Article

Using Expert Data to Inform the Use of Research Methods and Representations to Enhance Biochemistry Instruction and Textbook Design

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Abstract

Biochemistry textbooks often provide a disconnected, highly mathematical, and decontextualized 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. Toward 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 experts employ multiple representations and research methods to communicate how evidence can be used to understand phenomena. In contrast, textbooks explain what is known but seldom use representations to explain how it is known. Based on our findings, we suggest implications for instruction, including the design of textbooks, as well as potential instructional strategies to incorporate discussion of experimental methods and interpretation of representations during classroom activities. © 2019 International Union of Biochemistry and Molecular Biology, 47(5):513–531, 2019.

Keywords: *Protein folding; experimental methods; representations; gesture; instructional strategies; biochemistry*

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 [1–3]. Each resource helps characterize a different facet of disciplinary knowledge and, in combination with other resources, affords more holistic understanding of a phenomenon [4, 5]. 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 [1, 6–9]. Scientists translate between resources fluently, directly connecting the processes of science, scientific evidence, and practical contexts to the subject of science [10, 11]. Learning can be thought of as acquiring fluency in and across various discursive resources [12].

The investigation and modeling of molecular processes in biochemistry is impossible without these resources, especially ERs [12–16]. 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 [14, 17, 18]. Gestures are frequently combined

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with ERs to enhance discussion [19] and studies on gesture in professional environments [20–22] as well as the classroom [6, 23] 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 [1, 14, 24, 25]. However, the use of ERs in science courses and textbooks often appears to be disconnected from authentic disciplinary practices [11, 26], 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, which creates a disconnect between experiment, evidence, and "known" phenomenon [27].

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 article extends previous work, which modeled how four research scientists explain protein folding and dynamics research [28], 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 [16] 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.

Methods

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.* [28], 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).

Development and Description of Interview Protocol

As described in Jeffery *et al.* [28], the MACH model [29] 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. Semistructured interviews [30] 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.

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 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 [31] and constant comparison [32] 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 subcategories that emerged from the data.

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 subset of ERs and related transcript were randomly selected for analysis using the CRM model [16]. 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) [31]. 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 contain 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 using memoing [30] to sort the codes into categories and subcategories that were revised several times through constant comparison as category and subcategory descriptions crystallized (Fig. 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. 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 Fig. 2). Very few free energy-

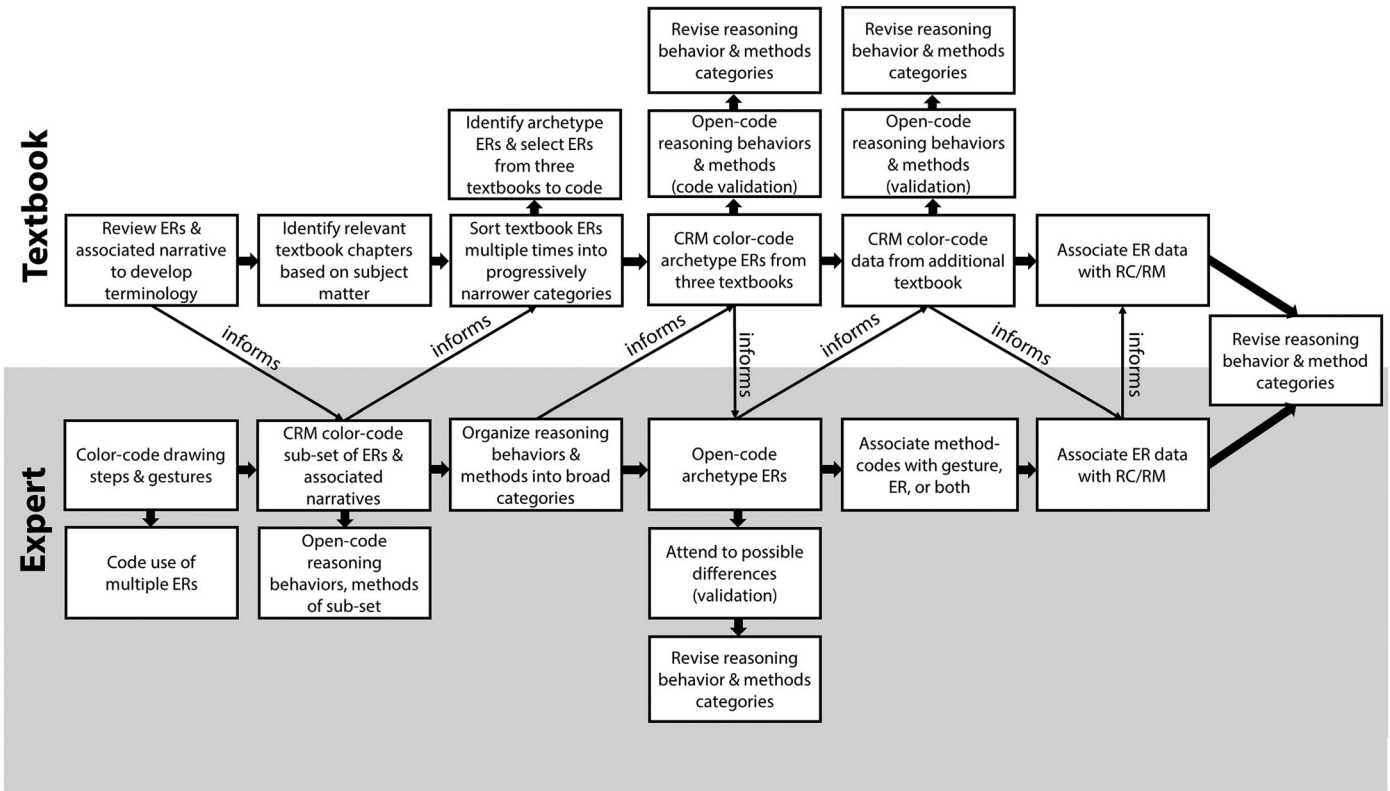


FIG 1

Simplified diagram of "CPC" 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 (gray) 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.

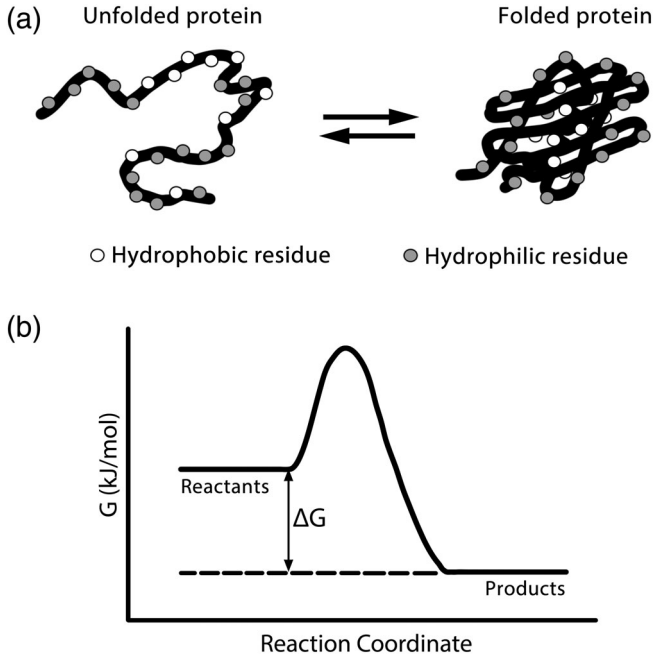


FIG 2

*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* $\Delta G^{\ddagger}_{uncat}$.

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.

ERs from four of the original eight biochemistry textbooks were selected for further analysis. The selected textbooks include those: (1) providing an overview of biochemical concepts for pre-med and allied health topics [33]; (2) focused on incorporating classical and current research on biochemistry [34]; (3) aimed at communicating fundamental principles of biological molecules to first-time biochemistry students [35]; and (4) aiming to balance new research findings with essential biochemical principles [36]. 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. 1).

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 I) and ways of discussing experimental methods (Table II), 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) [16]. At the end, we discuss the use of ERs in biochemistry textbook narratives, before comparing the expert and textbook data in the Conclusions.

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 3 contains a short excerpt from John's explanation (see "Speech" column in Fig. 3*c*), with drawing steps and gestures indicated in italics ("Gesture" column), and several examples of reasoning behaviors written as verb-noun pairs ("Reasoning" column in Fig. 3*c*; also see Table I). The excerpt provided in Fig. 3*c* begins after John completes the protein-folding cartoon in Fig. 3*a*, but we briefly describe the drawing of Fig. 3*a* to provide necessary context. John begins by drawing a cartoon of a folded protein (labeled "N" in Fig. 3*a*). 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"

TABLE I

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*	*Examples*
Identifies	*a. Physical* ...entities, subparts 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 subparts of entities; ...functions with entities or subparts 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 subpart 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 (fig. 14.4), a highly organized (low-entropy) entity..." (Textbook, Garrett & Grisham, 2013)
	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)

(Continues)

TABLE I *(Continued)*

Category	*Codes*	*Examples*
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)
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 labeling 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)

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). Where the excerpt in Fig. 3*c* begins, John integrates his explanation (see "Speech" column), 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. 3*b*). John repeatedly employs gesture and shapes (e.g. lines, circles; "Gesture" column in Fig. 3*c*) to tie the equations (Fig. 3*b*) to the cartoon (Fig. 3*a*). 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; see Figs. 3*b* and 3*c* "Speech" and "Gesture" columns). At the end of the excerpt in Fig. 3*c*, 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. In the interview, John continues to explain how those data are then used to determine the equilibrium constant (Fig. 3*b*, rearranged equation) and he 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 the free energy difference between the native and cleavable forms, and John draws an arrow from K_{unf} to $\Delta G^{\circ}_{C\text{-}N}$ to relate the equilibrium and free energy values (Fig. 3*b*). Referring to Fig. 4*a*, John explains that they can determine the $\Delta G^{\circ}_{C\text{-}N}$ for many different environmental conditions as well as the effect of mutations on $\Delta G^{\circ}_{C\text{-}N}$. He finally 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

TABLE II ***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	*Examples*	*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
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)	Plan The ability to analyze and process data.

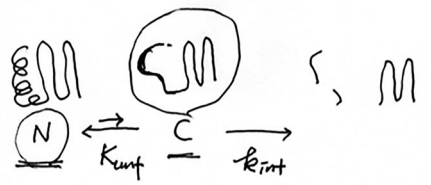
(Continues)

TABLE II *(Continued)*

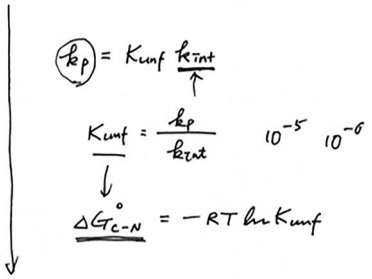
Category	*Examples*	*ACE-Bio Competencies**
	"...and then, in principle, using canonical ensembles, trying to estimate the relative free energies [points between two glutamates] of different conformations." (William) "And so you get a band at 1650 or so [moves mouse from bottom of dip down to x-axis] 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)	
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.*

(a)



(b)



(c)

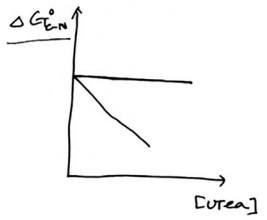
Speech	Gesture	Reasoning Behaviors / Discussion of Experimentation
This proteolysis reaction is irreversible.	*points between C form, fragments several times*	Identifies property of process[RC] Associates process with shift between graphical units[(C)RM]
Once it is digested, you know, peptide bond is gone, right?		
But this is dynamic equilibrium, right?	*points between N, C forms along arrows several times*	
So we define these.	*points between N, C forms along arrows*	
This is- we just call it K unfolding.	*labels equilibrium arrows with K_{unf}*	Associates symbols with arrows[RM] and with process[(C)RM]
And then this is- this is kinetic constant like k. We call it k intrinsic.	*labels k_{int} below unidirectional arrow*	
So it's intrinsic proteolysis rate.	*covers N form and equilibrium arrows*	
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.		Associates process with mathematical model[(C)RM]
The equilibrium constant times intrinsic proteolysis kinetics.	*writes $k_p = K_{unf}\, k_{int}$* *points at K_{unf} and k_{int} labels in reaction*	
Then... we monitor how fast protein is digested, then we determine this k_p.	*points at, circles k_p*	Describes outcome variables
And then we approximate this number, k intrinsic, using unstructured peptide. The same enzyme, right?	*points at k_{int} in reaction, points at k_{int} in equation*	
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

FIG 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 are indicated in superscripts at the end of the reasoning behavior.*

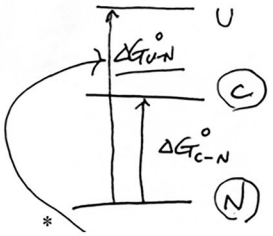
form—cannot be studied directly by any other typical method (e.g. nuclear magnetic resonance, circular dichroism, etc.).

As can be seen from this data, John combines a variety of gestures, ER-related reasoning behaviors, and ERs in his discussion. Table I 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" subcategories 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)

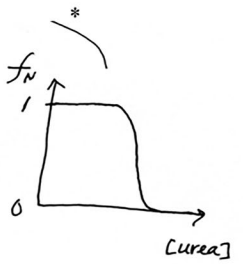
(a)



(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 3a points at N form in Figure 3a points generally at all drawn ERs*	Associates process with region^{RC}

FIG 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 d). For readability, alignments are approximate. Note that the asterisk connects the line in (c) to the 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 are indicated in superscripts at the end of the reasoning behavior.

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."

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

John combines several ERs (Figs. 4*a*–4*c*) with speech (Fig. 4*d* "Speech" column) and gestures and drawing (Fig. 4*d* "Gesture" column) to discuss methods he uses to study changes in free energy associated with protein folding. The explanation in Fig. 4*d* begins where John starts to draw Fig. 4*c*. However, we again briefly describe some of John's prior discussion to provide necessary context. 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 Fig. 5*a*). Other experts similarly used gesture to animate, as well as connect and quantify, their ERs (data not shown). John explains verbally 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. He initially represents this energy gap by vertically stacking his hands and decreasing the distance between his hands as he discusses

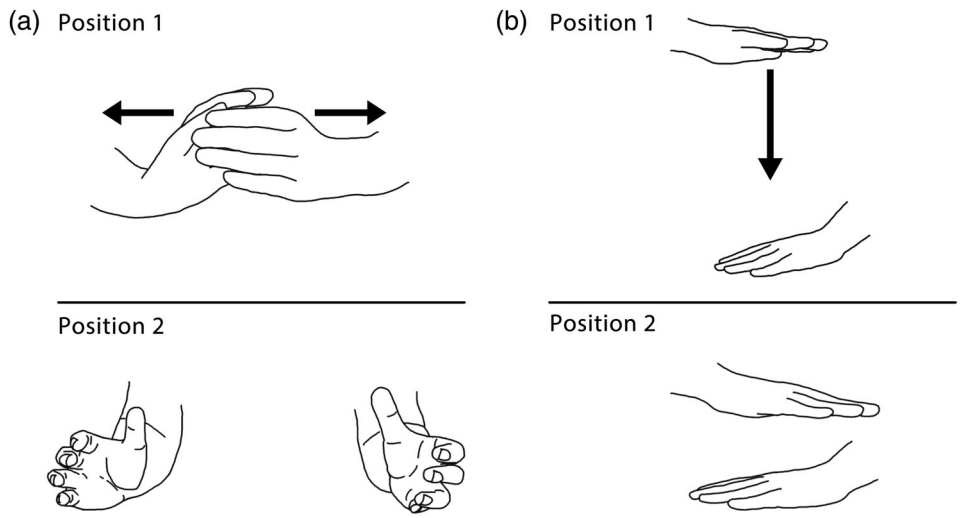


FIG 5

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).

monitoring the change in ΔG over time with changes to urea concentration (see Fig. 5*b*). As he tries to explain what "that slope" represents, John switches to using drawings, creating the axes of Fig. 4*a* before switching to create Fig. 4*b*.

In Fig. 4*b*, John identifies the relative energy levels of native (N) and cleavable (C) forms and associates the difference in energy with the symbol $\Delta G^{\circ}_{\text{C-N}}$ and an arrow. He explains how he plots $\Delta G^{\circ}_{\text{C-N}}$ data at different concentrations of urea (Fig. 4*a*) and associates the resulting slope with how much the protein unfolds, that is, the amount of structural difference between the native and cleavable forms. With Fig. 4*a*, 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 Fig. 3*a*, relating the two ERs. Later in his interview, where the excerpt in Fig. 4*d* begins, John returns to Fig. 4*b* to describe another experimental method used to characterize a protein's global stability. He explains that the globally unfolded form of a protein (U) exists at a higher energy state than the other states (see Fig. 4*b* and "Gesture" column in Fig. 4*d*), again associating the difference in energy with a symbol ($\Delta G^{\circ}_{\text{U-N}}$) and an arrow between the two states. Drawing the line on the plot in Fig. 4*c*, 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 those in Figs. 4*a*–4*c* and structural models, in order to represent the stability of the protein and regions that unfold.

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 II 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 [37, 38]. 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.

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

CPC of the textbook and expert data found similar categories of reasoning behaviors (Table I) in the textbook narratives and captions associated with ERs, as well as mentions of experimental methods or data in relation to ERs (Table II). 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 "Orders" category in Table I describes the order in which regions of proteins fold. The other textbook quotes in Table I in the "Associates a. 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 II). 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.

Conclusions

John's discussion and his use of ERs highlight 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.

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 II) [37, 38]. 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 II, 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 [35]. 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 II). 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.

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 I). 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 [26]. 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. 3*a*) to measured experimental variables (Fig. 3*b*). 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 (Figs. 4*a*–4*c*) and references the protein-folding cartoon in Fig. 3*a*. 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 [39–41].

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 [42], as discussed in the Implications for Teaching and Learning section.

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.

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 show, 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. 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.

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 [25, 26]. 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 [27]. 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 [28]. 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 III.

We wonder how the incorporation of methods in the instruction of biochemistry may affect student understanding, particularly in relation to abstract concepts like energy. In a previous study [28], we characterized how the same set of experts explained thermodynamic and kinetic concepts in different ways that aligned with their research methods. John, for example, used kinetics-based methods and considered 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 [28]. Complementing the characterizations provided in that work [28], the authors believe the data presented in this article 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 [43, 44], and could influence how students conceptualize physical phenomena in the same way linguistic choices do [43, 45]. As stated by the textbooks reviewed here, biochemical knowledge advances in parallel with the development of biochemical techniques [35, 36].

Other work [46] 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 [47, 48]. Scientists draw on sensorimotor experiences to understand abstract thermodynamic functions: for example, treating energy as a substance or a location [49–51]. 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 [44, 52, 53]. Very little research has been conducted to understand how different ERs help or hinder access to various disciplinary ways of knowing [12]. 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.

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

TABLE III *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 vs. 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]

[14, 54]. The CRM model [16] 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 IV), 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 V. We have provided an extensive list of suggestions in these

TABLE IV ***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, multicomponent 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]

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.

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 [42], and other tools that describe levels of visual literacy [24, 55, 56]. 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 Fig. 6, we provide an example of how an instructor might ask questions to help students interpret a fictitious data ER modeled on a figure from a protein-folding research study. Initial questions could be used to encourage lower-order skills like identifying states

TABLE V

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 III and IV; 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 multistep 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 vs. low entropy) or of data on plots/graphs (e.g. steep vs. shallow slopes mean…) to facilitate comparison
• For complex or multicomponent 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

or associating processes with specific mathematical terms (Table I). 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 [41].

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 [11].

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?

Interpret the representation below by answering the following questions:

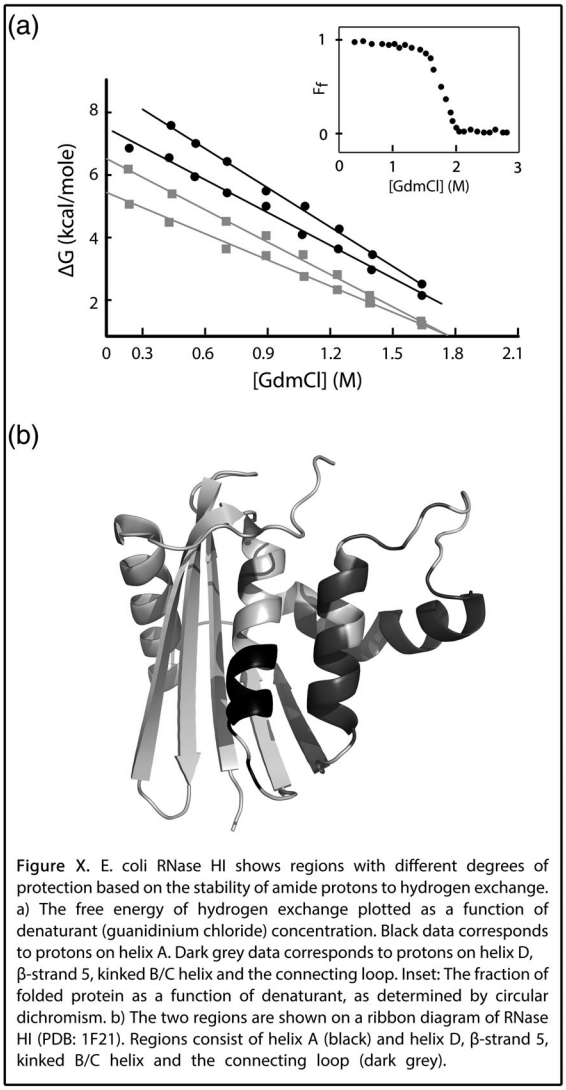


Figure X. E. coli RNase HI shows regions with different degrees of protection based on the stability of amide protons to hydrogen exchange. a) The free energy of hydrogen exchange plotted as a function of denaturant (guanidinium chloride) concentration. Black data corresponds to protons on helix A. Dark 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?

FIG 6

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 Escherichia coli RNase H [57, 58]. 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.

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REFERENCES

[1] Airey, J., Linder, C. (2009) A disciplinary discourse perspective on university science learning: Achieving fluency in a critical constellation of modes. J. Res. Sci. Teach. 46, 27–49.

[2] Amann, K., Cetina, K. K. (1988) The fixation of (visual) evidence. Hum. Stud. 11, 133–169.

[3] Woolgar, S. (1988) Time and documents in researcher interaction: Some ways of making out what is happening in experimental science. Hum. Stud. 11, 171–200.
[4] Kress, G. (2010) Multimodality: A Social Semiotic Approach to Contemporary Communication, Routledge, New York.
[5] Kress, G., Van Leeuwen, T. V. (2001) Multimodal Discourse: The Modes and Media of Contemporary Communication, Bloomsbury Academic, New York.
[6] Givry, D., Roth, W.-M. (2006) Toward a new conception of conceptions: Interplay of talk, gestures, and structures in the setting. J. Res. Sci. Teach. 43, 1086–1109.
[7] Lemke, J. L. (1998) Teaching all the Languages of Science: Words, Symbols, Images, and Actions, Conference on Science Education, Barcelona, Spain. pp. 1–13.
[8] Lemke, J. L. (2003) Mathematics in the middle: Measure, picture, gesture, sign and word. In: Anderson M., Sáenz-Ludlow A., Zellweger S., & Cifarelli V. V., Eds. Educational perspectives on mathematics as semiosis: From thinking to interpreting to knowing, Legas, Brooklyn, NY. pp. 215–234.
[9] Lemke, J. L. (2004) The literacies of science. In: Wendy Saul E., Ed. Crossing Borders in Literacy and Science Instruction: Perspectives on Theory and Practice, International Reading Association; NSTA, Newark, DE; Arlington, VA. pp. 33–47.
[10] Brewer, C., Smith, D. (2011) Vision and Change in Undergraduate Biology Education: A Call to Action, AAAS, Washington, DC.
[11] Bowen, G. M., Roth, W.-M., McGinn, M. K. (1999) Interpretations of graphs by university biology students and practicing scientists: Toward a social practice view of scientific representation practices. J. Res. Sci. Teach. 36, 1020–1043.
[12] Offerdahl, E. G., Arneson, J. B., Byrne, N. (2017) Lighten the load: scaffolding visual literacy in biochemistry and molecular biology. CBE Life Sci. Educ. 16, es1.
[13] Anderson, T. R., Schönborn, K. J. (2008) Bridging the educational research-teaching practice gap. Conceptual understanding, part 1: The multifaceted nature of expert knowledge. Biochem. Mol. Biol. Educ. 36, 309–315.
[14] Schönborn, K. J., Anderson, T. R. (2006) The importance of visual literacy in the education of biochemists. Biochem. Mol. Biol. Educ. 34, 94–102.
[15] Schönborn, K. J., Anderson, T. R. (2008) Bridging the educational research-teaching practice gap: Conceptual understanding, part II: assessing and developing student knowledge. Biochem. Mol. Biol. Educ. 36, 372–379.
[16] Schönborn, K. J., Anderson, T. R. (2009) A model of factors determining students' ability to interpret external representations in biochemistry. Int. J. Sci. Educ. 31, 193–232.
[17] Anderson, T. R., Schönborn, K. J., du Plessis, L., Gupthar, A. S., Hull, T. L. (2013) Identifying and developing students' ability to reason with concepts and representations in biology. In: Treagust D. F., Tsui C.-Y., Eds. Multiple Representations in Biological Education, Springer, Dordrecht, the Netherlands. pp. 19–38.
[18] Mnguni, L. E., Schönborn, K. J., Anderson, T. R. (2009) In: Hammann M., Waarlo A. J., Boersma K. T., Eds. The Nature of Research in Biological Education, Utrecht University, Utrecht, the Netherlands. pp. 185–197.
[19] Kendon, A. (2004) Gesture: Visible action as utterance, Cambridge University Press, Cambridge, MA.
[20] Becvar, L. A., Hollan, J., Hutchins, E. (2005) Hands as molecules: Representational gestures used for developing theory in a scientific laboratory. Semiotica 2005, 89–112.
[21] Goodwin, C. (2000) Action and embodiment within situated human interaction. J. Pragmat. 32, 1489–1522.
[22] Ochs, E., Gonzales, P., Jacoby, S. (1996) "When I come down I'm in the domain state": Grammar and graphic representation in the interpretive activity of physicists. In: Ochs E., Schegloff E., Thompson S., Eds. Interaction and Grammar, Cambridge University Press, Cambridge, UK. pp. 328–369.
[23] Roth, W.-M., Welzel, M. (2001) From activity to gestures and scientific language. J. Res. Sci. Teach. 38, 103–136.
[24] Arneson, J. B., Offerdahl, E. G. (2018) Visual literacy in bloom: Using bloom's taxonomy to support visual learning skills. CBE Life Sci. Educ. 17, 1–8.
[25] Krajcik, J. S., Sutherland, L. M. (2010) Supporting students in developing literacy in science. Science 328, 456–459.
[26] Roth, W.-M., Bowen, G. M., McGinn, M. K. (1999) Differences in graph-related practices between high school biology textbooks and scientific ecology journals. J. Res. Sci. Teach. 36, 977–1019.
[27] Rybarczyk, B. (2011) Visual literacy in biology: A comparison of visual representations in textbooks and journal articles. J. Coll. Sci. Teach. 41, 106–114.
[28] 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 Sci. Educ. 17, 1–15.
[29] Trujillo, C. M., Anderson, T. R., Pelaez, N. J. (2015) A model of how different biology experts explain molecular and cellular mechanisms. CBE-Life Sci. Educ. 14, ar20.
[30] Denzin, N. K., Lincoln, Y. S., Eds (2018) The SAGE Handbook of Qualitative Research, 5th ed., Sage Publications, Inc., Thousand Oaks, CA.
[31] Corbin, J., Strauss, A. (1990) Grounded theory research: Procedures, canons and evaluative criteria. Qual. Soc. 13, 3–12.
[32] Creswell, J. W., Creswell, J. D. (2017) Research Design: Qualitative, Quantitative, and Mixed Methods Approaches, 5th ed., Sage Publications, Inc., London, UK.
[33] Pratt, C. W., Cornely, K. (2014) Essential Biochemistry, 3rd ed., John Wiley & Sons, Inc., Hoboken, NJ.
[34] Voet, D., Voet, J. G. (2011) Biochemistry, 4th ed., John Wiley & Sons, Inc., Hoboken, NJ.
[35] Garrett, R. H., Grisham, C. M. (2013) Biochemistry, 5th ed., Brooks/Cole, Cengage Learning, Belmont, CA.
[36] Nelson, D. L., Cox, M. M. (2013) Lehninger Principles of Biochemistry, 6th ed., W.H. Freeman & Company, New York.
[37] Dasgupta, A. P., Anderson, T. R., Pelaez, N. (2014) Development and validation of a rubric for diagnosing students' experimental design knowledge and difficulties. CBE Life Sci. Educ. 13, 265–284.
[38] N. Pelaez, T. Anderson, S. M. Gardner, Y. Yin, J. K. Abraham, E. L. Bartlett, et al. (2017) The Basic Competencies of Biological Experimentation: Concept-Skill Statements. PIBERG Instructional Innovation Materials. Paper 4. Purdue ePubs, West Lafayette Indiana, USA, http://docs.lib.purdue.edu/pibergiim/4
[39] Bodemer, D., Ploetzner, R., Bruchmuller, K., Häcker, S. (2005) Supporting learning with interactive multimedia through active integration of representations. Instr. Sci. 33, 73–95.
[40] Kozma, R. B. (2003) The material features of multiple representations and their cognitive and social affordances for science understanding. Learn. Instr. 13, 205–226.
[41] Ainsworth, S. (2008) In: Gilbert J. K., Reiner M., Nakhleh M. B., Eds. Visualization: Theory and Practice in Science Education, Springer, Dordrecht, the Netherlands. pp. 191–208.
[42] Anderson, L. W., Krathwol, D. R., Airasian, P. W., Cruikshank, K. A., Mayer, R. E., Pintrich, P. R., et al. (2001) A Taxonomy for Learning, Teaching, and Assessing: A Revision of Bloom's Taxonomy of Educational Objectives, Abridged Edition, Longman, New York.
[43] Brookes, D. T., Etkina, E. (2015) The importance of language in students' reasoning about heat in thermodynamic processes. Int. J. Sci. Educ. 37, 759–779.
[44] Jeppsson, F., Haglund, J., Amin, T. G., Strömdahl, H. (2013) Exploring the use of conceptual metaphors in solving problems on entropy. J. Learn. Sci. 22, 70–120.
[45] Kaper, W. H., Goedhart, M. J. (2002) 'Forms of energy', an intermediary language on the road to thermodynamics? Part II. Int. J. Sci. Educ. 24, 119–137.
[46] Amin, T. G. (2009) Conceptual metaphor meets conceptual change. Hum. Dev. 52, 165–197.
[47] Lakoff, G., Johnson, M. (1980) Conceptual metaphor in everyday language. J. Philos. 77, 453–486.
[48] Lakoff, G., Johnson, M. (1999) Philosophy in the Flesh, Basic Books, New York.

[49] Amin, T. G., Jeppsson, F., Haglund, J., Strömdahl, H. (2012) Arrow of time: Metaphorical construals of entropy and the second law of thermodynamics. Sci. Educ. 96, 818–848.
[50] Close, H. G., Scherr, R. E. (2015) Enacting conceptual metaphor through blending: Learning activities embodying the substance metaphor for energy. Int. J. Sci. Educ. 37, 839–866.
[51] Dreyfus, B. W., Gupta, A., Redish, E. F. (2015) Applying conceptual blending to model coordinated use of multiple ontological metaphors. Int. J. Sci. Educ. 37, 812–838.
[52] Brookes, D. T., Etkina, E. (2007) Using conceptual metaphor and functional grammar to explore how language used in physics affects student learning. Phys. Rev. Spec. Top. - Phys. Educ. Res. 3, 1–16.
[53] Brookes, D. T., Etkina, E. (2009) Force, ontology, and language. Phys. Rev. Spec. Top. - Phys. Educ. Res. 5, 1–13.
[54] Schönborn, K. J., Anderson, T. R., Grayson, D. J. (2002) Student difficulties with the interpretation of a textbook diagram of immunoglobulin G (IgG). Biochem. Mol. Biol. Educ. 30, 93–97.
[55] Trumbo, J. (1999) Visual literacy and science communication. Sci. Commun. 20, 409–425.
[56] Mnguni, L. E., Schönborn, K., Anderson, T. R. (2016) Assessment of visualization skills in biochemistry students. S. Afr. J. Sci. 112, 1–8.
[57] Chamberlain, A. K., Handel, T. M., Marqusee, S. (1996) Detection of rare partially folded molecules in equilibrium with the native conformation of RNaseH. Nat. Struct. Biol. 3, 782–787.
[58] Hu, W., Walters, B. T., Kan, Z.-Y., Mayne, L., Rosen, L. E., Marqusee, S., Englander, S. W. (2013) Stepwise protein folding at near amino acid resolution by hydrogen exchange and mass spectrometry. Proc. Natl. Acad. Sci. 110, 7684–7689.