERs as meaning-making resources, using them to convince others of certain data interpretations, think about data, keep track of complex calculations, and simulate hypothetical situations (Latour, 1990; Phillips, Norris & Macnab, 2010). ERs in the laboratory serve to organize discussion (e.g. Ochs, Jacoby & Gonzales, 1994; Ochs et al., 1996). Amann and Knorr Cetina (1988), for example, describe how a group of scientists collectively manipulate and analyze autoradiograph data to

make sense of what they see, building off of others' knowledge or challenging interpretations of ER features. In another study, Woolgar (1988) describes how several scientists generate meaning by observing the line created by a pen-chart recorder and comparing its features to other ERs and what they had expected to see.

In the lab, scientists translate phenomena into ERs and then 're-present' that information through a variety of other ERs (e.g. Roth, Tobin & Shaw, 1997). Kozma et al. (2000), for example, describe how a chemist transforms an NMR spectrum via mathematical calculations into a structural diagram to evaluate the results of her synthesis. Scientists generate meaning by coordinating within and across multiple ERs. Trickett and colleagues (2007), for instance, characterize how meteorologists create and spatially transform mental models of ERs of weather data by mentally animating data, moving components around, or adding and deleting data. Another study of an interdisciplinary oceanographic research team describes how scientists interpreted data from a variety of tools and displays to build a model of seawater characteristics (Goodwin, 1995). The coordination of one's understanding with ERs, experimental tools, and/or other individuals has come to be known as distributed cognition, and is thought to simplify or lighten the burden of highly complex or abstract cognitive tasks (Hutchins, 1995; Osbeck & Nersessian, 2014; Zhang & Norman, 1994).

In this dissertation, experts' use of ERs in explanations of their research (Chapters 4-6) and students' use of ERs (Chapter 6) are investigated in order to understand how ERs, like cartoon drawings and graphs, are used to generate and communicate understanding of phenomena.

2.5.2 Gesture

Gestures are another important meaning-making resource in both every day and scientific contexts (e.g. Goodwin, 2000; Kress & Van Leeuwen, 2001; Roth, 2000). Research has found that gesture may indicate how individuals conceptualize abstract ideas (Scherr, Close, et al., 2013), reveal implicit understandings that are difficult to express (Broaders, Cook, et al., 2007), or help individuals keep track of where they are in a mental animation (Hegarty, Mayer, et al., 2005). Studies also suggest that using meaningful gestures when speaking facilitates thought and lightens cognitive load so that working memory resources can be directed towards other tasks (Goldin-Meadow, Nusbaum, et al., 2001; Iverson & Goldin-Meadow, 1998; Ping & Goldin-Meadow, 2010; Wagner, Nusbaum, & Goldin-Meadow, 2004). Studies on the use of gesture in professional

environments (e.g. Ochs, Gonzales & Jacoby, 1996; Goodwin, 2000), as well as the classroom (e.g. Givry & Roth, 2006; Roth & Welzel, 2001; Roth & Lawless, 2002), suggest that gesture during scientific tasks provides a bridge between laboratory experiences and scientific discourse about abstract entities by connecting perception and action to cognition. Ochs et al. (1996) proposed that scientists may come to their understandings by taking on the perspective of the object of their analysis and involving themselves in (re)enactments of physical events through speech and gesture, i.e. they embody the process they aim to explain. Examination of gestures made by biochemists in laboratory discussions similarly found that gesture had a significant role in how the group of scientists conceptualized and communicated theories about the interaction of two proteins involved in blood clotting, with one particular gesture ultimately becoming a representation that was conserved and recycled over six months (Becvar, Hollan, & Hutchins, 2005). The grounding of conceptual understanding in the physical world through gesture is another example of embodied cognition (Johnson, 1987). As gestures have been shown to be significant in conceptualizing scientific understanding and communicating scientific ideas, they are an important part of scientific explanation and were thus one of the meaning-making resources explored in this dissertation.

Gestures serve a variety of functions. Deictic gestures allow individuals to orient each other to the environment through pointing (Kendon, 2004). No equivalent verbal description (i.e. “this”, “that”) must accompany deictic gestures. In contrast, iconic gestures are used to describe concrete objects or events, often mimicking what is conveyed verbally (Kendon, 2004), but possibly expressing more meaning. Hands can represent material objects and distinct handshapes can be used to represent different types of objects (Emmorey, 2001). Imagine, for instance, an individual moving their hand up and down to describe “the molecules inside [a] syringe” (Givry & Roth, 2006). Despite no verbal description or reference to the motion, the listener can infer that the speaker means the molecules are moving or possibly being pushed, but without the gesture the speech takes on a different and incomplete meaning. Gesture can also have metaphoric character and give form to abstract or invisible ideas through motion and space (McNeill, 2002; Kendon, 2004). For example, gesture can be used to represent time spatially or to show the relations between concepts by assigning them to different locations (Emmorey, 2001).

Given these functions, gestures can therefore be used in conjunction with other resources to enhance meaning in communicative acts, and it is for this reason that Chapter 5 considers the use of these kinds of gestures in constructing meaning in experts' explanations.

CHAPTER 3. RESEARCH FRAMEWORKS

It is rarely possible to understand the complete meaning of a communicative act in science without collectively considering the meaning-making resources an individual employs. This has significant implications for the collection and interpretation of scientists' explanations of their research. This chapter provides an overview of the theoretical, methodological, and analytical frameworks that underlie this project. More detailed discussions of methods are contained within the relevant chapters.

3.1 Theoretical Framework

Qualitative research studies are frequently employed in the social sciences to explore how or why questions in-depth in order to obtain rich detail about individuals' experiences, knowledge, or opinions. A hallmark of good qualitative research is the use and explication of a theoretical framework. A theoretical framework is the system of goals, theories, and assumptions which guide a research project from the development of research questions, to design, and to the collection, analysis, and presentation of data (Bodner & Orgill, 2007). They allow the researcher to define their assumptions, justify their design choices, and make clear the different lenses through which they interpret their data. In this project, situated perspectives help frame the design and interpretation of my work.

3.1.1 Situated Perspectives: Disciplinary Discourse and Shared Ways of Knowing

Prior to the advent of constructivism, theories of knowledge assumed that there was a single objective reality which individuals could come to know, and which researchers could assess (Bodner, 1986; Lincoln, Lynham, & Guba, 2011). Early work moved psychology and cognition away from this perspective by taking a more explicit interest in how individuals engage with the world and make sense of their experiences (Duit & Treagust, 2003). As a theory of knowledge, constructivism essentially argues that knowledge is constructed in the mind of an individual (e.g. Bodner, 1986; Driver, Guesne, & Tiberghien, 1983; Piaget, 1964; Posner, Hewson, & Gertzog, 1982; von Glasersfeld, 1992). Thus, as a theoretical framework, constructivism assumes that realities are situated and co-constructed by individuals or groups of individuals through

experiences (Bodner, 1986; Lincoln et al., 2011; von Glasersfeld, 1995), and that is it possible to describe how knowledge is structured and how experiences produce and shape that knowledge (Cobern, 1993; Ferguson, 2007; Guba & Lincoln, 2005). These assumptions permit the existence of multiple, viable understandings created by individuals through their experiences, making it a useful lens through which to understand the ideas of the experts explored in this project (Bodner, Klobuchar, & Geelan, 2001).

Constructivism extends beyond consideration of an individual's personal experiences to consideration of the shared ways of knowing in a social setting. Social interaction can influence the exchange and creation of meaning, making context a crucial component of learning (Driver, 1989; Solomon, 1987). Situated learning theory argues that knowledge is inseparable from how it is learned and used; that is, it is 'situated' in the context within which it is learned (Brown, Collins, & Duguid, 1989). The physical and social characteristics of a setting (e.g. tools, activities, and culture) influence how individuals negotiate their understanding of the world, and how they see the world influences their understanding of the world and their tools (Brown et al., 1989; Greeno, 1998). 'Situated' is meant to imply that individual and context constantly influence each other, making it inappropriate to separate one from the other (Orgill, 2007). I use the phrase 'situated perspective' to capture the inseparability of meaning from context.

Situated perspectives are consistent with the idea of disciplinary discourse, which views knowledge and ways of knowing as distributed across multiple modes (Airey & Linder, 2009). Taken from social semiotics, modes are meaning-making resources that are shaped by the ways people use them in specific contexts (Jewitt, 2017), having evolved so that individuals might do things by making particular meanings (Lemke, 2002). Experiments and technologies, representations, activities, social culture, gestures, and even metaphor embedded in language, enable and influence how scientists of a particular discipline think and communicate about phenomena (e.g. Chue & Tan, 2012; Gooding, 1990; Norris & Phillips, 2003; Pickering, 1995). Without them, knowledge construction would be impossible. Thus, in short, we can consider human cognition as embedded in and including physical materials and practices. In the present dissertation, we adopt this perspective and accordingly consider – in addition to language – the role that experimental methods and tools, representations, and gestures play in experts' explanations (Chapters 4 and 5).

Distributed cognition and embodied cognition are situated perspectives. Distributed cognition is the idea that an individual coordinates their understanding with external representations, tools, or other individuals (e.g. Hutchins, 1995; Zhang & Norman, 1994). That is, cognition can be conceptualized as systems of humans and artifacts with external components, such as other researchers or computer modeling programs, which enable individuals to develop internal models for problem solving (e.g. Nersessian, 2009). In research, this means that an individual's thoughts should be analyzed in concert with external components, which is why this project attends to experts' use of ERs during their explanations. Another situated perspective, embodied cognition, is the idea that human cognition is grounded in perception and sensorimotor experiences (e.g. Johnson, 1987; Lakoff & Johnson, 1999; Varela et al., 1991). One example is the ability to successfully understand physics phenomena associated with 'pushes and pulls' because they can be experienced with one's body (White, 1993). The use of body-based experiences is also an unavoidable component of language in the form of conceptual metaphors (Lakoff & Johnson, 1999). Time, for example, is often expressed as though it is a thing that has motion or spatial position relative to the speaker (e.g. 'the future is ahead'). Gestures or bodily movement can similarly indicate how an individual conceptualizes something (e.g. Becvar et al., 2005; Close & Scherr, 2012). Because the components of an environment and conceptual understandings grounded in bodily experiences enable and constrain an individual's ability to think and represent ideas, they are of significant interest to research focused on meaning-making. It is for this reason this dissertation project attends to the use, and interaction of, language and gesture (see Chapters $ and 5).

Situated perspectives imply that learning by abstraction is not only impractical, but impossible. Individuals are strongly influenced by a discipline's ways of knowing through participation in disciplinary discourse; i.e., acculturation (Airey & Linder, 2009; Lave & Wenger, 1991). This has implications for research characterizing scientific practices and developing curriculum aimed at engaging students in disciplinary discourse. It suggests, for example, that the research and curriculum should consider how a discipline's physical and social characteristics – like tools and activities – influence meaning-making. Additionally, it implies that an effective learning environment exposes students to authentic disciplinary meaning-making resources so that they develop transferrable knowledge and ways of knowing (Bhattacharyya & Bodner, 2014; Brown et al., 1989). Thus, in this dissertation, I design curriculum materials that foreground the

use of experimental methods and representations in understanding phenomena, exploring the nature of students' knowledge and reasoning through these materials (Chapter 6).

Situated perspectives, in combination with ideas from constructivism and disciplinary discourse, thus provide a framework to consider the construction and use of knowledge by individuals, with particular attention to the various resources they use and combine to make meaning.

3.2 Methodology

The theoretical perspectives described in the previous section informed the study design and analysis. This section briefly describes the alignment between these perspectives, the methodology, and analytical frameworks used during analysis. Greater detail about these frameworks and methods are provided in the relevant chapters.

3.2.1 Methodological Framework

The methodology of this study is rooted in naturalistic inquiry which is sensitive to social processes and their contexts (Guba & Lincoln, 1982). Studying social processes requires a contextual richness that is not possible with rationalistic inquiry (Guba & Lincoln, 1982). Naturalistic inquiry, instead, asserts that realities are multiple and can only be studied holistically; that the inquirer and inquired influence each other during their interactions; that knowledge developed from this type of inquiry is case-dependent and context-bound; that multiple factors can explain an action; and that inquiry is always influenced by the values of the inquirer, the context, and the framework (Guba & Lincoln, 1982). Naturalistic inquiry therefore has an epistemology well-aligned with constructivist ontology and epistemology which similarly state that realities are multiple and co-constructed, and that findings are co-created through interactions of the inquirer and inquired (Guba, 1990; 1996; Guba & Lincoln, 1982; 2005). These core ideas underlie the hermeneutic and dialectic methodologies common to constructivism which aim to elicit individuals' understandings, refine meaning through sufficient discussion, and then convey informed and sophisticated reconstructions (Angen, 2000; Bodner & Orgill, 2007; Lincoln & Guba, 1985).

The main goals of this project include constructing rich accounts of individual experts' understandings of their research and to describe how experts use discursive resources to explain their research. Naturalistic inquiry asserts that realities are multiple and can only be studied

holistically, making it well-aligned with the project goals as well as with the constructivist and situated perspectives described previously, which view individual's meaning-making as inseparable from context. Naturalistic inquiry's sensitivity to social processes and contexts also make it especially well-suited for such studies of disciplinary discourse. Moreover, naturalistic inquiry is characterized by predominantly qualitative methods of inquiry, human interaction as the main research instrument, and purposeful sampling (Lincoln & Guba, 1985). In this project, qualitative methods of inquiry, including semi-structured interviews and content analysis of multiple document types, were employed. Detailed descriptions of the methods employed in each part of this dissertation can be found in the respective chapters.

3.2.2 Analytical Frameworks

3.2.2.1 Analyzing Multiple Modes

Given the assumption that knowledge and ways of knowing are distributed across multiple modes, interpretation of communication should account for language in conjunction with other information-carrying modalities (Gee, 1999; Goodwin, 2000; Roth, 2004; Kress, 2010). For this project, this means considering how expert participants used and integrated several modes – language, ERs, and gestures – to make meaning in explanations of their research. Investigative approaches that consider a range of communicational modes and their relationships, are said to be 'multimodal' (Jewitt, 2017). Four assumptions underpin multimodality: (1) meaning is made and remade through many representational and communicative modes, including language; (2) each mode does different communicative work, and these roles are not fixed but rather articulated and situated; (3) people orchestrate meaning by selecting and configuring various modes so the interaction between them is significant; and (4) the meanings of signs are shaped by social context (Jewitt, 2017).

Data analysis in this dissertation was inspired by multimodal interaction analysis in that the purpose of each mode was considered individually, as well as in relation to other modes whenever expert participants switched between or combined modes (Chapters 4 and 5). Multiple modes were also considered in the development of instructional materials and evaluation of student understanding as discussed in Chapter 6**.**

3.2.2.2 Coding Approach

Several analytical frameworks were employed as part of this project in combination with inductive analysis (Lincoln & Guba, 1985). Explicit coding frameworks (i.e. the MACH and CRM models described below) were combined with inductive analysis as part of the process of re-designing and generating new ideas about the categories of the current frameworks and their properties. A combined approach affords structure and flexibility for an exploratory and emergent project such as this. Explicit coding frameworks afford structure and systematicity in analysis, while an inductive approach provides flexibility and freedom to modify existing categories as well as develop new codes or categories grounded in data. The process of coding, categorizing, describing categories, and re-categorizing various data in this project resulted in the interpretations presented in the Results Chapters (4-6). I briefly describe the MACH and CRM coding frameworks below. Triangulation between different data types (e.g. interviews, textbooks, primary literature) was also employed in order to develop comprehensive understanding (Carter et al., 2014). More detailed descriptions of how the frameworks were used and combined with inductive analysis, can be found in Chapters 4-6.

3.2.2.3 The MACH Model

The study of expert explanations in the context of their research can provide valuable insight into the various meaning-making resources and activities experts employ. Based on case studies of expert biologists explaining cellular and molecular mechanisms relevant to their research, Trujillo et al. (2015) developed the MACH model which identified common components of mechanistic explanations. They found that the experts consistently interwove discussion of research methods (M), analogies (A), social or biological context (C), and descriptions of how (H) a phenomenon operates in their explanations of molecular mechanisms. The MACH model was used to structure the expert interview protocol (see Chapter 4 and Appendix B Table B.2) used in this dissertation. It was also initially employed as an analytical framework to code the expert interview data in Chapter 4, and was subsequently modified into the MAtCH model as discussed in Chapter 4 (Jeffery et al., 2018). In brief, the MAtCH model considers the integration of theoretical (t) knowledge of overarching scientific explanations and models with the previously described components of research explanations, and models the connections between the various components. The MAtCH model is also used as an analytical framework in Chapters 5 and 6.

3.2.2.4 The Concept-Reasoning-Mode (CRM) Model

External representations are essential meaning-making resources in biochemistry, allowing scientists to model the abstract and invisible world of molecular processes. Schönborn and Anderson (2009) developed the Concept-Reasoning-Mode (CRM) model, which is an empirically-based model of factors that affect an individual's ability to interpret ERs in a biochemistry context. Although based on student data, the CRM model can be used as a framework to structure analysis of experts' use of ERs (Anderson, Schönborn, du Plessis, Gupthar, & Hull, 2013). The model posits that successful use of an ER involves the application of appropriate Conceptual knowledge (e.g. biochemical concepts or theories) and Reasoning or sense-making abilities (e.g. think globally, spatially manipulate) to interpret the various symbolic markings which compose the representational Mode (Schönborn & Anderson, 2010). A Venn-logic model, the CRM allows reasoning behaviors to be applied to concepts (R-C), to an ER itself (R-M), or to both simultaneously (CRM). This conveniently lends itself to data analysis by means of generating verb-noun codes where conceptual knowledge can be associated with nouns or noun phrases, reasoning abilities can be associated with verbs, and modes can be associated with nouns referring to components and/or markings of the representations (Anderson et al., 2013). Verb + concept-noun or mode-noun pairs can thus serve as evidence of various reasoning behaviors. The CRM model was employed as an analytical framework in Chapters 5 and 6 of this dissertation, and its application is described in greater detail in Chapter 5 (Jeffery et al., n.d.).

3.2.3 Role of the Researcher

The role of the researcher in interpretivist qualitative research is significant because the collection and interpretation of data depends on the researcher co-creating findings through dialogue with their participants, and then exploring the data based on their values and understanding of the context (Guba & Lincoln, 1982; Lincoln et al., 2011). Rather than attempting to eliminate bias, philosophical hermeneutics, which underlies much constructivist methodology, contends that a researcher's position and interpretations are advantageous in the active production of complex meaning (Gadamer, 2000). This does not preclude the researcher from adopting techniques to enhance the trustworthiness of an analysis (Lincoln & Guba, 1986). Rather, reflexivity – that is, the act of reflecting on ones' past experiences and continually throughout the research process (Goodall, 2000) – is seen as essential to the integrity of qualitative research.

I have a Bachelor of Science degree in Biological Sciences for Secondary Education with minors in Integrated Science and Chemistry from Michigan State University in East Lansing, Michigan. Graduate-level coursework in chemistry, qualitative research methods, and education has also allowed me to gain knowledge and skills related to conducting education research in science. Furthermore, my background in biology, chemistry, and education uniquely situates me to conduct a research study like this, given its position at the intersection of several fields. Having experienced difficulties understanding thermodynamics as an undergraduate, I took special interest in physical chemistry education when I got involved with chemistry education research at Michigan State. I was a learning assistant for general chemistry at Lyman Briggs College at Michigan State for two years; I taught both biology and chemistry at the high school level to receive my teaching certificate; and I have also served as a graduate teaching assistant for a physical chemistry course for life science majors which covers thermodynamics and kinetics at Purdue University for three semesters. These teaching opportunities have given me experience in addressing student difficulties in thermodynamics and kinetics in biology and chemistry contexts.

To increase the trustworthiness of my analysis, I incorporated reflexive techniques like research memoing (Denzin & Lincoln, 2018), evaluating consistency across rounds of coding, and seeking out and paying attention to disconfirming evidence throughout the research process (Antin, Constantine, & Hunt, 2014; Merriam, 2009). I also employed member-checking at various points by soliciting feedback on my interpretations from my expert participants in order to enhance the accuracy of my characterizations (Merriam, 2009).

CHAPTER 4. HOW FOUR SCIENTISTS INTEGRATE THERMODYNAMIC AND KINETIC THEORY, CONTEXT, ANALOGIES, AND METHODS IN PROTEIN-FOLDING AND DYNAMICS RESEARCH: IMPLICATIONS FOR BIOCHEMISTRY INSTRUCTION

A version of this Chapter has been published as: Jeffery, K. A., Pelaez, N., & Anderson, T. R. (2018). How Four Scientists Integrate Thermodynamic and Kinetic Theory, Context, Analogies, and Methods in Protein-Folding and Dynamics Research: Implications for Biochemistry Instruction. CBE—Life Sciences Education, 17(1), ar13. Permission to use this article as a dissertation chapter has been granted, see Appendix A.

4.1 Abstract

To keep biochemistry instruction current and relevant, it is crucial to expose students to cutting-edge scientific research and how experts reason about processes governed by thermodynamics and kinetics such as protein folding and dynamics. This study focuses on how experts explain their research into this topic with the intention of informing instruction. Previous research has modeled how expert biologists incorporate research methods, social or biological context, and analogies when they talk about their research on mechanisms. We used this model as a guiding framework to collect and analyze interview data from four experts. The similarities and differences that emerged from analysis indicate that all experts integrated theoretical knowledge with their research context, methods, and analogies when they explained how phenomena operate, in particular by mapping phenomena to mathematical models; they explored different processes depending on their explanatory aims, but readily transitioned between different perspectives and explanatory models; and they explained thermodynamic and kinetic concepts of relevance to protein folding in different ways that aligned with their particular research methods. We discuss how these findings have important implications for teaching and future educational research.

4.2 Introduction

Recent calls for educational reform in the life sciences have repeatedly encouraged a greater focus on scientific competencies, including the modeling and analysis of complex systems, and the use of analytical and scientific reasoning in ways that are authentic and relevant to current research

(Howard Hughes Medical Institute and Association of American Medical Colleges [HHMI–AAMC], 2009; Brewer & Smith, 2011). This includes a call for greater integration of the physical and mathematical sciences into life sciences like biochemistry to support a deeper student understanding of fundamental scientific principles (National Research Council, 2003; HHMI–AAMC, 2009). In this way, students can develop the competence to address some of the many cutting-edge research questions pursued in the industrial, pharmaceutical, and medical fields that require application of biochemical knowledge and research methods to processes governed by thermodynamic and kinetic principles.

Toward partially addressing these calls for reform, the purpose of the present study was to investigate how selected experts, working at the cutting edge of protein folding and dynamics, use thermodynamics and kinetics with analytical and scientific reasoning and modeling to explain their research. This will, in turn, permit us to pursue our longer-term goal of using such expert data to inform the development of teaching activities in this cognitively demanding but crucial area of undergraduate biochemistry that is central to our understanding of many areas of biochemistry in general. Indeed, in so doing, we would hope that we would go a long way toward addressing the various well-documented conceptual difficulties that students exhibit with thermodynamics and kinetics in biochemistry (e.g., Sears et al., 2007; Wolfson et al., 2014), let alone other science contexts such as chemistry (see reviews by Bain et al., 2014; Bain & Towns, 2016), physics (e.g., Dreyfus et al., 2012; 2013), and engineering (e.g., Meltzer, 2007; Haglund et al., 2015). In the case of biochemistry, the thermodynamics and kinetics of complex, dynamic biochemical processes tend to be difficult for students to understand, a situation that can be exacerbated by the often confusing symbolic language, mathematical descriptions or models, and information-rich visualizations used to represent such processes (e.g., Liu et al., 2016). Thus, we believe that it is crucial to characterize how practicing scientists integrate theoretical and experimental knowledge of biochemical processes, like protein folding and dynamics, as only then will we be better prepared to help students master this complex topic, which is both an integral part of modern undergraduate biochemistry curricula and relevant to current research.

There are five major philosophical models of scientific explanations relevant to research and practice in science education (Braaten & Windschitl, 2011), but for the purposes of this study we broadly define “explanation” to include descriptions of observable phenomena; theoretical accounts of how phenomena progress according to any of the philosophical models; and/or the

process of clarifying ideas, reasoning, and findings regarding a phenomenon (Achinstein, 1983; Salmon, 1989; Knorr-Cetina, 1999). Some suggest that the model of scientific explanation that is most appropriate depends on the purpose(s) of an investigation and its explanatory aims (Van Fraassen, 1980; Craver, 2006; Brigandt, 2010; 2013). For instance, a researcher may provide a statistical–probabilistic explanation relating the occurrence of a disease to trends in environmental factors in order to make health recommendations. Although the underlying cause is not mentioned, the aim of the investigation is a predictive tool, so a mathematical account "suffices" as an explanation. In the life sciences, historical reconstructions and examinations of scientific discourse have enhanced our understanding of scientific explanation, especially of mechanistic processes where explanations specifically seek to establish causal links between agents and events (Machamer et al., 2000; Darden, 2008; Bechtel & Richardson, 2010). A growing number of problems in the life sciences also address emergent phenomena—like protein folding and dynamics—where the overall behavior of the system emerges from underlying random processes rather than a regular sequential mechanistic process (Chi et al., 2012). We aim to further characterize how scientists explain phenomena and their study, with a long-term goal of using the findings to inform the development of more authentic undergraduate life science educational materials to foster the integration of theoretical and experimental knowledge, the understanding of biochemical research methods, and the application of physical principles in the life sciences.

The idea of using expert knowledge to inform student learning is key to the philosophy underpinning this study. Not only is scientific research the primary source of scientific knowledge, but given the sophisticated nature of scientific problems, the study of expert scientific thinking can offer valuable insight into the higher-order cognitive processes educators desire to develop in their students. Research has, for example, shown that scientists employ distant analogies as explanatory devices (Dunbar, 2000) and that analogical reasoning is a crucial cognitive skill for expert biochemists, likely because biochemistry depends heavily on understanding the abstract world of molecular structures and processes (Anderson & Schönborn, 2008; Schönborn & Anderson, 2008, 2009). Previous studies have used information gleaned from the study of expert knowledge and reasoning practices to develop classroom activities, resources, and/or guidelines for connecting levels of biological organization (Van Mil et al., 2013; 2016), developing representational competence in chemistry (Kozma & Russell, 1997), and supporting students in monitoring their explanations of biological mechanisms (Trujillo et al., 2016a). Trujillo et al. (2015; 2016a; 2016b)

provide a detailed example of how knowledge from case studies of expert scientists can be brought into the classroom. Recognizing that science educators would benefit from a clear model of how biologists explain cellular and molecular mechanisms, Trujillo et al. (2015) asked several expert biologists to explain sequential causal mechanisms relevant to their research. They found that those scientists consistently interwove discussion of research methods (M), analogies (A), social or biological context (C), and descriptions of how (H) a phenomenon operates in their explanations of molecular mechanisms and used these themes to develop the MACH model of mechanistic explanations (Trujillo et al., 2015). An iterative design-based process was then used to adapt, test, and modify the MACH model to improve its function as an educational tool to help students construct explanations of biological mechanisms (Trujillo et al., 2016a; 2016b).

Although the MACH model helped students identify and incorporate its constituent components in explanations of mechanisms, Trujillo et al. (2016a) noted that students struggled to make connections between the MACH components and frequently overlooked research methods. The original MACH model does not describe how the components connect, or whether there is any pattern or sequence to their use. Driven by the overarching research question "How do experts explain their research related to protein folding and dynamics?," the present study used the MACH model as a guiding framework for data collection and analysis. The similarities and differences that emerged from analysis of interviews with four experts led us to make the following claims:

1. All four experts integrated their theoretical knowledge and their research context, methods, and analogies when they explained how protein-folding phenomena operate (MAtCH model, Figure 4.1), in particular by mapping phenomena to theoretical mathematical models.
2. All four experts explored different processes depending on their explanatory aims, but readily transitioned between different perspectives and explanatory models.
3. All four experts explained thermodynamic and kinetic concepts of relevance to protein folding in different ways that aligned with their particular research methods.

On the basis of these claims, we propose a revised version of the MACH model that includes the central role of theoretical knowledge. We offer the MAtCH model as a framework that can be used to analyze expert practice and to inform instruction.

4.3 Methods

4.3.1 Selection of Participants

A pool of expert participants from various science departments at a large Midwestern public research university were chosen purposefully based on two criteria used for theoretical sampling (Patton, 2015). Participant selection involved analyzing experts' research profiles to determine whether their current published research 1) is related to protein folding or dynamics and 2) considers kinetic and/or thermodynamic data. By protein folding and dynamics, we mean the physical processes by which a protein changes its three-dimensional structure, including both global (whole-structure) and local (single-atom and partial-structure) deviations in position over time. Once identified, the participants (N = 4) were approached and asked to participate in an approximately hour-long, semi-structured interview about their research. These participants will hereafter be referred to as "experts" or "expert scientists," and pseudonyms will be used to protect their identities. The current research was performed under the approval of the Purdue University Institutional Review Board (protocol #1511016694).

4.3.2 Development and Description of Interview Protocol

The MACH model (Trujillo et al., 2015) was used to structure the interview protocol (see Appendix B Table B.2) to focus on aspects previously identified as prevalent in experts' explanations. In the MACH model, M is operationally defined as the methods of research, including the experimental procedures, techniques, or instruments used to generate data that inform the explanation; A refers to the analogies that help make sense of the mechanism, including formal analogies, representations, and/or narratives; C encompasses the social or biological context that connects the explanation to an important situation in which it can be applied; and H describes how the entities of the phenomenon interact to produce changes of state, activities, and spatial and temporal organization involved with understanding how the phenomenon operates. With this guiding framework, the interview protocol was separated into artificial "phases" that began with a general question regarding the experts' research but then focused on probing the context and experimental methods used by these experts. Several probes were designed to ask experts whether and how they thought about specific thermodynamic concepts typically covered in undergraduate chemistry (e.g., entropy, free energy). As representations are also an integral part of scientific work and communication (e.g., Kozma, 2003), the interview also prompted

participants to draw or show any representations they felt would be useful to gain additional insight into their mental models.

The initial interview protocol was piloted with graduate students who were members of the research labs run by potential research experts. Pilot interviews were audio/video-recorded, and a record of protocol modifications with evidence and reasoning for each modification was updated after each pilot interview. This process allowed the interviewer (KAJ) to test, and if necessary improve, various phrasing and to become more familiar with the interview protocol. For the main portion of the final interview protocol, the participants were asked to explain their research as they would to a colleague or a scientist in a related or similar field. At the end of the interview, with considerations of future educational activities in mind, participants were also asked to explain their research and protein folding in general as they would to a junior or senior-level undergraduate student. Both types of data were collected to obtain a fuller characterization of these experts' explanations of protein folding and dynamics, including accessing any potential pedagogical content knowledge. The purpose and methods of the study were explained to the experts before their participation in the study. Semi-structured interviews were employed, as they allow an interviewer to explore individuals' ideas at great depth and to probe for additional details or clarifications in order to come to a shared understanding, just as might happen in a conversation between two investigators.

4.3.3 Data Processing and Analysis

Interviews lasted between 1 and 1.5 hours and were audio/ video-recorded. As expert use of representations was of interest, the production of representations or use of any computer-based representations was also video-recorded. Interviews were transcribed verbatim, and then portions of the text were aligned with provided representations by reviewing the video recordings. All drawing steps during the production of representations, gestures indicating parts of representations, and captured air gestures were described and inserted into the interview transcript. In this paper, only verbal data and a sample of the representations are examined. Gestures will be the target of future work. Interview transcripts were inductively analyzed (Lincoln & Guba, 1985, p. 203) to identify common concepts, representational modes, and analogies. The first round of analysis of the interview transcripts produced a master list of quotations that contained references to general concepts, and these were then sorted into a number of emerging categories. As the category

descriptions crystallized, categories with fewer quotations/excerpts were removed or merged into other larger categories. Representations were analyzed to describe all the modes of representation used by the experts. Interviews were then analyzed for analogies, which were similarly sorted into emerging categories and then aligned with the previously identified concept categories. This process resulted in the identification of the unique ways these four experts think about thermodynamic and kinetic concepts given their research goals and methods (claim 3), as well as similarities in how they applied knowledge of scientific theories to their research (claim 1). Several excerpts and representations from the interview transcripts were selected to create "expert research profiles" to showcase the unique way each expert approached his or her research. The excerpts were coded with the MACH components, using the operational definitions set forth by Trujillo et al. (2015) described above in the Introduction (Section 4.2). As an example, if the expert referenced the use of an experimental procedure, technique, instrument, or data, this was coded as "M." Initial case analyses were sent to the respective experts to check whether their thoughts were represented accurately. Two of the participants (John and Gertrude) responded, and sentences were revised per their suggestions. A constant comparison method in combination with MACH coding allowed us to characterize similarities and differences in how the four experts transitioned between the MACH components and their theoretical knowledge (claim 1), and how their research goals and methods influenced their explanations (claims 2 and 3). The patterns that emerged from this process are described in the Results and Discussion section (Section 4.4).

4.4 Results and Discussion

Analysis of the interviews revealed that the MACH model components feature prominently in all four expert explanations, with experts frequently connecting and integrating the components. Furthermore, each expert's explanation revealed clear connections between the MACH components and his or her knowledge of scientific theories. The amount of integration between the MACH components and theory made it difficult to organize the interview data in an easily understandable sequence. It became evident during analysis that all four experts integrated research context, methods, analogies, and how the phenomenon operates with their theoretical knowledge when explaining their research projects (claim 1). All four cases demonstrate this complex integration of components, but a general pattern of connections between the MACH components and theoretical knowledge emerged from the data. This pattern led us to propose a

modified MACH model, or MAtCH model (Figure 4.1), which incorporates a new component, "theory." By "theory," we refer to the experts' knowledge of overarching scientific explanations and models (e.g., collision theory or mathematical models of reaction kinetics) used by these experts when talking about their research. We situated the theory component at the center of the MAtCH model, because theoretical knowledge underpinned each of the MACH components and was used by the experts to mediate between the components. As the reader will see, the experts' use of theoretical knowledge was often implicit or tacit in their explanations, but at other times they made it explicit. We have left the "t" in lowercase to emphasize the foundational role of theoretical knowledge in each of these components and explanations. For reader convenience, we first present a diagram of the MAtCH model (Figure 4.1), after which we use our analysis to illustrate how the data support its structure.

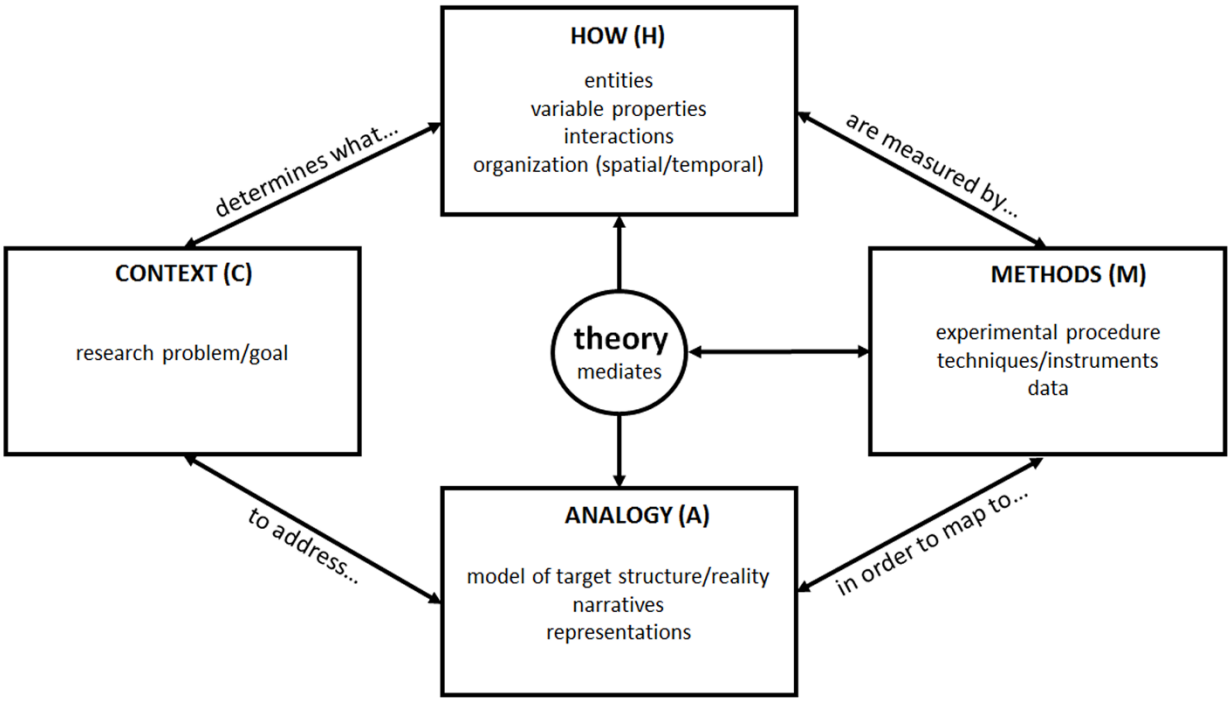


Figure 4.1 The MAtCH Model.

A simplified pattern of integration and connection between the MAtCH components was reflected in interviews of four experts who explained their protein-folding and dynamics research. The connections indicate a pattern, but the ways in which each connection was made differed for each scientist as highlighted in their research profiles. Please note that there is no specific direction or sequence, nor is there complete separation of components. The arrows are double-headed to reflect how the four experts moved back and forth between the components in their explanations, with the words near the arrows creating a sentence when read clockwise. This was done to simplify the relationships between components without making the diagram overly complex.

The structure of the MAtCH model will be used to introduce each of the expert research profiles, starting from their research context (C) and moving clockwise to first describe the entities they consider (H) and the methods by which they are measured (M) in their efforts to develop narrative or representational models (A) of a phenomenon. Although the research described in this paper could be considered very complex, we believe that following the order of the MAtCH model in Figure 4.1 allows us to make sense of these experts' explanations. In the same way that students might use the MAtCH model to follow a simplified story of complex research, this model is used here to guide a description of each scientists' research project while maintaining the connections between the MACH components and the theoretical knowledge the scientists used. For enhanced

readability, the original MACH components will be indicated with the appropriate letter in parentheses in the analysis.

Throughout our analysis, we will also highlight the different ways in which the experts demonstrate the inseparability of the components of the MAtCH model in explanations of their research. We will indicate where the experts use knowledge of scientific theories and models (the "t" in MAtCH) to mediate between the MACH components, particularly by mapping phenomena to mathematical models. By this, we mean the way these experts interpret symbols, theoretical concepts (often represented symbolically), or formulas (A) through knowledge of physical systems such that they represent entities, states, processes, and/or measurable variables (H/M). Furthermore, we will use these four cases to illustrate how the experts explain thermodynamic and kinetic concepts in different ways closely aligned with the research methods they employ (claim 3). In this section, we present all four cases separately. The last two cases (Gertrude and William) are summarized, and full analyses can be found in Appendix B. We then return to our three main claims in the Summary and Conclusions section (Section 4.5) , where we briefly compare the experts' explanations, reflecting on their similarities (claim 1) and differences (claims 2 and 3).

4.4.1 Beaker Elucidates Enzyme Mechanisms

Beaker and his research group focus on how enzymes recognize substrates in order to design drugs and enzymes (C). One of their broad aims is to understand how a protein recognizes and catalyzes a reaction with a substrate (H). Because their focus is on understanding mechanisms (H), they collect data on structure and structural movement through techniques like x-ray crystallography, site-directed mutagenesis, and stroboscopic methods (M). These data (M) are used to map out the positions and movements of specific amino acid residues or protein domains in the active site along a reaction trajectory to propose a mechanism (H/A). In his discussion, Beaker focuses mainly on structural relations like proximity, orientation, and angle (H), consistently using theoretical knowledge of mathematical models of reaction kinetics, steric effects, and interactions to interpret data (M) and to explain the organization and activities of entities in the proposed mechanism (H/A). Beaker's first excerpt in Figure 4.2a showcases how he uses theoretical knowledge to mediate between the H and M components in the MAtCH model to propose a mechanism via a narrative (A). We can also see how Beaker assigns meaning to mathematical models and symbols (A) during this process.

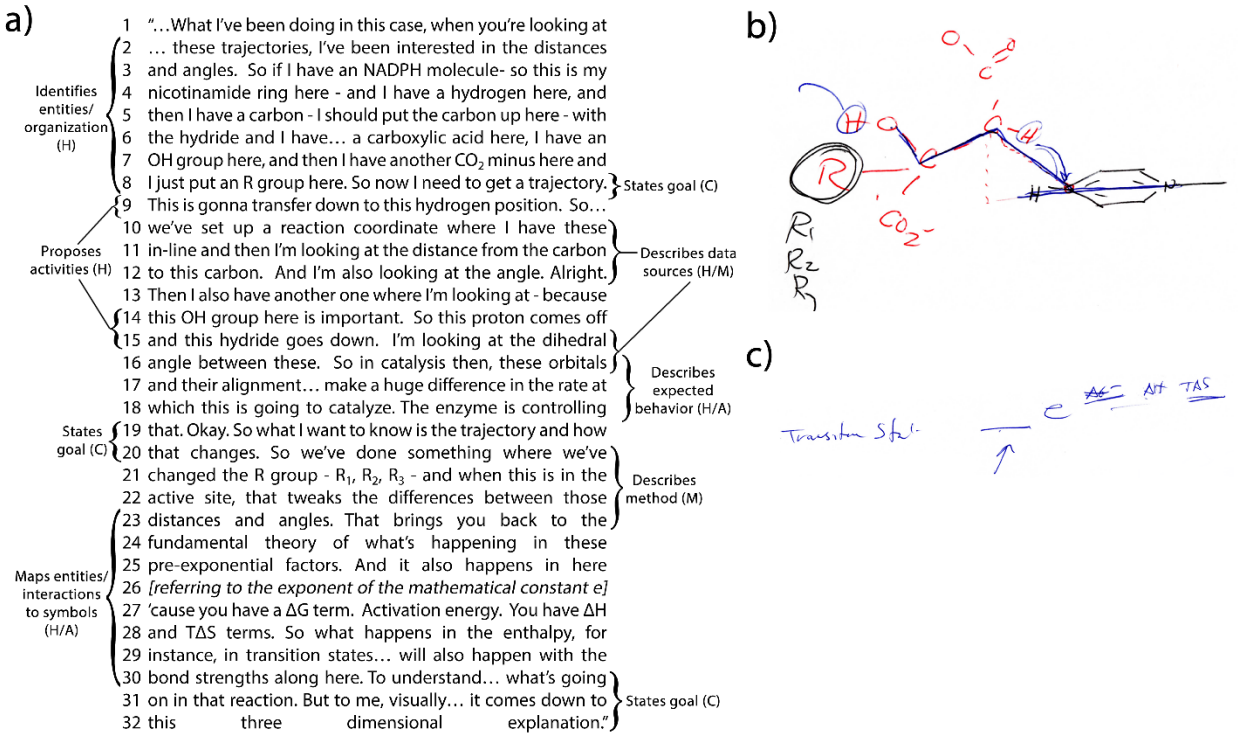


Figure 4.2 Beaker draws and explains the structural data he collects and interprets to determine reaction mechanisms.

Beaker first constructs a representation of a protein active site with several residues (drawn in red) and a ligand (NADPH, black ring to the right) pictured in b. The line bisecting the ring of the NADPH molecule serves as a reaction coordinate. In a, Beaker mentions his research goal is to understand the trajectory of a reaction, and this remains implicit as he discusses research methods until it is mentioned again. He describes some of the activities that will take place along that trajectory, for example, circling two hydrogens in b in blue and using a line or arrow to show their movement. Beaker describes the types of data he will collect so that he can model the reaction trajectory, such as the dihedral angle indicated in b by the blue line tracing from the carbon on the nicotinamide ring to the carbon with the hydroxyl group or distances indicated by the dotted red lines. He uses theoretical knowledge of orbital alignment and reaction kinetics to support his use of research methods focused on determining angles and distances between the entities in b. Beaker also explains how the modification of R groups (black R is changed to R1, R2, etc., at the left side of b) will affect those distances and angles, and thus the rate of catalysis, which will enable him to better model the reaction trajectory. He explicitly relates all of these data back to theoretical knowledge and mathematical models of kinetics and thermodynamics, represented by formulas such as the one in c. He explains how distance and angle are included in pre-exponential factors (the underlined area indicated by an arrow in c), and how enthalpy (ΔH) and entropy (ΔS) are included as factors that affect activation energy in the exponent of e.

In his excerpt, Beaker starts by describing the organization of residues in the active site and the NADPH molecule (H, lines 2–8). He represents each of these physical entities and their organization in a drawing (A, Figure 4.2b). Then Beaker connects the H and M components of the MAtCH model as he describes what he measures about these particular entities (i.e., distance, angle; H/M, lines 10–12, 15–16). At this point, Beaker enters a cycle wherein he uses his theoretical knowledge to constantly mediate among the H, M, and A components of the MAtCH model in his efforts to understand the reaction mechanism (C, lines 8, 19–20). In his narrative (A), he proposes activities for some of the entities (H, lines 9, 14–15) and interprets data (M) in light of his theoretical knowledge about orbitals, reaction kinetics, and the importance of alignment to make a general claim regarding how he expects the system will behave (H/A, lines 16–18). After making this claim, Beaker restates what he wants to measure about the system (H) and describes a specific R group modification method for doing so (M, lines 20–23). As stated elsewhere in his interview, these data will allow him to construct a model of the active site and the reaction trajectory (H/A), furthering his understanding of the reaction mechanism (C, lines 30–32). As part of this cycle, Beaker uses his theoretical knowledge to explicitly connect the measurable and molecular worlds through the interpretation of mathematical models of reaction kinetics and thermodynamics as represented by formulas (A, lines 23–30). He does this by assigning meaning to the mathematical models by mapping entities and interactions (H) to particular symbols (A; e.g., lines 23–25; see Figure 4.2c, where alignment information is represented in pre-exponential factors).

Although Beaker mentions thermodynamic quantities in the excerpt, as a result of his focus on elucidating mechanism, he does not assign much significance to thermodynamic values. He states later that this is because they only indicate that something has happened, but not what or how. Therefore, it seems appropriate that Beaker focuses on collecting data (M) that will inform causal mechanistic explanations, and he thinks about the theoretical concepts of enthalpy and free energy in ways important to mechanisms by considering bond and interaction strengths (H). We can see evidence for this at the end of the excerpt (lines 21–23) as well as elsewhere in the interview when Beaker uses a dose of ibuprofen for treating a headache as a formal analogy (A) to explain the difference in ΔG values of different states (see Appendix B).

Throughout his interview, Beaker consistently makes similar connections between theoretical thermodynamic and kinetic concepts and the interactions of entities (H). He does this

by transitioning between narrative about generic models based on his knowledge of scientific theories and mathematical models, and more specific models (A) of interacting entities and their organization in a system (H). One such example is found in the next excerpt we discuss. This particular excerpt was chosen because Beaker devoted a significant amount of his discussion to the importance of spatial organization (H) and reaction kinetics (M) in elucidating enzyme mechanisms (C). To contextualize this excerpt, Beaker was claiming that there are very few examples of how enzymes work in detail, that is, their motions, distances, and angles along a reaction trajectory (H/M). In his opinion, this is partly because enzyme mechanisms have typically been studied using indirect methods (M) and partly because scientists over the years have reinterpreted and "rediscovered" the original model Linus Pauling proposed (Pauling, 1946)—that enzymes work by binding to the reaction transition state. In the excerpt in Figure 4.3a, Beaker essentially makes an argument for the concepts of proximity, orientation, and complementary binding (H) underlying Pauling's original model through the use of a representation and narrative of a hypothetical two-substrate reaction mechanism (A).

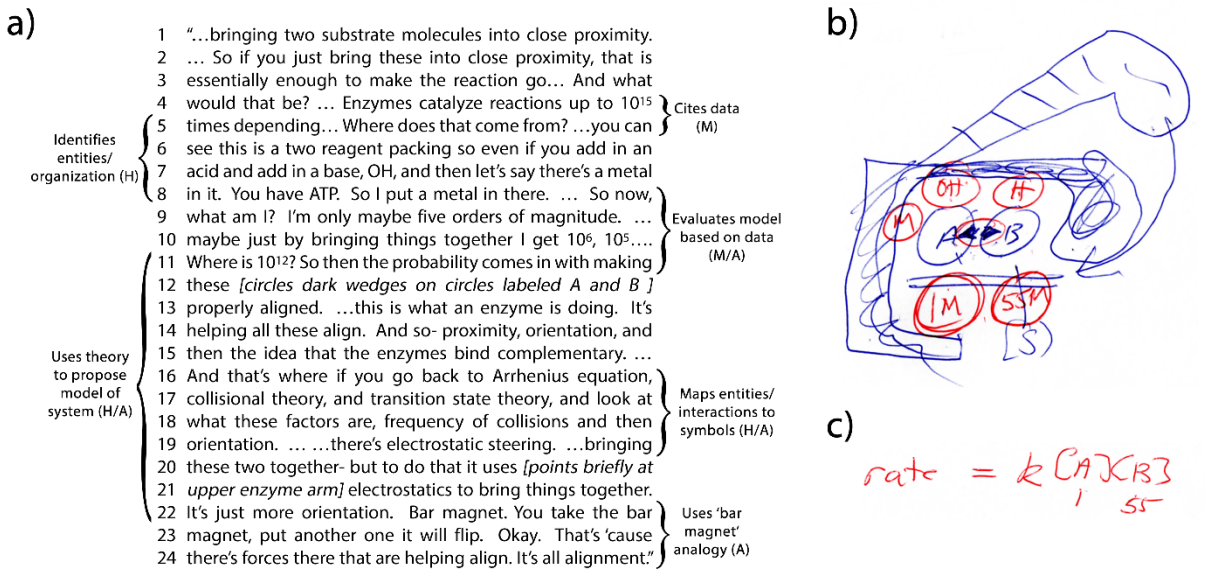


Figure 4.3 Beaker explains rate enhancement in an enzyme active site.

In a, Beaker begins by citing the most basic conditions for a reaction according to theory: bringing two reactants, like A and B in the blue circles in b, into proximity. Using a generic form of a rate law shown in c, Beaker assumes 1 M and 55 M concentrations for reactants A and B to illustrate that bringing reactants into close proximity provides a rate enhancement that is negligible in comparison to data for enzymes. Beaker then uses the rate law in c to estimate rate enhancement after the addition of multiple other reagents (red circles H, OH, and M in b) at 55 M each to again illustrate that rate enhancement is a negligible 105 or 106 in comparison with enzymatic data at 1012. Thus, enzyme rate enhancement cannot be due to concentration alone. Using theoretical knowledge of factors that increase reaction rate, like probability, proximity, and orientation represented by mathematical formulas elsewhere (e.g., Figure 4.2c), he proposes a model in which the cartoon enzyme in b uses its upper arm to bring the reactants together and appropriately orient them (where the darkened blue triangles on reactants A and B in b represent the structural parts that must be aligned). Beaker uses a bar magnet analogy to explain how the cartoon enzyme uses electrostatic forces to aid the alignment of the reactants. Thus, he uses this excerpt to explain that enzymatic rate enhancement is ultimately the result of purposeful spatial organization by the enzyme leading to specific orientations and interactions.

In this excerpt, Beaker once again uses his theoretical knowledge to mediate between the H, M, and A components of the MAtCH model as part of a cycle. He begins by connecting the H and A components as he describes the organization of a variety of entities (H, lines 5–8) in a hypothetical two-substrate reaction mechanism (A; see also Figure 4.3b). He then uses his theoretical knowledge of mathematical models of reaction kinetics, represented by a rate law equation (A; see Figure 4.3c), to model this hypothetical reaction and to illustrate that concentration alone cannot account for the observed enhancement of enzymatic rate from 10^5 or 10^6 to 10^{12}. Because data on observed rates (M) cannot be mapped onto such a simple mathematical model, the equation is insufficient to represent reality (A, lines 8–11). Beaker then uses theoretical knowledge to propose that if, however, the function of the model enzyme is to bring the appropriate substrates into proximity with the appropriate orientation/alignment in order to react (H), as suggested by transition-state theory and mathematical models like that represented by the Arrhenius equation (A), then he has a reasonable model of the system (H/A, lines 11–22). In this process, Beaker again uses theoretical knowledge to connect the measurable and molecular worlds, namely by assigning meaning to mathematical models of reaction kinetics by mapping entities and interactions (H) to equations and symbols (A). There are additional instances in his interview when Beaker makes similar connections. For example, Beaker indicates that he always thinks about the Henderson-Hasselbalch equation (A) and pK_a values when considering an active site, because the ionization state, and thus the structure, of certain residues can differ (H) depending on the pH (i.e., entities have variable properties). Beaker also uses theoretical knowledge of mathematical models to relate the energetics of steric hindrance, interaction strength, and structure (H) with functionality (C). For example, by determining the actual distance between residues (M), he can use mathematical models of electrostatics (A) to reason about why the system behaves a certain way (H). All of these considerations provide him with rich data that he can use to inform enzyme and drug design. We see in the next case that John similarly assigns meaning to mathematical models of reaction kinetics to think about both his research methods and how protein-folding phenomena operate.

4.4.2 John Investigates Protein Stability with Proteolysis Kinetics

John is interested in how globular proteins lose their structure in order to understand more about protein rigidity and longevity and to engineer more robust proteins for function in harsher

conditions or for longer shelf-lives (C). John's research group investigates how partially unfolded nonnative protein conformations that are in equilibrium with native proteins lose their structure (H). In his interview, John focuses on the use of proteolysis kinetics (M) to measure how often a protein loses its structure (H). If, for example, the addition of a mutation changes which region of a protein is digested or alters the rate of proteolysis (M), this suggests that the mutation has changed the relative stability of the partially unfolded forms of the protein (H). From this kinetic data (M), John derives change in free energy values to estimate the relative stabilities of folded and partially unfolded proteins (H) and maps such results onto different representations (A). In the excerpt in Figure 4.4a, John provides a simplified narrative (A) of his proteolysis kinetics method, in which we can see how he uses kinetic theory to integrate the H and M components of the MAtCH against the backdrop of several related representations (A; Figure 4.4b). We also see evidence of how John assigns meaning to mathematical models by linking symbols (A) to entities or measurable variables (H) and of his unique understanding of thermodynamic and kinetic concepts.

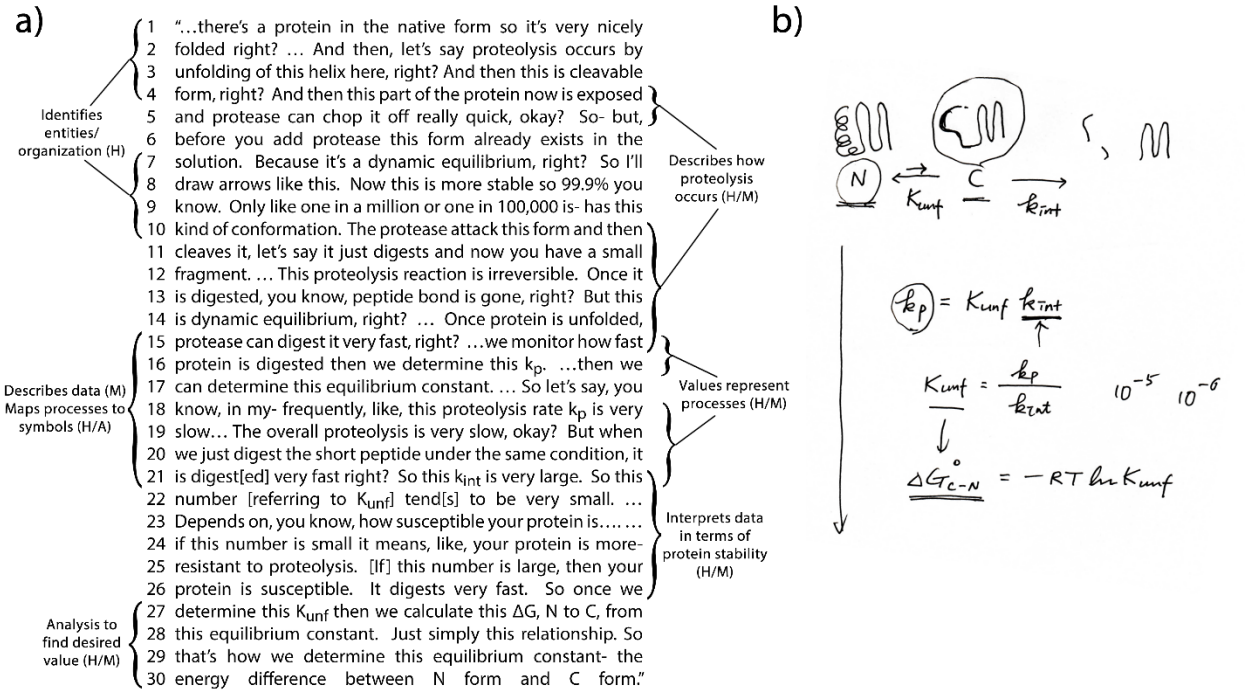


Figure 4.4 John outlines his method to determine the energy difference between folded and partially unfolded proteins.

John first describes two processes undergone by proteins in his proteolysis kinetics method: the folding–unfolding equilibrium of native folded protein (N) to cleavable partially unfolded protein (C), and the proteolysis of the cleavable form. He represents these processes with cartoons and several constants at the top of b. The size of the equilibrium arrows represents the relative populations of each form, while the unidirectional arrow represents the irreversible proteolysis reaction. John explains that they monitor the proteolysis reaction that produces fragments (later measured by gel electrophoresis) and use these data to determine K_{unf}, the equilibrium constant for unfolding; k_{int}, the intrinsic rate constant for proteolysis; and then the product of these two variables, which represents the overall proteolysis rate, k_p. k_{int} is approximated using the unstructured peptide or a generic peptide substrate if the sequence is unknown. Toward the end of the excerpt in a, John provides an example of how digestion rates relate to relative values of variables, providing examples of small K_{unf} values on the right (10^{-5} and 10^{-6}) of b. The series of mathematical formulas in b shows how John uses theoretical knowledge to relate kinetics and kinetic data to thermodynamics to calculate ΔG and to numerically describe the susceptibility/stability of the protein.

In the excerpt in Figure 4.4, John uses his theoretical knowledge of kinetics and equilibrium to repeatedly cycle through the H, M, and A components of the MAtCH model. He seamlessly moves between a description of the interacting entities and their activities in his method (H/M), his data measuring that process (M), and a mathematical model of the system as represented through a series of equations (A; see Figure 4.4b). John first draws connections between the H and M components. He describes the dynamic equilibrium that naturally exists between the native and nonnative conformations of a protein (an entity with variable states, H, lines 1–4, 7–10), and intersperses this description with a discussion of how proteolysis occurs (H/M, lines 4–5, 10–15) and how his method gives several kinetic values (M, lines 15–21). Each of these values represents a different process in the system (H/M, lines 15–16, 18–21; also see Figure 4.4b). We can see that John assigns meaning to mathematical models by using his theoretical knowledge of kinetics and equilibrium to map processes in the system (H) to particular symbols (A) and to connect their relative measured values (M) to what they imply about the susceptibility or stability of the protein (H, lines 21–26). This enables John to use these equations (A) to mediate between the interacting molecular entities of the folded–unfolded–digested protein system (H) and the measurable world of data (M). It is also significant to note that John closely intertwines kinetic and thermodynamic theoretical concepts during his explanation. This unique integration is critical to how John relates the variable states of protein molecules to the abstract idea of their relative stability (H). The excerpt in Figure 4.5a provides additional evidence of the unique way John does this.

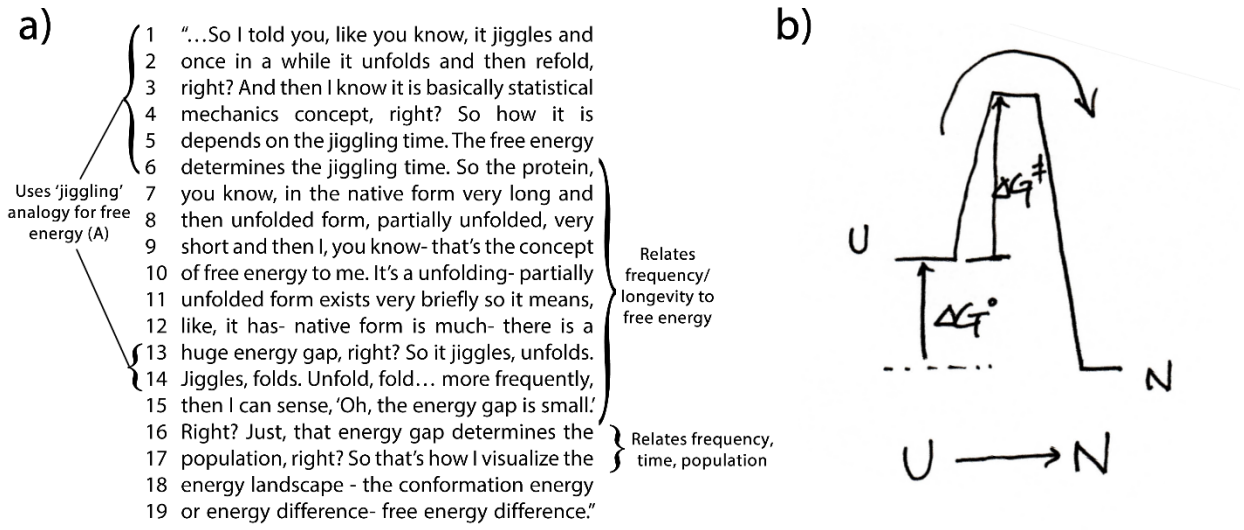


Figure 4.5 John describes how he relates protein movement to free energy.

John describes how a protein will "jiggle," relating the concept of free energy to the time it takes for it to "jiggle" in or out of a particular conformation. He also states that the time a protein spends in a particular form and the frequency at which a protein changes form are representative of the difference in free energy between conformations. This difference determines the populations of the conformations. In an earlier part of the interview, John used the kinetic barrier diagram in b to similarly relate speed of protein folding to the concept of free energy. $\Delta G°$ represents the difference in free energy between the unfolded (U) and native (N) protein conformations as environmental conditions change. $\Delta G^{‡}$ canonically represents activation energy. For this diagram, John explains that the time it takes for the protein to fold (the kinetics), which he represents with an arrow over the top of the diagram, determines the height of the barrier (the energy difference). The excerpt in a and the picture in b serve as further evidence of how John closely intertwines kinetic and thermodynamic concepts in a way that aligns with his experimental methods.

Throughout his interview, John talks about how proteins "jiggle" or have a "jiggling time," which he relates to free energy (A, lines 1–6, 13–14). To John, the frequency at which a protein loses its structure and/or its longevity in a particular form appear to be physical manifestations of free energy (lines 6–15). John also interweaves frequency, time, and relative population of protein conformations (lines 16–17 in this excerpt) with the concept of free energy through statements like "This is a rare conformation so its free energy is much higher," or "How frequently that would happen... Is it one of one million? Or one of ten thousand?" This temporal way of thinking about free energy (A; see also Figure 4.5b) aligns with John's use of proteolysis kinetics (M) to estimate ΔG values. It is not apparent whether John's conception of free energy is influenced by the methods he uses or whether he chose those methods because of his understanding of thermodynamics and kinetics. Thus, the kinetic data that John collects allow him to better understand the energetics of partial unfolding in proteins in order to engineer improved proteins. In the following case, we see how Gertrude uses a fundamentally similar method to study the stability of protein drugs.

4.4.3 Gertrude Investigates Protein Drug Shelf-Life

Gertrude is interested in the physical and chemical modification processes undergone by lyophilized (i.e., freeze-dried) protein drugs in order to improve drug formulations and enhance shelf-life (C). These drug formulations include excipients, which are inactive substances that serve as vehicles for delivering drugs or other active ingredients. Her research group considers the extent to which protein drugs unfold and how they aggregate when they are unfolded or partially unfolded (H). The degree of unfolding is determined by hydrogen–deuterium exchange (HDX): lyophilized protein powders are exposed to deuterium vapor and the resulting peptide mass is measured with a mass spectrometer (M). These data are then used to create representations (A) reflecting deuterium incorporation, indicating what regions of the protein drugs remain protected during unfolding (H). Gertrude's case provides a clear example of the presence and integration of the MACH model components and the implicit role of theory in her explanations (see Appendix B for full analysis).

Gertrude makes distinct connections between the data collected (M), how they are represented (A), what entities and interactions are described in the system (H), and what that implies about functionality (C). As she cycles through components in her discussion, she explicitly and implicitly employs theoretical knowledge of protein structure, inter- and intra-molecular

interactions, and equilibrium. For example, she explains how an increase in mass via HDX (M) allows her to measure the exposure/protection of regions of protein structure (H), mapping data directly onto three-dimensional representations of protein structure (A). These data can then be correlated with a drug's stability as a dry solid (C). Gertrude also examines the interactions of protein drugs and their organization in space and over time (H), employing her theoretical knowledge to suggest a hypothetical model (in narrative form) of what may occur in a protein–excipient system (H/A). During this process, she integrates knowledge of a suggested "hydrogen bond replacement theory" from her field and connects her hypothetical model of the protein–excipient system (H/A) to her research goal of predicting good excipients (C). Gertrude uses her theoretical knowledge to closely relate HDX (M) to the scale of unfolding and interactions between entities in the phenomenon (H) through a narrative story (A).

Gertrude also uses representations (A) as backdrops during her discussion. For example, her research group also investigates protein aggregation, because proteins that become partially unfolded after lyophilization have a tendency to form aggregates (H) when they are reconstituted and potentially cause immune responses in patients (C; e.g. see Ratanji et al., 2013). The kinetics and equilibria underlying the episodic incorporation of deuterium into the partially unfolded proteins are particularly important, as the amount of deuterium that is incorporated over time (M) reflects how fast residues become buried in the aggregated form and where residues are buried (i.e., the aggregation interface; H). As before, theoretical knowledge plays a critical role in this process by allowing Gertrude to mediate between the representation (A) of the measurable world of HDX data (M) and the molecular world of interacting entities (H). Gertrude's research enables her to more quickly make inferences regarding which peptide drug formulations will have longer shelf-lives through the application of HDX methods. We can see in the following case how William's efforts similarly aim to improve predictions but address an entirely different research problem.

4.4.4 William Simulates Protein Dynamics to Improve Drug Metabolism Prediction

William's work focuses on incorporating protein dynamics into computational models (M/A) in order to improve predictions about where drug candidates are metabolized and by which enzymes, so as to aid the development of more metabolically stable drugs (C/H). Unlike the other experts interviewed, William's goal is the development of a predictive method to model possible drug and

protein movements and interactions (M/A), which is validated and trained using experimental site metabolism data (M). The end product of his research—a process incorporating a variety of techniques like molecular dynamics simulations, molecular docking, and statistical techniques (M/A)—can then be used to produce data of its own (M). By considering protein dynamics (which he defines as the trajectories of atoms and residues in a protein [H]), he can produce an ensemble of protein structures to represent the multitude of possible conformations and average them to suggest the most likely preferred conformation (M/A). This conformation can then be used in the simulated docking of drug candidates to make predictions (M/A). Because of his research goal, and the computer model-based nature of his research, the H, M, and A components are completely integrated in William's discussion and his understanding of thermodynamics similarly appears to intertwine or align with his simulations (A). The MAtCH model allows us to make sense of this complexity by focusing on the connections (see Appendix B for additional analysis).

In his interview, William describes how the structural components of proteins might change their spatial organization to accommodate drug compounds (H). He argues that, because alternative structural states (i.e., dynamics) can affect the prediction of a compound's distance in relation to the catalytic center (M/H), including dynamics in simulations (M/A) is critical to improving the predictive capabilities of current methods (C). William's tacit use of theoretical knowledge allows him to productively mediate between the measurable world (M) and what it implies about the molecular world of (simulated) protein structures and their interactions (H/A). William's discussion shows that he relates residue flexibility to protein dynamics and that he also has a unique way of assigning meaning to theoretical thermodynamic concepts. William's understandings of enthalpy, entropy, and free energy appear to align with his simulations (M/A) and are mapped to entities, interactions, and states of a protein system (H). For example, he makes the concept of entropy tangible as "How much an object is moving. How dynamic it is..." (i.e., structural flexibility) and he connects it to temperature and the velocity of particles (H). He describes enthalpy as internal or potential energy but also associates it with the sum of interactions and interaction strength (H). William states that both entropy and enthalpy must be considered to determine the actual preferred state of the system and explains how, in his simulations (A), temperature can be "turn[ed] on" to allow protein dynamics (entropy), and the resulting different states have different kinds of interactions (enthalpy; H). William explains that, if protein dynamics

are ignored, “you don’t have entropy, you’re not calculating ΔG’s,” and the result is incorrect predictions for ligand binding (M/A) and unreliable predictions about drug candidates (C).

Throughout his discussion, William assigns meaning to mathematical models by mapping entities, interactions, and variable states (H) to particular symbols in formulas and graphs (A). At one point, William discusses the difficulties his students seem to have interpreting data (M). He explains how, to him, a change in free energy on a graph (A) reflects underlying changes in structural movement and/or the formation of new interactions (H) in the simulation (A). It also indicates he must look at the simulated protein system (A) to interpret the possible structural cause (H/A) of the data (M). According to William, while producing a numerical or graphical output is doable for students in his lab, interpreting and making connections between the data (M) and the underlying (simulated) physical causes (H/A) are not as obvious. Thus, a combination of experimental and simulated data enables William to improve current methods used to predict the metabolism of drug candidates.

4.5 Summary and Conclusions

The present study explored how four scientists integrate thermodynamic and kinetic theories, analogies, and research goals and methods in explanations of research projects related to protein folding and dynamics. What differentiates our study from extant accounts of expert explanatory practices is that it compares how several experts understand their work in the context of their research goals and methods as they work on projects at the intersection of physical and biological sciences. Within this context, our study attends to the structure of these experts’ explanations, as well as the central and underlying role of thermodynamic and kinetic theories that are typically covered at the undergraduate level. Current research has begun to characterize components of explanations but does not examine how data from particular research contexts are incorporated as evidence with the intent to inform instruction. Four explanations of research projects were analyzed, ranging in context from enzyme mechanism elucidation (Beaker), to globular protein stability (John), to protein drug shelf-life (Gertrude), and protein dynamics simulations (William). From these data we make the following claims, which we briefly discuss below:

- All four experts integrated their theoretical knowledge and their research context, methods, and analogies when they explained how protein-folding phenomena operate (MAtCH

model, Figure 4.1), in particular by mapping phenomena to theoretical mathematical models.

- All four experts explored different processes, depending on their explanatory aims, but readily transitioned between different perspectives and explanatory models.
- All four experts explained thermodynamic and kinetic concepts of relevance to protein folding in different ways that aligned with their particular research methods.

Claim 1: All four experts integrated their theoretical knowledge and their research context, methods, and analogies when they explained how protein folding phenomena operate, in particular by mapping phenomena to theoretical mathematical models. Experts' common integration of the MACH components and theoretical knowledge in their explanations led us to propose the MAtCH model (Figure 4.1). For the purpose of simplifying our data analysis, we attempted to separate the experts' explanations into the individual components, though in reality there was no clear separation of these components, nor was there any specific sequence in which the components were used by each expert. We found that, by attempting to separate the experts' explanations into the MACH components, we were able to track the complex connections between what they study (H), how they study it (M/A), why it is important (C), and the theoretical knowledge (t) underpinning the components according to how the experts mediated among them. Whereas the original MACH model identified components of expert explanations of cellular and molecular mechanisms, the MAtCH model provides a framework that can be used to recognize the role of theory in tying the components together in explanations of research. Overall, we found that these experts address the social or biological importance of their research in their opening statements and do not appear to immediately move from statements of research goals (C) to experimental methods (M), but rather do this by way of interacting entities (H) or models of entities involved (A). In constructing their explanations, the experts consistently use knowledge of scientific theories and mathematical models to cycle between the how, methods, and analogy components, integrating that knowledge with experimental data (M) and various models of reality in narrative and representational forms (A) to discuss the interacting entities of the phenomenon (H). For example, both John's and Gertrude's research methods rely on knowledge of mathematical models of kinetics and equilibrium, and this knowledge allows them to relate their methods (M) and representations of data (M/A) to specific interacting entities (H) involved in those

processes. Theoretical knowledge and mathematical models in particular are key to how these experts mediate between a molecular-level description of a phenomenon (H) and the measurable world of data and data representations (M/A). To do this, the experts map entities, states, interactions, and processes (H) to formulas (A) representing mathematical models via measurable variables (M). For instance, Beaker connects the collision of entities to variables in rate laws and the Arrhenius equation, whereas William connects particle movement and protein flexibility to entropy and temperature. As Schuchardt and Schunn (2016) suggest, and as our case studies support, it is the context behind the mathematical representation that determines whether it is seen as a model of a phenomenon or a calculated procedure. The integrated nature of the MAtCH components suggests that explaining how a phenomenon operates (H) in practice may be inseparable from how we measure it (M) and the theories (t), mathematical concepts, and analogies (A) we bring to bear on it (see also Boumans, 1999).

Claim 2: All four experts explored different processes depending on their explanatory aims, but readily transitioned between different perspectives and explanatory models. Analyzing the explanations according to the MAtCH model also helped us consider how scientists' research goals influenced their methods and types of explanations. We found that, despite all four experts addressing research problems involving protein folding and dynamics, they did so in different ways and for different reasons. Differences in research goals (C) led the experts to explore different types of processes (H) and to collect data (M) for different explanatory aims (Brigandt, 2013). We found that the experts considered protein folding and dynamics from both emergent and sequential perspectives, depending on their research goals. Beaker, the only expert who was chiefly concerned with mechanism in our study (C), mainly focused on methods to observe and perturb a system in order to seek underlying cause–effect relationships (causal explanation) and describe the order of events in an enzyme mechanism (a sequential process). The other three experts—John, Gertrude, and William—focused their discussion on describing causal relationships in emergent processes (H) or methods (M) based on emergent processes, making inductions from trends in data (statistical– probabilistic explanation). The latter is a decidedly different research goal (C) from establishing causation. For example, John and Gertrude used proteolysis kinetics and HDX, respectively, to make inferences about structural stability. Seeking the underlying causes of events was not their predominant research goal, possibly because their projects focused on emergent processes, which cannot be reduced into sequences of subevents.

While John, Gertrude, and William, like Beaker, described causal relationships among entities, properties, and interactions for emergent processes, they did so without suggesting a cause–effect chain of events. Instead, they described the actors (entities) and their roles (interactions) without an order to events, as one would expect in a narrative. They made references to multiple states of the system. Furthermore, all four experts had instances in which they transitioned between statistical–probabilistic and causal explanations, or between describing sequential and emergent processes as part of explaining their methods (M) or the phenomena (H) they study. We believe this highlights that these experts used and combined a variety of explanatory models; which model is employed in a particular instance depends on the nature of the process being explained and the explanatory aims of the research. John, for example, offered a sequential–causal explanation to describe his proteolysis kinetics method (M), but his description of the equilibrium between folded and cleavable forms of a protein (H) reflected the "collective summing" characteristic of emergent processes (Chi et al., 2012). In regard to his research goals (C), he focused on what kinetic data (M) imply about protein stability (H) rather than on establishing causation, which is characteristic of a statistical–probabilistic explanation (Braaten & Windschitl, 2011). As another illustration, Beaker referenced diffusion and collision frequency (emergent processes) in determining reaction rate, but such processes are secondary to the importance of proximity and orientation (H) in determining a mechanism of enzyme catalysis (sequential process). In a sense, the emergent processes operated at a hierarchical level (Machamer et al., 2000) below where Beaker's research goals (C) and methods (M) were concerned, but he pulled them into his explanation where appropriate.

Talanquer (personal communication) offers another perspective on this, suggesting that there are three levels to mechanistic explanation: the macroscopic–phenomenological, particulate–mechanistic, and particulate–structural, and it is possible for explanations to be hybrids of more than one level. From the perspective of the MAtCH model, the explanations here suggest something similar: experts interweave discussion of measurable (M) system behavior (macroscopic–phenomenological) with discussion of collisions and forces (particulate–mechanistic) and interactions or properties resulting from structure (particulate–structural; H), and do so for both sequential and emergent processes. For example, in one of his excerpts, Beaker explained how the (measurable) enhancement of a reaction rate by an enzyme (macroscopic–phenomenological) cannot be explained entirely by frequency of collisions (particulate–

mechanistic) but must consider how structures in the active site orient reactants in close proximity (particulate–structural). The properties of entities, or the "particulate–structural level," were repeatedly highlighted in these experts' explanations as they used structure or structural properties to make predictions even when they did not have a particular mechanism in mind, regardless of whether they were considering the phenomenon from an emergent or a sequential perspective. For example, William and Beaker discussed the significance of entities' properties (e.g., charged, hydrophobic) on interactions in the system. Whether they focused on emergent or sequential processes, structure appears to be a powerful predictive tool for these experts.

Claim 3: The four experts explained thermodynamic and kinetic concepts of relevance to protein folding in different ways that were aligned with their different research methods. The data also revealed that the experts explained thermodynamic and kinetic concepts in multiple, functionally useful ways, closely aligned with their research methods. Beaker remarked that thermodynamic data do not provide mechanistic information about *how* something occurred, only that something may have changed, so he devotes less attention to thermodynamics. Even so, Beaker's discussions of entropy and enthalpy reflect a focus on structure and mechanism: enthalpy is connected to interactions, and entropy is connected to the movement of molecules from a more organized or restricted state to one of greater disorder (e.g., the displacement of water from an active site). John's aim was to measure a thermodynamic property (free energy), but he used kinetics-based methods that led him to consider free energy and stability from a temporal perspective. John was interweaving frequency, time, and population by discussing the frequency at which a protein "jiggles" into partially unfolded conformations or its longevity in a particular conformation. He avoided breaking free energy into enthalpy and entropy components, because he considered it too difficult to compare their magnitudes. On the other hand, William looked at entropy and enthalpy separately in developing simulations. He connected enthalpy to interactions and made entropy tangible as flexibility or particle movement, which can be "turned on" through temperature. Given the practical and descriptive orientation of her research, Gertrude devoted little attention to thermodynamic variables but directly connected the idea of stability to measurements of mass (i.e., amount of deuterium incorporation) and rigidity to the extent of hydrogen-bonding interactions. This relates to how she represented her HDX data. Gertrude, John, and William's explanations of thermodynamic concepts particularly show how they integrated theoretical knowledge with their research methods and data representations so intricately that they cannot be

isolated from one another. We believe this further supports the integrated nature of the MAtCH components. It also suggests that theoretical concepts of significance to the study of protein folding and dynamics can be explained in many different ways and with a basis in authentic research methods. Rather than a single definition of entropy or free energy, there are multiple practical definitions, each of which emphasizes different aspects of a phenomenon and varies in degree of usefulness depending on the research context. This aligns with Brigandt's (2013) remark that scientific models and explanations—and we add analogies—are not all-purpose tools but serve specific purposes and explanatory aims. These experts provided other verbal and visual analogies that will be the focus of later studies.

4.6 Limitations

As with any qualitative study, there are important limitations to consider. First, the original intention was for participants in this study to address the interviewer as a colleague in a similar or related field, but this was difficult, and the authors acknowledge that the explanations provided to the interviewer were directed more at the level of a graduate student with some knowledge of the field. However, this was actually advantageous, as the semi-structured nature of the interviews still allowed the participants and interviewer to develop a mutual understanding of the research at a level that shows application of thermodynamic and kinetic concepts students would learn in undergraduate science courses. This serves the long-term goal of this research. Furthermore, while these results only represent the ideas and work of a small sample of four experts currently conducting research related to protein folding and dynamics and therefore cannot be generalized across all experts in this area, the results do provide an opportunity for a deeper analysis of expert explanation than would be obtainable through a larger sample size study. The authors would argue that, while the specifics would change from research project to research project, it is probably commonplace for experts to integrate components of explanations (as per Figure 4.1) and shift between types of explanatory approaches and perspectives when appropriate. Similarly, while we cannot claim from this study that the ways these experts think about thermodynamic and kinetic concepts are shared by other individuals working on similar research projects, the findings do indicate that experts' ideas may align with their research methods.

4.7 Implications for Instruction

Given the previously stated pedagogical importance of protein folding and dynamics to the undergraduate curriculum and current research, we suggest that these findings can inform the following:

- Development of educational materials to support students' ability to use research methods, data, and theoretical knowledge to explain protein-folding phenomena;
- Use of mathematical models in biochemistry courses; and
- Examples or case studies based on the expert research described in this paper, including a range of ways to conceptualize thermodynamic and kinetic concepts used in protein-folding and dynamics research.

These pedagogical implications are discussed in greater detail in the following paragraphs.

First, findings can inform the development of educational materials to support students' ability to use research methods, data, and theoretical knowledge to explain protein-folding phenomena. The cases here suggest that the blending of MACH components guided by theoretical knowledge (i.e., MAtCH) is critical to research projects of social impact. We believe that the findings of this study highlight the importance of bringing both research contexts (C) and methods (M) into the science classroom to provide a more holistic and practical understanding of natural phenomena and the process by which they are understood. Students are often not prompted to consider or integrate the MAtCH components in their course work. Although the original MACH model was used to help undergraduates think about components of mechanistic explanations (Trujillo et al., 2015; 2016a), students still struggled to make connections *between* the MACH components—especially between the phenomenon (H) and how it is measured (M)—which resulted in disjointed explanations (Trujillo et al., 2016a; 2016b). While we did not investigate student learning in this study and therefore cannot make any claims regarding the use of the MAtCH model in the classroom, we found it was helpful for making sense of the complex interconnected components and theoretical knowledge important to complex cutting-edge research projects. Similarly, we believe that instructors can use the MAtCH model as a tool to design or modify curricula for life science courses to create contextualized content with activities and assessments structured to emphasize the MAtCH components and their connections. By using the

MAtCH model to systematically check for the presence of components and connections, instructors can critique course objectives and materials based on expert practice, thus identifying strengths and limitations or gaps in coverage, so that they may make informed decisions regarding design and implementation to ensure that the curricula expose students to more authentic and practical science. By emphasizing the components and connections, our objective is to help students not only gain knowledge of procedures and data-processing techniques (M), but also to enable them to use underlying theoretical knowledge to develop models and representations of a system (A) and to discuss data (M) in terms of what they measure about the interacting entities of the system (H) as well as the social or biological implications (C). As an illustration, we employed the MAtCH model to briefly review and suggest possible modifications for three protein-folding and dynamics educational materials published this year (Helgren & Hagen, 2017; Lipchock et al., 2017; McLaughlin, 2017; see Appendix B Table B.1). Lipchock et al. (2017), for example, provide a 10-week research-like laboratory module in which students use various techniques to explore the effect of mutagenesis on enzyme structure and function using protein tyrosine phosphatase 1B (PTP1B). Evaluation of the materials using MAtCH suggests a strength of the module is its in-depth discussion and use of different techniques (M) that involve or result in a variety of representations (A). However, the module does not explicitly help students interpret their data and/or data representations in terms of the interacting entities of the system (M/H, A/H). To address this, prompts like the following could be included in the module:

- What information about PTP1B can be obtained from your stained gel? What cannot? (A/H)
- Compare and contrast the methods used in this project with other methods for studying protein structure and dynamics. What can each of those methods tell you about the protein you are studying? What can they not tell you? (M/H)

Modified prompts like these, which elicit more integration of the MAtCH components, may enhance student learning by supporting meaningful interpretation of (multiple) representations, by scaffolding discussion of data in terms what they measure about a system so that they can be used to develop a model, and by directing students' attention to the limitations of methods and representations, thereby supporting the development of a more authentic understanding of scientific practice. By including more opportunities for students to integrate

MAtCH components (such as the M/H and A/H connections above) instructors can encourage students to think in ways that are more similar to experts in the field.

Our second implication concerns the instruction and use of mathematical models in biochemistry courses. Previous research has found that many students seem to engage with thermodynamic and kinetic formulas solely as algorithmic exercises (e.g., Carson & Watson, 2002; Hadfield & Wieman, 2010; Bektasli & Çakmakci, 2011). Students can demonstrate mathematical proficiency without conceptual understanding and often struggle to interpret physical meaning from mathematical expressions and/or to produce mathematical expressions from physical situations (e.g., Thompson et al., 2006; Hadfield & Wieman, 2010; Becker & Towns, 2012). As Bain et al. (2014) point out, if educators expect students to develop an understanding of thermodynamics through mathematical relationships and representations, they must be taught what those mathematical concepts mean in a thermodynamics context. Too often mathematics in science becomes a summary of data or a calculated procedure that is manipulated, with little link to scientific phenomena or processes (Schuchardt & Schunn, 2016). The findings here underscore the importance of mapping entities, interactions, and processes to mathematical formulas and symbols in scientific practice. The MAtCH model demonstrates that, to address current scientific research problems, one must be able to use mathematical models to mediate between methods, data, and ever-developing models of interacting entities in a phenomenon. The findings provide several examples of how mathematical models related to thermodynamics and kinetics serve as key theoretical tools for interpreting data and reasoning about complex processes. We believe the MAtCH model can be used by instructors to reflect on how they might better connect mathematical models to scientific phenomena and research methods in the life sciences.

The third pedagogical implication of this study is a broader range of ways for educators to conceptualize thermodynamic and kinetic concepts used in protein-folding and dynamics research, including how they may be integrated with each other and with research methods. We believe that, if educators intend to support students in understanding scientific practice and knowledge, it is necessary to develop educational materials that scaffold the integration of research methods and conceptual knowledge in the ways that expert scientists do. In the traditional biochemistry classroom, thermodynamics and kinetics are taught separately, with little emphasis on experimental methods and significant focus on calculation and interpretation of various plots (e.g., Lineweaver-Burk plots). Contrary to this, the experts in our study used a variety of analogies and

employed unique descriptions of theoretical concepts to explain their research. We believe our findings support the argument Haglund (2012) provides in his work on entropy: that instead of abandoning several distinct meanings for a single "scientifically correct" concept, educators should take into account "the perceptual embodied nature of our cognition [and] the pragmatic, contextual circumstances in which any act of reasoning is performed." He notes that different models can highlight different aspects of a phenomenon to create richer descriptions and allow for varying degrees of idealization within different knowledge traditions. Not only do the experts provide examples with language and analogies that at times seem hardly "scientific" at all—for example, using analogies like electrostatics as magnets and free energy as "jiggling," which could be powerful tools for instructors—but the heterogeneity in these experts' conceptions demonstrates that context and pragmatics have a notable influence on reasoning and explanation. The apparent alignment between these experts' conceptions and their research methods indicates that research methods can directly influence the ways in which these scientists think about phenomena, implying that understanding research practice may be an important part of functional scientific knowledge. Therefore, it may be useful to incorporate several case studies based, for example, on the four experts' research projects described in this study, in order to make the thermodynamics and kinetics of protein folding and dynamics more tangible to the learning of biochemistry.

4.8 Implications for Future Research

Frameworks to evaluate scientific explanations began, in part, with consideration of how expert scientists work and communicate, and it is critical to continue investigating how experts explain complex research projects and processes so that these can be better communicated to students. We identify at least two main avenues for future research. First, this study offers only a preliminary characterization of several experts' explanatory practices connected to specific phenomena. Significantly more work is required to untangle the complexity inherent to explanations of scientific research projects in order to develop pedagogical strategies and materials that help students integrate course content with practice (e.g., understanding research methods or connecting experimental findings to processes governed by theories that students are learning in the classroom). A second major avenue for future research concerns the critical role of analogical models in scientific communication and reasoning. Past research has shown that the interpretation of models can be extremely difficult for students and can lead to a range of conceptual difficulties

that impact learning, especially when students must interpret representations of theoretical concepts (Schönborn et al., 2002; Schönborn & Anderson, 2006). As with mathematical formulas, students can demonstrate competence at answering graph-related questions, but without understanding or referencing its meaning in the natural world (e.g., Bowen et al., 1999). By characterizing how experts use analogical models to explain protein folding and dynamics in a research context, such studies may inform the design of educational materials aimed at scaffolding the development of students' explanatory skills in this cutting-edge area of biochemistry.

4.9 Acknowledgments

We especially thank the pilot and expert participants of our study for their time and willingness to share with us their knowledge and insights into their research areas, as well as members of our VIBE research group for their contributions to the progress of the study. We also thank our reviewers for their excellent feedback, which we believe greatly contributed to the quality of our article. This work was partially supported by the ACE-Bio project (NSF grant 1346567) and the BASIL project (NSF grant 1503798). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

CHAPTER 5. WHERE TEXTBOOKS MISS THE MARK: USING EXPERT DATA TO INFORM THE USE OF RESEARCH METHODS AND REPRESENTATIONS TO ENHANCE BIOCHEMISTRY INSTRUCTION

A version of this Chapter has been submitted for publication. Jeffery, K. A., Pelaez, N., & Anderson, T. R. Where textbooks miss the mark: Using expert data to inform the use of research methods and representations to enhance biochemistry instruction.

5.1 Abstract

Biochemistry textbooks often provide a disconnected, highly mathematical, and de-contextualized treatment of thermodynamic and kinetic principles, which renders topics like protein folding difficult to teach. This is concerning given that graduates entering careers, like the pharmaceutical industry, must be able to apply such knowledge and related research methods to solve biochemistry research problems. Thus, it is essential that instructors have strategies to incorporate research methods and representations to help students understand the source of such scientific knowledge. Therefore the goal of our work is to examine expert practice and use the findings to identify instructional strategies to incorporate more cutting-edge research and authentic ways of knowing into science classrooms and textbooks. Towards this goal, we examined how four scientists explain protein-folding and dynamics research, focusing on the interaction of spoken language and representations, including gesture. Our analysis indicates that textbooks explain what is known but seldom use representations to explain how it is known, whereas experts employ multiple representations and research methods to communicate how evidence can be used to understand phenomena. Based on our findings, we suggest implications for the design of instructional materials, including textbooks, as well as potential instructional strategies to incorporate discussion of experimental methods and to support student interpretation of representations during classroom activities.

5.2 Introduction

Scientific practice relies on a combination of disciplinary resources to create and communicate meaning, including spoken and written language, mathematics, gestures, external representations

(ERs), experimental tools, and activities (e.g. Airey & Linder, 2009; Amann & Cetina, 1988; Woolgar, 1988). Each resource helps characterize a different facet of disciplinary knowledge and, in combination with other resources, affords more holistic understanding of a phenomenon (Kress, 2010; Kress & Van Leeuwen, 2001). To gain expertise requires knowledge of disciplinary theories and models, understanding of how they are represented, and the ability to productively coordinate and translate between multiple, irreducible, meaning-making resources (Airey & Linder, 2009; Givry & Roth, 2006; Lemke, 1998; 2002; 2004). Scientists translate between resources fluently, directly connecting the processes of science, scientific evidence, and practical contexts to the subject of science (Brewer & Smith, 2011; Bowen, Roth, & McGinn, 1999). Learning can be thought of as acquiring fluency in and across various discursive resources (Offerdahl, Arneson, & Byrne, 2017).

The investigation and modeling of molecular processes in biochemistry is impossible without these resources, especially ERs (Offerdahl et al., 2017; Anderson & Schönborn, 2008; Schönborn & Anderson, 2006; 2008; 2009). ERs are visible or tangible representations (as opposed to internal, mental models) and include, for example, data outputs from experimentation, graphs used to summarize or transform complex data sets, and two- and three-dimensional models of phenomena to support visualization and communication. ERs often employ discipline-specific conventions or stretch across multiple levels of complexity or abstraction, so effective use requires conceptual knowledge, knowledge of modes (i.e. symbolic markings and conventions), and the ability to combine and apply cognitive skills to perceive, process, and express ERs (Schönborn & Anderson, 2006; Anderson et al., 2013; Mnguni, Schönborn, & Anderson, 2009). Gestures are frequently combined with ERs to enhance discussion (Kendon, 2004) and studies on gesture in professional environments (Becvar, Hollan, & Hutchins, 2005; Goodwin, 2000; Ochs, Gonzales, & Jacoby, 1996) as well as the classroom (Givry & Roth, 2006; Roth & Welzel, 2001) suggest that gesture in scientific practice allows individuals to embody the processes they aim to explain, acting as a bridge between laboratory experiences and thought.

Supporting the development of visual and discursive fluency in students requires understanding how discursive resources like ERs and gestures are used in scientific practice (Airey & Linder, 2009; Schönborn & Anderson, 2006; Arneson & Offerdahl, 2018; Krajcik & Sutherland, 2010). However, the use of ERs in science courses and textbooks often appears to be disconnected from authentic disciplinary practices (Bowen et al., 1999; Roth, Bowen, & McGinn, 1999), leaving

students to learn only what is known, not how it is known. For example, textbooks seldom contain ERs of actual data and oversimplify research methods, failing to illustrate how authentic scientific evidence is visualized and communicated, and creating a disconnect between experiment, evidence, and 'known' phenomenon (Rybarczyk, 2011).

We believe it is important to incorporate more cutting-edge research and authentic ways of knowing into science classrooms and textbooks to help students understand where scientific knowledge comes from. Therefore, the overarching goal of the work presented here is to examine expert practice and use the findings to identify useful instructional strategies. This paper extends previous work, which modeled how four research scientists explain protein-folding and dynamics research (Jeffery, Pelaez, & Anderson, 2018), by exploring how the same scientists use spoken language in concert with ERs and gestures to describe their research. Using the Conceptual-Reasoning-Mode (CRM) model (Schönborn & Anderson, 2009) as a framework, we focus on the use of two archetypical ERs' related to protein folding and dynamics, found in biochemistry textbooks. We characterize how one scientist describes research methods for investigating protein folding and dynamics, in order to illustrate the reasoning behaviors and ways of discussing experimental methods which emerged from the data from all four experts. We discuss and provide examples of potential instructional actions based on the data.

5.3 Methods

5.3.1 Participants

We interviewed four expert scientists whose current research is related to protein folding or dynamics, and involves kinetic and/or thermodynamic data. They are hereafter referred to as 'experts,' or by pseudonyms. The experts' research projects, described in Jeffery et al. (2018), stretch from elucidating enzyme mechanisms (Beaker), understanding globular protein stability (John), improving protein drug shelf-life (Gertrude), and developing protein dynamics simulations (William). The current research was approved by an Institutional Review Board (#1511016694).

5.3.2 Development and Description of Interview Protocol

As described in Jeffery et al. (2018), the MACH model (Trujillo, Anderson, & Pelaez, 2015) was used to structure an interview protocol to focus on four aspects previously identified in scientists' explanations of mechanisms: research methods, analogies, context, and how the phenomenon

operates. Semi-structured interviews (Denzin & Lincoln, 2018) were employed to explore individuals' ideas in greater depth and probe for additional details or clarifications.

In brief, the experts were first asked to describe their research as they would to a colleague or a scientist in a related field, then their research context and experimental methods were probed. They were then asked to explain their research and protein folding as they would to an upper-level undergraduate student, including thermodynamic concepts typically covered in undergraduate chemistry (e.g. entropy, free energy). The interviews, including the production of ERs or use of any computer-based ERs, were audio/video-recorded. Interviews were transcribed verbatim, images of ERs were aligned and embedded in the transcript, and all drawing steps during the production of ERs, gestures referring to ERs, and captured air gestures were described and inserted into the transcript.

5.3.3 Selection and Analysis of Expert and Textbook Data

Although we describe analysis of the expert and textbook data separately, actual analysis consisted of moving between the two data sets as part of a novel analytical method which we call 'constant parallel comparison' (CPC). Figure 5.1 provides a summary of the process developed during analysis of the data sets. We introduce the expert and textbook lines of analysis separately because they began as two distinct collections of data which naturally came to relate to one another as the study progressed. Open coding (Corbin & Strauss, 1990) and constant comparison (Creswell & Creswell, 2017) occurred first within a single data set. Then the developed codes informed, but were not used to restrict, the coding of the other data set. This enabled open coding and constant comparison within, as well as between, both data sets. Thus, the expert and textbook data were analyzed as discrete data sets in 'parallel' with 'constant comparison' between and across the data sets. In this way, our coding method revealed the myriad of ways a reasoning behavior or method could be communicated, informing future coding and producing categories populated with diverse instances. Additionally, this method allowed the researcher to validate the contents and descriptions of the categories and sub-categories that emerged from the data.

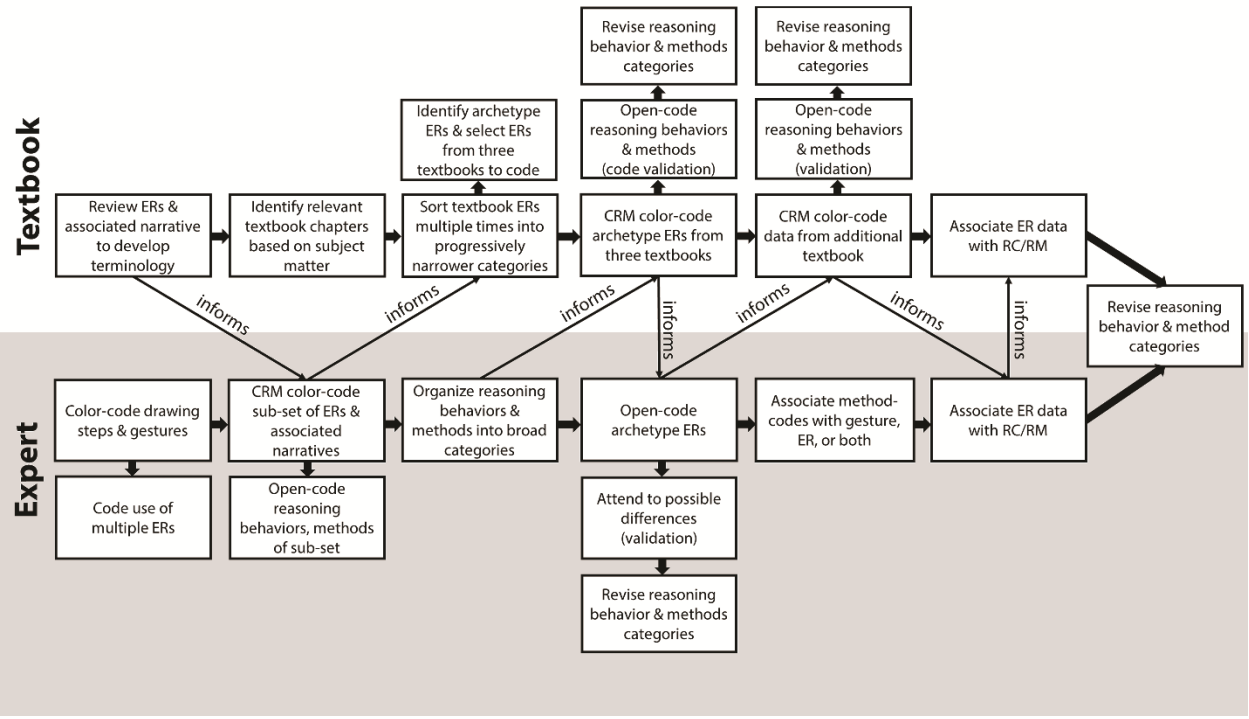


Figure 5.1 Simplified diagram of 'constant parallel comparison' analytical method developed to code expert and textbook data.

Boxes in the upper half (white) indicate steps related to the processing of textbook data; boxes in the lower half (grey) indicate steps involving expert data. The thick black arrows should be interpreted as providing a loose order to the steps, as some occurred concurrently and others asynchronously. The vertical stacks of boxes are meant to illustrate some of the smaller steps that occurred during a round of coding. Thin arrows crossing between the data sets suggest times when the knowledge produced in one data source informed the other.

Initial coding of all four expert interviews identified instances of drawing, use of gestures, and use of multiple ERs (i.e. where experts referred to previously drawn ERs, and/or switched between ERs in the course of their discussion). A sub-set of ERs and related transcript were randomly selected for analysis using the CRM model (Schönborn & Anderson, 2009). The CRM model describes the factors that affect an individual's ability to interpret ERs in a biochemistry context. The conceptual factor (C) represents relevant conceptual knowledge; the reasoning factor (R) represents reasoning or sense-making abilities needed to interpret a representation; and the mode factor (M) characterizes the external nature of the representation, such as symbolic markings. Reasoning behaviors can be applied to concepts (RC), to a representation itself (RM), or to both

simultaneously (CRM). By associating reasoning abilities with verbs, conceptual knowledge with nouns or noun phrases, and modes with nouns referring to components and/or markings of ERs, verb + concept-noun or mode-noun pairs can serve as evidence of a reasoning behavior.

Reasoning (verbs), concepts (nouns), and modes (nouns) were color-coded to draw the coder's (KAJ) attention to the parts of speech during analysis. The selected transcripts were then analyzed line-by-line to identify and describe possible instances of reasoning behaviors (open coding; Corbin & Strauss, 1990). During this process, the surrounding context was considered so as to preserve the participants' meanings and to enable coding at larger granularities; that is, we coded pairs of words, then considered the sentence, then multiple sentences. A single phrase could be coded for multiple behaviors. For example, consider the text, "…so even if you add in an acid [draws red circle with 'H' in it]…." This data contains a reasoning-verb phrase ('if you add in'), a concept-noun ('an acid'), and the use of symbolic markings on a representation (red circle, H). Possible descriptions of the behaviors suggested by this data are 'manipulates representation to discuss a hypothetical situation' and 'associates symbolism (red circle/H) with an entity (acid).' A master list of instances of reasoning behaviors was produced this way. The phrasing was refined by sorting the codes into categories and sub-categories, with memoing (Denzin & Lincoln, 2018), which were revised several times through constant comparison as category and sub-category descriptions crystallized (Fig. 5.1). Following a similar process, the entire transcripts of all four experts were analyzed to describe instances of discussion of experimental methods, and the resulting codes were sorted and refined into categories (Fig. 5.1). Each of these instances was also coded to indicate if it was associated with a gesture, an ER, or a combination of both.

To select textbook ERs, eight biochemistry textbooks were reviewed to identify chapters with thermodynamics and protein folding content. ERs from these chapters were sorted into general categories based on the ER, its caption, and associated body text. ERs were sorted multiple times into a number of narrower categories (e.g. protein dynamics). This led to the identification of textbook ERs which shared similarities with each other and some ERs from the expert interviews. Abstracting features from these ERs, enabled us to identify two 'archetypical ERs' which represent 1) equilibria between folded and unfolded protein states, and 2) free energy change for a reaction, often along a reaction coordinate (see Figure 5.2). Very few free energy-coordinate diagrams were present in the chapters initially reviewed, so the index and table of contents were used to identify relevant ERs in enzyme and catalysis chapters for inclusion.

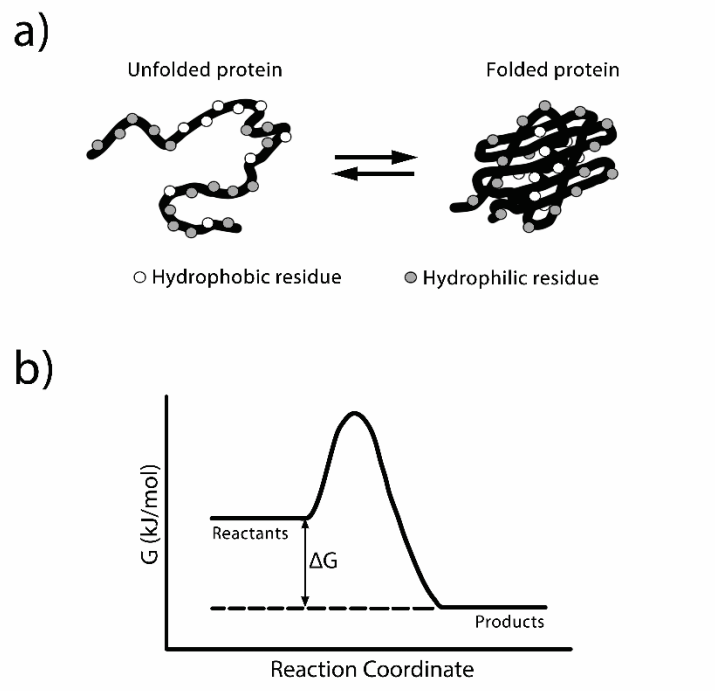


Figure 5.2 Archetypical ERs abstracted from review of the expert and textbook data.

a) Archetypical ER of equilibrium between protein structural states. Variations on this ER include protein denaturation equilibria; unfolded to folded protein ERs showing hydrophobic collapse; models of folding pathways (i.e. formation of secondary elements, hydrophobic collapse); examples of computer simulations of folding pathways; pathways to improperly folded proteins; and pathways involved in maintaining proteostasis. b) Archetypical ER of free energy change for a process. Variations on this ER typically display free energy (G) on the vertical axis and may or may not include a horizontal axis labeled 'reaction coordinate'; indicate reactant, product, and possibly intermediate or transition states; may designate values such as ΔG_r, $\Delta G^{\ddagger}_{cat}$, or $\Delta G^{\ddagger}_{uncat}$.

ERs from four of the original eight biochemistry textbooks were selected for further analysis. The selected textbooks include (1) a textbook providing an overview of biochemical concepts for pre-med and allied health topics (Pratt & Cornely, 2014); (2) a textbook focused on incorporating classical and current research on biochemistry (Voet & Voet, 2011); (3) a textbook aimed at communicating fundamental principles of biological molecules to first-time biochemistry students (Garrett & Grisham, 2013); and (4) a textbook aiming to balance new research findings with essential biochemical principles (Nelson & Cox, 2013). All of the selected ERs from these

four textbooks were coded line-by-line in the same manner as the expert interviews. The four expert interviews were then reviewed again to identify ERs like the archetypical ERs and coded (or re-coded) using the same process. The coding of old and new expert and textbook ERs served to validate the codes previously produced (see Fig. 5.1).

5.4 Results and Discussion

In this section we examine how one expert, John, explains his research by combining language, ERs, and gesture. John investigates how globular proteins lose structure with the aim of better understanding protein rigidity and longevity and therefore how to engineer proteins for function in harsher conditions or to improve shelf-life. We discuss two of John's ERs because they provide clear illustrations of several different reasoning behaviors and ways of integrating discussion of experimental methods and data. Other experts' narratives were comparable. We present a list of reasoning behaviors (Table 5.1) and ways of discussing experimental methods (Table 5.2), which emerged from all four expert interviews and the textbook data. In the data excerpts contained in the figures, we highlight examples of reasoning behaviors written as verb-noun pairs. However, it should be noted that use of an ER always involves simultaneous reasoning with both conceptual knowledge (RC) and mode (RM) (Schönborn & Anderson, 2009). At the end, we discuss the use of ERs in biochemistry textbook narratives, before comparing the expert and textbook data in the Conclusions.

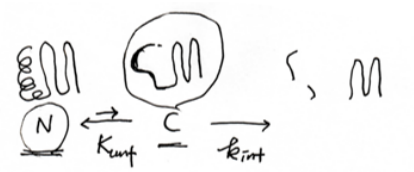
5.4.1 John Combines a Protein Folding Cartoon with Equations and Gestures to Explain Proteolysis Kinetics

John uses proteolysis kinetics to understand the energetics of globular protein unfolding. Figure 5.3 contains a short excerpt from John's explanation with drawing steps and gestures indicated in italics, and several examples beside the excerpt of reasoning behaviors written as verb-noun pairs (also see Table 5.1) and instances of discussing methods. John begins by drawing a cartoon of a folded protein (Fig. 5.3a). Speaking and drawing simultaneously, John first identifies the entities and processes he investigates by describing how a native protein, N, can partially unfold into a cleavable form, C, with an exposed alpha helix which can be digested by proteases. He uses differently sized arrows to show that, prior to adding protease, a dynamic equilibrium exists between the forms of the protein where the native form is more stable and therefore "99.9%" of

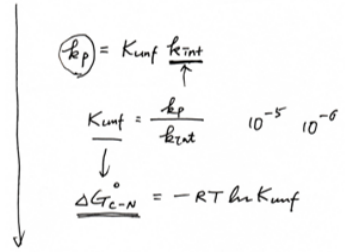
the protein is in that conformation. By doing this, he associates the process of equilibrium with the arrows, as well as a property of the system with the arrow length. John repeatedly points to the different cartoon forms as he compares their stabilities, thereby anchoring his discussion of populations to the drawn entities. He then explains how protease can irreversibly digest the cleavable form into small fragments, transforming the 'C form' by drawing a third cartoon of the digested protein components. Other experts similarly modified, covered up, or described manipulating ERs in their explanations (data not shown). In Figure 5.3, John integrates his explanation, gesture, and multiple ERs to define the processes and properties significant to proteolysis kinetics. He does this in part by associating the processes with specific symbols (e.g. k_{int} as intrinsic proteolysis rate), variables in equations, and his cartoon ER by labeling the arrows. John goes on to explain that he uses an equation to relate the processes to the overall rate of proteolysis, k_p, associating the entire process with a mathematical model (Fig. 5.3b). John repeatedly employs gesture and shapes (e.g. lines, circles) to tie the equations (Fig. 5.3b) to the cartoon (Fig. 5.3a). For example, by pointing first to a symbol in the cartoon and then to the same symbol in an equation, John relates a process to an experimental variable, indicating the processes and values significant to proteolysis kinetics.

John goes on to describe the data he collects and calculates, such as speed of digestion to determine the overall rate of proteolysis (k_p). He integrates discussion of strategizing within experimental constraints by using a generic peptide to approximate k_{int} if the sequence is unknown, and the inherent limitation and error associated with doing so. This data is then used to determine the equilibrium constant (Fig. 5.3b, rearranged equation) and John offers examples of the magnitude of K_{unf}, placing them next to the equation. He explains that the overall rate of proteolysis is usually very slow (small k_p) while digestion of a short peptide is very fast (large k_{int}). The K_{unf} value therefore describes the susceptibility of the protein to proteolysis. K_{unf} can be used to find free energy difference between the native and cleavable forms, and John draws an arrow from K_{unf} to $\Delta G°_{C\text{-}N}$ to relate the equilibrium and free energy values. Referencing Figure 5.4a, John explains that they can determine the $\Delta G°_{C\text{-}N}$ for many different environmental conditions as well as the effect of mutations on $\Delta G°_{C\text{-}N}$. Finally, John situates this information within the context of his research goals and other experimental methods by explaining how he uses the data to make structural models of cleavable states, which – because they are so rare in comparison to the native

a)



b)



c)

Speech	Gesture	Reasoning Behaviors / Discussion of Experimentation
This proteolysis reaction is irreversible.	*points between C form, fragments several times*	Identifies property of process[RC] Associates process with shift between graphical units[(C)RM]
Once it is digested, you know, peptide bond is gone, right? But this is dynamic equilibrium, right? So we define these.	*points between N, C forms along arrows several times* *points between N, C forms along arrows*	
This is- we just call it K unfolding. And then this is- this is kinetic constant like k. We call it k intrinsic. So it's intrinsic proteolysis rate.	*labels equilibrium arrows with K_{unf}* *labels k_{int} below unidirectional arrow* *covers N form and equilibrium arrows*	Associates symbols with arrows[RM] and with process[(C)RM]
Once protein is unfolded, protease can digest it very fast, right?	*points between C, fragment forms several times*	Draws causal link between structural state and event[RC]
So then overall proteolysis rate is a product of these two variables. The equilibrium constant times intrinsic proteolysis kinetics.	*writes $k_p = K_{unf}\, k_{int}$* *points at K_{unf} and k_{int} labels in reaction*	Associates process with mathematical model[(C)RM]
Then... we monitor how fast protein is digested, then we determine this k_p. And then we approximate this number, k intrinsic, using unstructured peptide. The same enzyme, right?	*points at, circles k_p* *points at k_{int} in reaction, points at k_{int} in equation*	Describes outcome variables
And then if it has the same sequence that's better,	*runs pen over unfolded portion of C form*	Associates sub-region of protein with part of graphical unit[(C)RM]
but sometimes we don't know the sequence so we use just... generic peptide substrate and we determine this number. And then it's an approximation, you know. Maybe there is some error…	*briefly points at the unfolded portion of the C form* *underlines k_{int} in equation*	Describes strategizing within constraints, error associated with method

Figure 5.3 John describes the method of proteolysis kinetics.

The ERs John produced for his explanation are shown in (a) and (b). An excerpt from John's discussion of proteolysis kinetics is provided in (c). John's gestures, including drawing steps, are aligned with his speech (see italics, second column in c). For readability, alignments are approximate. A few examples of reasoning behaviors (written as verb-noun pairs) and discussion of experimental methods are provided in the third column. Reasoning with modes (RM), concepts (RC), or both (CRM) are indicated in superscripts at the end of the reasoning behavior.

form – cannot be studied directly by any other typical method (e.g. nuclear magnetic resonance, circular dichroism, etc.).

As can be seen from this data, John combines a variety of gestures, ER-related reasoning behaviors, and ERs in his discussion. Table 5.1 provides descriptions and examples of the major categories of reasoning behaviors which emerged from the expert data. The most prevalent categories were the "identifies" and "associates" categories as the experts frequently first identified what they were discussing, drawing, or pointing at, before associating properties, processes, etc. with particular molecular components or graphical units. For these two categories, we include "physical" and "modal" sub-categories in an attempt to distinguish reasoning behaviors that address the components of the molecular system as opposed to graphical units in the ERs themselves; that is, we attempt to distinguish RC and RM behaviors. This is an artificial separation as effective use of an ER involves both (CRM). Consider the following: associating a property (e.g. hydrophobicity) with an entity (e.g. amino acid residue) is an RC behavior particular to the "physical" system, while associating a property (e.g. hydrophobicity) to symbols (e.g. hatching, color) is particular to the "modal" system and thus an RM behavior. Several other categories that emerged from the data are "orders", "compares", and "draws (causal) link." These behaviors involve more than one entity, state, etc. or graphical unit, so they are slightly more complex reasoning behaviors than "identify" or "associate."

Table 5.1 Examples of categories of reasoning behaviors demonstrated by experts and textbooks.

Reasoning behaviors are written as verb + noun pairs, and are accompanied by example quotes. Categories emerged from grouping similar reasoning behavior codes. The categories and codes presented here are not comprehensive, nor are each of the categories equally represented in the data. The source of the example quotes are provided in parentheses at the end of each quote.

Category	Codes	Example
Identifies	*a. Physical* …entities, sub-parts of an entity, or emergent structures; …properties of entities, interactions, or processes; …interactions; …states of a process, system, or entity; …the environmental conditions; …spatial organization, location, or orientation; …the purpose or function of an entity; …processes or events.	*Identifies entities/sub-parts; identifies spatial organization* "…I have a carboxylic acid… an… arginine over here… maybe I have a water molecule here… a backbone carbonyl over here…" (Beaker) *Identifies state of entity* "So (the) protein is in the unfolded state…" (John) *Identifies interactions between entities* "...the hydrogen bonds that the molecule is making with itself…. …hydrogen bonds it's making with matrix…." (Gertrude)
	b. Modal …graphical units in a representation; …what entity is described by a plot; …graph or plot features (e.g. large changes in line shape); …groups of bars/lines via color or proximity; …or indicates a particular feature using an arrow, line, or shape.	*Identifies graphical units in a representation* "…I have a carboxylic acid [draws C with H] and I maybe have an NH_3 group here [draws NH_3^+]… an arginine [writes N]… a backbone carbonyl over here [draws line to O, writes C=O]…" (Beaker) *Identifies a graph or plot feature* "Really tall bars [moves mouse across bars from left to right] up to 60% [briefly points at tallest bar]." (Gertrude)
Associates	*a. Physical* …properties with entities or sub-parts of entities; …functions with entities or sub-parts of entities; …properties or states of a system with entities, their interactions, and organization.*	*Associates property with entity* "…here are some hydrophobic residues…" (William) *Associates function with a sub-part of an entity* "…this tyrosine can be a hydrogen bond donor [points at H on –OH of Tyr]…" (Gertrude) *Associates property of a system with entities and their organization* "The entropy loss arises from the formation of the ES complex (Figure 14.4), a highly organized (low-entropy) entity…" (Textbook, Garrett & Grisham, 2013)

Table 5.1 continued

	b. Modal …entities or sub-parts of entities with symbols; …states, changes in state, interactions, or properties with symbols (e.g. hydrophobicity shown by hatching); …motion or process with a symbol (esp. arrows); …a state or process with plot shape; …properties of an entity or state with a plot feature; …variables (e.g. time) with axes on a plot; …a process or state with a mathematical expression or term; …a mathematical expression or term with the behavior of a plot (e.g. line shape); …different symbols or representations (i.e. horizontal translation).	*Associates sub-part of entity with symbol (number)* "…as a function of peptide…. …that's what all these weird numbers are." (Gertrude) *Associates process with arrow* "And proteolysis usually it occurs through just one step [points at N to C arrow in reaction]…" (John) *Associates structural state with plot shape* "[…over data lines on plot] are taking up more deuterium which suggests that there's a more open structure of the aggregate." (Gertrude)
Compares	…states, entities, or environmental conditions; …the magnitude of a property or change; …features of plots or graphs.	"If you put it in the solid state… those same groups are now interacting not with water…" (Gertrude) "10 to the fifth, 10 to the sixth [writes in exponent '5', then '10^6']. Where is 10 to the 12th?" (Beaker) "…the scale is the y-axis is the same [points briefly at y-axis label], but you can see that everything is suppressed…" (Gertrude)
Orders	…events or states in a process.	"And then you'll go through a transition state…. …that takes place first…. Then…" (Beaker) "The core (or some part of it) folds in a β sheet before the rest of the protein folds correctly…" (Textbook, Nelson & Cox, 2013)

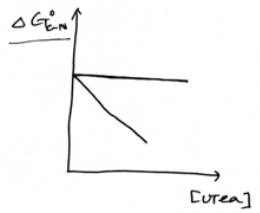
Table 5.1 continued

Draws (causal) link	…between events or interactions and changes in states or properties; …between changes in interactions and emergent structures; …or indicates lack of relationship between events or interactions and changes in states or properties.	"…the labelling process…. …what the deuterium incorporation is telling you not only what's solvent exposed on the protein… but it also gives you some information about dynamics…" (Gertrude) "So this folded form….is probably folded in part because of its hydrogen bonding interactions… to itself… that make its structure…" (Gertrude) "…when this is in the active site that tweaks the differences between those distances and angles." (Beaker) "…the presence of the hydrophobic groups interrupts the hydrogen-bonded network of water molecules." (Textbook, Pratt & Cornely, 2014)

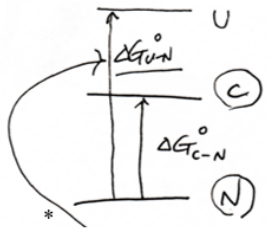
5.4.2 John Combines Protein Denaturation Graphs and a Free Energy Diagram to Describe Ways to Study Protein Folding

John combines several ERs (see Fig. 5.4a-c) to discuss the ways he studies changes in free energy associated with protein folding. The short excerpt in Figure 5.4d begins where John starts to draw Figure 5.4c. When previously prompted to describe his methods in more detail, John begins by explaining that in addition to investigating proteolysis of partially folded proteins under native conditions, he sometimes changes the experimental conditions by adding urea. John draws a causal link between the presence of urea and the change in the energy required for a protein's conformational change: that is, less energy is required for the protein to unfold when urea is added, and this decrease in energy correlates with an increase in the observed kinetics. He adds a sense of dynamics to his discussion by cupping his hands together to represent a globular protein and moving them apart when he mentions unfolding (see Figure 5.5a). Other experts similarly used gesture to animate, as well as connect and quantify, their ERs (data not shown). John explains that adding urea can also be used to determine the energy gap ($\Delta G°_{C\text{-}N}$) between the native and partially folded cleavable states. John initially represents this energy gap by vertically stacking his hands and decreasing the distance between his hands as he discusses monitoring the change in ΔG over time with changes to urea concentration (see Figure 5.5b). As he tries to explain what "that slope" represents, John draws the axes of Figure 5.4a before switching to create Figure 5.4b.

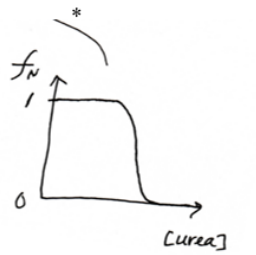
a)



b)



c)



d)

Speech	Gesture	Reasoning Behaviors / Discussion of Experimentation
...sometimes we monitor global unfolding... let's say, this is fraction of native and this is urea concentration. Because we need to know how...	*draws axes of plot (c); labels y-axis and x-axis*	Associates axes with entity state and experimental conditions$^{(C)RM}$
...here we determine the energy gap between N and C.	*points to N to C transition in diagram (b)*	Describes output variable
But up there, there is a U. U is globally unfolded form.	*draws U line*	Associates structural state with symbol and line$^{(C)RM}$
And then we can determine this energy.	*draws arrow from N state line to U state line*	Describes output variable
So this is delta G, N to U... And this is what people say global stability of protein stability. And then we determine this too.	*labels $\Delta G_{U\text{-}N}°$*	
So we monitor, like, the change of fraction of native, so under native condition, this number is one. It means 100% protein is folded.	*labels 1 on graph in (c) draws flat line*	Describes experimental conditions Identifies structural state^{RC}, associates state with line$^{(C)RM}$
And then when we increase urea concentration, eventually protein loses its structure and fraction of native protein goes to zero. ...	*draws downward curve, draws line to meet x-axis*	Describes changing conditions Associates change in structural state with shape of line$^{(C)RM}$
And we fit this data and we calculate this delta G, U to N. So we frequently add this type of plot too.	*draws arrow pointing from plot in (c) to $\Delta G_{U\text{-}N}°$ on diagram in (b)**	
And then delta C-N, delta G N to C, is determined from kinetics.... Proteolysis kinetics.		Indicates method that produces output variable
...in the discussion section I try to add, like, the structural model. You know. What- which region unfolds. So- in results section too. In results section too we try to represent this experimental data on a structure. ...and that helps people to visualize the result....	*points at N, C forms in Figure 5.3a points at N form in Figure 5.3a points generally at all drawn ERs*	Associates process with region^{RC}

Figure 5.4 John describes different methods used to explore protein folding energetics.

The ERs (a-c) John uses in his explanation are provided along with a short excerpt where he describes several methods he uses to explore free energy changes associated with protein folding (d). John's gestures, including drawing steps, are aligned with his speech (see italics, second column in c). For readability, alignments are approximate. Note that the asterisked line in (c) connects to the asterisked arrow in (b). This drawing step is similarly indicated in (d). A few examples of reasoning behaviors (written as verb-noun pairs) and discussion of experimental methods are provided in the third column. Reasoning with modes (RM), concepts (RC), or both (CRM) are indicated in superscripts at the end of the reasoning behavior.

In Figure 5.4b, John identifies the relative energy levels of native (N) and cleavable (C) forms and associates the difference in energy with the symbol $\Delta G°_{C\text{-}N}$ and an arrow. He explains how he plots $\Delta G°_{C\text{-}N}$ data at different concentrations of urea (Fig. 5.4a) and associates the resulting slope with how much the protein unfolds, i.e. the amount of structural difference between the native and cleavable forms. With Figure 5.4a John explains the meanings of two instances: no slope indicates that the native and cleavable forms are similar and conformational change depends very little on urea, whereas a large slope suggests a large structural change when the protein changes form. Each time he mentions a structural difference or change, he points back and forth between the N and C forms in Figure 5.3a, relating the two ERs. Later in his interview, John returns to Figure 5.4b to describe another experimental method used to characterize a protein's global stability (where the excerpt in Fig. 5.4d starts). He explains that the globally unfolded form of a protein (U) exists at a higher energy state than the other states, again associating the difference in energy with a symbol ($\Delta G°_{U\text{-}N}$) and an arrow between the two states. Drawing Figure 5.4c, John explains that a protein in its native state eventually loses its structure as the concentration of urea increases and they can monitor the change in the fraction of native protein. In this way, John draws a link between changing environmental conditions and changing structural state, associates change in structural state with the shape of the plot, and identifies the data collected. John ends by describing how this experimental data is manipulated, calculated, combined, and then represented with ERs like Figure 5.4a-c and structural models to represent the stability of the proteins and regions that unfold.

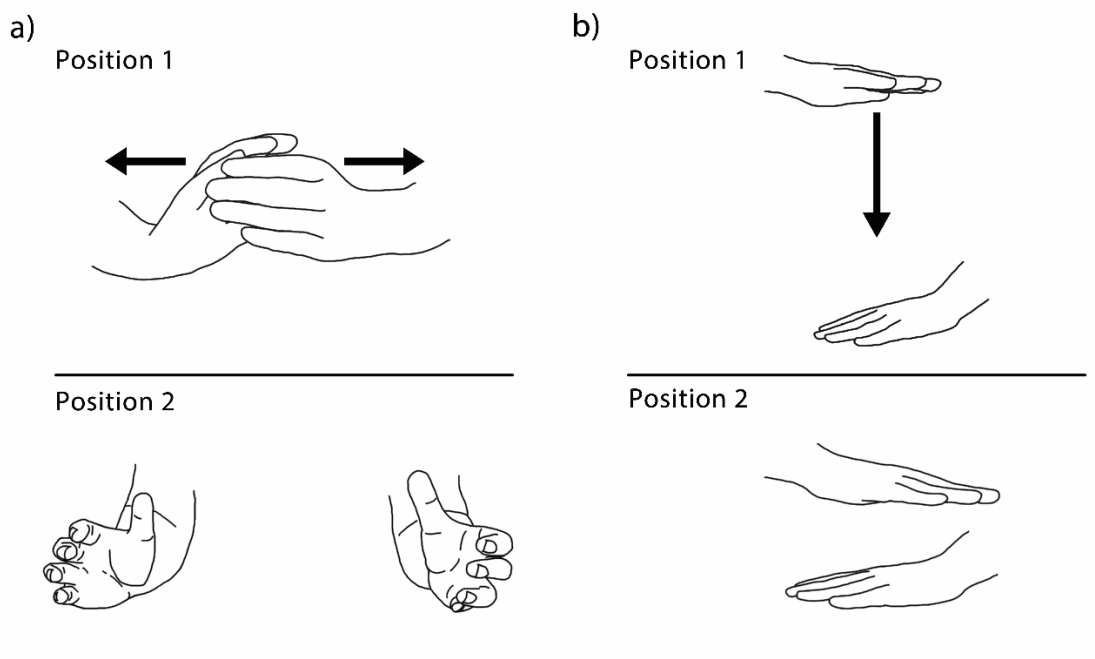


Figure 5.5 Two gestures used by John as he discusses his research on protein folding energetics. In panel (a), John uses cupped hands to illustrate a folded globular protein. He then moves his hands apart (see arrow) as he talks about the protein unfolding, adding a sense of motion that is not present in his speech or ER. This movement was often repeated more than once. In panel (b), John represents the relative energy gap between native and partially unfolded protein states using his hands. John then decreases the distance between his hands (see arrow) as he describes monitoring the change in free energy over time. Other experts similarly used gesture to animate, as well as connect and quantify, their ERs (data not shown).

The excerpts discussed here provide examples of the ways in which the experts integrate ERs with experimental information, such as limitations of methods and estimates of data values. Table 5.2 provides descriptions and examples of the major categories related to experimental methods which emerged from the data. Given that the interviews focused on explanations of research, the largest categories reflect some of the main considerations of experimentation: purpose, limitations of methods, treatment variables, and outcome variables (Dasgupta, Anderson, & Pelaez, 2014; Pelaez et al., 2017). Experts integrated experimental information by directly representing it on the ERs in the form of, for example, variables to be collected or calculated, data estimates, or labels and symbols indicating environmental conditions, as well as by pointing to indicate or relate different components of ERs.

Table 5.2 Five ways in which experts and textbooks integrated discussion of experimental methods and data with ERs.

The categories and codes presented here are not comprehensive, nor are each of the categories equally represented in the data. The source of the example quotes are provided in parentheses at the end of each quote.

Category	Example	ACE-Bio Competencies*
Identifies or describes method or method purpose	"…I can make it [palms facing each other, misaligned] hold them apart [twists both hands so palms face away from each other] so that they never react. So that's theoretically possible to do with an enzyme too. So I could do that by [indicates region of cartoon enzyme] changing the orientation, etc." (Beaker) "…if you dissolve your protein in D_2O, amide backbone proton(s are) exchanged with deuterium in solution [waves hand back and forth generally] and then that rate is determined exactly same way [points at cartoon of conversion N to C]." (John) "…it starts with what we call MD simulations…. …in principle, simulate over a course of, typically-nowadays, typically on the order of 50-100 nanoseconds [writes '50-100 ns'] the full dynamics of the protein [hand makes sweeping motion], including water and including ions, cofactor, etc.…" (William) "Yet dialyzing away the urea and exposing the resulting solution to O_2 at pH 8 yields a protein that…" (Textbook, Voet & Voet, 2011)	The ability to generate a research **question** and formulate hypotheses. **Plan** feasible and ethical experiments to answer research questions
Describes treatment variables	"…compare it to the histidine formulation [shows histidine plot]… …the ones with sucrose and trelose, the sucrose is… …whereas… …for mannitol..." (Gertrude) "…expose it to D_2O [points at asterisk below dish with powder] in the vapor phase at controlled temperature, relative humidity. … …and then at a particular temperature…" (Gertrude) "…and we can change the condition [draws arrow] and then protein starts to fold [writes N underneath], right?" (John) "…in the presence of a small amount of β-mercaptoethanol with gentle warming…" (Textbook, Garrett & Grisham, 2013)	**Plan**

Table 5.2 continued

Describes outcome variables (the type of data a method collects or has collected). May provide an example of data.	"…the rate of the reaction will depend upon how the enzyme [points at wedge on A circle] can orient these two correctly." (Beaker) "These two [draws square bracket line from first to second red circle] when we did the RMS deviation are almost identical. [labels bracket 'RMS Deviation Identical']" (Beaker) "…and then, in principle, using canonical ensembles, trying to estimate the relative free energies [points between two glutamates] of different conformations." (William) "And so you get a band at 1650 or so [moves mouse from bottom of dip down to x-axis] that corresponds to alpha helix [mouse traces over dip in dotted line]…" (Gertrude) "Thermodynamic calculations indicate that lowering $\Delta G^{\ddagger}$ by about 5.7 kJ • mol^{-1} accelerates the reaction 10-fold." (Textbook, Pratt & Cornely, 2014)	**Plan** The ability to **analyze** and process data.
Describes limitations of methods/techniques, error associated with methods, or limitations in data representation. May compare to another method to illustrate limitations.	"…I used hydrogen exchange. Hydrogen exchange requires, like, NMR or mass spec, right? But proteolysis doesn't require anything pretty much. Protease and SDS PAGE gel, right? So it's very simple." (John) "So you take your compound and you want to have the answer in a minute or five minutes. If you do this like this [taps pen at ligands in active site of enzyme diagram], you're waiting several days for each compound." (William) "…this C state [points at cartoon C form], again, this partially unfolded form is very rare, right? It's just one out of a million. So there's no way to monitor- to determine the structure of this C directly. Impossible. If you use NMR or CD or fluorescence or whatever spectroscopic method, this N form is dominant form. …99.99% so the signal you get… is from this N [circles N] and then it's hard to get information on C…." (John)	**Plan** The ability to **conclude** about data with inferences that are limited to the scope inherent in the experimental design. The ability to **communicate** research work in professionally appropriate modes, including visual, written, and oral formats.
Compares data to expectations or to other work in the field	"Kinetic(s) might (have an) important role [points at heme and porphyrin drawing]- it's less studied or not studied at all from (a) computation(al) aspect." (William) "They're probably more theories than there are examples where people have 'quantitated' [indicates drawing of substrate in active site] what's happening in there." (Beaker)	**Identify** gaps or limitations in current research knowledge.

*In cases where more than one ACE-Bio competency (Pelaez et al., 2017) is relevant, the competency is only defined the first time it appears.

5.4.3 Textbooks Explain What Is Known But Seldom Use ERs to Show How It Is Known

Constant parallel comparison of the textbook and expert data found similar categories of reasoning behaviors (Table 5.1) in the textbook narratives and captions associated with ERs, as well as mentions of experimental methods or data in relation to ERs (Table 5.2). In general, very few references were made to the components of ERs in the associated textbook narrative and the text very rarely prompted the reader to draw connections between ERs. Additionally, few of the reviewed textbook ERs combined different types of representations in the same figure (e.g. graphs and cartoons). Many of the items coded as reasoning behaviors in the reviewed textbooks were statements that characterized entities, properties, or interactions, and drew relationships between them. For instance, the example textbook quote in the "order" category in Table 5.1 describes the order in which regions of proteins fold. The other textbook quotes in Table 5.1 in the "associates – physical" and "draws (causal) link" categories similarly read as statements of disciplinary knowledge, rather than explanations of what evidence has led to a particular understanding or how that knowledge is known. Correspondingly, references to experimental methods in the reviewed textbooks tended to include identifying methods, experimental conditions and possibly outcome variables (see example textbook quotes in the first three categories of Table 5.2). In the reviewed textbooks, experimental methods and evidence were mainly utilized to support disciplinary knowledge statements rather than demonstrate how research leads to the development of disciplinary knowledge.

5.5 Conclusions

John's discussion and his use of ERs highlights how he connects experimental methods and data to ERs to understand protein-folding and dynamics phenomena. Furthermore, it highlights how his understanding is informed by coordinating several different kinds of ERs. We briefly discuss our findings before showing how they can inform the translation of experimental methods into the classroom, and the design of educational materials to support student interpretation of ERs, in the Implications section.

5.5.1 Experts Used ERs to Explain How Research Methods Provide Evidence About Phenomena

Experts used ERs to describe their experimental methods and show how the methods informed their understanding of phenomena. Our analysis produced five categories related to experimental methods. These categories align with concepts and skills of experimentation identified elsewhere, such as the ACE-Bio competencies (see Table 5.2; Dasgupta et al., 2014; Pelaez et al., 2017). The experts' discussions included evidence of all seven ACE-Bio competency areas, including the ability to conduct an investigation which, although it is not indicated in Table 5.2, is accounted for in their performance as research scientists and in statements about taking measurements and troubleshooting. In comparison, few of the reviewed textbook ERs prompted students to consider the methods behind the information presented. References to experimental methods in the reviewed textbooks were limited mostly to brief descriptions of methods. For example, reviewed textbook ERs of protein folding equilibria commonly labeled arrows with denaturants or mentioned the use of computer simulation in generating models. Computer simulation data was also associated with some textbook free energy-coordinate ERs, but only one of the reviewed ERs included an actual numerical value for a bond angle (Garrett & Grisham, 2013). Compared to the expert data, there was less discussion of how an experimental method provides evidence about a phenomenon, why a method was chosen, or inherent limitations or error (see Table 5.2). This is unsurprising given that most textbooks aim to communicate established disciplinary knowledge, but it nevertheless obscures how scientific knowledge depends on evidence from experimentation.

5.5.2 Experts Used Multiple ERs and Gestures to Communicate Their Understanding

The experts employed a variety of reasoning behaviors when they produced and used their ERs (see Table 5.1). By far the largest categories were "identifies" and "associates" because these had to occur first in order to engage in other behaviors, like comparison or drawing (causal) links. The same kinds of reasoning behaviors were identified in the textbook data. Though no quantitative comparison was made, it appears that in comparison to experts, very few references were made to components of ERs in the associated text and/or the captions. Labels are probably intended to play this identifying role for ERs, however identification of a graphical component without directing attention to it in associated text limits opportunities to connect the ER to the surrounding discussion and enhance meaning (Roth et al., 1999). The frequent use of gesture allowed experts to integrate their discussion with various ERs. Experts connected their speech, components of a single ER, and

different ERs. Take, for example, John's frequent use of pointing to associate the processes represented by his cartoon drawing (Fig. 5.3b) to measured experimental variables (Fig. 5.3c). The reviewed textbooks seldom used language or labels to explicitly draw the same kinds of repeated connections, and it is probably for this reason that the text seemed disconnected from textbook ERs. The experts also frequently combined different types of ERs into composite ERs. In the last excerpt, for example, John combines three different data representations (Fig. 5.4b-d) and references the protein folding cartoon in Fig. 5.3. In comparison, the reviewed textbook ERs seldom combined graphs of experimental data, equations, and/or cartoons in the same figure, nor did their associated text prompt the reader to refer to one ER and then another. This is problematic for textbooks as our evidence indicates that meaning is generated by coordinating features within and across multiple ERs (Bodemer et al., 2005; Kozma, 2003; Ainsworth, 2008).

Furthermore, when they initially generated ERs, experts employed simpler or more concrete behaviors – such as identifying part of a graphical unit as a protein domain or associating a property like hydrophobicity with an entity. After they established relevant components, the experts continued on to consider the context of their research, at which point they began to use more complex reasoning skills, such as strategizing given experimental constraints or evaluating and synthesizing data across ERs. Further research is necessary, but this could have implications for the design of textbooks and curriculum aimed at scaffolding the development of higher-order cognitive skills (Anderson et al., 2001), as discussed in the Implications section.

5.6 Limitations

This was an exploratory, qualitative study of four experts and selected textbook ERs. The results represent only the behaviors identified in relation to these ERs and therefore cannot be generalized across all ERs. However, the limited sample enables deeper analysis than would be obtainable through a larger study. We do not claim that the results presented here are comprehensive, but it is likely that these behaviors are exhibited by other experts and illustrated in textbooks, and that the results have implications that are useful for a broader audience. By design, we considered a limited number of ERs in the textbooks we selected and are not making inferences about their entire content, nor are we ranking or rating them in any manner.

5.7 Implications for Teaching and Learning

Scientists can bring their research into the biochemistry classroom to impart authenticity to the subject matter and to expose students to cutting-edge research and methods. As our data shows, students can be shown the relevance of a topic like protein folding by learning how it is known (i.e. experimental method) and why it matters in a social context (e.g. to improve stability and shelf-life of protein drugs). The findings of this study can inform the translation of cutting-edge biochemical research methods into the classroom and textbooks, as well as the design of educational materials to support student interaction with ERs. Others can use the process (Fig. 5.1) applied in this study to evaluate and enhance instruction for other cutting-edge research topics. We briefly discuss these implications and suggest instructional actions.

5.7.1 Incorporating Research Methods in Instruction

Past research has indicated that the representations used in science courses and textbooks are often disconnected from authentic scientific research practices (Krajcik & Sutherland, 2010; Roth et al., 1999). For example, textbooks often do not contain representations of actual data and use oversimplified diagrams to explain methods, thus failing to illustrate how authentic scientific evidence is visualized and communicated (Rybarczyk, 2011). It would therefore be unsurprising to find students struggling to interpret ERs of actual data or to understand how experimental methods elucidate phenomena. The authors recognize that using an ER to communicate experimental methods serves a different purpose compared to textbook ERs which typically aim to communicate knowledge about structures or processes. However, during analysis we were struck by how these experts easily combined discussion of experimental methods and data with ERs like those found in textbooks to relate phenomena to their social and experimental contexts (see also Jeffery et al., 2018). We believe this process was likely made easier by the narrative-like structure of the experts' explanations, which is quite different from the often dense writing of textbooks. None of the descriptions or representations provided by the experts were particularly complex, which suggests that incorporating ERs that are better connected to current, authentic research practices and contexts, is doable. Potential instructional actions to incorporate methods in the classroom are provided in Table 5.3.

Table 5.3 Examples of potential actions for instructors to incorporate discussion of experimental methods into their teaching.

The actions are listed in no particular order and organized by category for convenience. The highest Bloom's taxonomy level that could be associated with a particular potential action is indicated as a superscript at the end of each action (1-Knowledge; 2-Understand; 3-Apply; 4-Analyze; 5-Evaluate; 6-Create).

Category	**Potential Actions for Instructors**
Identifies or describes method or method purpose	• Describe the goal and desired output of a method[2] • Describe how a method modifies a particular system (e.g. slows reaction rate, changes angles between residues, increases unfolding)[3] • Use ERs (e.g. a schematic) to associate the steps of experimental processes with the research subject/phenomenon[4] • When possible, show how seemingly dissimilar methods can achieve similar goals (e.g. piecing together processes or creating new methods based on principles underlying other methods)[6]
Describes treatment variables	• Construct multiple ERs/panels to show different treatments/conditions, labeling the conditions on each[2] • Prompt students to identify and compare treatment variables[4] • Compare experimental *(in vitro, in silico)* conditions to cellular (*in vivo*) conditions[5] • Associate changes in treatments/conditions with plot axes[2] • Discuss what variables and/or conditions are possible to represent in *in silico* models (related to limitations below)[6]
Describes outcome variables	• Provide examples of data, including graphs or plots, relative magnitudes or estimates of values, etc. • Explain how data are manipulated to create a "complete picture" of a phenomenon[5] • Prompt students to associate data values with ERs representing abstract concepts (e.g. reaction coordinates, energy level diagrams)[3] • Use narratives to frame the types of data collected as part of piecing together a larger "model" or "story"
Describes method limitations, error, or limitations in data representation	• Describe the properties of a phenomenon that make it easier or more difficult to study[5] • Compare two methods to illustrate the limitations and affordances of each[5] • Identify approximations or sources of error inherent to a particular method[5] • Model evaluation and creation of ERs in terms of communicating data (e.g. compare 'realistic' and 'schematic' ERs in terms of accuracy v. clarity)[6] • Discuss data resolution and how to represent resolution[6] • Prompt students to evaluate/develop methods given constraints[6]
Compares data to expectations or to other work in the field	• Frame the use of specific experimental methods in terms of the information they provide about a phenomenon[5] • Discuss the types of methods and data that have been used to study a phenomenon[2] • Describe what avenues of research have not been pursued or only pursued in a limited manner[2]

We wonder how the incorporation of methods in the instruction of biochemistry may affect student understanding, particularly in relation to abstract concepts like energy. Jeffery et al. (2018) characterized how the same set of experts explained thermodynamic and kinetic concepts in different ways aligned with their research methods. John, for example, uses kinetics-based methods and considers free energy and stability from a temporal perspective, interweaving time, frequency, and population with the idea of a protein 'jiggling' in and out of particular conformations. The authors believe the data presented in this paper demonstrate a way to communicate abstract ideas by grounding them in experiences, specifically experimental methods. We propose that as disciplinary meaning-making resources, experimental methods and activities could serve as knowledge resources that help ground knowledge and reasoning about abstract concepts (Brookes & Etkina, 2015; Jeppsson et al., 2013), and could influence how students conceptualize physical phenomena in the same way linguistic choices do (Brookes & Etkina, 2015; Kaper & Goedhart, 2002). As stated by the textbooks reviewed here, biochemical knowledge advances in parallel with the development of biochemical techniques (Garrett & Grisham, 2013; Nelson & Cox, 2013).

Other work (Amin, 2009) also suggests that experiential resources can support the design of ERs by pulling on embodied conceptions (i.e. conceptions based in sensorimotor experiences) which are used to conceptualize abstract concepts by referring to more familiar or concrete ideas (Lakoff & Johnson, 1980; 1999). Scientists draw on sensorimotor experiences to understand abstract thermodynamic functions: for example, treating energy as a substance or a location (Amin et al., 2012; Close & Scherr, 2015; Dreyfus, Gupta, & Redish, 2015). Furthermore, using multiple ERs to model concepts from a variety of perspectives reflects the flexible nature of scientific concepts and is more consistent with the nature of scientific expertise (Jeppsson et al., 2013; Brookes & Etkina, 2007; 2009). Very little research has been conducted to understand how different ERs help or hinder access to various disciplinary ways of knowing (Offerdahl et al., 2017). The authors would like to encourage future research in this area, particularly as institutions adopt educational approaches that foster experimentation, such as course-based undergraduate research experiences (CUREs).

5.7.2 Supporting Student Interpretation of Representations

Previous research has shown that biochemistry students struggle with interpreting ERs due to the complex and abstract nature of the phenomena they are meant to represent, as well as students' own lack of content knowledge, unfamiliarity with symbolism, and limited reasoning skills (Schönborn & Anderson, 2006; Schönborn, Anderson, & Grayson, 2002). The CRM model (Schönborn & Anderson, 2009) proposes that successful interpretation of an ER requires retrieval of appropriate conceptual knowledge (C), recognition of the symbols and icons present (M), and application of the necessary reasoning skills (R). In an instructional context, this means students should receive scaffolding that targets each of these components when they encounter unfamiliar ERs.

The findings of this study suggest ways in which instructors and textbooks might support student interpretation of ERs. We identified a number of reasoning behaviors employed by experts which suggest potential actions for instructors (Table 5.4), such as using gesture to repeatedly relate ERs of entities or processes to mathematical equations, or prompting students to compare features of data representations like line shape. The identified reasoning behaviors can also inform activities to help students interpret ERs and/or draw the connections missing in textbooks. We provide specific recommendations for how textbooks might support interpretation of ERs as well as incorporate research methods in Table 5.5. We have provided an extensive list of suggestions in these two tables, but it would obviously be too challenging to incorporate all of these simultaneously into one course. Instead, we suggest that instructors survey the suggested instructional actions and decide which could be incorporated as a priority into their classroom as part of their teaching. As different actions are successfully incorporated, an instructor can phase in additional actions.

Table 5.4 Examples of potential actions for instructors to model and/or scaffold interpretation of ERs during instruction.

The actions provided for each category are listed in no particular order. The highest Bloom's taxonomy level that could be associated with a particular potential action is indicated as a superscript at the end of each action (1-Knowledge; 2-Understand; 3-Apply; 4-Analyze; 5-Evaluate; 6-Create).

Category	**Potential Actions for Instructors**
Identify/Associate	• Point to identify the processes, entities, etc. represented in the ER being discussed • Point to indicate where entities in ERs "have motion" and use gestures to demonstrate the type of motion if possible • When introducing a new ER, prompt students to first identify symbols and icons, then identify conceptual knowledge about the entities, properties, etc. the symbols are meant to represent[2] • Draw or trace a plot to associate its shape with changing properties, processes, etc.[3] • Use simple shapes to indicate changes to the entities represented on an ER (e.g. use an 'X' to indicate which residue is mutated) • Combine gestures with descriptions of processes or two-dimensional symbols (e.g. arrows, letters) to add structural information or motion • Point to associate different kinds of ERs, particularly drawings, with mathematical expressions, terms, and graphs of data • Combine speech and drawing when introducing ERs with complex, multi-component states (i.e. present entities then interactions successively)
Compare	• Use different colors and shapes to emphasize or draw attention to significant features of ERs • Add quantitative and qualitative comparisons directly on ERs (e.g. use arrows of different lengths to show differences in magnitude) • Cover up or reveal portions of ERs to support comparison and discussion of hypothetical situations (e.g. cover $T\Delta S$ when ignoring the effect of T on ΔG of a system) • Prompt students to compare the states and properties of the entities represented[4] • Prompt students to compare features of data (e.g. line shape, bar height, bar groupings) and axes on plots[4] • Juxtapose ERs to support comparisons • Compare the components of mathematical expressions and terms[4] • Prompt students to predict how modifying a mathematical term affects the expression/outputs (e.g. will the output be larger? Smaller?)[5]
Order	• Combine speech with pointing when walking through the events/steps in a process • Use different colors and shapes to emphasize or draw attention to events/steps • Use labels to define events/steps and aid discussion about the order of events
Draw (causal) links	• Point to indicate what events or interactions are affecting states or properties when drawing causal links • Use arrows to indicate relationship(s) or lack of relationship(s) between ideas or ERs • Relate mathematical expressions to each other through variables and purpose[4] • Describe the relative magnitudes of variables or terms in mathematical equations and relate their values to mathematical outputs and physical meaning (e.g. "If this term is small, the overall value will…")[5]

Table 5.5 Recommendations for textbooks writers.

Recommendations regarding how to incorporate research methods and support student interpretation of ERs. These recommendations are similar to some of the potential actions listed in Tables 5.3 and 5.4, however they are more specific to the organization and composition of text and ERs in textbooks.

Body Text / Caption
• Identify and refer to the graphical units, symbols, steps, etc. of an ER throughout the associated text (as opposed to a single reference) • Identify significant features of numerical or graphical data and describe their meaning; possibly compare the interpretation to an alternative case • Associate mathematical expressions, terms, or operations with specific processes, states, interactions, etc., as well as the behavior of plots • Prompt horizontal translation by associating different types of ERs which represent the same entities, processes, etc. (e.g. Lewis structures, ribbon) • Use narrative to walk the reader through ERs, particularly when discussing multi-step processes or drawing links between steps • Prompt consideration of multiple ERs simultaneously, highlighting the information provided by each (e.g. the graph indicates at what concentration the protein begins to unfold, while the protein structure indicates where unfolding first occurs) • Whenever a specific experiment or research method is identified, describe the purpose (i.e., what will an experiment reveal about the phenomenon? Or how will an experiment alter the research subject?) • Discuss treatment variables and their effect on a phenomenon, especially if they change over time (e.g. protein denaturation) • Describe outcome variables for particular methods and/or provide examples of data including exact numerical values, comparisons of relative magnitudes, and/or qualitative descriptions or comparisons • Comment on limitations of methods, data, or data ERs when possible • Indicate uncertainty about phenomena, the limits of current research methods, and/or questions or areas that remain unexplored • Associate methods and phenomena with a specific biological or social context wherever possible, particularly when interpreting data or data ERs, to emphasize the relevance of the phenomenon to students
Representations
• Use proximity to combine different types of ERs (e.g. mathematical formulas, cartoon graphics) to connect mathematical terms and operations, with cartoons of processes, states, etc. • Explicitly associate arrows with movement or properties through the use of labels (and refer to them in the text) • Provide examples of alternative cases of states (e.g. high entropy v. low entropy) or of data on plots/graphs (e.g. steep v. shallow slopes mean…) to facilitate comparison • For complex or multi-component systems, use a series of panels to "build up" to the final diagram (i.e. successively identify entities, then interactions, then discuss properties of the system, etc.) • If digital material is included with the textbook, create ERs that animate simple movements • Illustrate how specific experiments or research methods affect or alter the research subject at particulate and macroscopic (measurable) levels. This is related to what data/outcome variables reveal about a phenomenon. • Use multiple panels/ERs to compare different treatment variables or treatment over time, labeling each • Use actual data, in addition to or rather than idealized examples of data

After engaging in simpler reasoning behaviors to establish the content of ERs, the experts employed more complex reasoning behaviors as they began to consider the context of their research. This hierarchy of behaviors aligns with the idea of lower-order and higher-order cognitive skills as described by the revised Bloom's taxonomy (Anderson et al., 2001), and other tools that describe levels of visual literacy (Arneson & Offerdahl, 2018; Trumbo, 1999; Mnguni, Schönborn, & Anderson, 2016). The use of seemingly more complex cognitive skills when considering the surrounding research context may have implications for structuring the presentation of material and questions in textbooks and curriculum. In Figure 5.6, we provide an example of how an instructor might ask questions to help students interpret a fictitious data ER modeled on figure from a protein folding research study. Initial questions should encourage lower-order skills like identifying states or associating processes with specific mathematical terms (Table 5.1). These kinds of questions prompt students to decode symbols and icons, which is the most basic skill of ER interpretation, as well as access pertinent conceptual knowledge (Ainsworth, 2008).

Interpret the representation below by answering the following questions:

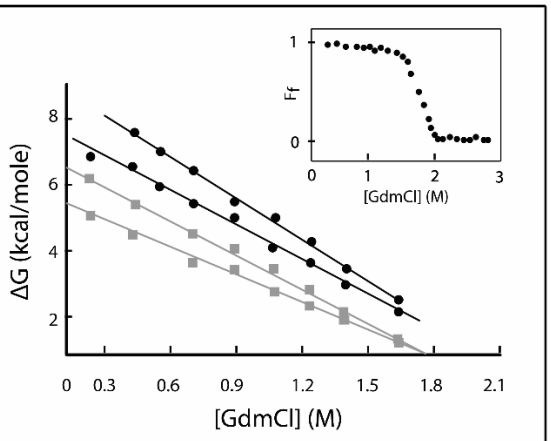


Figure X. E. coli RNase HI shows regions with different degrees of protection based on the stability of amide protons to hydrogen exchange. a) The free energy of hydrogen exchange plotted as a function of denaturant (guanidinium chloride) concentration. Black data corresponds to protons on helix A. Dark grey data corresponds to protons on helix D, β-strand 5, kinked B/C helix and the connecting loop. Inset: The fraction of folded protein as a function of denaturant, as determined by circular dichromism. b) The two regions are shown on a ribbon diagram of RNase HI (PDB: 1F21). Regions consist of helix A (black) and helix D, β-strand 5, kinked B/C helix and the connecting loop (dark grey).

1. Identify what each of the axes and symbols on plot (a) is meant to represent in the physical world (e.g. Do they describe entities? Properties? Environmental conditions?). What do large and small values on each of these axes indicate?

2. Identify the protein regions associated with each of the sets of colored lines on the ΔG v. [GdmCl] plot.

3. Compare the location of the sets of colored lines to each other and to the ΔG axis. What does their height imply about the stability of each of the protein regions?

4. Under what environmental conditions does the protein unfold according to the inset F_f plot? What features of the plot indicate this?

5. Based on the data presented, construct an energy level diagram comparing the difference in the ΔG of unfolding for the different protein regions (helix A; helix D, β-strand 5, kinked B/C helix, and the connecting loop) and the native structure of the protein.

6. Based on the relative ΔG values for the different regions of the protein, in what order might you expect the protein to fold (i.e. which region would fold first? Second?)? Explain your reasoning.

7. Imagine that you need to engineer this protein so that it can function under higher temperatures. Describe, in general, what you might do to enhance this protein's stability. What other factors do you have to take under consideration and how will you show, experimentally, that you have accounted for them?

Figure 5.6 An example of how an instructor might scaffold a series of increasingly complex questions to support interpretation of an ER of experimental data.

This figure contains fictitious data, but the format of the ER and the data used to create the ribbon diagram is based on published data exploring the folding of E. coli RNase H (Chamberlain, Handel & Marqusee, 1996; Hu et al., 2013). Questions 1-4 focus on decoding the meaning of the symbols by relating them to the experimental system; relating the graphs and ribbon diagram (component ERs); and comparing the values of the plotted data. Question 5 prompts students to compare the relative free energy data and transform the data into another kind of ER. Question 6 prompts students to draw a causal link between free energy change and degree of unfolding, in order to create a model of the protein folding process. Question 7 then aims to extend students' consideration beyond this specific ER to the practical application of the data and other experimental techniques.

After students have accessed the knowledge needed to effectively interpret an ER, instructors can pose more complex questions which require students to engage in higher-order cognitive skills, such as critiquing choice of research method or estimating experimental outcomes. This requires that the content presented in textbooks and instruction are situated within a research context which allows students to consider experimentation and social significance, like the experts in this study. Understanding of the practical context is so fundamental to understanding data ERs that "…students should not be taught interpretive resources independent of actual interpretations…" that is, independent of practical contexts (Bowen et al., 1999).

In summary, we have provided strategies to improve instruction with biochemical research methods and representations, but the question remains: are students being given the chance to become fluent with representations like those used by experts in cutting-edge research?

5.8 Acknowledgments

We especially thank our expert participants for their time and willingness to share their knowledge and insight into their research areas, as well as Stefan Irby of the VIBE Research Group for acting as a sounding board and his frequent feedback on this paper. A portion of this work was supported by the ACE-Bio Network project (NSF grant 1346567) and the BASIL project (NSF grant 1503798). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

CHAPTER 6. HOW TO TRANSLATE PROTEIN-FOLDING AND DYNAMICS RESEARCH INTO THE BIOCHEMISTRY CLASSROOM: USING EXPERT RESEARCH KNOWLEDGE AND REPRESENTATIONS TO INFORM CURRICULUM DESIGN

A version of this chapter is being prepared for submission as a stand-alone manuscript: Jeffery, K. A., Pelaez, N. J., & Anderson, T. R. How to Translate Protein-Folding and Dynamics Research Into the Biochemistry Classroom: Using Expert Research Knowledge and Representations to Inform Curriculum Design.

6.1 Abstract

For disciplines like biochemistry that focus on studying abstract and complex systems, interpreting cutting-edge experimental work and representations is an essential part of constructing meaning and engaging in the discipline. In contrast, biochemistry textbooks often contain dated information, supply representations that have been decontextualized or modified by publishers, and seldom discuss how experimental work is used to investigate complex biochemical phenomena, such as protein folding and dynamics. Thus, in order to keep biochemistry instruction relevant and to equip students with the skills they need to address research problems, undergraduate curricula must teach experimental research methods and make representations a major part of classroom discussions. Accordingly, the goal of this study was to demonstrate how expert research can be used to inform the design and implementation of instructional materials aimed at developing biochemistry students' understanding of protein folding and dynamics. To address this goal, we attempted to answer the following research questions: How can we use expert research knowledge and representations to develop and organize anticipated learning outcomes (ALOs) about protein folding and dynamics (RQ1); how can we used expert research knowledge and representation to design a curriculum module to design a curriculum module to develop biochemistry students' understanding of protein folding and dynamics (RQ2); and what is the nature of understanding developed by students during the curriculum module (RQ3)? Here, we report on the process of analyzing interviews with expert research scientists, primary literature, and textbook and policy learning objectives to inform the design of a curriculum module on the use of hydrogen-deuterium exchange mass spectrometry (HDX-MS) to study protein structure and dynamics in the development of biopharmaceuticals (RQs 1 and 2). Components of the curriculum module were

piloted in an undergraduate biochemistry course for the health sciences to explore the understandings developed by students through the materials (RQ3). Through this process, we demonstrate that expert research can be used to develop ALOs relevant at the undergraduate level which support the incorporation of cutting-edge research contexts, experimental methods, and authentic scientific activities, like representation interpretation and data analysis, into the classroom. We provide evidence of students' abilities and difficulties in explaining HDX-MS, in applying their understanding to interpret representations, and in discussing protein structure, bonding, and folding. We believe the findings of this study suggest that significant potential remains untapped when expert data sources are not considered in the development of curriculum. Compared to what is typically presented in textbooks, we believe the ALOs developed in this study more accurately reflect how knowledge of protein folding and dynamics is created, applied, and represented in an authentic research context. We demonstrate that students can learn about foundational biochemistry concepts and experimental methods through case studies of relevant research contexts. We argue through this study that future education research and instruction can design curricular materials that incorporate relevant case studies of expert research and take advantage of experimental methods and representations in order to better teach complex, poorly covered, or out-of-date topics.

6.2 Introduction and Motivation

Scientific practice is characterized by the use of multiple resources to study, understand, and communicate phenomena, including language, external representations (ERs), activities like modeling, and experimental work (Airey & Linder, 2009). Fluency with these different resources – that is, an understanding of how concepts are known through scientific activities like experimental work, how concepts are represented, and how to productively coordinate and translate between multiple resources – provides holistic access to disciplinary ways of knowing (Airey & Linder, 2009; Lemke, 1998; 2002; 2004). Thus, just as these resources are a critical part of scientific practice, they should also be an important part of the teaching and learning of science (e.g. Adadan, 2013; Ainsworth, 2008; Airey & Linder, 2009; Kozma, 2003; Kozma & Russell, 2005; Oliveira, Justi, & Mendonça, 2015).

However, science education research has primarily focused on studying the effect of single resources on student understanding (e.g. use of a particular type of ER), rather than the collective

role of language, ERs, activities, and experimental work in developing understanding (e.g. Matuk, 2018; Oliveria et al., 2015). Previous work, for example, has established the difficulties science students face when interpreting ERs (e.g. Pinto & Ametller, 2002; Schönborn, Anderson, & Grayson, 2002; Schönborn & Anderson, 2006), the importance of explicit instruction to enhance representational competence (e.g. diSessa, 2010; Stieff, 2011), as well as the importance of ERs in developing student understanding of complex scientific concepts (e.g. Ainsworth, 2008). Many studies have also compared the role of physical and virtual laboratories on student performance (e.g. Brinson, 2015; de Jong, Linn, & Zacharia, 2013; Irby, Borda, & Haupt, 2018), but very few studies explicitly focus on the role of experimental work in developing student understanding. Experimental work and tools are often treated as merely part of providing concrete experiences to students in the lab (e.g. Trumper, 2003), but experimental work is actually the primary source of most scientific knowledge and may shape the nature of an individual's understanding of the content matter (Bernhard, 2007; 2010). In exploring the affordances of various technologies in physics labs, Bernhard (2018) for instance, found that using different measurement technologies influenced what students could experience in the laboratory and therefore affected their discourse. Other studies similarly suggest a close relationship between the development of conceptual understanding and the experimental process (e.g. Bernhard, 2018; Nersessian & Chandrasekharan, 2009), including our previous work (Jeffery, Pelaez, & Anderson, 2018) which found that the ways in which experts discussed thermodynamic concepts (e.g. entropy) were aligned with the experimental methods they employed in their research. This suggests that there is much to be gained from investigating how ERs and experimental work are used by experts to understand phenomena, with particular consideration for how such expert research knowledge can be used to inform the design of modern undergraduate curricula – the focus of the present study.

For disciplines like biochemistry that focus on representing abstract and complex systems, interpreting experiments and ERs is an essential part of constructing meaning and engaging in the discipline. However, the experimental work and ERs used to investigate biochemical phenomena are often glossed over or not discussed in undergraduate instructional materials, contributing to a disconnect between disciplinary knowledge and disciplinary ways of knowing (e.g. Rybarczyk, 2011). In the design of curriculum, this issue is further compounded by the use of potentially inaccurate multimedia resources (e.g. Goodsell & Johnson, 2007), as well as the use of textbooks which may be dated, third-hand, and contain ERs that have been decontextualized, modified by

publishers, and/or separated from other ERs that supported meaning-making (Bowen & Roth, 2002; Roth & Bowen, 1999; Roth, Bowen, & McGinn, 1999).

Consider protein folding and dynamics – the context of the present study. Protein folding and dynamics are foundational concepts of central importance to the life sciences. In opening up a biochemistry textbook, one will almost certainly find discussion of Anfinsen's experiment and protein folding funnels. However, while important to protein folding, these examples stand in great contrast to current expert research in protein folding, which uses experimental methods like NMR spectroscopy (e.g. Kleckner & Foster, 2011), mass spectrometry, and *in silico* molecular dynamics simulations (e.g. Compiani & Capriotti, 2013), in contexts such as protein engineering and biopharmaceuticals. In addition, the information necessary for a complete description of protein folding and dynamics, is often located in different sections of a textbook (e.g. under protein structure and bioenergetics topics), leaving it to the reader to create an integrated explanation of the phenomenon which, as stated earlier, may still be affected by out-of-date information. Therefore, compared to other available sources, expert research knowledge is the primary and most rigorous source to understand how experimental work and ERs are used to create and represent disciplinary knowledge, and was thus the target of the present study.

6.3 Research Questions

The overarching goal of this study was to demonstrate how expert research can be used to inform the design and implementation of instructional materials aimed at developing student understanding about complex phenomena, like protein folding and dynamics, and the experimental methods that underpin such knowledge. Towards this goal, we addressed the following research questions (RQs):

1) How can we use expert research knowledge and representations to develop and organize anticipated learning outcomes about protein folding and dynamics?
2) How can we use expert research knowledge and representations to design a curriculum module to develop biochemistry students' understanding of protein folding and dynamics?
3) What is the nature of understanding developed by students during the curriculum module?

Our approach to addressing these RQs was similar to that of Trujillo, Anderson, and Pelaez, (2016b) in that we used expert knowledge and a backwards design approach (Wiggins & McTighe, 2005) to develop instructional materials based on expert knowledge. Backwards design is based on the principle that curriculum design begins with identifying desired or anticipated learning outcomes (what students should know, understand, and be able to do) and determining what is acceptable evidence that such learning has actually occurred (Wiggins & McTighe, 2005). To explore expert research knowledge, we interviewed four scientists about their research related to protein folding and dynamics, and analyzed these interviews along with related primary literature.

Here we report on the process of using expert data to inform the design of curriculum that translates cutting-edge research into the biochemistry classroom by elaborating on the design of curriculum module that aims to develop student understanding of hydrogen-deuterium exchange mass spectrometry (HDX-MS; see, for example, Marcsisin & Engen, 2010) as a method to study protein structure and dynamics (RQ1/2), and subsequent assessment of student understanding (RQ3).

6.4 Description of the Curriculum Module

The HDX-MS curriculum module discussed here includes a pre-reading and two activities (see Appendix C). Its purpose is to teach students how HDX-MS can be used to determine information about protein structure and dynamics (e.g. extent of denaturation), which is of particular relevance to the development and storage of protein drugs produced by biopharmaceutical companies. In addition, it aims to support student interpretation of complex data representations from relevant primary literature. The pre-reading provides an introduction to HDX-MS and its use as a method to investigate protein structure and dynamics, situated in the context of drug discovery and development. Written in a narrative-like format, the pre-reading walks students through the interpretation of several representations. Illustrative representations are used to explain the phenomenon of hydrogen-deuterium exchange. Representations of data, reflecting representations from primary literature (e.g. Campobasso & Huddler, 2015), are also presented to prepare students for the interpretation of similar ERs in the curriculum module's activities. For the purposes of this study, the pre-reading was also adapted into an active learning format for the classroom, taking the place of one 50-minute lecture in an undergraduate biochemistry course. In this format, the instructor presented an overview of the main points from the pre-reading through PowerPoint, and

embedded several questions in the presentation to facilitate discussion between the students and check for understanding. The students were then introduced to the research context for Activity 1 (see below) and had approximately half of the class time to work on it in small groups.

The HDX-MS curriculum module also includes two classroom activities to be completed after covering the pre-reading material. Each activity is based on a different study taken from recent biopharmaceutical literature. Activity 1 is based on a study by Moorthy et al. (2014) which is introduced through a brief summary of its research purpose and methods. In this activity, students are guided through the interpretation of a table and two figures from the article using sequential, scaffolded questions. The figure around which much of the questions center is a more complex version of one of the data representations (i.e. a heat map) discussed in the pre-reading. In Activity 2, the students are introduced to a second study (Hsu et al., 2013) and guided through the interpretation of several multi-component figures. One of these figures combines the two main data representations discussed in the pre-reading (i.e. heat maps and residue uptake plots) into one compound figure. The other figure, despite appearing very different at the surface level, incorporates features of both discussed data representations.

6.5 Methods

Figure 6.1 presents an overview of the process used to develop and organize the ALOs (RQ1), design the HDX-MS curriculum module materials (RQ2), and assess student understanding (RQ3). Accordingly, the Methods section is divided into several phases which correspond to each of the research questions. As in Figure 6.1, in Phase 1 (Section 6.5.2) we describe how we analyzed data from expert interviews, primary literature, and textbook and policy objectives in order to develop, refine, and organize ALOs. These ALOs guided the development of the HDX-MS curriculum module, which is described in Phase 2 (Section 6.5.3). The design of materials in Phase 2 was also informed by expert research and expert-based models (Figure 6.1). Finally, in Phase 3 (Section 6.5.4), we discuss the implementation of components of the HDX-MS curriculum module and assess student responses. Phase 3 is informed by and informs the development of ALOs and the design of the module materials, as indicated by the vertical arrows leading to and from the boxes in Figure 6.1. The overall process we engaged in to develop instructional materials from expert research was recursive within and across phases. For readability, we discuss our methods as discrete phases.

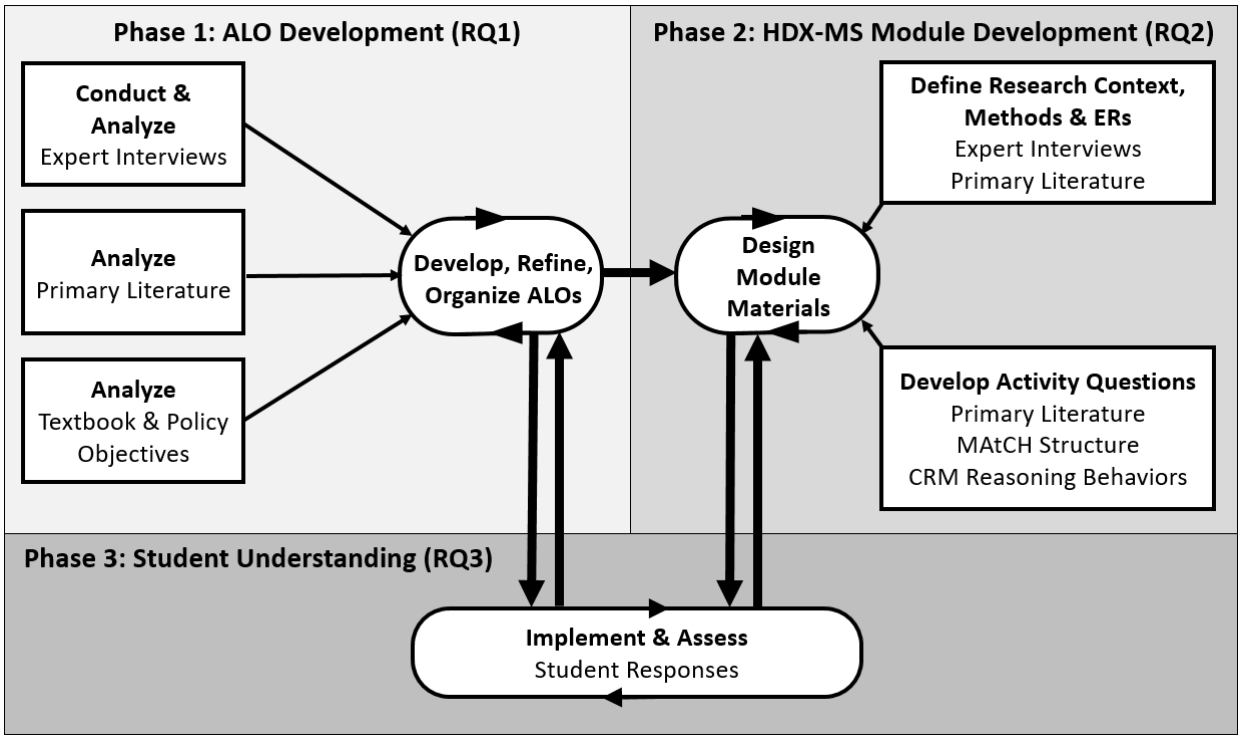


Figure 6.1 Overview of the process in which expert research was used to inform curriculum design. Overview of the process to identify anticipated learning outcomes (ALOs), design the HDX-MS module, and evaluate the nature of student understanding. Bolded text in each of the boxes summarizes the step of the process, while plain text indicates the data sources and expert-based models (e.g. MAtCH) employed. The boxes with curved edges surrounded by arrows indicate the iterative nature of refining the ALOs, designing module materials, and modifying the materials after conducting field tests with students.

6.5.1 Guiding Frameworks for Data Analysis and Curriculum Design

In this study, expert research was used to inform undergraduate instruction (e.g. Trujillo, Anderson & Pelaez, 2016b) of protein folding and dynamics. Our understanding of expert work in this area was informed by previous work (Jeffery et al., 2018; n.d.), as well as exploration of additional expert data sources as described below. Findings were used to inform the content and structure of the curricular materials. The materials were then explored, refined, and extended through field tests with students to improve the materials.

Two guiding frameworks were used to characterize the data: the MAtCH model of Jeffery et al. (2018) and the conceptual-reasoning-mode (CRM) model of Schönborn & Anderson (2009).

The MAtCH model identifies components of expert explanations and relationships between those components, including research methods, analogies, knowledge of scientific theories and models, research context, and how a phenomenon operates. The MAtCH model is an extension of the original MACH model (Trujillo, Anderson & Pelaez, 2015), which was developed from expert explanations of mechanisms in the life sciences, and has been used in the classroom to support undergraduate students in explaining molecular or cellular mechanisms (Trujillo, Anderson & Pelaez, 2016a).

The CRM model describes knowledge and skills required to effectively interpret external representations, providing a way to account for concepts (C) and modes (M) present in representations and characterize ways of reasoning (R) with concepts (RC) and modes (RM; Schönborn & Anderson, 2009; 2010). It has previously been used to identify visual skills in biochemistry (Schönborn & Anderson, 2009; 2010), to design assessments of student reasoning (Anderson, Schönborn, du Plessis, Gupthar, & Hull, 2013; Dasgupta, Anderson, & Pelaez, 2016), to guide research looking at scientists' use of evolutionary trees (Kong, Thawani, Anderson, & Pelaez, 2017) and explanations of molecular and cellular mechanisms (Trujillo et al., 2015), and to construct ability statements (ALOs) for a course-based undergraduate research experience (Irby, Pelaez, & Anderson, 2018a; b). We also previously applied the CRM model to characterize how representations are used by experts and textbooks in explanations of protein folding and dynamics, ultimately identifying a variety of reasoning behaviors (Jeffery et al., n.d.). The MAtCH model, CRM model, and identified reasoning behaviors were used to guide data analysis and activity design in this study.

6.5.2 Phase 1: Using Expert Resources to Develop Anticipated Learning Outcomes (RQ1)

In this section, we describe how several data sources were used to develop and organize ALOs from expert biochemistry research (RQ1). Three main sources informed the development of ALOs, their organization into a series of modules, and the design of the HDX-MS module (see Phase 2, Section 6.5.3): interviews with four research scientists, primary literature related to their research, and textbook and policy learning objectives. All of these data sources were triangulated (e.g. Carter et al., 2014) to develop ALOs for a series of modules on using experimental methods to study protein folding and dynamics (see Appendix C Table C.1), however we use examples from a single

module to illustrate the process of developing curriculum materials around complex expert resources.

6.5.2.1 Expert Interviews

Four research scientists, hereafter referred to as 'experts' or by pseudonyms, were interviewed about their research related to protein folding and dynamics (see Jeffery et al., 2018 for more detail). The research projects explained by the experts ranged from structure-based approaches to elucidate enzyme mechanisms, to developing and training computer simulations of drug metabolism. Concise descriptions of the experts' research goals and examples of their experimental methods are provided in Table 6.1.

Table 6.1 Brief descriptions of expert research goals and examples of experimental methods employed. All names are pseudonyms.

Expert	Research Goal	Experimental Methods
Beaker	To elucidate enzyme mechanisms	X-ray crystallography, enzyme kinetics
Gertrude	To (better) evaluate protein drug formulations	Hydrogen-deuterium exchange mass spectrometry
John	To investigate the relationship between protein energetics and function	Proteolysis kinetics, site-directed mutagenesis, spectroscopic methods
William	To improve computer prediction in drug binding and metabolism	Molecular docking and dynamics simulations

Each interview was transcribed verbatim, aligned with any external representations (ERs) shown or drawn during the interview, and drawing steps and gestures were described and inserted into the transcript. Each expert interview was then coded by the same researcher (KAJ) using the MAtCH model as a framework (Jeffery et al., 2018; Trujillo et al., 2015). Explicit skills described by or taken by the experts (e.g. "…then we use this and perform docking…"), as well as both explicitly-mentioned and implicitly-required concepts (e.g. orbitals, equilibrium), were identified and inductively coded with short, descriptive statements. To aid data analysis, the interview transcripts and their associated codes were divided into short segments. A separate document was

created to keep track of emerging patterns in the codes, such as those described below, ultimately producing the preliminary set of ALOs.

Analysis of the expert interviews served several purposes. First, it made it possible to identify phenomena, methods, and contexts that could serve as topics in a curriculum. For example, two of the experts in this study conduct research on protein drugs in order to understand and improve their stability, but utilize different experimental methods to do so. Curriculum materials based in a biopharmaceutical context are appropriate for a range of majors, such as pre-med or nursing, pharmaceutics, or biochemistry majors. The experts' different experimental methods are also appropriate topics for any of those majors, as well as for analytical chemistry or biotechnology majors.

Second, analysis of expert interviews also enabled identification of implicit or 'ancillary knowledge' employed by the experts. In this regard, Reif and colleagues note in their work on problem solving that the mere definition or statement of a concept is meaningless without ancillary knowledge to make the concept useful in a context; such as knowledge needed to apply the concept in specific instances or familiarity with the basic implications or applications of a principle (Reif & Heller, 1982; Reif, 1983). In the process of analyzing the expert interviews, the coder used words or short statements to identify explicit and implicit ideas. Table 6.2 provides a quote from an expert interview with examples of ancillary knowledge. Identifying ancillary knowledge helped specify topics to be addressed in a curriculum and/or pre-requisite knowledge that students would need or be assumed to have.

Table 6.2 Quote from an expert interview with examples of ancillary knowledge.

Ancillary knowledge makes the explicitly-stated concepts or principles useful in addressing complex problems.

Quote from Expert Interview	Ancillary Knowledge
"Most of all, for hydrogen exchange you need to reduce the pH. Okay? So typically people use pH 5 or 5.5. pH 7, now hydrogen exchange too fast because, you know, hydrogen exchange is catalyzed by hydroxide ion- So, you know, it's proportional. When you increase pH by 10- pH by 1, reaction rate is increased by 10. So at some point, you know- hydrogen exchange is too fast and it doesn't equilibrate for H ion any more. But proteolysis can be done in any pH so we can run proteolysis pH 8 or 7 so there's no real- especially with- we pick a protease which is relatively insensitive to the pH change, right? So we can use, like, many different pHs. Also, hydrogen exchange gives too much data. Sometimes it's so complex. It tells us so many different conformation changes and then- I- sometimes it's hard to sort those things out." (John)	• Appropriate pH ranges for employing HDX • Relationship between pH and concentration of hydronium/hydroxide ions • Chemical reactions have a range of rates and reaction rate is related to/can be controlled by reaction conditions (e.g. concentration) • Equilibrium involves opposing processes occurring at the same rate and initial reaction conditions or perturbation affect the ratio of the concentrations of reactants and products • Proteolysis as a reaction and experimental technique, and limitations/affordances as a technique • Effect of environmental conditions on protein structure (e.g. titration of amino acid residues) • Interactions/structural differences of proteins that lead to structural rigidity/stability • Type, amount, and analysis of data, and information about a phenomenon obtained from HDX • Existence/examples of protein conformational change

Third, analysis of the expert interviews provided examples of the types of representations used by researchers to create and communicate their understanding of phenomena, as well as characterizations of how they use them (i.e. reasoning behaviors; see also Jeffery et al., n.d.). This included simple diagrams of more complex processes, representations of data, and gestures used to convey both concrete and abstract ideas (see, for example, Box A in Table 6.3). In this way, experts' explanations of their research provided accessible summaries of important aspects of their research which could then be fleshed out through triangulation with other data sources (Carter et al., 2014).

6.5.2.2 Primary Literature

Several articles from primary literature on protein folding and dynamics were also coarsely coded using the MAtCH model. Two of the four experts were contacted via email for suggestions of proteins or protein drugs that they (or others) have researched which may be good candidates for a case study in the biochemistry classroom. We reviewed the suggested proteins and selected three articles related to RNase HI to code (e.g. Chamberlain et al., 1996). Each paragraph was coarsely

coded in order to indicate discussion of methods (M), analogies (A), theoretical knowledge (t), research context (C), or how the phenomenon occurred (H). Analysis of primary literature using the MAtCH model provided an additional way to identify phenomena, contexts, and methods addressed in protein-folding and dynamics research. For example, an article may state in their introduction or discussion section how their work may inform protein engineering efforts by revealing regions that are functionally significant or structurally weak (e.g. see Chang & Park, 2009). Thus, protein engineering could be an appropriate context around which to develop curricular materials related to protein structure, folding, dynamics, or function.

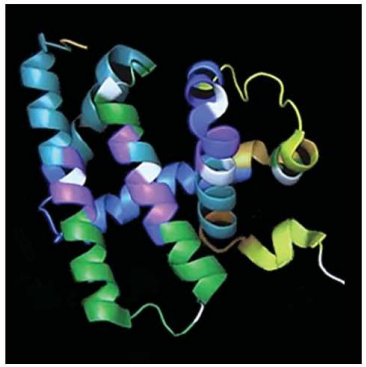
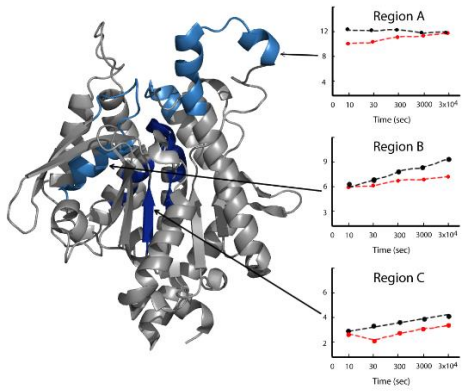
Given a particular research context, phenomenon, or experimental method (e.g. biopharmaceutical research or HDX-MS), analysis of relevant primary literature can also be used to identify common types of ERs and how they are interpreted and used. In reviewing primary literature, we attended to where/how the authors used and interpreted data representations in the article narrative and representation captions (e.g. how they connected features of a representation (A of MAtCH) to the phenomenon (H) or implications for the research context (C)). To illustrate, read the following caption:

> "RNase H* separates into three regions based on the stability of protons to hydrogen exchange. *a,* The free energy of hydrogen exchange (ΔG_{HX}) is plotted as a function of denaturant concentration. Protons shown are: Met 47, Gln 102 and Ala 110 in blue squares, triangles, and diamonds (helices A + D)…. *b.* The three regions with differing stabilities shown on a ribbon diagram of the RNase H crystal structure… The regions consist of: helices A and D (blue), helix B and strand 4 (green), and the remaining protons in helices C, E, and strands 1, 2, 3, and 5 (red)." (Chamberlain et al., 1996, p. 783)

While a figure its self suggests ER(s) that may be important to address in curriculum on protein folding and dynamics, the caption explicitly indicates what features a reader (or student) must decode in order to interpret the ER. Another example, taken from Hsu et al. (2013) which is used in the curriculum module designed in this study, is provided in Box B of Table 6.3. In this quote, Hsu et al. (2013) use a representation (A of MAtCH) to describe differences in hydrogen-deuterium exchange for sets of residues in the active site of a protein when a ligand is present or absent (M and H). In these two sentences, the authors refer the reader to another figure in the article, identify protein regions of interest, and comment on data patterns. To understand the ER within its research context, students will need to successfully handle each of these tasks and thus

they serve as guidelines for developing ALOs and questions aimed at scaffolding student interpretation of ERs. Identifying the ways in which primary literature used ERs helped further develop and refine preliminary ALOs (e.g. modifying language slightly in order to better match ER use), particularly by supporting the development of ALOs targeting skills related to interpreting ERs.

Table 6.3 Example of alignment of expert interviews, primary literature, and textbook and policy objectives, to develop an ALO.

A. Expert Interview	**B. Primary Literature**
 "The difference is that we can get... …a heat map that shows extent of deuterium incorporation…. So cool are less deuterium incorporated, hot colors are more. And so now with this HDX method, we can map what parts of the molecule incorporated a lot of deuterium and what parts incorporated none. So you can see right here these two alpha helices are… protected from exchange. Whereas this loop over here is brighter colored, hotter colored and so it incorporates a lot more deuterium." *	 "The significant deuteration changes upon PHFK binding are located in the catalytic domain of $iPLA_2$ (Figure 5). In particular, five regions surrounding the active-site dyad, residues 483-493, 516-525, 544-549, 631-655, and 773-778, showed the most significant decrease of deuteration (Figure 6), while none of the regions showed an increase in H/D exchange." (Hsu et al., 2013, p. 1333) †

C. Examples of Related Content from Textbook Objectives or Summaries	The peptide bond is uncharged, which allows proteins to form tightly packed globular structures having significant amounts of the backbone buried within the protein interior. (Berg et al., 2015, p. 60) Structural bioinformatics is concerned with the storage, visualization, analysis, and comparison of macromolecular structures. (Voet et al., 2013, p. 173) Without this flexibility, enzymes would be nonfunctional. … The exchange of a protein's internal protons with solvent requires its transient local unfolding. Hydrogen exchange studies therefore demonstrate that proteins have a great variety of infrequently occurring internal motions. (Voet & Voet, 2011, p. 318-319)
D. Examples of Related Content from ASBMB Foundational Concepts‡	Students should be able to compare and contrast the primary, secondary, tertiary and quaternary structures of proteins and nucleic acids. Students should be able discuss the time scales of various conformational effects in biological macromolecules and design appropriate experiments to investigate ligand induced changes in conformation and dynamics Students should be able to either propose experiments that would determine the quaternary structure of a molecule or be able to interpret data pertaining to tertiary and quaternary structure of molecules.

Table 6.3 continued

E. Anticipated Learning Outcome	Students should be able to interpret HDX data in the form of residue plots and/or heat maps in order to identify and compare protein regions with different amounts of exchange.

*This image was digitally modified to protect the confidentiality of the expert's research data. See also Jeffery et al. (2018).
†This image is an example of a type of representation found in primary literature. It is based off of Figure 6 in Hsu et al. (2013) and uses fictitious data.
‡These objectives are taken from the ASBMB Foundational Concepts on macromolecular structure & function (ASBMB, 2018).

Further review of primary literature can also be used to deepen the knowledge of the instructor and/or identify potential primary sources, such as introductory or seminal literature in the field, to bring into the classroom as part of an activity. In this study, we chose to develop a module on HDX-MS, and so reviewed a number of articles describing the basic theory, applications, and limitations of HDX-MS (e.g. Deng et al., 2016; Marciano et al., 2014; Campobasso & Huddler, 2015; Marcsisin & Engen, 2010; Wei et al., 2014). This also supported refinement of the ALOs (RQ1), as well as inspired the design of the ERs in the pre-reading (RQ2; see Phase 2).

6.5.2.3 Textbook and Policy Learning Objectives

Knowledge and reasoning skills identified during analysis of the expert interviews and primary literature were used to develop preliminary ALOs central to one or more expert explanation. These preliminary ALOs were then evaluated for pre-requisite knowledge a student would need in order to achieve an ALO, such as knowledge of secondary structures or the role of non-covalent forces in protein-protein interactions.

To aid identification of pre-requisite knowledge, the preliminary ALOs were also compared to learning objectives related to protein folding and dynamics in introductory biochemistry textbooks and biochemistry policy documents. Generally, biochemistry textbooks provide a simple treatment of the topic of protein folding/dynamics early on and then re-visit some ideas in the context of catalysis. Thus, for this study, stated learning objectives and summaries from textbook chapters on thermodynamics, protein structure and function, and catalytic mechanisms were reviewed and sorted into broad categories (Appling, Anthony-Cahill & Mathews, 2016; Berg, Tymoczko, Gatto & Stryer, 2015; Garrett & Grisham, 2013; McKee & McKee, 2012; Nelson & Cox, 2013; Pratt & Cornely, 2014; Voet & Voet, 2011; Voet, Voet & Pratt, 2013). Because most textbooks offer a simple treatment of protein folding and dynamics, they lack detailed objectives directly relevant to protein folding and dynamics specifically. Many objectives were instead related to foundational knowledge of protein structure, composing categories like ‘non-covalent structure-defining forces’ or ‘influence of environment.’ However, some of the reviewed textbook learning objectives did directly address protein folding and dynamics or the methods used to study them. See, for example, the objectives provided in Box C of Table 6.3. The first objective listed is a typical statement regarding how a particular structural

property of proteins affects protein folding. In order for a student to engage in discussions about changes to protein structure and methods to study those changes, students would need pre-requisite knowledge about higher-level protein structure like that stated. Thus, in this study, it is expected that students have learned this information before the first curriculum module. The second and third objectives listed in Box C inform the development of ALOs and/or learning objectives related to the representation and study of proteins, respectively. The second objective, for example, suggests that students should have or gain some understanding of how proteins can be visualized, analyzed, and compared, which is one of the overarching goals of the HDX-MS curriculum module presented here.

In addition, stated learning objectives from the American Society for Biochemistry and Molecular Biology's (ASBMB) Foundational Concepts (Tansey et al., 2013; White et al., 2013) were also reviewed and compared to both the preliminary ALOs and textbook objectives. The objectives put forth by the ASBMB represent foundational concepts and skills that biochemistry and molecular biology (BMB) educators believe BMB undergraduates should be equipped with upon graduation. Comparing the textbook objectives and our preliminary ALOs to the objectives put forth by the ASBMB allowed us to confirm pre-requisite or foundational knowledge (e.g. the first ASBMB objective listed in Box D of Table 6.3). Additionally, it enabled us to align our ALOs with current expectations of the BMB education community. For example, the second ASBMB objective listed in Box D of Table 6.3 states that students should be able to design experiments to investigate changes in conformation and dynamics. Thus, both of the activities in the HDX-MS curriculum module presented here explore how experimentation can reveal information about protein folding and dynamics. Additionally, Activity 2 is intentionally based around a study which used HDX-MS to explore the binding of a ligand to a protein. The third objective in Box D also states that students should be able to interpret data pertaining to higher-order structure. Thus, both activities are built around the interpretation data ERs to draw conclusions about protein structure. Other ALOs in subsequent modules (see Section 6.5.2.4 and Appendix C Table C.1) similarly state that students should be able to design experiments or interpret data to address questions about protein function, folding, or stability.

As described above, analysis of textbook and policy learning objectives helped us develop a more complete understanding of the learning outcomes currently expected of biochemistry students. We compared these objectives to the preliminary ALOs in order identify what pre-

requisite knowledge would be needed for each ALO, whether an ALO needed to be modified, or if additional ALOs that addressed particular content matter needed to be added. This process was important because biochemistry knowledge that is implicit for an expert may not have been captured in a preliminary ALO, and it is highly likely that students will need to be taught this information explicitly. For example, to understand how protein structure limits exchange of deuterium, students must have prior knowledge of the different levels of protein structure and the types of interactions that produce them. It is for this reason, as discussed in the following section, that organization of ALOs into modules was a critical part of the development process.

6.5.2.4 Development, Refinement, and Organization of ALOs into Curriculum

The data from the abovementioned sources (Sections 6.5.2.1 to 6.5.2.3) was triangulated (Carter et al., 2014) to develop and refine the ALOs. Table 6.3 provides an example of the alignment of expert interview data, primary literature, and textbook and policy learning objectives to develop a single ALO (Box E). As described previously, preliminary ALOs were developed from analysis of the expert interviews and further developed or refined from analysis of selected primary literature. In Boxes A and B in Table 6.3, the expert sources interpret HDX data in the form of heat maps (A and B) and residue uptake plots (B). Textbook and policy objectives, such as some of the examples provided in Boxes C and D, suggest that biochemistry students should have knowledge of and be able to visualize, compare, and interpret data about higher-order protein structure. Thus, the refined ALO in Box E states that students should be able to interpret HDX data, as presented in two types of ERs, in order to talk about protein structure.

As demonstrated in the preceding paragraphs, development of the ALOs from the expert data was a recursive process. Part of the refinement process also involved organizing the ALOs into a series of modules to make up a larger curriculum (see Appendix C Table C.1 for the expanded curriculum). Table 6.4 outlines the series of modules and provides an example of an ALO for each topic. While the exploration of this larger curriculum is an area for future research, the purpose – and importance – of organizing the ALOs was two-fold. First, it enabled the establishment of foundational knowledge needed for each successive ALO, allowing us to further refine the ALOs and add necessary ALOs. To illustrate this point: the expanded curriculum assumes that students have prior knowledge about levels of protein structure and that these levels of structure are obtained through folding caused by interactions like hydrogen bonding,

hydrophobic interactions, disulfide bonds, and salt bridges. Loosely based on the idea of a spiral curriculum (Harden, 1999), the curriculum is designed to revisit concepts, relating new learning to previous learning and providing increasing levels of difficulty in order to develop student understanding from an intuitive understanding of free energy as 'stability' to a more formal description of free energy through the Gibbs energy equation. This approach to developing student understanding is similar to Vygotsky's (1978) "zone of proximal development" which defines the distance between what a learner can do independently and what they can achieve with guidance or scaffolding. These understandings are built through and against a backdrop of experimental methods and representations employed by experts in the development of the same types of understandings. Thus, while general understandings of protein structure and chemical kinetics are appropriate for explaining protein stability in the first module, by the fourth module students are expected to be able to qualitatively relate the components of the free energy equation and use formal mathematical models to discuss stability.

The second reason for organizing the ALOs into a larger curriculum is that it allowed us to identify research contexts and experimental methods around which we could unify sets of ALOs to create 'modules,' and which could inform the development of module activities (RQ2). It would be unfeasible to incorporate all ALOs into a single lesson, unit, and/or research context, as well as impractical for others to attempt to incorporate an unorganized list of ALOs and contexts into their course.

Table 6.4 Outline of the module topics of an expanded curriculum about protein folding and dynamics.

Beside each module topic is an example of an ALO developed from the data sources. Please refer to Appendix C Table C.1 for the expanded curriculum, including additional ALOs and examples of classroom activities.

Module Topic	**Example ALO**
1. Proteins are dynamic molecules	Students should be able to explain how protein dynamics and unfolding lead to variable, measurable hydrogen-deuterium exchange (HDX) across different regions of a protein.
2. Proteins exist as an ensemble of states	Students should be able to describe and represent, at a macromolecular level, the equilibria that exist between folded, partially unfolded, and globally unfolded forms of a protein, and the relative population of different forms.
3. How the environment drives protein folding	Students should be able to explain and represent how interactions within a macromolecule and with molecules in the surrounding environment lead to emergent structure and degree of dynamics.
4. Structure v. flexibility	Students should be able to use HDX data and/or relative ΔG values of protein regions to construct a probable folding pathway for a protein.
5. Experimental techniques to study the function of residues and flexibility	Students should be able to identify residue (and/or ligand) properties and interactions based on their structure and environment, in order to predict possible functions.
6. Connecting kinetics to free energy	Students should be able to mathematically and qualitatively relate kinetic, equilibrium, and free energy values in order to map measurable data to conclusions about stability.

The use of the MAtCH model to code the expert data for knowledge and skills primed us to attend to the use of experimental methods and ERs to understand phenomena in various research contexts, which was useful in defining potential ‘boundaries’ for a module. The ALOs developed from this analysis therefore focus on how experimental methods are used to study and develop models of protein folding and dynamics. While experimental methods and ERs have been largely ignored in past learning objectives, recent education efforts have led to a much greater focus on understanding experimental competencies (Pelaez et al., 2017; Irby, Pelaez, et al., 2018a; b) and teaching science and engineering practices (NGSS, 2013). The ALOs presented in Table 6.4 are based on the expectation that students will develop skills that enable them to explain how and what

experimental methods measure or characterize about a phenomenon (so-called M-t-H connections in the MAtCH model); to represent a phenomenon or interpret data in the form of a representation to describe a phenomenon (M-A or H-A connections); and to use representations or models of phenomenon to address social or biological problems (A/H-C connections). Thus, for example, the ALOs associated with the first module topic, 'Proteins are dynamic molecules,' are unified around the use of hydrogen-deuterium exchange mass spectrometry to study protein structure and dynamics. In the next section, we illustrate how the ALOs and expert sources were used to design module activities (RQ2).

6.5.3 Phase 2: Designing Module Activities Using Expert Sources and Expert-Based ALOs (RQ2)

We chose to focus on developing a curriculum module on the use of HDX-MS to determine information about protein structure and dynamics in the context of drug development (RQ2). The module also intends to support student interpretation of data representations from primary literature. The specific ALOs for the HDX-MS curriculum module are presented in Table 6.5. We chose to situate the HDX-MS module in a biopharmaceuticals context based on the expert interviews (Table 6.1) and recent primary literature that employs HDX in the context of drug discovery and development.

Table 6.5 ALOs for the HDX-MS curriculum module.

1. Students should be able to explain how protein dynamics and unfolding lead to variable, measurable hydrogen-deuterium exchange (HDX) across different regions of a protein.
2. Students should be able to interpret HDX data in the form of residue plots and/or heat maps in order to identify and compare protein regions with different amounts of exchange.
3. Students should be able to use HDX data in order to draw conclusions about the spatial organization, stability, or function of different regions of a protein structure.

The HDX-MS module materials consist of a pre-reading and two activities (see Appendix C). These materials were developed through an iterative process, beginning with the pre-reading. In order to inform our explanation of how HDX-MS is used to study protein structure and dynamics, we reviewed several expert sources, including the expert interviews, biopharmaceutical studies that employed HDX-MS, and articles on the application of HDX-MS in drug development. As mentioned previously in Phase I, this included a number of articles describing the basic theory, applications, and limitations of HDX-MS (e.g. Deng et al., 2016; Marciano et al., 2014; Campobasso & Huddler, 2015; Marcsisin & Engen, 2010; Wei et al., 2014). The content of the interviews and primary literature was used to outline a general description of the experimental method (HDX-MS), to identify common ERs (heat maps and residue uptake plots), and to inspire the development of ERs for the pre-reading. Once outlined, a storyboard was drafted and modified through several iterations. The pre-reading and its series of ERs were then drafted and edited, with particular attention to composing a narrative which walked its readers through interpretation of the representations. Although we created ERs inspired from the literature, an instructor could use or adapt existing representations (see, for example, Fig. 2 in Deng et al., 2016).

Data representations from two studies (Hsu et al., 2013; Moorthy et al., 2014) were selected as the focus of the module Activities. The selection of these studies influenced the contents and ERs of the pre-reading because we needed to introduce and discuss ERs that would be the same as or similar to what students would later interpret in the Activities (i.e. heat maps, residue uptake plots). For each Activity, we composed short introductions to situate students to the context of the study by summarizing the research purpose and methods.

We then used two expertise-based models to guide the development of questions to help students interpret the selected representations in Activities 1 and 2 (Appendix C). The CRM reasoning behaviors identified in Jeffery, Pelaez, and Anderson (n.d.) were used to sequentially scaffold ER interpretation, and the MAtCH connections (Jeffery et al., 2018) were used to frame questions that prompted students to relate research context, method, and phenomenon. Sequentially scaffolded questions typically followed a pattern of: identify/associate, compare, order, draw (causal) links, and relate to broader experimental method or context. Figure 6.2 provides examples of generic questions that would fall under each of these categories and, reading from left to right, one can see an increasing degree of complexity.

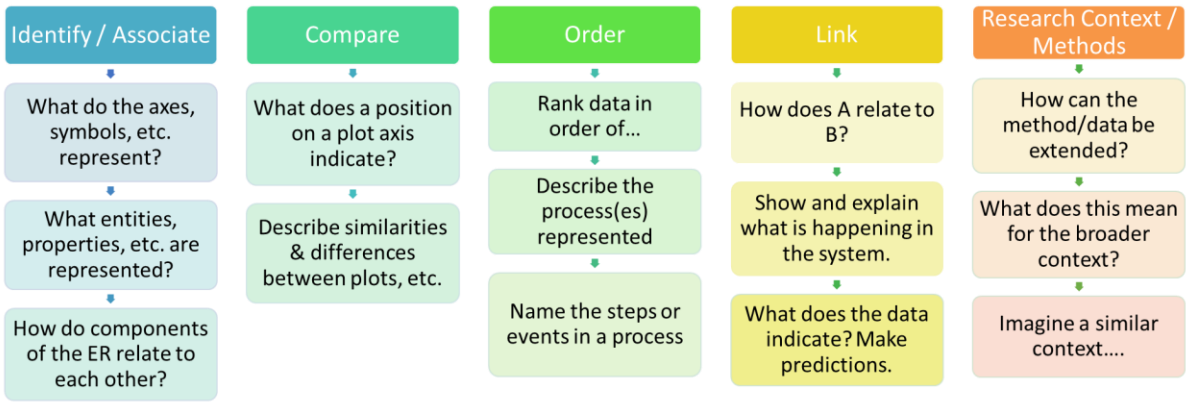


Figure 6.2 Example of the types and order of questions to sequentially scaffold interpretation of ERs and multiple representations (MRs).

Suggested questions are based on the categories of expert reasoning behaviors identified in Jeffery et al. (n.d.) and the MAtCH model (Jeffery et al., 2018). Briefly, questions prompt students to first decode symbolism and associate it with the physical system/process (identify/associate); evaluate differences in data if presented (compare); relate different data sets or describe a process (order); relate components or events in a system or process (draw links); and relate the ER to a phenomenon, method, or context.

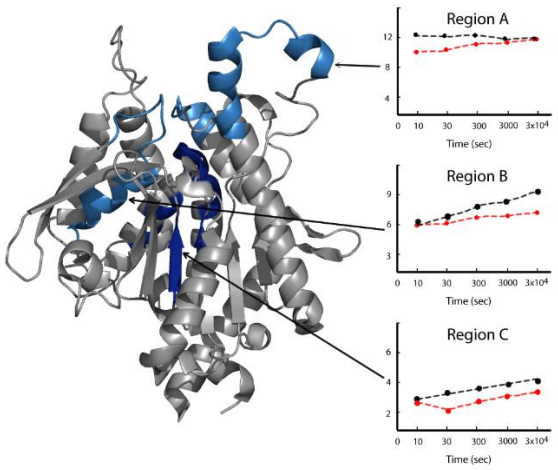
In Table 6.6 we provide an example of several scaffolded questions taken from Activity 2, which are aimed at supporting student interpretation of a figure from Hsu et al. (2013). Each question is annotated with its purpose according to the reasoning behaviors and MAtCH connections (see right column). For example, initial questions prompt students to decode symbolism and associate symbols with the physical system ("identify" and "associate"; e.g. question 3), while later questions prompt students to describe the components and relations of the physical system based on the data (e.g. question 5). Although each of the questions prompts students to consider particular MAtCH connections (indicated in parentheses in the annotation column), questions can be written to prompt students to consider more generally how the experimental method reveals the phenomenon (e.g. by describing or drawing the method; H-M; see Activity 1 in Appendix C) and/or to situate the figure in the research context or future studies by making inferences from the represented data (A/H-C; question 6 of Activity 2 in Table 6.6).

In developing the Activity questions, the ways in which the primary literature discussed ERs in the text of their articles helped guide question development; for example, by suggesting

comparisons between certain data points or information contained in separate figures. To illustrate, in the quote from primary literature in Box B of Table 6.3, the authors use an ER to describe differences in hydrogen-deuterium exchange for sets of residues in the active site of a protein when a ligand is present or absent (Hsu et al., 2013). When designing the questions to scaffold interpretation of this ER in Activity 2, several questions prompt students to use the HDX data to suggest a potential binding site (e.g. question 6 in Table 6.6).

Table 6.6 Example of scaffolded questions taken from Activity 2.

Annotations regarding the purpose of each question, whether to prompt particular reasoning behaviors (bolded) or highlight MAtCH connections (bolded, in parentheses), is provided in the column to the right of each question. For illustrative purposes, an image using fictitious data is shown. This image is based off of Figure 6 in Hsu et al. (2013) which is explored in Activity 2 (see Appendix C). The caption provided is for Figure 6 in Hsu et al. (2013).



"Figure 6. Regions of H/D exchange most affected by PHFK binding. The deuteron number is shown for each fragment in the presence (red circles) and absence (black squares) of inhibitor. The black and red curves indicate the number of H/D exchanges at six time points corresponding to 10, 30, 100, 300, 1000, and 3000 s. Color coding of structure is as shown in Figure 5."*

Questions	Annotation
1. According to the caption, what data is presented in Hsu et al. (2013) Figure 6?	Students are prompted to **identify** the entity and process **(H)** for which data **(M)** are presented in the figure **(A)**.
2. Identify the six color-coded regions on the protein by describing their structure (e.g. loop, alpha helix, etc.).	To orient them to the protein's features, students are prompted to **identify** structural elements of the protein **(H)** highlighted in the figure **(A)**.
3. Examine the legend for Figure 5 in Hsu et al. (2013). The color coding indicates the effect of PHFK binding on the amount of hydrogen/deuterium exchanged during a time period. The percentages beside each color represent the percent by which the deuteration level increased or decreased as a result of the presence of the inhibitor. (Hint: Just like you calculated in the previous question #4.) a. Using the deuteration level change legend from Hsu et al. Figure 5, identify the approximate percent change in deuteration level for each region.	Students are prompted to **associate** color **(A)** with amount of hydrogen-deuterium exchange **(M)** and with it **identify** change in deuteration for parts of the structure **(M/H)**. Students must refer to multiple representations.

Table 6.6 continued

4. Decode the symbolism on Hsu et al. (2013) Figure 6. a. What do the x- and y-axes of the six inset plots represent? Associate each axis with the physical property or process it represents. b. What do the black and red curves represent? What does a higher position on the y-axis indicate?	To support their interpretation of symbolism, students are prompted to **associate** the axes, lines and shapes, and relative position on a plot **(A)**, with the measured properties and processes of the physical system **(M/H)**.
5. Compare the red and black curves on each of the six plots: a. What does the relative position of the black and red curves indicate about hydrogen/deuterium exchange for those regions when the PHFK inhibitor is present vs. absent? b. Compare the scaling of the y-axis across the six inset plots. Keeping the different scales in mind, which plot(s) show the greatest difference in deuterium incorporation in the presence vs. absence of PHFK?	Students are prompted to **compare** the positions of the curves relative to each other on and across the plots **(A)**, and then **associate** the curves' positions with properties of the physical system **(M/H)**.
6. Consider both the structure and six inset plots. a. Given the change in deuteration level for the different regions and your general knowledge of enzyme structure, which regions would you predict to be part of the binding site? Indicate on Figure 6 where you think PHFK may bind. b. Use a series of sketches and captions to show and explain what is happening in this region to cause a change in deuterium incorporation. That is, explain how the binding of the PHFK inhibitor can affect the incorporation of deuterium. You can use simple shapes to represent iPLA2 and PHFK.	Students are prompted to **causally link** changes in deuterium incorporation as presented in the data **(A)** to a possible binding site **(H/C)** by **comparing** data across the different regions. Students are prompted to **causally link** measurable changes in deuterium incorporation **(M)** to changes in spatial organization of the protein-inhibitor system **(H)**.

*A correction to the caption for Figure 6 in Hsu et al. (2013) was published. The provided caption is the corrected version.
†This image is an example of a type of representation found in primary literature. It is based off of Figure 6 in Hsu et al. (2013) and uses fictitious data.

6.5.4 Phase 3: Module Implementation and Assessment of Student Understanding (RQ3)

In this section, we discuss the implementation of the pre-reading and Activity 1 of the HDX-MS module (see Appendix C) in an undergraduate biochemistry course for health sciences, and explore the nature of student understanding developed by students (RQ3). We briefly describe the methods used to code student responses to the assessment questions, before discussing modifications made to Activity 1 based on the field tests and evidence of student understanding.

6.5.4.1 Course and Student Context

The students were mainly upper division undergraduate students in an introductory biochemistry course for the health sciences. The course was customized to cater to the level and requirements of life science, health science, and agricultural majors, with a focus on developing the foundational principles and problem-solving competencies that are essential to practice in those areas. Protein structure, protein folding, and protein diseases were covered early in the semester, prior to the incorporation of the HDX-MS module materials. The ALOs of the HDX-MS curriculum module are well-aligned with the general and specific ALOs of the introductory biochemistry course, as outlined in Table 6.7. For example, one of the general ALOs of the course includes using visualizations to represent and explain abstract biochemical structures and processes, which aligns with all three module ALOs and is one of the overarching goals of the HDX-MS curriculum module. Furthermore, all three of the HDX-MS module ALOs necessitate that students be able to distinguish between different levels of protein structure and understand how covalent and non-covalent bonds stabilize protein structure, which are two specific course ALOs.

Table 6.7 Examples of general and specific ALOs from the introductory biochemistry course.

This course served as the context for the study. The numbers in the second column indicate how the HDX-MS curriculum module ALOs (see Table 6.5) align with the biochemistry course's ALOs.

Biochemistry Course ALOs	**Module ALO Alignment**
General ALOs	
• Understand how to use the listed knowledge and skills to inform yourself about biochemistry and to empower you think like a biochemist working in the health or life sciences	1-3
• Be able to illustrate specific learning outcomes with appropriate examples from the health/life sciences	1-3
• Be able to interpret and use representations (e.g. diagrams, molecular models) to represent and explain abstract biochemical structures, processes, and systems	1-3
Specific ALOs	
• Identify the amino and carboxyl groups, the α-carbon atom, and the amino acid side chains	1
• Explain and distinguish between the different levels of protein structure: primary, secondary, tertiary, and quaternary and how they are formed	1-3
• Understand how the various non-covalent and covalent bonds stabilize the different types of protein structure	1-3
• Recognize and distinguish beta pleated sheet and alpha helix secondary structures	1-3
• Understand the influence of protein structure and conformation on function and how small changes can drastically affect structure and function	3
• Interpret and distinguish between ball-and-stick, space-filling, and ribbon representations of peptides and proteins	2-3
• Explain the effects of specific denaturing conditions (e.g. detergents) on proteins, including the role of specific amino acids in the denaturation process	1-2

6.5.4.2 Field Tests of Module Components

The pre-reading and Activity 1 from the HDX-MS module (see Appendix C) were integrated into the biochemistry course near the end of the first unit, which focused mainly on protein structure. The pre-reading was adapted into an active learning format for the classroom, and in-class presentation modified slightly according to what material had been previously covered in the course. During the field tests, the presentation portion of the class lasted for approximately 30 minutes and was interspersed with several questions to formatively assess student understanding of several main ideas. Then the students were introduced to the Moorthy et al. (2014) study and given the last 20 minutes of class time to work on a shortened version of Activity 1. The instructors and teaching assistant walked around and assisted students during this time. At the end of the class, students were asked to turn in their worksheets, which were returned later to them with feedback. The pre-reading was also provided to the students online through the course management system. To assess student learning, questions were added to the unit exam and final exam (see Appendix C Table C.2). In short, the questions asked students to explain how HDX-MS is used to study protein conformational change (ALOs 1 and 3, Table 6.5) and/or to interpret HDX-MS data (ALO 2, Table 6.5). Student responses to the activity and exam questions in field tests were collected for analysis (protocols #1812021400; #1901021503).

6.5.4.3 Coding and Analysis of Data

Written student responses were analyzed inductively and deductively. Student responses were first coded for evidence of correct and incorrect student understanding. The MAtCH and CRM models were also applied to the data in order to explore how students reasoned with HDX-MS as an experimental method to explore protein structure and dynamics, as well as how they reasoned with ERs they produced or ERs that were part of the assessment questions.

6.6 Results

In this section, we briefly describe the kinds of modifications made to the pre-reading and Activity 1 based on instructor reflections, and student responses to the Activity 1 and exam questions. We also discuss preliminary patterns in student understanding and difficulties as revealed through the

activities and assessments (RQ3). Data analysis is ongoing and additional discussion of student reasoning will be the focus of future research.

6.6.1 Modifications to Activity

Interactions with students during class, as well as review of student responses to Activity 1, indicated several areas where modifications needed to be made. Many of these modifications related to wording, such as prompting students to 'Look at the information across Figure 4 and Table 1' or better directing their attention to decode the meaning of words like 'formulations' in the context of the study. Responses also revealed that most students could not discern the difference between the space-filling heme groups and their associated myoglobin ribbon structures due to the location of the heme (in the interior and partially obscured by the 3D ribbon structure) and color. Student responses on the exam questions also indicated that some ideas needed to be made more explicit in-class and in the pre-reading, such as which kinds of hydrogen atoms undergo measurable exchange.

6.6.2 Evidence of Student Understanding and Related Difficulties

We used student responses to Activity 1, an open-ended unit exam problem using HDX-MS data from a third study (Zhang, Banks, He, Treuheit, & Becker, 2015), and an open-ended question on the final exam to explore student knowledge and reasoning (see Appendix C Table C.2). In these assessments, students were required to explain how HDX-MS can be used to study protein folding and dynamics, and/or had to interpret HDX-MS data. On the unit exam, for example, students were asked in part (a) to explain how different protein regions can undergo different amounts of hydrogen-deuterium exchange, using drawings to compare are least two different regions. Part (b) of the question asked students to interpret a graph displaying percent deuterium uptake for residues 16-32 of an alpha helix in the protein rhGCSF under three conditions: rhGCSF alone, rhGCSF with sucrose, and rhGCSF with benzyl alcohol (Zhang et al., 2015). Students were asked to identify which formulation results in the most unfolding, citing evidence from the plot and how HDX-MS works. Part (c) of the question provided students with a ribbon structure of rhGCSF and asked them to explain what other information is needed to decide which formulation is better. Here, we discuss preliminary patterns in student understanding and difficulties as revealed through the unit exam problem. Analysis of student responses to the unit and final exam questions is ongoing.

Responses show that some students were able to correctly describe how protein structure affected the exchange of hydrogen and incorporation of deuterium in different regions of a protein (ALO 1). Students commonly drew representations of unstructured protein regions compared to alpha helices in order to show regions where there would be high vs. low exchange, respectively (Table 6.8, Row 1). They discussed how amount of exposure or protection to the environment, the role of hydrogen bonds or other intramolecular interactions, steric hindrance, and "breathing" motions of secondary structure could affect the rate and amount of incorporation of deuterium.

However, several areas of confusion were also evident. Some students reversed the meaning of deuterium exchange, stating that higher amounts of deuterium indicate more structure or less unfolding or vice versa (Table 6.8, Row 2). Additionally, some students – both those who were correct about what exchange indicated and those who were not – talked incorrectly about which hydrogens underwent exchange despite explicitly addressing this in class. For example, students provided examples and drawings showing exchange occurring with the hydroxyl group on a single carboxylic acid despite the fact that such a hydrogen would not be present along a peptide backbone (Table 6.8, Row 3). A few students also structured their sentences in ways that suggested that hydrogen-deuterium exchange was the *cause* of protein unfolding, although this could just indicate unrefined communication skills or unfamiliarity with explaining how experimental evidence elucidates phenomena.

Students were also capable of interpreting the data on the residue uptake plot (ALOs 1 and 2) in part (b) in order to correctly identify which formulation resulted in the most unfolding of rhGSCF. Some students described the change in the lines over time and/or relative to one another, even citing specific time stamps ("In 10 minutes…") and percentages ("60% of the hydrogens are replaced with deuterium"). A few students suggested reasons for why the exchange may have been different between the two formulations, including changes in the amount of "breathing" by the protein in one formulation vs. the other (Table 6.8, Row 4), and hydrogen bonding between the excipient and potential sites of exchange on the protein.

Table 6.8 Examples of student responses to the Unit Exam problem.

Brief notes regarding the responses are provided in italics beside each quote. Problem part is indicated in parentheses below the notes.

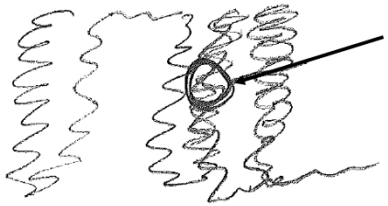
Examples of Student Responses	Notes
The more available a protein region is to accept hydrogen-deuterium [sic], the more it will exchange. These regions include regions that are on the outside of the protein, free to exchange.  A protein strand outside of the alpha-helices would be a great strand to have a lot of exchange, since it's easy access. Another example. This is where a great location would be for exchange of hydrogen-deuterium [sic] due to it being out in the "open" away from the coils and tangles.  This area would be a poor place for exchange of deuterium as it is coiled and packed tightly with the other strands. There is not really any good location for the exchange to occur as the protein is faced inward of [sic] the alpha helices. (Exam 1-4a)	*Student focuses on the idea of solvent accessibility in exchange and does not explicitly discuss the role of hydrogen bonding. Note that the student mentions the exchange of 'hydrogen-deuterium' as a single entity more than once.* *(Unit Exam Problem, part a)*
rhGCSF with sucrose results in most protein unfolding. From the plot, we can find that, in sucrose present, deuterium uptake is lower than the uptake when there is only rhGCSF peptide. While in benzyl alcohol, peptide uptake more deuterium [sic] than normal situation. If protein is more activated, the uptake of deuterium should be higher, which means the most protein unfolding needs the lowest uptake of deuterium. From the [chart], peptide in sucrose has lower uptake and it has the most protein unfolding. (Exam 2-2b)	*Student reverses the meaning of more/less deuterium exchange, indicating that lower uptake implies greater unfolding. Note that the student does correctly decode and compare the data provided in the residue uptake plot, but incorrectly interprets it through a misunderstanding of the relationship between hydrogen-deuterium exchange and protein structure.* *(Unit Exam Problem, part b)*

Table 6.8 continued

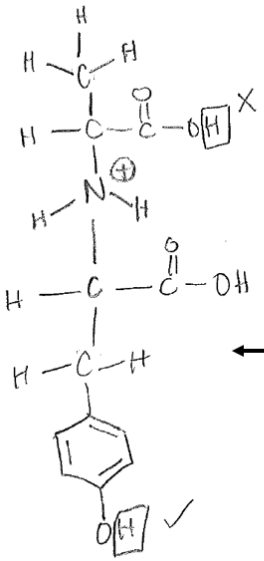
 When there is a lot of steric hindrance in parts of the protein, this makes it hard for the deuterium exchange to occur because there's not enough space. In this sequence it would be easier for deuterium to make an exchange on the hydroxyl on phenylalanine rather than the hydroxyl on carboxylic acid because there's more steric hindrance in that area. So more deuterium exchange occurs in areas with low steric hindrance, less rigidity, and more flexibility. (Exam 1-6a)	*Student explains how steric hindrance affects the exchange of deuterium. Note that both of the hydrogens indicated by the student with boxes are not the type of hydrogens exchanged and measured with HDX-MS (i.e. not amide hydrogens). Also note that the molecule drawn by the student is not a peptide; the amino acid components (of which only one is correctly drawn) are not connected correctly through a peptide bond.* *(Unit Exam Problem, part a)*
The higher the percentage of deuterium present in the α-helix, the more the protein unfolds. Because there is a higher percentage of deuterium uptake present in the rhGCSF with benzyl alcohol, there is more protein unfolding present in the drug formulation that uses benzyl alcohol. This configuration leads to more deuterium exchange, most likely because that α-helix "breathes" more than that of rhGCSF and rhGCSF with sucrose. As percentage of deuterium uptake increases, the stability of the protein decreases, leading to more protein unfolding. (Exam 2-6b)	*Student correctly interprets residue uptake plot to identify which formulation results in the most unfolding of the peptide. Note that the student suggests a possible reason is that the alpha-helix 'breathes' more. Also note how the structure of the last sentence reads like deuterium exchange/uptake is the cause of protein unfolding.* *(Unit Exam Problem, part b)*
…it would be necessary to study the protein in these solutions at different temperature, pH, and environmental conditions to see which solution prevents or causes denaturing of the protein. More information is also needed about the chemical properties of benzyl alcohol and sucrose and the concentrations necessary to see how they interact with other proteins and structures. Drug formulations need to be safe. And finally, it might come down to cost. Which is more efficient and at a lower cost to produce? Answers to all these questions would help decide which is best. (Exam 1-10c)	*Student suggests a variety of potentially necessary pieces of information, even mentioning cost which is certainly considered in a pharmaceutical industry context.* *(Unit Exam Problem, part c)*

Table 6.8 continued

Although the graphical data tells how "open" the protein structure is and how resistant or not it may be to change of its environment, finding the best storage solution is not as simple as considering the levels of deut. exchange [sic]. It is also important to know the charge of the protein at neutral pH, allowing one insight on whether its [sic] overall acidic or basic or neutral in nature. An acidic protein may be better suited to be placed in an acidic solution as to not promote any side reactions with the sugar. However, a basic protein may not be considered w/ an acidic soln. [sic] as the same issues of side reactions, denaturing, electric imbalance, apply. … …important to consider electrical charge (acidity, basicity) of the protein. (Exam 7-3c)	*Student argues that the graphical data provides some information about protein stability, however, identifying the best solution for storage also requires an understanding of potential interactions between the environment (storage solution) and the protein's inherent properties.* *(Unit Exam Problem, part c)*

The purpose of part (c) of the open-ended exam problem was to have students think about what other information is needed beyond what the uptake plot in part (b). It was hoped that students might recognize that the plot represented data for only one alpha helix from rhGSCF and would cite the need for data about the rest of the protein. Several students did indicate this. Recognizing the relationship between structure and function, some students wanted data related to exchange at the active site or wanted to test the function (activity) of the protein to determine if any unfolding would be detrimental to the function of the protein drug (Table 6.8, Row 5). One student desired additional representations to know the overall exchange as well as which regions had the most exchange since scientists may "want to alter/fix this region." Other students stated that helpful information would include knowing if the formulation included any other compounds that might affect storage (i.e. like the compounds in the table they interpreted in Activity 1), as well as examining the proteins over a longer time period as there might be a difference in deuteration between the formulations given more time. Students suggested a variety of other types of information which could be useful, including protein polarity, ionic state, bonding angle, electronegativity, electric imbalance, charge at neutral pH, protein acidity/basicity, etc. Some of these ideas suggest somewhat limited consideration of their application to the context (see also Heisterkamp & Talanquer, 2015), but others suggest that students were thinking about the influence of the environment at a (macro)molecular level (Table 6.8, Row 6).

6.7 Conclusions

In this study, we used interviews with four expert scientists about their protein-folding and dynamics research, primary literature, and textbook and policy objectives to develop anticipated learning outcomes (ALOs; RQ1) and design an HDX-MS curriculum module usable at the undergraduate level (RQ2). We implemented this module in a biochemistry course for health sciences majors and explored student understanding of the use of HDX-MS to study protein folding and dynamics (RQ3). In this study, we demonstrate that:

- Expert research can be used to develop ALOs relevant at the undergraduate level which support the incorporation of experimental methods and authentic scientific activities, such as representation interpretation and data analysis;

- Cutting-edge research, including research contexts, methods, and representations, can be translated into the classroom through curricular modules, which we illustrate through the development of a module on HDX-MS;
- Students were capable of explaining HDX-MS and applying their understanding of the method to interpret representations from primary literature; and
- The assessments revealed both sound and unsound understandings of the HDX method, as well as of protein structure, bonding, and folding.

Previous work brought expert case studies into the classroom by using a model of components found in expert explanations of molecular mechanisms as a cognitive tool to help students construct explanations of mechanisms (Trujillo et al., 2015, 2016a, 2016b). This model helped students identify and incorporate the components in their explanations of mechanisms, however students struggled to make connections between the components and frequently overlooked the research methods used to study the phenomenon (Trujillo et al., 2016a). In this study, the ALOs developed from the expert data sources highlight the role of experimental work, as well as representations, in understanding and communicating about protein folding and dynamics. Research has established the difficulties science students face when interpreting ERs (e.g. Schönborn, Anderson, & Grayson, 2002; Schönborn & Anderson, 2006), but there are comparatively few studies in science education regarding student understanding of complex topics in biochemistry or the role of experimentation in developing conceptual understanding (e.g. Bernhard, 2018). Accordingly, in this study, we brought research methods and representations to the forefront by centering the module on HDX-MS and representations of HDX-MS data, using the findings of our previous work to design the materials and questions (Jeffery et al., 2018; n.d.).

Many of the ALOs developed in this study would not be found in biochemistry textbooks because they are closely tied to the development or application of protein-folding and dynamics knowledge, the interpretation of ERs, or the use of experimental methods and data. In other words, these ALOs are more closely related to developing fluency with various discursive resources than the mostly ‘fact-based’ outcomes that are listed in textbook chapters on protein structure and folding. We do not mean to suggest that any and all ALOs developed in this study, or in future work with experts, should be immediately included as part of a biochemistry curriculum. However, by investigating expert sources on this topic, we believe we have developed ALOs that better

reflect how knowledge about protein folding and dynamics is created, applied, and represented in different ways. We feel the incorporation of research methods into curricula is critical as the means by which nature is investigated appears to influence the development of conceptual understanding and discourse (e.g. Bernhard, 2007; 2010; 2018; Nersessian & Chandrasekharan, 2009; Jeffery et al., 2018). As pointed out by Bernhard (2018), instructors fail to fully exploit experimental work for learning when unaware of the role of experimental technologies in developing understanding. Moreover, if we neglect or ignore the role of experimentation in science, we present students with a distorted and decontextualized view of science (Passmore, Gouvea, & Gierre, 2014) and may produce students with naïve understandings of how experimental tools influence scientific thought (Ihde, 1991). We believe the ALOs developed in this study can be used to support biochemistry instruction and instructional materials, including previously published curricula and activities related to this topic (e.g. Anthony-Cahill, 2001; Helgren & Hagen, 2017; Jones, 1997).

We implemented this activity with a narrow sample of health science students in an undergraduate introductory biochemistry course. The activities and assessments revealed a range of student understandings and reasoning skills, as discussed previously. Students were capable of drawing correct conclusions about protein stability from heat maps and residue uptake plots taken from primary literature (ALO 2, Table 6.5). However, some students demonstrated difficulties, for example, reversing the meaning of more/less deuterium exchange which led to misinterpretation of the ERs. Students were also able to describe qualitatively how hydrogen-deuterium exchange mass spectrometry can be used to study protein folding and dynamics (ALOs 1 and 3, Table 6.5), but some explanations revealed student difficulties with identifying which hydrogens exchanged during HDX, or difficulties related to protein structure, bonding, and folding (e.g. incorrectly drawn amino acids or small peptides). Given that our assessments revealed evidence of both sound student understanding and difficulties related to the module ALOs, we feel confident about their validity. The student data analyzed thus far therefore suggests that the HDX-MS curriculum module ALOs may be verified learning outcomes (VLOs; Irby, Pelaez, et al., 2018a), however further research and development will be required in order to fully validate them and establish the nature of student learning from the activities.

6.8 Implications

This study serves as a proof of concept regarding the feasibility of transferring cutting-edge research contexts into the undergraduate classroom. Protein folding and dynamics are foundational concepts in the life sciences and remain an area of intense research in cutting-edge science. The biopharmaceutical context adopted for the activity is both relevant to the health science student population with which we piloted our activity, as well as timely for their future careers as biologic drugs grow in use.

Furthermore, we believe this study suggests that significant potential remains untapped when expert data sources are not considered in the development of curriculum, particularly in a rapidly growing field like biochemistry. The expert interviews revealed how researcher scientists' applied theoretical knowledge and research methods to better understand phenomena and address research goals (see also Jeffery et al., 2018). Although we interviewed four experts in this study, any number of interviews could be conducted to begin characterizing research in order to translate it into the classroom. Primary literature also provided examples of how experts represented and communicated their results, as well as characterizing research methods. As in this study, only a small number of articles were needed in order to identify common ERs and the ways they are used. We believe that engaging in an extensive review of the literature base is unnecessary as it would impede timely translation of research into the classroom and make this process impractical for the majority of instructors. For instructors who wish to adapt their own research into the classroom, seminal literature in their field will likely provide an ample source of research contexts, methods, and representations. The information gleaned from both expert interviews and primary literature can be used in the design of curricular materials and/or directly incorporated as readings; both of which we did here (see also the expanded curriculum in Appendix C Table C.1).

We invite other instructors to test, modify, and further develop the ALOs and HDX-MS curriculum module in their own classroom contexts. Additionally, we encourage interested instructors and science education researchers to use the method we describe to address other complex, poorly covered, or out-of-date topics.

6.9 Acknowledgments

First, we would like to thank our four expert participants whose time and insight into their research made this work possible, as well as the biochemistry students who engaged with our instructional materials. We would also like to thank Stefan Irby for numerous conversations and feedback throughout the development of this study, as well as Katherine Wehde for taking time to answer questions about mass spectrometry. This work was partially supported by the ACE-Bio project (NSF grant 1346567) and the BASIL project (NSF grant 1503798). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

CHAPTER 7. FINAL DISCUSSION AND IMPLICATIONS

The goals of this dissertation were to investigate how expert researchers integrate theoretical knowledge of thermodynamics and kinetics with experimental methods and representations when explaining their research into protein-folding and dynamics, and how such expert knowledge can be used to inform the development of instructional materials in this area of biochemistry. To address these goals, the following research questions were explored:

1. How can we model experts' explanations of their research related to protein folding and dynamics? (RQ1; Chapter 4);
2. How do experts use representations to explain their protein-folding and dynamics research? (RQ2; Chapter 5); and
3. How can we use expert research to inform the design and implementation of instructional materials aimed at developing biochemistry students' understanding of protein-folding and dynamics? (RQ3; Chapter 6)

In response to each of these RQs, the findings of this research permit us to make the following overarching claims:

1. In explanations of their research into protein folding and dynamics, experts use theoretical knowledge to integrate and relate research context, experimental methods, analogies, and how phenomena operate; and these factors could be formulated into a model, which we term the MAtCH Model (RQ1; Chapter 4);
2. Experimental methods and multiple external representations play central roles in how experts understand and explain their research into protein folding and dynamics, of which many concepts are relevant at the undergraduate level (RQ2; Chapter 5); and
3. Expert research knowledge and representations can be effectively used to inform the design and implementation of instructional materials related to protein folding and dynamics; and preliminary evidence suggests that undergraduate students can use experimental methods and representations to talk about this topic, but still demonstrate a range of difficulties (RQ3; Chapter 6).

To address RQ1 (Chapter 4), we used interviews with four experts to develop the MAtCH model of how experts explain protein-folding and dynamics research, and to characterize how they explain thermodynamic and kinetic concepts typically covered at the undergraduate level. Four research project explanations were analyzed, ranging in context from enzyme mechanism elucidation (Beaker), to globular protein stability (John), to protein drug shelf-life (Gertrude), and protein dynamics simulations (William). What differentiates the study in Chapter 4 from extant accounts of expert explanatory practices is that it compares how several experts understand their work in the context of their research goals and methods as they work on projects at the intersection of physical and biological sciences. All four experts used and combined a variety of explanatory models (Braaten & Windschitl, 2011), transitioning between different perspectives to match the nature of the process being explained and/or the explanatory aims of their research (Brigandt, 2013). We found that all four experts explained thermodynamic and kinetic concepts of relevance to protein folding in different ways which aligned with their particular research methods. Whereas the original MACH model (Trujillo et al., 2015) identified components of expert explanations of cellular and molecular mechanisms, the MAtCH model provides a framework that can be used to recognize the role of theory in tying the different components together in explanations of research. Notably, the integrated nature of the components of the MAtCH model suggests that, in practice, explaining how a phenomenon operates (H) may be inseparable from how it is measured (M) and the theories (t), mathematical concepts, and analogies (A) brought to bear on it (e.g. Boumans, 1999). The experts' explanations of thermodynamic concepts particularly show how they integrated theoretical knowledge with their research methods and data representation so intricately that they could not be definitively isolated from one another. These findings have implications for the development of educational materials to support students' ability to use research methods, data, and theoretical knowledge to explain phenomena. One major implication of this study is that the blending of MAtCH components is critical in addressing current scientific research problems of social impact, and therefore it is important to bring research contexts and methods into the science classroom so that students may develop a more holistic and practical understanding of natural phenomena and the processes by which they are understood. To address such problems, students must be able to use theories and mathematical models to mediate between research methods, data, and continually-developing models of interacting entities in a phenomenon. Furthermore, the apparent alignment between these experts' conceptions and their research methods indicates that

practical contexts have a notable influence on reasoning and explanation, affecting the ways in which these scientists think about phenomena. This implies that understanding research practice may be an important part of functional scientific knowledge. In this respect, and keeping in mind that different models serve different purposes (Brigandt, 2013; Haglund, 2012), future teaching should abandon the idea of teaching a single "scientifically correct" concept and instead consider incorporating research methods in the classroom in order to make concepts more tangible to students. As a curricular tool, the MAtCH model can be used by instructors to design or modify life science curricula in order to create contextualized content with activities and assessments structured to emphasize the MAtCH components and their connections, such as how underlying theoretical knowledge (t) can be used in tandem with particular research methods (M) to measure and develop models of a phenomenon (H). By including more opportunities for students to integrate the MAtCH components, instructors can encourage students to think in ways that are more similar to experts in the field.

Pursuing a deeper understanding of the role of ERs in the explanations of protein folding and dynamics (RQ2), Chapter 5 illustrates the use of the CRM model to analyze ERs from expert interviews and biochemistry textbooks, comparing how experts and textbooks use ERs and experimental research methods to discuss how evidence can be used to understand a phenomenon. Our analysis revealed that experts used ERs to show how their methods informed their understanding of phenomena. The ways in which they discussed experimentation aligned with concepts and skills of experimentation that have been identified elsewhere (e.g. Dasgupta et al., 2014; Pelaez et al., 2017). In comparison, discussion of experimental methods in the reviewed textbooks was mostly limited to brief descriptions of methods, with little discussion of how they provide evidence about a phenomenon; that is, textbooks typically explained what is known, but not how it is known. Additionally, unlike the reviewed textbooks which made few in-text references to or between ERs, the experts frequently used gesture to integrate their verbal explanations with ERs, to indicate components of ERs, to relate different ERs, and to show dimensionality and movement which could not be captured in their 2D, static ERs. In producing and using their ERs, the experts employed a variety of reasoning behaviors, starting with more concrete behaviors to establish the meaning of the ER components before employing more complex reasoning as they considered the meaning of the ER within their research context. This work is one of a few examples that demonstrate how an expert's understanding is informed by the

coordination of several different kinds of ERs. Notably, in making a comparison between expert and textbook discussion of ERs and experimental methods, this work reveals a problematic gap in textbook design. Our evidence, as well as others, shows that meaning is generated by coordinating features within and across multiple ERs (Bodemer et al., 2005; Kozma, 2003; Ainsworth, 2008), and by failing to connect ERs to each other, to the surrounding discussion, and to the practical context, opportunities to enhance meaning are missed (Bowen et al., 1999; Roth et al., 1999). Additionally, research indicates textbook ERs fail to illustrate authentic scientific practices and evidence, often using idealized data, presenting oversimplified diagrams of methods, or offering only simplistic descriptions and interpretations (Krajcik & Sutherland, 2010; Roth et al., 1999; Rybarczyk, 2011). However, our study shows that experts were able to easily relate phenomena to their experimental and social contexts by using ERs and narrative-like discussions (see Chapters 4 and 5). Thus, a major implication of this work for education is the modification of textbooks and instruction to incorporate greater discussion of experimental methods and to scaffold the use of multiple ERs in ways similar to expert use in order to enhance the development of student understanding and reasoning (e.g. Kozma, 2003; Schönborn & Anderson, 2006). In this regard, instructional actions for textbooks and instructors are provided in Chapter 5. Just as explored in Chapter 4, experimental methods could serve as knowledge resources that can support conceptualization and reasoning about abstract concepts by grounding them in experience (e.g. Brookes & Etkina, 2015; Lakoff & Johnson, 1999). Conceptions based in sensorimotor experiences are often used to understand abstract concepts in science, whether through language (e.g. Brookes & Etkina, 2009; 2015; Lancor, 2012), ERs (e.g. Amin, 2009; Dreyfus et al., 2015), or gesture (e.g. Becvar et al., 2005), and reflect the flexible nature of scientific concepts consistent with scientific expertise (e.g. Jeppsson et al., 2013). This is a critical direction for future education research as very little is known about how different disciplinary resources help or hinder access to disciplinary ways of knowing (Airey & Linder, 2009; Offerdahl et al., 2017).

Building on the findings of Chapters 4 and 5, Chapter 6 pursued an answer to RQ3, attempting to use expert research to inform the design of instructional materials focused on developing biochemistry students' understanding of protein folding and dynamics. We addressed this question by using expert interview data, primary literature, and textbook and policy objectives, to develop anticipated learning outcomes (ALOs) and design an HDX-MS curriculum module which we implemented in a biochemistry course for health sciences majors. This study attempted

to foreground the role of experimental work and ERs in understanding phenomena related to protein folding and dynamics. As pointed out by others, neglecting the role of experimentation in science fails to take advantage of how experimental work or technologies may support understanding (e.g. Bernhard, 2018; Jeffery et al., 2018), potentially produces students with naïve understandings of how experimental tools influence scientific thought (Ihde, 1991), and presents a distorted and decontextualized view of science (Passmore et al., 2014). Additionally, previous research suggests that despite the importance of ERs in understanding of complex scientific concepts (e.g. Ainsworth, 2008; Kozma & Russell, 2005), science students face many difficulties when interpreting ERs (e.g. Schönborn et al., 2002; Schönborn & Anderson, 2006). This study demonstrated that expert research can be used to inform undergraduate curriculum to support instruction related to experimental methods and the interpretation of ERs. This was illustrated through the development of a curriculum module on the use of HDX-MS in protein drug development, and analysis of student responses to related activities and assessments. These assessments revealed that students could explain and apply their understanding of HDX-MS to interpret complex representations, in addition to revealing unsound understandings of protein structure, bonding, and folding. This research therefore adds to the almost non-existent literature bases on student understanding of protein folding and dynamics; student understanding of biochemical research methods; and student interpretation of complex representations from primary literature and how to support that process. Thus, the ALOs and materials developed in this study can support previously published instructional materials related to protein folding and dynamics, such as those mentioned in previous chapters (e.g. Anthony-Cahill, 2001). Yet perhaps the most significant contribution of this work to biochemistry education research and instruction is that it demonstrates that, with scaffolding, expert knowledge and ERs from cutting-edge research contexts can be translated into the undergraduate classroom. Through a relevant and timely research context (biopharmaceuticals), health and life sciences students learned about foundational biochemistry concepts (protein folding and dynamics) and a widely used experimental method to study them (HDX-MS), even gaining experience with interpreting complex representations from primary literature. Moreover, this study indicates that significant potential remains untapped when expert data sources (i.e. interviews and primary literature) are not considered in the development of curriculum, particularly in rapidly growing fields like biochemistry. By investigating expert sources, we believe we have developed ALOs that better reflect how knowledge about protein

folding and dynamics is created, applied, and represented through discursive resources, which stands in contrast to many of the 'fact-based' ALOs that are found in biochemistry textbooks. These ALOs are more closely related and could better support the important task of developing students' fluency with various discursive resources (Airey & Linder, 2009). Significantly more research must be conducted regarding the specific ALOs put forth in this study, as well as ALOs related more generally to the development of discursive fluency.

Overall, our findings demonstrate that practitioners of science combine a variety of disciplinary resources to conduct and communicate their work. Educational activities in undergraduate science courses should therefore reflect the multimodal nature of disciplinary knowledge and provide opportunities for students to practice using disciplinary ways of thinking (e.g. Airey, 2006; Airey & Linder, 2009; Kittleson & Southerland, 2004; Krajcik & Sutherland, 2010; Metros & Woolsey, 2006). Given that disciplines, like biochemistry, require knowledge and skills to investigate, model, and manipulate an abstract and invisible world (Schönborn & Anderson, 2006), activities that provide students authentic experiences with disciplinary knowledge and discursive resources must therefore be created carefully in order to support intended learning outcomes (e.g. Kozma et al., 2000). Through this dissertation, we have shown that it is possible to study and use expert practice to inform our understanding of what knowledge and skills instructors should aim to develop in their science students so that they will be able to navigate their discipline on their own.

Education research has barely begun to scratch the surface when it comes to understanding and teaching how concepts are understood in the context of and through experimentation, as well as how to assist students in developing the ability to productively coordinate and translate between discursive resources. Furthermore, very little research has investigated how different discursive resources relate to one another and build upon each other, in either educational (e.g. Oliveira, Justi, & Mendonça, 2015) or authentic research contexts (e.g. Chandrasekharan & Nersessian, 2014). As no single resource itself is capable of fully communicating a disciplinary way of knowing, lacking the ability to use discursive resources prevents an individual from gaining holistic and meaningful understanding (Airey & Linder, 2009; Lemke, 1998, 2002; 2004; Offerdahl et al., 2017). Despite science education's consistent rallying to include more 'inquiry-based practices' or the rapid development and rising popularity of experiences like Course-based Undergraduate Research Experiences (CUREs), in-depth education research on actual scientific practices and how

they are taught and learned, remains surprisingly lacking. Frameworks to evaluate scientific explanations, for example, began, in part, with consideration of how expert scientists work and communicate, but significant work remains (e.g. Braaten & Windschitl, 2011). This dissertation offers only a preliminary characterization of several experts' explanatory practices connected to a specific phenomenon and adds to a remarkably limited literature base. Significantly more work must be completed to untangle the inherent complexity of explanations of scientific research so that we may develop instructional strategies and materials that help students integrate course subject matter with authentic science practices (e.g., understanding research methods or connecting experimental findings to processes governed by theories that students are learning in the classroom). With the rapid development of scientific technologies and research problems that increasingly cross multiple disciplinary lines, one of the most significant challenges facing science education is understanding and developing strategies to help students cope with different and evolving ways of knowing and of representing. Future science education research must address this issue by investigating how experts and students handle discursive resources as they understand, create, and communicate scientific knowledge.

In conclusion, this work expands our understanding of how experts employ multiple discursive resources in explanations of their research, and demonstrates that cutting-edge expert research can be used to inform the teaching and learning of biochemistry. In so doing, our findings have made, in our view, a small but important contribution to the process of educational reform in the life sciences, as strongly advocated in various national reports and seminal papers, including those by AAMC-HHMI (2009), Brewer and Smith (2011), Brewe, Pelaez, and Cooke (2013), Airey and Linder (2009), and Krajcik and Sutherland (2010). In all cases there has been a strong emphasis placed on developing knowledge and skills of relevance to modern scientific research and careers, including recent advances in foundational scientific knowledge and general and disciplinary-specific practices (e.g. Brewer & Smith, 2011). The life sciences, in particular, have been under pressure to develop curricula that reflect a rapidly changing knowledge base and take into account the increasingly close relationship of the physical and mathematical sciences with life science research (e.g. AAMC-HHMI, 2009; Brewe, Pelaez & Cooke, 2013). For biochemistry and the molecular life sciences, understanding and transforming instruction to foster student competency in areas such as experimentation and visualization, as we did here, is a critical objective of current and future education research and practice.

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APPENDIX B. CHAPTER 4 SUPPLEMENTAL MATERIAL

BEAKER DOSE ANALOGY

"…'The ΔG of this state. The ΔG of that state.' What does that mean? ... What does 2 kilocalories mean? Okay. Now I can easily explain what 1.3 kilocalories... …means in drug discovery. What does 1.3 mean in binding constants? 1.3 kilocalories. So let's go with something that people understand: dose. You take- do you take over the counter prescription or prescription medications from time to time? Ibuprofen? Got a headache? You take Ibuprofen right? So what does 1.3 kilocalories mean? So if I have a headache and I take a pill and that particular pain medication is weak for me- or just weak in general - it's not working. Alright. I need to increase the dose by ten-fold so I go from 1 ibuprofen to 10 pills because the interaction strength between the drug and the protein target is weaker. I want to strengthen it by ten-fold. That's 1.3 kilocalories. The ΔΔG between - association constant, you know, going from 10- or dissociation constant- going from 10 micromolar to 100 micromolar is ten-fold. At room temperature that's 1.3 kilocalories. That all the sudden means something now."

EXPANDED CASE: GERTRUDE INVESTIGATES PROTEIN DRUG SHELF-LIFE

Gertrude is interested in the physical and chemical modification processes undergone by lyophilized (i.e. freeze-dried) protein drugs in order to improve drug formulations and enhance shelf-life (C). These drug formulations include excipients, which are inactive substances that serve as vehicles for delivering drugs or other active ingredients. Her research group considers the extent to which protein drugs unfold and how they aggregate when they are unfolded or partially unfolded (H). The degree of unfolding is determined by hydrogen-deuterium exchange (HDX): lyophilized protein powders are exposed to deuterium vapor and the resulting peptide mass is measured via mass spectrometer (M). This data is then used to create representations (A) of deuterium incorporation like structural maps (e.g. see Fig. B.1), indicating what regions of the protein drugs remain protected during unfolding (H). Gertrude's first excerpt in Figure B.1a below provides a clear example of the presence and integration of the MACH model components, and the implicit role of theory in her explanation.

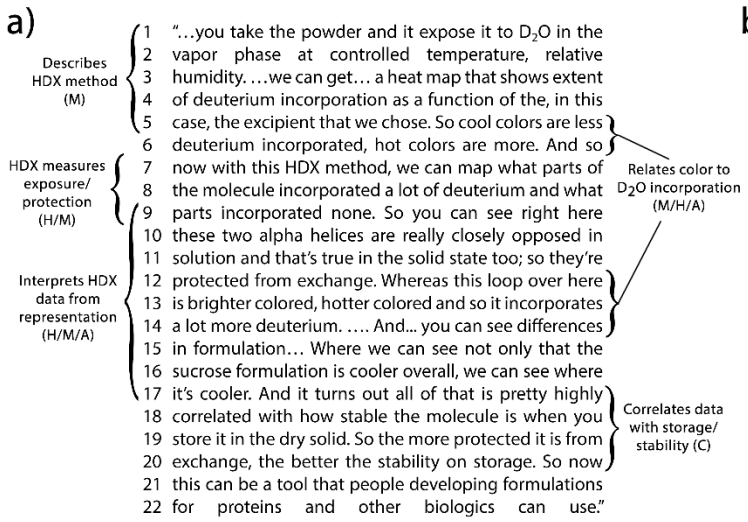

Figure B.1 Gertrude maps hydrogen deuterium exchange (HDX) data to a protein drug structure to make predictions about drug stability.

Gertrude first outlines how a lyophilized protein drug-excipient powder is exposed to deuterium in the HDX method. Unprotected hydrogens in the protein are exchanged with deuterium and the mass of the protein drug is measured via a mass spectrometer. This data is used to make heat maps of the protein like that in panel b, where gradations of color are used to represent the extent of deuterium incorporation (purple represents 0-10% deuterium uptake; dark blue 11-20%; light blue 21-30%; aquamarine 31-40%; dark green 41-50%; light green 51-60%; yellow 61-70%). Gertrude indicates different parts of the heat map in b as she interprets what parts of the protein are protected from exchange. For example, the two parallel alpha helices on the left display cooler colors, which indicates they are not significantly exposed to deuterium in this formulation. On the upper right, the hotter yellow-colored loop indicates significantly more deuterium incorporation in that region. She explains that this data is correlated with stability on storage so they can compare differences in formulations and make judgments about the stability of drug formulations in a shorter time period. The image in panel b was digitally modified to protect the confidentiality of research data.

In this excerpt, Gertrude makes distinct connections between the data collected (M), how it is represented (A), what entities and interactions it describes in the system (H), and what that implies about functionality (C). Using the MAtCH model (Chapter 4, Figure 4.1) as a framework, her discussion generally follows an M-H-C pattern against a backdrop of how she interprets one kind of representation (A). During this process, she implicitly uses theoretical knowledge of protein structure and equilibrium. She begins by describing the procedure of exposing the protein drug powder to deuterium which results in data in the form of degree of deuterium incorporation (M, lines 1-5). Then, connecting the M and H components, she explains that the HDX method (M)

allows her to measure the exposure/protection of regions of protein structure (H, lines 7-9). Portions of the protein molecule (entity) have a certain amount of protection and this can be measured by an increase in mass through the replacement (interaction) of hydrogen with deuterium (entities) (H/M). The M and H components are highly integrated in Gertrude's discussion, with protection from HDX exchange seemingly synonymous with degree of unfolding. As the backdrop for this discussion, Gertrude uses a representation (Fig. B.1b) which maps HDX mass spectrometry data (M) directly onto a 3D protein structure where color (A, e.g. lines 5-6, 'brighter' and 'hotter') indicates degree of deuterium incorporation (M) and she can thus interpret degree of exposure/unfolding (H, lines 9-17). This demonstrates how Gertrude cycles between the M, H, and A components of the MAtCH model (lines 6-17), using her theoretical knowledge of protein structure and the HDX process to mediate between them. After establishing the connections between these three components, Gertrude states how the representation (A) of where and how much the protein is protected from exchange (H/M) is correlated with the drugs' stability as a dry solid (C, lines 17-20). Thus, she transitions between the A and C components, and cannot only visually (A) compare the relative stability of the entities in different formulations side by side to address her research problem, but she can also do it in weeks rather than year(s)-long shelf studies (C). Although Gertrude does not explicitly use terms like thermodynamics or kinetics in this excerpt, she tacitly employs theoretical knowledge of equilibrium in her discussion of deuterium incorporation.

Gertrude also examines protein drugs from another perspective by looking at their interactions and organization in space and over time (H). The temporal dimension is a significant part of Gertrude's research, from the context of protein drug shelf-life (C), the kinetics of HDX exchange (M), and folding-unfolding and aggregation equilibria (H). The following excerpt in Figure B.2a provide another example of how Gertrude integrates the MACH components and theoretical knowledge. Specifically, Gertrude uses her theoretical knowledge to mediate between the H and M components of the MAtCH model, describing what interactions (H) she believes are measured through deuterium exchange (M). Through the use of a narrative (A), she suggests a hypothetical model of a protein drug in solid (lyophilized) form as in Figure B.2.

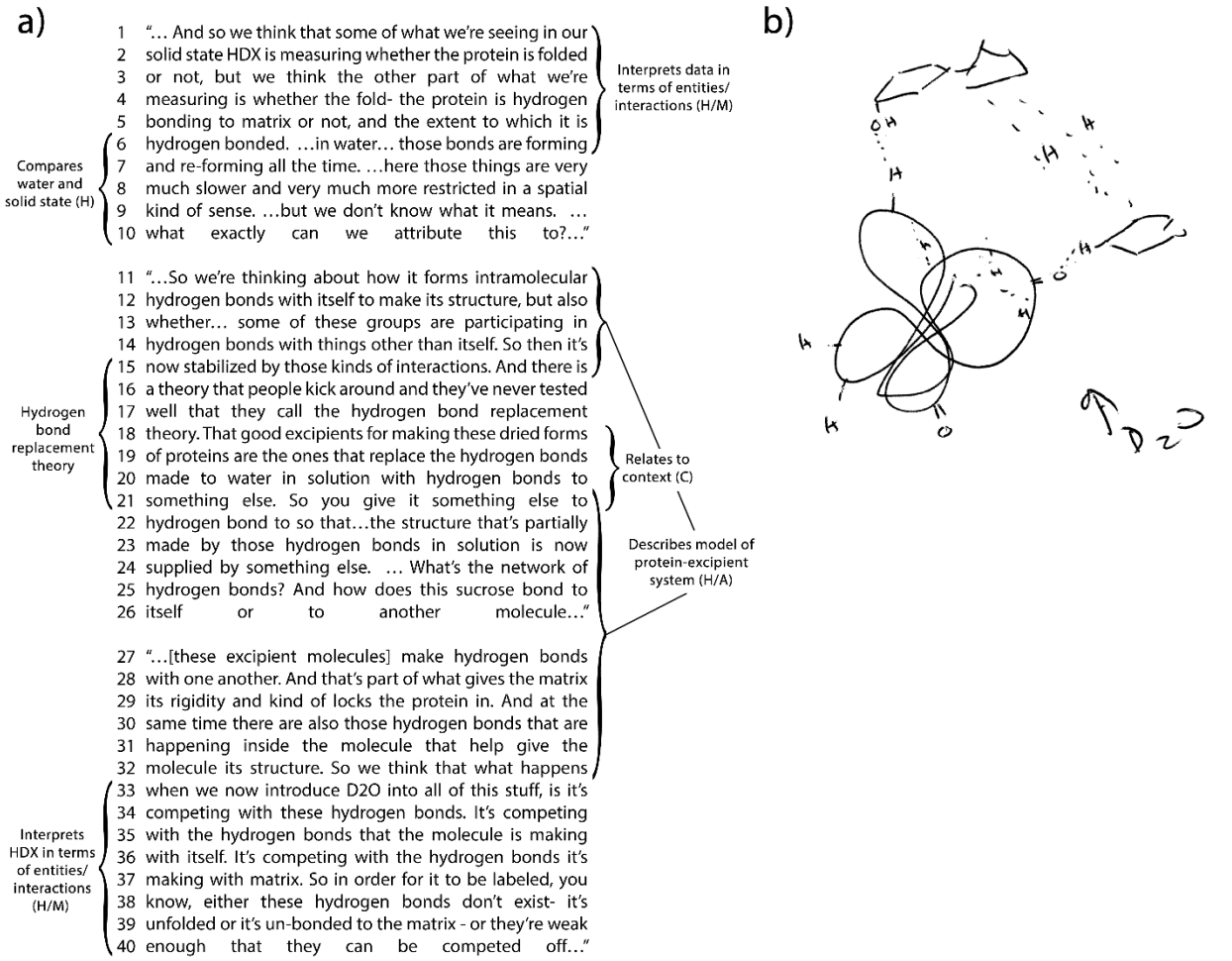

Figure B.2 Gertrude proposes a possible model of the interactions between protein drugs and excipients in the powdered (solid state) form.

She explains that beyond the HDX data indicating if a protein is folded, they believe the data might also indicate if the protein drug is hydrogen bonding to the matrix. Referring to previous water absorption experiments and plots of percent deuterium incorporation v. time, Gertrude explains that while they can interpret the information in terms of protein dynamics (data not presented), they cannot explain at a molecular level why they see a difference in the liquid and solid states. She elaborates on what they think might be happening in the solid state, using an online image of the pentapeptide Leu-enkephalin to explain hydrogen bond donors and acceptors (not pictured). Gertrude then draws a "cheater picture of what's in [her] head" for the liquid (not pictured) and solid states (panel b). The large scribble represents the backbone of a folded protein drug with several hydrogen bond donor and acceptor groups. The ring-like structures on the top and right represent excipient molecules, like sucrose, with donor and acceptor groups. Dotted lines represent possible hydrogen bonding interactions between excipient molecules, between excipient molecules and protein drug, and within the protein drug itself. Gertrude references a 'hydrogen bond replacement theory' in panel a, thus proposing that good excipients protect hydrogen bond donors and acceptors from deuterium exchange, and possibly chemical degradation in general, by participating in hydrogen bonding interactions that would normally be made to water. The arrow

labeled 'D_2O' in panel b corresponds to Gertrude's final explanation of what they believe happens when deuterium is introduced to the solid state and what it indicates about the system.

In the excerpt provided above, Gertrude cycles through the M, H, and A components of the MAtCH model. We see from Gertrude's initial remarks (lines 1-5) that she interprets the HDX data (M) in light of her theoretical knowledge of the HDX process and theoretical knowledge about hydrogen bonding (interactions), and water, protein, and excipient structures (entities with properties) (H). After establishing the connection between what entities (H) are being measured (M), Gertrude uses her theoretical knowledge to suggest a hypothetical model (in narrative form) of what may occur in the protein-excipient system (H/A, lines 11-15, 21-29). Gertrude integrates theoretical knowledge of a "hydrogen bond replacement theory" which has been suggested in her field (lines 15-21) with other theoretical knowledge to construct her hypothetical model (A, lines 21-32). She provides a drawing to assist her explanation (A, Fig. B.2b). We can also see that Gertrude connects her hypothetical model of the protein-excipient system (H/A) to her research goal of predicting good excipients (C, lines 18-21). As with the first excerpt, it is possible to see from this discussion and representation how Gertrude uses her theoretical knowledge to closely tie her research methods (M) to a hypothetical model of the physical process (H/A). Both implicitly throughout, and at times explicitly (lines 1-5, 33-40), we can see that Gertrude discusses HDX (M) in terms of what it can measure about the scale of unfolding (H), as well as what it implies about the interactions between different entities in the system and the relative strengths of those interactions at the molecular level (H).

In other parts of her interview, Gertrude provides additional examples of how she transitions between the MACH components using theoretical knowledge, often against the backdrop of a representation (A). For example, Gertrude's research group also investigates protein aggregation because proteins that become partially unfolded after lyophilization have a tendency to form aggregates (H) when they are reconstituted, which can cause immune responses in patients (C) (see for example Ratanji, Derrick, Dearman, & Kimber, 2013). Through the use of episodic exposure to deuterium (M), Gertrude can measure what protein regions appear to participate in exchange or are buried during aggregation (H/M). The kinetics and equilibria underlying the episodic incorporation of deuterium into the partially unfolded proteins are particularly important as the relative amount of deuterium that is incorporated over time (M) reflects how fast residues

become buried in the aggregated form, as well as where residues are buried (that is, the aggregation interface) (H). As before, theoretical knowledge plays a critical role in this process by allowing Gertrude to mediate between the representation (A) of the measureable world of HDX data (M) and the molecular world of interacting entities (H). However, in this instance, Gertrude uses a special type of line graph called a butterfly plot where information about aggregation (H/M) is not mapped directly onto a protein structure (A) as with the Figure B.1, but some structural information in the form of residue number (H) is still provided and combined with percent deuterium incorporation (M). This is sufficient for Gertrude to interpret what the representation (A) implies about the protein system (H). Thus, Gertrude's research enables her to more quickly make inferences regarding which peptide drug formulations will have longer shelf-lives through the application of HDX methods. We can see in the following case how William's efforts similarly aim to improve predictions, but address an entirely different research problem.

EXPANDED CASE: WILLIAM SIMULATES PROTEIN DYNAMICS TO IMPROVE DRUG METABOLISM

William's work focuses on incorporating protein dynamics into computational models (M/A) in order to improve predictions about where drug candidates are metabolized and by what enzymes, so as to aid the development of more metabolically stable drugs (C/H). Unlike the other experts interviewed here, William's goal is the development of a predictive method to model possible drug and protein movements and interactions (M/A), which is validated and trained using experimental site metabolism data (M). The end product of his research – a process incorporating a variety of techniques like molecular dynamics simulations, molecular docking, and statistical techniques (M/A) – can then be used to produce data of its own (M). By considering protein dynamics (which he defines as the trajectories of atoms and residues in a protein (H)), he can produce an ensemble of protein structures to represent the multitude of possible conformations and average them to suggest the most likely preferred conformation (M/A). This conformation can then be used in the simulated docking of drug candidates to make predictions (M/A). Because of the goal and computer model-based nature of his research project, the H, M, and A components are extremely integrated in William's discussion and his understanding of thermodynamics similarly appears to intertwine or align with his simulations (A). The MAtCH model allows us to make sense of the

complexity by focusing on the connections. In the following excerpt in Figure B.3, we can see how William connects the components, as well as how his understanding of thermodynamics aligns with his simulations (A).

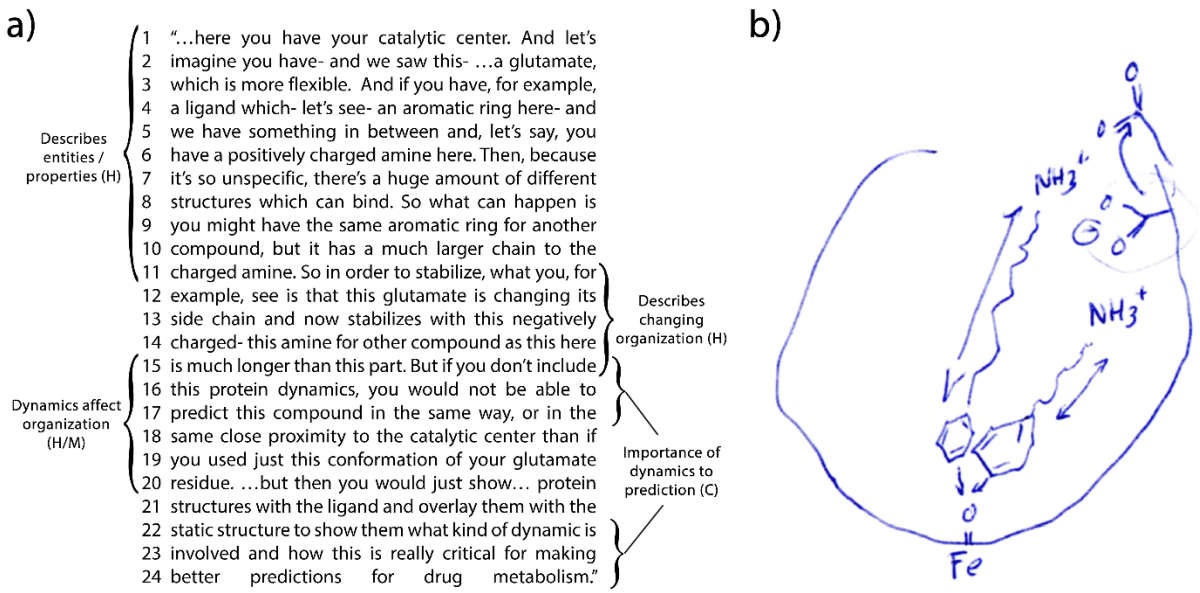

Figure B.3 William provides an example of the binding of two different drug compounds.

To illustrate the significance of including protein dynamics in simulations, William asks to imagine a binding site, pictured in panel b, which has a specific flexible glutamate residue some distance away from the catalytic center. He explains that the glutamate residue can change its conformation to stabilize different drug compounds (aromatic rings with hydrocarbon chains of different lengths ending in amine groups). William argues that if protein dynamics – like the changing conformation of the glutamate residue – are not included, it is not possible to predict how the ligand interacts with the catalytic center. Thus, in papers, William shows how the inclusion of protein dynamics in simulations leads to better drug prediction by overlaying images of predicted protein-with-ligand structures over the static structure of the protein.

In this particular excerpt, William's discussion generally follows an H-M/A-C pattern against the backdrop of a representation (A, Fig. B.3b). William begins by describing the significant structural components of the binding site, their properties, and two hypothetical drug compounds (H, lines 1-11). He then explains how those residues might change their spatial organization to accommodate different drug compounds (H, lines 11-15; see Fig. B.3b) and thus alter a compound's distance in relation to the catalytic center (H/M, lines 15-20). He argues that

because of this, including dynamics in simulations (M/A) is critical to improving the predictive capabilities of current methods and thus aiding the drug discovery process (C, lines 15-17, 22-24). William's tacit use of theoretical knowledge allows him to productively mediate between the measurable world (M) and what it implies about the molecular world of (simulated) protein structures and their interactions (H/A). We also begin to see in the above excerpt that William relates residue flexibility to protein dynamics, but what is not yet apparent is his unique way of assigning meaning to theoretical thermodynamic concepts. The following excerpt in Figure B.4a provides an example of how William maps meaning onto mathematical models and symbols (A), as well as how he applies his theoretical knowledge of thermodynamics, particularly of enthalpy, entropy, and free energy, to the context of developing protein dynamics simulations (M/A):

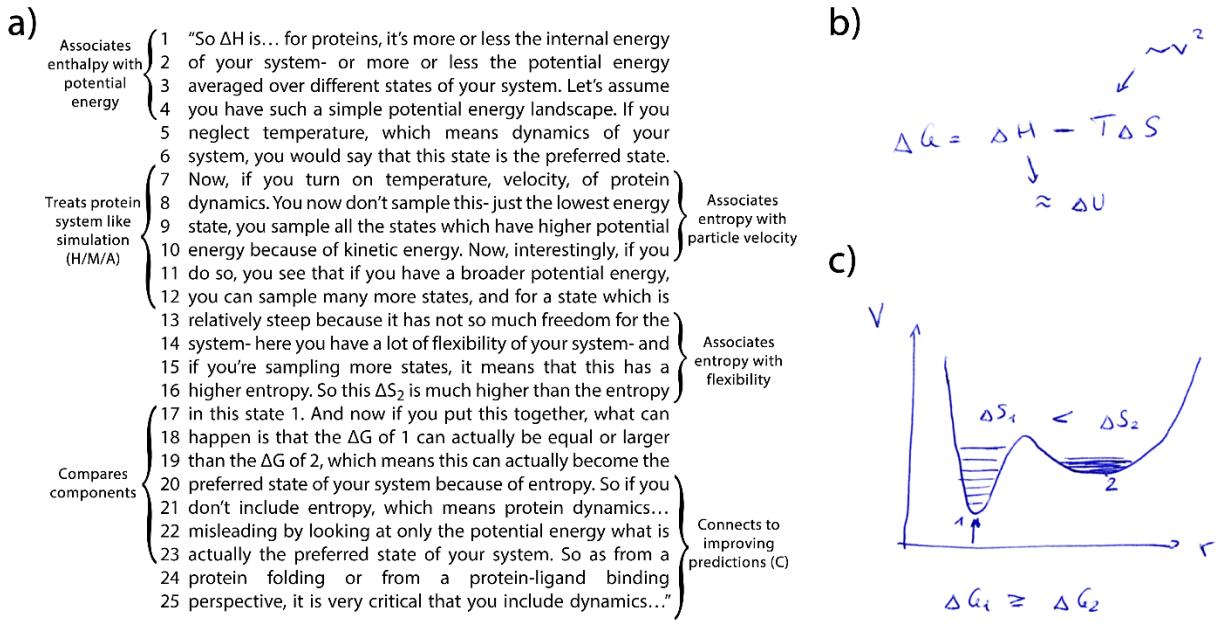

Figure B.4 William explains the influence of entropy on free energy in a two-state protein system.

William begins by substituting variables in the Gibbs' free energy equation in panel b with other variables that have physical meaning: ~v2 to indicate particle movement for the TΔS term and ΔU for averaged potential energy or interactions (strengths) for the ΔH term. He proposes looking at a simple energy landscape of a two-state protein system, shown in panel c, and explains that the preferred state of the system can change if you ignore the dynamics of the protein by 'turning off' temperature; that is, you ignore the TΔS term of the formula in panel b. If temperature is considered, there are many more states that can be sampled. These states are represented as lines within the wells of the graph in c. William explains that the widths of the wells in panel b are a function of

greater particle velocity or kinetic energy due to temperature: the greater the breadth of the well, the more possible states can exist, which reflects greater flexibility or freedom of movement. So-called "steep" states have limited flexibility and thus limited states to sample. In the second half of the excerpt, William considers entropy and compares the different ΔG values of the two protein states to illustrate how disregarding entropy in simulations can be misleading and thus the inclusion of dynamics in predictive methods is critical.

Although William integrates the H, M, and A components extensively, using the MAtCH model allows us to make sense of this excerpt by semi-isolating the components. It is first important to note that throughout this excerpt William structures his explanation around two representations (A, see Fig. B.4b & c) in addition to talking about a protein system (H) like it is one of his simulations (M/A, e.g. lines 7-12). He uses his theoretical knowledge of thermodynamics to seamlessly map a description of the states of a protein system (H) to his representation/ simulation (M/A). In doing so, William assigns meaning to mathematical models by mapping entities and their variable states (H) onto particular symbols in the formula and graph (A, e.g. lines 1-3, 16-20 ; also see Fig. B.4b & c). William's understandings of enthalpy, entropy, and free energy appear to align with his simulations (M/A) and are mapped to the entities, interactions, and states of a protein-drug system (H). He makes the concept of entropy tangible as "How much an object is moving. How dynamic it is…" (i.e. structural flexibility; lines 13-16) and connects it to temperature and the velocity of particles (H, lines 7-10). He describes enthalpy as internal or potential energy in this excerpt, but also associates it with the sum of interactions and interaction strength (H) in other parts of the interview. In the above excerpt, states both entropy and enthalpy must be considered in order to determine the actual preferred state of the system (lines 17-23). That is, free energy involves "compensation" between interaction strength and protein flexibility. In his simulations (A), temperature can be "turn[ed] on" to allow protein dynamics (entropy) and the resulting different states have different kinds of interactions (enthalpy) (H). William explains that if protein dynamics are ignored, "…you don't have entropy, you're not calculating ΔG's… and ΔG finally determines what… states you observe in nature 'cause we're not living at 0 Kelvin", resulting in incorrect predictions for ligand binding (M/A). Without a method that approximates reality well (M/A), William cannot make reliable predictions about drug candidates (C, lines 20-25).

William also discusses the difficulties students in his research group seem to have interpreting data from simulations (M) and how he must guide them to relate the trajectories of simulated atoms (H/A) to what they might reveal about the simulated system (A):

> "…if you get the statistical analysis out, they stop looking at the- at trajectory- at the atoms moving itself completely. So I have constantly students who will say, 'Oh! I have run the MD simulation. Here's the free energy,' or 'Here's the free energy profile.' And then… so you have your simulation and then they'll look at the ΔG over time and say, 'Oh yeah it goes first up.' And then they see a jump. And then it's equilibrium and then I'll say, 'Wow. What is this jump to you?' And they'll say, 'I don't know.' But didn't you look at the trajectory? Didn't you look really qualitatively at what is happening in the movie? And the structure. And they'll, 'No, I didn't. I just did the analysis.' … Once students have the feeling that they have an analysis to it and they get the values out, they're happy with this. They don't look back- and I don't know if it's hard for them to look at the trajectory and identify what is going on there, or if they're just happy that they have a good quantity coming out? But it is surprising. So I always say, 'Look at the structure. Really look at the- Look at the raw data. Look at the raw data to explain what is going on in the system.' …. It's important and it's valuable information but I want to understand what is the basis of getting this data. And people forget analyzing this part of- so it's in principle like, you're doing an experiment, you do the analysis and you're not really interested in what could happen in the experiment because certain… things [can] be wrong. But if you don't look at the raw data you don't see it."

For William, connecting the H, M, and A components is obvious. He tacitly uses his theoretical knowledge of thermodynamics to mediate between the measurable world by interpreting the data (M) in terms of what it implies about the (simulated) protein system (H/A). He explains how a change in free energy on a graph (A) reflects underlying changes in structural movement and/or the formation of new interactions (H) in the simulation (A). It also indicates he must look at the simulated protein system (A) in order to interpret the possible structural cause (H/A) of the data (M). According to William, while producing a numerical or graphical output is doable for students in his lab, interpreting and making connections between the data (M) and the underlying (simulated) physical causes (H/A) is not as obvious. Thus, a combination of experimental and simulated data enables William to improve current methods used to predict the metabolism of drug candidates.

EXAMPLES OF MODIFICATIONS TO EDUCATIONAL MATERIALS USING THE MAtCH MODEL

Table B.1 Examples of ways in which selected protein folding and dynamics educational materials could be modified using the MAtCH model as a guiding framework.

Both manuscript and relevant supplementary material were considered. The most relevant components or connections that the example questions address are indicated in parentheses, although it should be noted that most questions require the application of some amount of theoretical knowledge and may elicit other components.

MAtCH Evaluation	**Possible Modifications**
Exploring protein structure and dynamics through a project-oriented biochemistry laboratory module *Lipchock, Ginther, Douglas, Bird & Loria (2017)*	
• Provides a social context although module does not ask questions in terms of it (C) • Provides protocols and describes theory of how they work, but does not discuss limitations or alternative methods (M) • Representations are produced and analyzed, but little time is spent discussing purposes, affordances, or limitations (A) • Students are given a hypothesis, rather than producing their own (C)	• What other appropriate methods exist for studying protein structure and dynamics? (M) • Discuss similarities and differences between the acid loops and P-loops of the five protein tyrosine phosphatase sequences you aligned. (H) • What information can be communicated through the ribbon structure of PTP1B you develop in Experiment 1? What are its limitations? (A) • Design the forward and reverse primer sequences for the site-directed mutagenesis of PTP1B. (H/M) • Explain how melting temperature is calculated (H/M/A). • Explain why commercial vectors often contain *lac repressor* sequences. What other kinds of repressor/operator systems are used and in what research contexts? (M, C) • Often the DNA produced through transformation and amplification of a PCR product is sent for sequencing to confirm synthesis of the desired mutation. Why is this necessary? What issues are associated with PCR? (M) • How does one decide on the ratio of bisacrylamide and acrylamide for a polymerization reaction? (M) • What are the purposes of each of the four buffers used in purification of PTP1B? (M) • How does purification of a soluble, well-folded protein differ from purification of natively insoluble or unfolded proteins? Briefly explain the theory behind at least two different methods. (H/M)

Table B.1 continued

MAtCH Evaluation	Possible Modifications
• Practice-oriented, no discussion of thermodynamics and only moderate discussion of kinetics (t)	• Describe the process of creating a Bradford calibration curve with BSA. Explain your choice of wavelength, standard concentrations, and any decisions you made while creating your graph. (H/M/A) • What is the purpose of each of the samples loaded into your gel for SDS-PAGE analysis in Experiment 8? Is there any reason for their order? (M/A) • What information about PTP1B can be obtained from your stained gel? What cannot? (A/H) • Discuss error inherent in kinetic analysis of PTP1B. How is this error summarized in representation of your average reaction rates? (M, A) • Discuss the fit of your data to the Michaelis-Menten equation. (M/A) • How does the data you obtained over the course of this project extend characterization of PTP1B catalysis? (M/C/H) • If you were to conduct further studies on PTP1B (or a similar PTP), what would you do? Explain why you chose those research goals. (C/H/M) • Using literature, identify another enzyme for which protein motions have been shown to be important for function. What is currently known about this enzyme and what research problems or goals currently exist? (H/C) • To what other research could you apply the methods you used in this project? (C, M) • If your aim was to understand more about how PTP1B interacts with its substrate, what would you study (e.g. properties)? What current theories or models would you consider? (H)

Table B.1 continued

MAtCH Evaluation	Possible Modifications
Demonstration of AutoDock as an educational tool for drug discovery *Helgren & Hagen (2017)*	
• Provides opportunity to explore 3D structures of CDK2 and CDK2 inhibitors (A) • Use docking software and apply fragment growth to hit molecules (M/A) • Situates methods in context of drug discovery practices and a specific target molecule, CDK2, but little specific background regarding the latter (M, C) • Introduces a variety of methods used over the course of the drug discovery process (M) • Discusses how models for use in AutoDock are developed/modified (A/M)	• What interactions/distances are significant to your reasonable docking poses? (H/M/A) • What kinds of modifications can be applied compounds to affect their binding affinity? Explain. (H/M) • Make a recommendation for a compound based on the docking poses you produced. (A/C) • What additional experiments are appropriate after identifying a viable compound(s)? (M/H/C) • What other research problems or contexts employ computational methods as part of their methodologies, and at what point(s) are they used? (C, M) • Explain how dissociation constant and inhibitory concentration resulting in 50% activity reduction (IC_{50}) are related. (H/M) • Explain what information about entities and interactions can be obtained from the methods you used. (H/M) • Discuss any similarities or differences across the possible inhibitors and their interactions with the CDK2 protein. (H) • You modified the CDK2 receptor prior to docking. Discuss these modifications in terms of how well the AutoDock model represents the cellular or *in vivo* environment. (A/H) • How are docking scores calculated? What concepts and/or mathematics underlie score calculation? (M/A) • Explain how variability in ligand and receptor conformations during docking can affect your predictions. Are there implications for your research problem/goal? (M/A/C) • Docking runs can predict highly-scored but physically impossible docking poses, and duplicate docking runs can produce different results. What factors lead to this and how can you 'trust' your results? (M)

Table B.1 continued

MAtCH Evaluation	Possible Modifications
• Thorough description of how to use software like AutoDock and AutoGrid to modify the protein model (M/A) • Software produces a variety of representations carrying information about the receptor (A) • Limited discussion of affordances and limitations of models/representations (A)	• Explain the implications of measuring a binding affinity that is overly high or overly low. (M/H/C) • How do *in vivo*, *in vitro*, and *in silico* drug discovery efforts differ? (M/A/C)
Understanding structure: A computer-based macromolecular biochemistry lab activity *McLaughlin (2017)*	
• Introduces origin of X-ray crystallographic images, but provides little social or biological context (C) • Draws connections between electron density maps (A) and amino acid residues (H), but no discussion of how electron density data is measured (M)	• What is the biological significance of the incorrect residues in the mutated model? How might those mutations affect the structure? (C/H) • What are the limitations of the methods used in this activity? What can and what can they not tell you about a protein? (H/M) • What does it mean for a residue to lack electron density? How can such residues be differentiated from mutated residues? (H/M/A) • What are other reasons protein structure refinement software is used? (M/A/C) • What is the purpose of crystallizing proteins to develop protein models? / How can protein models developed from crystallization be used? (M/A/C) • Are there any other strategies to aid crystallization? (M)

Table B.1 continued

MAtCH Evaluation	Possible Modifications
• Produce images of corrected amino acid residues (A), but do not analyze changes in terms of social or biological implications (C) • Limited discussion of the limitations of X-ray crystallography (M) and the theory (t) behind it • Limited discussion of the accuracy of X-ray crystallography and computer protein models (M/A) • Provides practice using PyMol and *Coot* (M/A), but limited discussion about what these models (A) can describe about interactions and functions (H)	• How accurately do PyMol and X-ray crystallography models represent the *in vivo* or native state of the protein they represent? (H/A) • What is the purpose of the crystallization solutions used in preparing your crystal tray? (M) • With references, describe two contexts where protein models are used to address research goals. (A/C) • Identify a current area of research which employs similar methods and describe what entities and interactions it investigates. (C/H/M)

INTERVIEW PROTOCOL

Table B.2 Semi-structured interview protocol used to explore expert explanations of research.

Purpose	Interview Question
Phase 1: Exhaust description of research; freeform explanation	**1. Explain your research as you would to a colleague, somebody who is in a related or similar field. Feel free to sketch or show any representations during your explanation. (Let them answer/draw/etc. freely.)** a. Why did you choose to study that (topic of interest)? b. Is there a particular way you want to apply this research? {For clarification: That is, why is this research important, such as to organisms or to society?} **2. What is the role of the living environment** (i.e. the in vivo) in your research?
Phase 2: Probe description of research methods, data, and how data is processed	**1. Can you explain in detail how you study this? (For clarification: …in terms of your data collection, your methods, etc.? How do you actually *do* the science that you do?) (Answer freely.)** a. What kind of data do you collect? i.e. Where does your data come from? **(data source)** Do you use data from other sources (e.g. PDB files) to supplement your own data? If so, where from and how? b. Do you take thermodynamic or kinetic measurements? c. What experimental methods do you use to collect data? **(data collection)** **2. (So) What kind of information do each of those techniques give you? What kind of information do you get from those sources?** **3. Do you use any sort of modeling in studying your protein? (What do you do? How do you use them?)** a. (If applicable) At what stages do you use those models (source, collection, analysis)? b. (If applicable) Can you draw or show the model(s) and describe how you use them? Can you explain how the information for your models/simulation(s) develops from your data OR how your simulation is used as data to explain the phenomenon you study? c. (Limitations) What is this model useful for and what is it not useful for? (What can it do or not do?) **4. How do you analyze your data? (data analysis)** a. When you analyze your data, how does that data help you develop an explanation? How do you piece together the data that you collect and the theoretical aspects of your work? b. How do you represent that data? If you're writing up a paper and in the results section, what sort of data would you present (to communicate your findings)? Do you use (indicate previous drawings) or…? Can you draw an example? i. For the representations that you use (to think about what you do or in publications), what sorts of limitations do they have? Do they communicate too much, too little…?

Table B.2 continued

Phase 3: Probe for additional representations	**1. When you think about your research or when you're trying to explain it, what do you visualize? What do you picture in your mind or draw? Can you draw it for me? (Answer freely).** a. (Clarification) Do you use this/that as a tool for thinking about it during experimentation? Or as a representation for publication? **2. Let's see, you mentioned... (summarize to confirm that you understood their drawings). Apart from those examples, do you use any other visuals in your explanations?**
Phase 4: Research explanation to an upper-level undergraduate student	**1. Could you explain your research like you would to an upper-level undergraduate student (specifically to student in a 300-400-level course)?** **2. Could you tell me a bit more about how you would explain protein folding in general to a student?** a. Would you use entropy to explain (protein folding/dynamics)? If so, how? (Feel free to draw.) b. Would you use enthalpy to explain? If so, how? (Feel free to draw.) c. Would you use free energy to explain? If so, how? (Feel free to draw.) d. You mentioned the concept _____. Can you draw and explain how you would explain that concept in the context of protein folding? e. (If necessary) How would you describe the methods used to get your data in the classroom? Feel free to draw any pictures you would use. **3. That covers everything I wanted to ask. Is there anything else you would like to tell me?**

APPENDIX C. CHAPTER 6 SUPPLEMENTAL MATERIAL

HDX-MS CURRICULUM MODULE PRE-READING

Pre-Reading

Instructions: Read the following before attending the class period on hydrogen-deuterium exchange mass spectrometry (HDX-MS). You may find it helpful to refer to this reading when completing the in-class activity, as well as additional readings by Marcsisin & Engen (2010); Marciano, Dharmarajan, & Griffin (2014); and Campobasso & Huddler (2015). References are included at the end of this reading.

Drug discovery and development: Understanding protein structure and dynamics

In the past several decades, biopharmaceuticals have become increasingly prevalent and are expected to account for the majority of newly approved drugs in the near future. Biopharmaceuticals are drugs developed from biological sources using biotechnology. They include drugs such as monoclonal antibodies (mAbs) which are used in cancer treatments, recombinant protein therapeutics like human insulin for treating diabetes, or small peptides like cyclosporine which is used to prevent organ transplant rejection. Unlike small molecule drugs (e.g. aspirin, Benadryl) which are organic compounds with well-defined structures, biopharmaceuticals are often proteins and, as such, have higher-order structure and undergo dynamic conformational changes which are critical to their biological activity and thus intimately linked to their effectiveness as therapeutic agents. Due to their complexity and flexibility, protein drugs can be made to bind their targets strongly and more specifically, but these same properties make them vulnerable to environmental stressors that may affect their structure, and thus safety and effectiveness. Discovering, developing, and manufacturing protein drugs with consistent higher order structures for commercial use is therefore critical to the biopharmaceutical industry.

How does a particular manufacturing process affect the structure of a protein drug? How can a company validate storage formulations or ensure quality control between different batches? How does a scientist even identify if and where a drug binds to a target receptor? Answering these questions requires analytical methods capable of characterizing higher order protein structure, dynamics, and interactions. Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS) is one of the most sensitive analytical methods that can probe protein structure and dynamics. Like other routine biopharmaceutical methods (e.g. circular dichroism, FTIR spectroscopy), HDX-MS reveals changes to the overall 'global' structure or conformation of proteins and, therefore, whether a protein has retained its native structure and activity during manufacturing and storage. However, it can also characterize minor changes at the level of a single amino acid. HDX-MS is therefore one of the most powerful analytical methods available to support drug discovery and development.

HDX-MS reveals higher-order structure and dynamics

Proteins have complex structures as a result of interactions between the amino acids in their primary sequence or interactions between multiple protein sub-units. Although many representations of proteins suggest they are static, proteins are often highly dynamic. Proteins experience small local fluctuations often described as "breathing". They also undergo large movements such as shifts of entire protein domains.[1] The structure and dynamics of proteins are intimately related to their function in that just small changes in conformation can either promote protein activity or lead to a loss of function. Thus, monitoring protein dynamics is crucial in the drug industry. HDX-MS is an important technique that can reveal information

[1] Visit YouTube to check out simulations of protein movements like this one (https://bit.ly/2LL6WSl) showing small, local fluctuations, or this one (https://bit.ly/2OBhC3E), which gives an example of larger-scale movements related to function.

about the structure and dynamics of proteins, such as what regions of proteins are exposed to or protected from solvent, what regions are involved in interactions, or what regions move or shift more frequently.

HDX-MS works by taking advantage of a chemical reaction unique to hydrogens in proteins. If we look at the backbone of a protein, there are three kinds of hydrogen atoms (**Figure 1**):

1. Hydrogens attached to alpha carbons (green squares)
2. Hydrogens attached to side chains (purple circle); and
3. Backbone amide hydrogens involved in hydrogen-bonding in secondary structure elements (red pentagon)

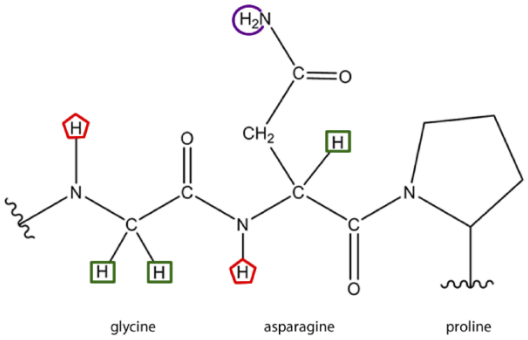

Figure 1 Three kinds of hydrogen atoms in a peptide

In aqueous solutions, both the amide hydrogens along the protein backbone and the side chain hydrogens continuously exchange with the hydrogens in water molecules. The hydrogens attached to alpha carbons do not exchange. Side chain hydrogens exchange too quickly to be detected, but the amide hydrogens exchange at a measurable rate. **Figure 2a** illustrates the exchange of an amide hydrogen (dark blue) with a hydrogen from water (light blue).

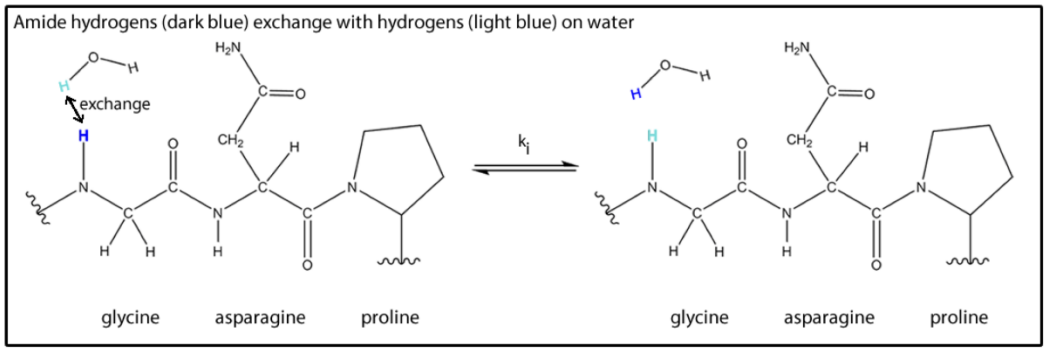

Figure 2 Amide hydrogen exchange for a short peptide

Like all chemical reactions, the rate of hydrogen exchange depends on the environment. Recall from introductory chemistry that the rate of a reaction can be described by a rate law, which has the general form rate=k*[A]. In a completely unfolded peptide, the rate at which an amide hydrogen exchanges with a solvent hydrogen depends only on temperature, pH, and electrostatic or steric effects from nearby residues. The dependence of the exchange rate on surrounding residues has been measured for any given peptide. The rate of a single amide hydrogen exchange is called the "intrinsic rate of exchange" and the rate constant 'k' is used to relate the overall rate of the reaction to the reactant concentration at a particular temperature. The rate constant for an amide hydrogen exchange is often represented by k_i ('i' for "intrinsic") as above the arrow in **Figure 2**.

If a protein is placed in a solution where the solvent contains an isotope of hydrogen, like deuterium, the exchange of hydrogen for deuterium by the backbone amides can be measured. Recall that deuterium has an additional neutron and is therefore heavier than hydrogen; this difference in mass can be measured. **Figure 3** shows the exchange of an amide hydrogen (dark blue) for a deuterium atom ('D', red) from deuterated water (D_2O).

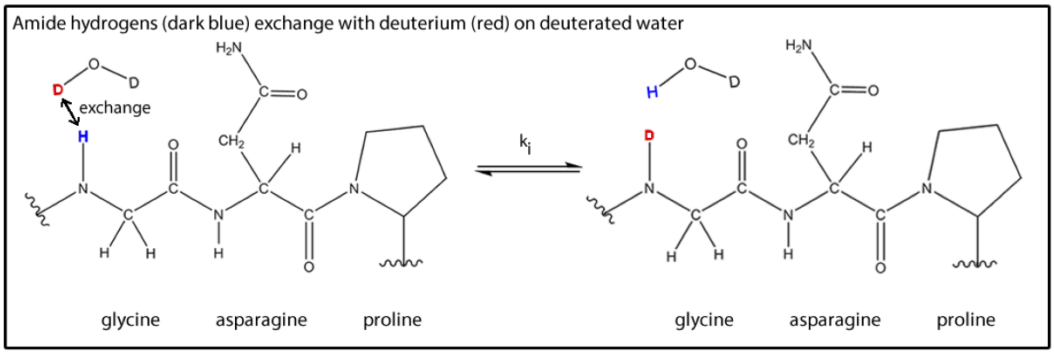

Figure 3 Deuterium exchange with an amide hydrogen for a short peptide

Every backbone nitrogen has an amide hydrogen, meaning that every amino acid along the length of a protein can be studied. The only exception is proline, one of the residues in the peptide in **Figure 3**, which has no amide hydrogen. Of course, it is just as possible for a deuterium atom to exchange back. The bi-directional equilibrium arrow reflects how these processes can occur simultaneously during HDX. In order to ensure that hydrogens will be replaced, and therefore 'labeled', by deuterium, proteins are placed into solutions which are often 90% deuterated water. The excess deuterium drives the forward reaction, favoring the labelling of the proteins with deuterium. The back exchange of deuterium is negligible in comparison.

At pH 7.0 and 25 °C, most amide hydrogens in an unfolded peptide exchange on the order of milliseconds to seconds. However, an unstructured peptide is different from a folded protein. Structure can slow the intrinsic rate of exchange up to 100,000,000 times by 'protecting' amide hydrogens from exchange. The rate of exchange for an amide hydrogen in a folded protein is often writer as k_{ex}. Two main structural factors dictate the rate of exchange in a folded protein:

1) The extent to which an amide hydrogen is exposed to the solvent (i.e. its "solvent accessibility");
2) Whether or not an amide hydrogen participates in a hydrogen bond that maintains secondary structural elements (i.e. alpha helices and beta-sheets).

Let's imagine, for example, several residues in a protein. **Figure 4** is a ribbon diagram of RNase HI. Residues 80-88 (pink) are located in an alpha helix on the exterior surface of RNase HI. Residues 104 and 107 (blue) are located in an alpha helix in the protein's interior.

If you examine the overall structure of RNase HI and the colored residues, which residue set would you predict to be more "protected" from the solvent? A residue's amount of "solvent protection" or its opposite ("solvent accessibility") depend on the location of the residue, such as whether it is in the interior of a protein's 3D structure or exposed on the exterior. In **Figure 4**, the most protected residue set would probably be blue (residues 104 and 107), followed by pink (80-88), and then bright green (96-99).

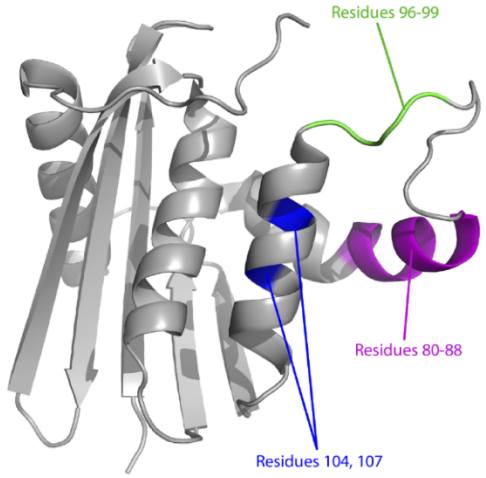

Figure 4 Ribbon diagram of RNase HI with colored residue sets

Solvent accessibility affects the amount and rate of deuterium incorporation when a protein is placed in a solution like deuterated water. The more accessible a residue or region of a protein is, the faster deuterium can be incorporated. An amide hydrogen located at the surface of a protein can exchange rapidly, whereas those buried in the interior are more difficult for the solvent to reach. More time must pass before an interior amide hydrogen undergoes exchange. Therefore, in a given time period, the residues indicated in blue (104 and 107) in **Figure 4** would likely undergo less exchange compared to the other residues.

The presence of secondary structure also dictates the rate of exchange in a protein. Consider residues 80-88 (pink) and residues 96-99 (green) in **Figure 4**. Both of these residue sets could be considered to be on the exterior of the protein. However, the pink residue set is part of an alpha helix whereas the green residue set composes a flexible loop region. Which residue set would you predict to become deuterated more quickly?

The more flexible or dynamic a region is, the easier it is for the amide hydrogens to exchange with deuterium atoms. Conversely, if secondary structural elements, like alpha helices, are present, those residues' amide hydrogens are involved in hydrogen bonds to stabilize the secondary structures, and will take longer to exchange. Backbone amide hydrogens in dynamic regions which are not involved in hydrogen bonding, or only involved in weak hydrogen bonds, can exchange more rapidly than amide hydrogens involved in stronger hydrogen bonds with other residues in order to make secondary structure. One would therefore expect that the amide hydrogens of residues 96-99 (green) in **Figure 4** would more easily exchange with deuterium than the amide hydrogens of residues 80-88 (pink). In a given time period, residues 96-99 (green) would therefore be deuterated quickest because their lack of structure means that their amide hydrogens are "less protected" from exchange.

However, the existence of secondary structure does not mean hydrogen-deuterium exchange can never occur. The innate "breathing" fluctuations of proteins can briefly interrupt hydrogen bonds involved in stabilizing protein structure. **Figure 5** shows the stretching of a single alpha helix, which results in the opening and closing of its hydrogen bonds (black dashed lines).

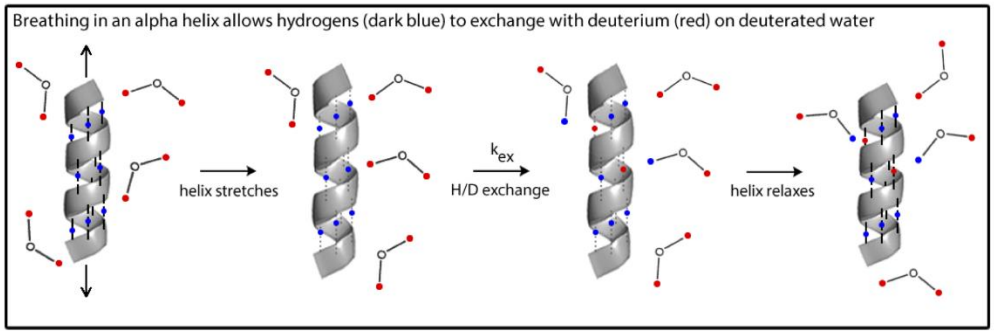

Figure 5 Hydrogen-deuterium exchange in an alpha helix due to 'breathing' fluctuations

When the hydrogen bonds are 'open' (light gray dotted lines), a deuterium atom (red dot) in the surrounding solution can exchange with a hydrogen atom (blue dot) that was originally part of the alpha helix. This innate motion means that even rigid or stable secondary structural elements can undergo hydrogen-deuterium exchange, it will just happen more slowly. Hydrogen-deuterium exchange is therefore an ideal way to probe protein structure and dynamics. If there is a change in the solvent accessibility or the hydrogen-bonding network, the rate and location of deuterium incorporation will be altered.

Because the time it takes for a particular amide hydrogen to exchange can vary dramatically depending on its location and involvement in bonding, HDX is often tracked over multiple time periods, ranging from seconds to months. **Figure 6**, for example, shows deuterium uptake for two residue sets in a protein at two time points. The dynamic and exposed regions of the protein will undergo exchange first. After a longer period of time, the more protected regions will exchange. The amount of deuterium that is incorporated for a set of residues (e.g. residues 7-9 labeled in Figure 6) at the different time points can be recorded and graphed on residue uptake plots, such as those in **Figure 6b**. In these plots, the amount of deuterium is measured in terms of mass in atomic mass units (amu) or Daltons (Da, y-axis) over several time points (x-axis). Comparing the deuterium levels to each other and over time, reveals that residues 60-62 incorporate more deuterium and do so more quickly than residues 7-9. This suggests that residues 60-62 are more exposed and/or less structured.

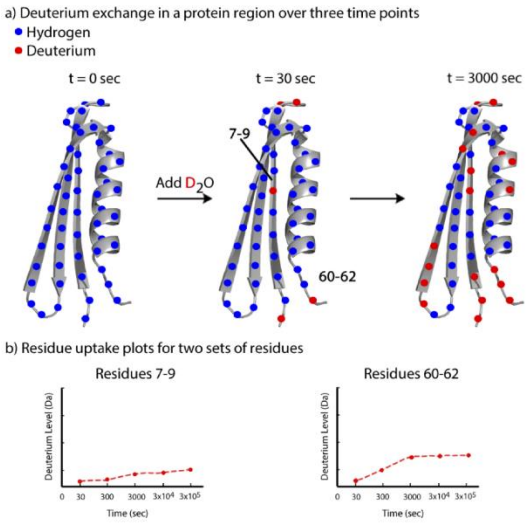

Figure 6 Example of deuterium uptake across several time points and residue uptake plots for two residue sets

Figure 7 shows a simplified diagram of the steps in a "continuous labeling" HDX-MS experiment used to investigate the effect of two drug formulations on protein dynamics during storage (**Panel 1**). In continuous labeling experiments, a protein drug sample is placed in a deuterated solvent and allowed to undergo hydrogen-deuterium exchange for various amounts of time. Time zero (t=0) represents a protein drug sample that has not been treated with deuterium and can serve as a reference. If the drug formulations have different effects on the protein dynamics (e.g. protect the structures or prevent partial unfolding differently), the amount and location of deuterium incorporation will be different, as can be seen by comparing the two structures in **Panel 2**. At various times (e.g. t=30 sec), the labeling reaction is "quenched" by lowering the temperature to 0°C and dropping the pH. Doing this essentially stops the exchange of deuterium and hydrogen. The protein samples can then be denatured and digested with a protease like pepsin to obtain peptide fragments (**Panel 3**). Pepsin cleaves peptide bonds between certain amino acids, resulting in peptide fragments of various lengths and sequences. The peptide fragments can then be ionized and a mass spectrometer can be used to detect the amount of deuterium incorporated. If you compare the peptide fragments in **Panel 3**, you can identify several residues where the dynamics of the protein changed as a result of the ligand binding. Mass spectrometers detect the masses of the individual peptide fragment ions which can be compared to reference peptides in order to determine the amount of deuterium incorporated for a particular fragment. By matching the amino acid sequences of the peptide fragments to the overall sequence, the fragment masses can be mapped onto the protein structure to show the amount and location of deuterium incorporation. This data analysis can transform the outputs from the mass spectrometer into representations such as residue uptake plots, like those in **Figure 6**, or structural heat maps (**Fig. 7, Panel 4**). Structural heat maps use shades of color to show the amount of deuterium uptake, making it possible to visually identify protein regions that have increased or decreased protection from exchange. Red regions on the heat map in **Panel 4**, for example, indicate increased deuterium uptake or 'deuteration level'. This suggests that the binding of the ligand affects the dynamics of these regions.

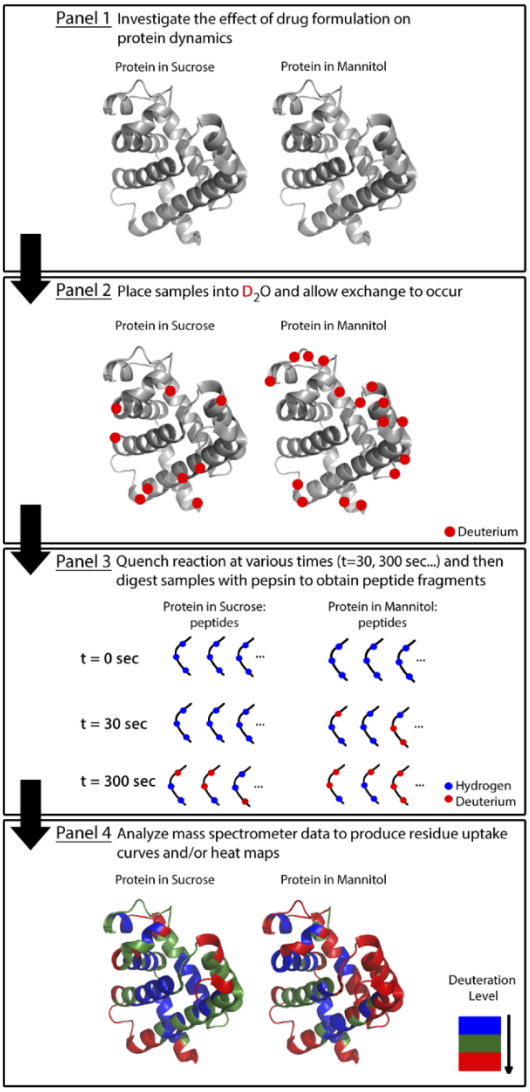

Figure 7 Overview of an HDX-MS experiment to characterize the effect of different drug formulations on protein dynamics.

A change in solvent accessibility or hydrogen-bonding detectable by HDX-MS could result from a variety of events. Proteins may have very flexible regions with a lot of small fluctuations which allow for more deuterium to be incorporated. Alternatively, proteins may undergo conformational changes due to interactions with other proteins or ligands, or as a result of partial or global unfolding. This means that HDX-MS can assess changes to protein structure that could affect protein drug quality, such as denaturation during storage.

HDX-MS has also been used to study the effect of ligands binding to proteins. Because proteins are dynamic molecules, binding a ligand in one region of a protein may cause structural changes elsewhere. HDX can be therefore used to reveal if and where proteins may change conformation as a result of binding a ligand. Additionally, when a ligand is bound to a protein, it "protects" the residues it interacts with from exchange, and thus prevents the incorporation of deuterium. The amount of deuterium in particular regions or sets of residues can therefore reveal information about which regions may (or may not) be involved in binding interactions. Identifying and characterizing potential binding sites can aid the discovery and development of new small molecule drugs, new vaccines, and diagnostic tests.

Additional Readings[i]

Campobasso, N., & Huddler, D. (2015). Hydrogen deuterium mass spectrometry in drug discovery. *Bioorganic & Medicinal Chemistry Letters*, 25, 3771-3776.

Marciano, D. P., Dharmarajan, V., & Griffin, P. R. (2014). HDX-MS guided drug discovery: small molecules and biopharmaceuticals. *Current Opinion in Structural Biology*, 28, 105-111.

Marcsisin, S. R., & Engen, J. R. (2010). Hydrogen exchange mass spectrometry: what is it and what can it tell us?. *Analytical and Bioanalytical Chemistry*, 397, 967-972.

[i] Representations in these articles inspired the design of several of the representations contained in this pre-reading.

HDX-MS CURRICULUM MODULE ACTIVITY 1

Activity 1

Instructions: The purpose of the following activity is to develop your understanding of HDX-MS as an experimental method to study protein folding and dynamics, and to support your ability to interpret complex representations like those found in research articles. You may find it helpful to refer to the pre-reading and the additional readings mentioned therein.

The following activity uses representations from:

Moorthy, B.S., Schultz, S.G., Kim, S.G., and Topp, E.M. (2014). Predicting protein aggregation during storage in lyophilized solids using solid state amid hydrogen/deuterium exchange with mass spectrometric analysis (ssHDX-MS). *Molecular Pharmaceutics*, 11, 1869-1879.

Using ssHDX-MS to assess protein drug formulations

Let's evaluate what the data presented in Table 1 and Figure 4 from a study by Moorthy et al. (2014) reveals about structural changes in different lyophilized (freeze-dried) protein drug formulations. Lyophilization (freeze-drying) attempts to prevent the aggregation or denaturation of protein drugs during long-term storage. Aggregation and denaturation can reduce the effectiveness of the protein drug or cause the body to mount an immune response. The formulation of the protein drug (i.e. the protein, as well as the type and amount of additives) and the lyophilization processing conditions affect a protein drug's stability; less stable formulations are more likely to denature and aggregate over time. Traditionally, assessing the stability of protein drug formulations requires trial-and-error studies occurring over months or years. However, experimental methods like HDX-MS have the potential to relate the measurable properties of formulations to the rate of denaturation or aggregation. Solid state HDX-MS (ssHDX-MS) is a special HDX technique that is used with solid samples – like freeze-dried protein drugs – and can be used as a screening tool to design stable formulations of lyophilized protein drugs.

Take a look at Moorthy et al. (2014) Table 1 and Figure 4.

1. What protein, represented in Figure 4, is used in this study?

2. Compare the information provided in the columns and rows of Moorthy et al. (2014) Table 1. Briefly summarize the differences that exist between the information contained in the "MbA, MbB, etc." columns. It may help to use an example.

3. The Figure 4 caption states that "13 nonredundant pepsin digest fragments were mapped onto the [holoMb] structure." Break down this sentence by providing an explanation of how HDX alters a protein, how the protein is prepared for analysis by MS, and how MS data is mapped onto a protein structure.

4. Compare the data for the protein in each of the five formulations represented in Figure 4:
    a. Examine the legend and the five protein structures. What does the color coding represent?

    b. Are there any protein **regions** (e.g. loops, alpha helices, etc.) which show greater deuterium incorporation across all five formulations? Are there any **regions** which have typically lower incorporation? Use whatever markings you need to indicate and label these on the figure.

    c. In the center of each of the protein structures, there is a dark red/brown structure. According to the legend, how much deuterium incorporation has occurred here? Does this make sense given the location? If so, explain. If not, what else might this structure be?

    d. What does the amount of deuterium uptake in a region indicate about rigidity or amount of structure in that area?

e. Which drug formulation(s) result in the overall greatest deuterium uptake for the protein? The least? Explain what the deuterium uptake indicates about the overall stability of the protein molecules in each formulation.

f. Based on your assessment in question 4e, predict which drug formulation would have the best shelf life. Which would you expect to have the worst? Cite data from Figure 4 in your prediction.

5. Take a look at Figure 3B from Moorthy et al. (2014) which shows deuterium uptake for the drug formulations over a time period of approximately 100 hours. Compare differences between the drug formulations over time, as well as any differences in overall drug formulation stability. How do the predictions you made in question 4f compare to the data displayed in Figure 3B?

6. Protein aggregation occurs when improperly folded proteins cluster together. When proteins are improperly folded, regions that are exposed can form non-covalent interactions with other exposed regions, causing proteins to 'stick' together.
    a. Based on the data in Figures 3B and 4, predict which drug formulation you would expect to have the most protein aggregation after a year of storage. Support your prediction using evidence from the figure, as well as a description of how the HDX-MS method would reveal if proteins were misfolded.

7. Protein drug formulations contain protein molecules as well as other additives. Look at Table 1 and consider the ingredients of the drug formulations you identified as “least stable” and “most stable” in question 5e.
    a. Suggest a possible mechanism at the atomic/molecular level to explain how the additives in the drug formulations prevent the protein molecules from being deuterated. Think about what factors affect exchange in protein molecules and what interactions might occur between the additives and the protein molecules.

HDX-MS CURRICULUM MODULE ACTIVITY 2

Activity 2

Instructions: The purpose of the following activity is to develop your understanding of HDX-MS as an experimental method to study protein folding and dynamics, and to support your ability to interpret complex representations like those found in research articles. You may find it helpful to refer to the pre-reading and the additional readings mentioned therein.

The following activity uses representations and information from:

Hsu, Y. H., Bucher, D., Cao, J., Li, S., Yang, S. W., Kokotos, G., ... & Dennis, E. A. (2013). Fluoroketone inhibition of Ca2+-independent phospholipase A2 through binding pocket association defined by hydrogen/deuterium exchange and molecular dynamics. *Journal of the American Chemical Society*, 135(4), 1330-1337.

Hsu, Y. H., et al. (2013). Correction to "Fluoroketone Inhibition of Ca2+-Independent Phospholipase A2 through Binding Pocket Association Defined by Hydrogen/Deuterium Exchange and Molecular Dynamics". *Journal of the American Chemical Society*, 135(4), 1330-1337.

Using HDX-MS to study the binding of drug molecules to proteins

Let's evaluate the data presented in two figures from a study by Hsu et al. (2013). Hsu and colleagues used HDX-MS and simulations of molecular dynamics to understand how fluoroketone (FK) inhibitors interact with the active site of the Group IV Ca^{2+}-independent phospholipase A_2 enzyme (GVIA $iPLA_2$, hereafter simplified as $iPLA_2$). Recent studies have demonstrated that the function of the $iPLA_2$ enzyme is associated with several neurological disorders and may affect cancer cell growth, making it an important drug target. Drugs developed to target $iPLA_2$ have been capable of inhibiting the enzyme, but some of their effects and interactions with other proteins made them highly toxic. One FK inhibitor, PHFK, shows great potential as a potent and highly selective compound to treat $iPLA_2$-related diseases. Understanding how PHFK fits into and interacts with the binding site of $iPLA_2$ informs how molecules like PHFK can be chemically altered in pursuit of designing and testing a variety of inhibitors.

In their study, Hsu and colleagues prepared samples of the $iPLA_2$ enzyme with PHFK and without PHFK, and then placed these samples into D_2O buffer solutions and incubated them for several time periods. Let's evaluate what the HDX-MS data in the following figures reveals about the binding of PHFK to $iPLA_2$.

Take a look at Hsu et al. (2013) Figure 4.

1. Look at the caption. What entities and process are described by the data in this figure?

2. Decode the symbolism in Figure 4:
    a. Identify the purpose of the numbers and letters that make up the top two lines of each 'row'.

    b. There are two colored bars underneath each row of numbers and letters. What experimental conditions are represented by each of these bars?

3. Examine the caption, the legend on the right, and the time stamps in the upper right.
    a. What does the color coding in the legend indicate?

    b. Explain how the time stamps are related to the HDX method (in general) and the colored bars in the figure.

4. Look at the set of residues from approximately 516-525.
    a. Examine the color change over time for this set of residues under the -PHFK and +PHFK conditions. What does the color change indicate about deuterium incorporation over time for these residues under each of the different experimental conditions?

    b. Estimate the difference in the amount of deuterium exchanged in the absence versus presence of the inhibitor for residues 516-525. Does exchange increase or decrease, and by how much? Give your estimate as a percent.

5. Consider the entire figure.
    a. Identify two 'groups' of residues where deuterium exchange changes over time. Describe and then compare how deuterium exchange changes over the duration of the experiment for the residue sets you chose in both the presence and absence of the inhibitor.

    b. Does the presence of PHFK appear to affect deuterium exchange for either of the groups of the residues chose? If yes, explain how the data shows this and what it might indicate about the binding of PHFK. If not, explain what data pattern would indicate that the presence of PHFK has an effect. (Hint: Recall the purpose of the Hsu et al. (2013) study.)

Now examine Hsu et al. (2013) Figure 6. (***Important:*** The caption for Figure 6 was incorrect in the original article and was corrected in a Correction Notice.)

1. According to the caption, what data is presented in Hsu et al. Figure 6?

2. Identify the six color-coded regions on the protein by describing their structure (e.g. loop, alpha helix, etc.).

544-549:

631-650:

516-525:

710-730:

773-778:

483-493:

3. Examine the legend for Figure 5 in Hsu et al. (2013). The color coding indicates the effect of PHFK binding on the amount of hydrogen/deuterium exchanged during a time period. The percentages beside each color represent the percent by which the deuteration level increased or decreased as a result of the presence of the inhibitor. (Hint: Just like you calculated in the previous question #4.)
    a. Using the deuteration level change legend from Hsu et al. Figure 5, identify the approximate percent change in deuteration level for each region.

4. Decode the symbolism on Hsu et al. (2013) Figure 6.
    a. What do the x- and y-axes of the six inset plots represent? Associate each axis with the physical property or process it represents.

    b. What do the black and red curves represent? What does a higher position on the y-axis indicate?

5. Compare the red and black curves on each of the six plots:
    a. What does the relative position of the black and red curves indicate about hydrogen/deuterium exchange for those regions when the PHFK inhibitor is present vs. absent?

    b. Compare the scaling of the y-axis across the six inset plots. Keeping the different scales in mind, which plot(s) show the greatest difference in deuterium incorporation in the presence vs. absence of PHFK?

6. Consider both the structure and six inset plots.
    a. Given the change in deuteration level for the different regions and your general knowledge of enzyme structure, which regions would you predict to be part of the binding site? Indicate on Figure 6 where you think PHFK may bind.

    b. Use a series of sketches and captions to show and explain what is happening in this region to cause a change in deuterium incorporation. That is, explain how the binding of the PHFK inhibitor can affect the incorporation of deuterium. You can use simple shapes to represent $iPLA_2$ and PHFK.

Additional Readings

Cao, J., Burke, J. E., & Dennis, E. A. (2013). Using hydrogen/deuterium exchange mass spectrometry to define the specific interactions of the phospholipase A2 superfamily with lipid substrates, inhibitors, and membranes. *Journal of Biological Chemistry*, *288*(3), 1806-1813.

EXPANDED CURRICULUM

Table C.1 Expanded curriculum related to protein folding and dynamics.

Potential ALOs developed from expert research are organized according to module topics. Potential activities, including examples of primary literature to review, are provided in the right-most column.

Module Topic	Anticipated Learning Outcomes (ALOs)	Potential Activities
Prerequisite Knowledge	• Structure of water (dipoles, types of interactions) • Basic protein structure (e.g. amino acids, primary through quaternary structure, types of interactions, existence of active sites or binding regions, structure-function relationship) • Basic ideas related to kinetics/equilibrium (from introductory chemistry)	
Proteins are dynamic molecules	• Students should be able to explain how protein dynamics and unfolding lead to variable, measurable hydrogen-deuterium exchange (HDX) across different regions of a protein.* • Students should be able to interpret HDX data in the form of residue plots and/or heat maps in order to identify and compare protein regions with different amounts of exchange.* • Students should be able to use HDX data in order to draw conclusions about the spatial organization, stability, or function of different regions of a protein structure.*	• Explore animations/simulations of small and large protein movements • Explore literature on HDX and its application (e.g. Deng et al., 2016; Marciano et al., 2014; Campobasso & Huddler, 2015; Marcsisin & Engen, 2010; Wei et al., 2014) • Interpret HDX heat maps, residue uptake plots (e.g. Moorthy et al., 2014, Hsu et al., 2013) • Represent exchange at the atomic and macromolecular level • Relate HDX incorporation to protection and stability • Become familiar with Foldit (Cooper et al., 2010; Farley, 2013; FoldIt, 2018)

Table C.1 continued

Proteins exist as an ensemble of states	• Students should be able to describe and represent, at a macromolecular level, the equilibria that exist between folded, partially unfolded, and globally unfolded forms of a protein, and the relative population of different forms. • Students should be able to explain and represent, on molecular and macroscopic levels, how changing environmental conditions (particularly the addition of denaturants) affects protein structure. • Students should be able to relate enthalpy to the number and strength of interactions in a system in order to make predictions about flexibility and binding affinity. • Students should be able to relate relative values of **enthalpy,** entropy, and free energy to a qualitative description of the dynamic nature/motion of a protein. • Students should be able to explain and represent how interactions within a macromolecule and with molecules in the surrounding environment lead to emergent structure and degree of dynamics. • Students should be able to predict and explain how the addition of a mutation affects a protein's stability macroscopically (i.e. measurable) and at the molecular level • Students should be able to predict and explain how physical or chemical modification of a protein may lead to aggregation.	• Interpret and create representations of partially unfolded forms, population ratios • Use a simulation to explore and produce representations of micro-states • Use HDX data (e.g. 'strong, medium, and weak' protection levels) to predict or identify partially unfolded forms and compare their stability (e.g. from Start2Fold database of Varadi (2015) and Pancsa et al. (2016)) • Interpret denaturation curves • Use a simulation to explore the effect of temperature on protein flexibility • Compare interactions of protein regions in the presence/absence of denaturants • Interpret site-directed mutagenesis data to investigate the function of interactions on higher-order structure • Discuss the role of non-covalent interactions in protein aggregation diseases (e.g. Stefani & Dobson, 2003) • Work on introductory Foldit puzzles (e.g. backbone packing, hydrogen bonding, hydrophobics and hydrophilics)

Table C.1 continued

How the environment drives protein folding	• Students should be able to relate relative values of enthalpy, **entropy**, and free energy to a qualitative description of the dynamic nature/motion of a protein.† • Students should be able to compare the degree of protein dynamics/protein flexibility under different environmental conditions, including variable temperature. • Students should be able to explain and represent how interactions within a macromolecule and with molecules in the surrounding environment lead to emergent structure and degree of dynamics. • Students should be able to relate entropy to structural and emergent states (i.e. degrees of freedom, conformational variability/flexibility, and molecular order/microstates). • Students should be able to explain the hydrophobic effect by comparing and representing the flexibility/freedom of water molecules, ligands, and proteins in bound and unbound states. • Students should be able to describe environmental conditions relevant to protein folding and dynamics, including those which can and cannot be modeled during experimentation. • Students should be able to explain and represent models of the protein folding process, including ideas related to cooperativity, hydrophobic collapse, foldons, hierarchical order, etc.	• Interpret and create representations of interactions in water, water with unfolded protein, water with folded protein • Interpret and create representations of protein folding pathways based on burying of hydrophobic residues • Work on introductory foldit puzzles (e.g. backbone packing, hydrogen bonding, hydrophobics and hydrophilics) • Use representations to compare changes in entropy • Predict “simple” folding pathways based on HDX data regarding ‘early, intermediate, and late’ folding (e.g. from Start2Fold) • Explore case studies of simple (e.g. two-state, three-state) folding pathways; case studies on rapid folding in cold shock proteins (Perl et al., 1998) or unfoldons in maltose-binding protein (Bertz & Rief, 2008) • Explore folding-site prediction with EFoldMine case study (Raimondi et al., 2017) • Explore how simulations have been used to model the effect of solvent and intramolecular forces on the folding process (e.g. Chen, Im, & Brooks, 2006)

Table C.1 continued

		• Interpret and create storyboards of protein-ligand interactions involving solvent • Explore how chaperones influence protein folding (e.g. Bechtluft et al., 2007) • Extension – Exploring the Frontier of the Protein Folding Problem: Review cutting-edge research (e.g. Schönfelder et al. (2016) Multiple pathways revealed through mechanical unfolding) • Extension – Using simulations to explore protein folding and dynamics (e.g. Brooks et al. (2009) CHARMM and examples provided therein)
Structure v. Flexibility	• Students should be able to relate relative values of enthalpy, entropy, and free energy to a qualitative description of the dynamic nature/motion of a protein. • Students should be able to compare the degree of protein dynamics/protein flexibility under different environmental conditions, including variable temperature. • Students should be able to discuss free energy changes in protein structure and desolvation in terms of a balance in changes to enthalpy, entropy, and the physical components they represent. • Students should be able to use HDX data and/or relative ΔG values of protein regions to construct a probable folding pathway for a protein. • Students should be able to link conformational variability (of residues) and/or dynamic protein regions to functions.	• Interpret and use the free energy equation • Compare balancing of thermodynamic factors in ligand/protein binding, DNA helix formation, etc. • Explore the effects of single-point mutations on overall protein stability (e.g. Bigman & Levy (2018) Role of long-range contacts) • Interpret and create representations of protein/residue flexibility when binding ligands • Use a simple toy model to represent possible conformations/interactions and create a simple folding funnel

Table C.1 continued

	• Students should be able to relate enthalpy to the number and strength of interactions in a system in order to make predictions about flexibility and binding affinity. • • Students should be able to relate entropy to structural and emergent states (i.e. degrees of freedom, conformational variability/flexibility, and molecular order/microstates). • Students should be able to use a toy model of a protein to construct a simple folding funnel and use it to explain conformational diversity and interactions.	• Interpret and create energy well diagrams • Relate idea of partially unfolded forms, etc. to locally stable/meta-stable states (i.e. other than the globally stable form) • Interpret HDX data to determine order of folding (e.g. from Start2Fold; Chamberlain et al. 1996 case study) • Extension – Exploring the Frontier of Protein Folding: Review cutting-edge research (e.g. Lim, Bolin, & Marqusee (2018) Monitoring folding pathways over evolutionary time with HDX-MS)
Experimental techniques to study the function of residues and flexibility	• Students should be able to predict and explain how the addition of a mutation affects a protein's stability macroscopically (i.e. measurable) and at the molecular level. • Students should be able to relate enthalpy to the number and strength of interactions in a system in order to make predictions about flexibility and binding affinity. • Students should be able to identify residue (and/or ligand) properties and interactions based on their structure and environment, in order to predict possible functions. • Students should be able to connect protein structure and properties to protein function. • Students should be able to describe the role/purpose of structural changes during binding and/or catalysis. • Students should be able to explain the role/importance of interactions in an active site in order to predict how changes in distance (proximity) and angle (orientation/alignment) of residues affect catalysis.	• Interpret HDX data to determine effect of mutation on flexibility/interactions • Discuss complementarity, orientation, proximity in designing drugs • Explore the functions of different amino acids in an active site (packing, proton donation, etc.) • Predict functions of residues based on their properties, location, theories like orbital steering, etc. • Evaluate reaction rates with and without enzyme, mapping particulate representations to mathematical models (esp. rate constant) • Design experiments to determine residue function • Work on Foldit puzzles (e.g. sequences, protein design)

Table C.1 continued

Extension: Connecting kinetics to free energy	• Students should be able to design an experiment to determine the function of residues in protein function (in an active site) and protein stability (dynamic regions). • Students should be able to interpret/use reaction coordinate diagrams in order to relate energetic barriers and structural changes. • Students should be able to mathematically and qualitatively relate kinetic, equilibrium, and free energy values in order to map measurable data to conclusions about stability. • Students should be able to use graphs of denaturant concentration vs. kinetic or thermodynamic values (ΔG, ln k, etc.) to discuss protein stability. • Students should be able to use reaction coordinate diagrams and protein folding funnels to relate energetic barriers and structural changes.	• Explore how epitopes are mapped (e.g. Malito et al., 2013) • Explore how x-ray crystallography can be used in antidepressant drug development (e.g. Coleman et al., 2016) • Relate rate, equilibrium, and free energy through experimental methods like HDX/proteolysis kinetics (e.g. Park & Marqusee, 2005) • Explore mathematical modeling of HDX kinetics (e.g. EX1/EX2 kinetics) • Interpret and create denaturant versus ln k or ΔG graphs • Interpret and create free energy diagrams based on relative stabilities of structural states • Explore how protein engineering and kinetic experiments are used to characterize folding energetics (e.g. Matouschek et al., 1990; Fersht et al., 1992) • Explore folding and refolding trajectories (e.g. Samelson et al., 2018)

*ALOs have been refined through review of primary literature and student data appears to support their designation as Verified Learning Outcomes (VLOs) from the HDX-MS Module

†Several ALOs are present in connection with multiple Module Topics as the ALO is revisited from a slightly different perspective.

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EXAM QUESTIONS

Table C.2 Questions provided on the unit and final exams. Expectations regarding answers are provided in italics.

Unit Exam

1. Imagine a concerned patient asks you to explain how protein drug formulations are tested for quality and safety. You know that protein drug stability is a major concern in biopharmaceutical development, and can be studied using hydrogen-deuterium exchange mass spectroscopy (HDX-MS).

   Show with pictures and **explain with words** how different protein regions can have different amounts of hydrogen-deuterium exchange. Make sure to compare **at least two** different protein regions.

   Students should include a rough sketch (or multiple sketches) of a protein, preferably with some structures (e.g. alpha helices).

   Students should indicate at least two different protein regions, using by some type of label (e.g. arrows, circles, letters) or which are described in writing in enough detail that they can be identified on the pictures.

   In a complete explanation, students should mention the effect of 1) solvent accessibility and 2) participation in hydrogen bonding as part of secondary structure, on the ability of amide hydrogens to exchange with deuterium in the solvent. For solvent accessibility, students should explain how hydrogens on the interior regions of a protein are protected from the solvent which makes exchange difficult. For hydrogen bonding, students should explain how hydrogens in more flexible/dynamic regions with weaker hydrogen bonds can more easily exchange than hydrogens that are part of strong hydrogen bonds making up more stable regions. Students should indicate that hydrogen bonds in secondary structural elements must break before this exchange can occur. The instructor should consider their preferences as well as student answers across the course when assigning a point value related to the level of detail.

2. The effect of two drug formulations on the dynamics of the protein rhGCSF were compared. The two formulations were "rhGCSF with sucrose" and "rhGCSF with benzyl alcohol (BA)".
   a. The following plot* shows the percent deuterium uptake for residues 16-32 in an alpha helix of rhGCSF. Based on the plot, which drug formulation results in the most protein unfolding? Explain your reasoning. Cite evidence from the plot and how the HDX-MS method works.

      Students should state that the formulation with benzyl alcohol results in the most unfolding.

      Students should explain that the line for the BA formulation indicates that a higher percentage of deuterium is incorporated into the protein over the time period studied. Must briefly explain that in order for a higher amount of deuterium to be incorporated, the protein must be losing structure, either by unfolding so there is greater solvent accessibility (deuterium can reach previously protected/interior regions) or by the breaking/weakening of hydrogen bonds, making it easier for deuterium to exchange to with hydrogens that were part of hydrogen bonds maintaining secondary structure.

      Students could indicate that ultimately the protein (GCSF) by itself undergoes just as much unfolding as the formulation with BA by the final time point. There is nothing wrong with this statement, though they should have some discussion that the BA formulation incorporates more deuterium early on so it results in faster degradation of the drug compared to the protein alone.

Table C.2 continued

b. The protein rhGCSF (shown below)† is a bundle of alpha helices. In addition to the plot provided in (a), what other information is necessary to decide which formulation ("rhGCSF with sucrose" or "rhGCSF with benzyl alcohol (BA)") is better? Explain your reasoning.

Students should state that data needs to be collected for the entire protein structure.

Students should explain that without all of this data it is not possible to determine which formulation is better as the formulations could stabilize the other helices/protein regions differently (i.e. the BA formulation may result in less unfolding for the other three helices than the sucrose formulation).

Final Exam

1. Protein conformational change is a key indicator of protein drug aging. Demonstrate with diagrams and molecular structures what changes to the structure and bonding in proteins occur when they unfold and how protein conformational change can be studied using hydrogen-deuterium exchange mass spectrometry (HDX-MS).

Students should include sketches of protein structures (e.g. folded v. unfolded globular protein, alpha helices, beta sheets). Students may provide an overview of the four levels of protein structure. In either case, students should describe (through pictures and/or words) the differences in the interactions that lead to the formation/loss of structure when transitioning between levels. Students should discuss how interactions such as peptide bonds, hydrogen bonding, hydrophobic interactions, etc. contribute to different levels of structure. Ideally, students should mention that environmental changes (e.g. change in pH, high temperature, addition or removal of chemical agents) can cause proteins to unfold,

Students should explain the basic mechanism behind hydrogen-deuterium exchange, ideally discussing the effect of 1) solvent accessibility and 2) participation in hydrogen bonding on the ability of amide hydrogens to exchange with deuterium in the solvent. Students should include some sort of representation showing this exchange. A complete answer will indicate that HDX-MS measures the difference in the masses of proteins/peptides as a result of the incorporation of heavier deuterium atoms, but the instructor should consider student answers across the course in determining a point value regarding this. Ideally, students should also include a representation or describe how hydrogen-deuterium exchange differs for different regions/structure of a protein.

*Figure 5a from: Zhang, J., Banks, D. D., He, F., Treuheit, M. J., & Becker, G. W. (2015). Effects of Sucrose and Benzyl Alcohol on GCSF Conformational Dynamics Revealed by Hydrogen Deuterium Exchange Mass Spectrometry. Journal of Pharmaceutical Sciences, 104:1592-1600.
†Protein structure from: PDB #1GNC

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PUBLICATION

Jeffery, K.A., Pelaez, N., & Anderson, T.R. (2018). How four scientists integrate thermodynamic and kinetic theory, context, analogies, and methods in protein-folding and dynamics research: Implications for biochemistry instruction. *CBE–Life Sciences Education*, *17*(1), ar13.