Article

Students' Understanding of External Representations of the Potassium Ion Channel Protein Part II: Structure–Function Relationships and Fragmented Knowledge*

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Research that has focused on external representations in biochemistry has uncovered student difficulties in comprehending and interpreting external representations. This study focuses on students' understanding of three external representations (ribbon diagram, wireframe, and hydrophobic/hydrophilic) of the potassium ion channel protein. Analysis of the interview data demonstrates that students were able to use the ribbon structures and polarity of the cell membrane to help support claims about the protein's orientation and interactions within the cell membrane. Students expressed fragmented understandings of the interactions between the potassium ion and the aqueous solution outside/inside of the cell membrane. Suggestions for instruction are to probe student understanding to help students activate prior knowledge and to help them build a more connected set of concepts pertaining to protein structure and function.

Keywords: Visual literacy, tertiary education, scholarship of teaching and learning.

Biochemistry students must interpret and use external representations to understand large, complex biochemical macromolecules. Whereas our first article in this two-part series focused on how students interpret three types of external representations of the potassium ion channel, this article describes how students use external representations to support their claims about channel's structure and function. Schönborn and Anderson [1] have identified factors that affect students' ability to interpret and use external representations in biochemistry, including:

- Ability to make sense of and read the external representation
- Ability to select and retrieve conceptual knowledge of relevance to the external representation
- Understanding of the concepts of relevance to the external representation

Schönborn and Anderson [1–3] state that a discussion of the nature of external representations needs to be part of all biochemistry curricula because of the diversity of types of external representations that students need to be familiar with and because of the factors associated with successful interpretation of external representations in biochemistry. Petre and Green [4] agree with this notion writing, "what a reader sees is largely a matter of what he or she has learned to look for [p. 42, 4]."

Schönborn and Anderson wrote, "only limited empirical research exists on students' interpretation and visualization of ERs (external representations) in our science [p. 347, 2]." Our studies aim to expand the evolving empirical research base in biochemistry education research. In Part II of our study of the student understanding of representations of the potassium ion channel, we focus on protein structure and function. Here, we show how students use external representations (ribbon, vines, and hydrophobic/hydrophilic) to make claims about protein orientation, function, and the environment of the potassium ion. The research question guiding the study is, how do students use external representations to consider the potassium ion channel protein's structure and function?

METHODS

A qualitative research approach [5] was used to discover how students understand three external representations of the potassium ion channel and create meaning. The methods used in the research originate from the social sciences and emphasize building a rich, thick description of participant understanding through interviews, observations, and written artifacts. Qualitative research is a valuable approach when one wishes to understand a particular phenomenon in detail, such as how students understand external representations and use them to make claims about structure and function.

Sampling and Participants

Maximum variation sampling methods were used to capture the central themes that cut across study participants from a variety of biochemistry courses in the chemistry and biochemis-

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TABLE I
Course name and abbreviation, the number of participants, and a description of the course

Course name (abbreviation)	Number of participants	Description
Chemistry 333 (CHM 333)	2	"Principles of biochemistry" is a three-credit course offered by the College of Science for health science majors. The material covered concentrates on the structure and function of biologically important molecules.
Chemistry 533 (CHM 533)	4	"Introductory Biochemistry" is a three-credit course offered by the College of Science for students majoring in chemistry.
Biochemistry 100 (BCHM100)	4	"Introduction to Biochemistry" is a two-credit course offered by the College of Agriculture providing a survey of biochemistry.
Biochemistry 307 (BCHM 307)	9	"Introduction to Biochemistry" is a three-credit course offered by the College of Agriculture designed for life science majors. The course focuses on an introduction to the chemistry, function, and metabolism of compounds found in living organisms.
History 151 (HIST 151)	2	"American history to 1877" is a three-credit undergraduate-level course offered by the College of Liberal Arts.

try departments [5]. The 21 participating students were from a large Midwestern research university and were enrolled in four different biochemistry courses or one history course, which are described in Table I.

Interview Structure

At the beginning of each interview, participants were given a brief tutorial about FirstGlance in Jmol and were allowed to use the program on another protein to familiarize themselves [6]. Every protein representation used in the interviews was shown on a computer screen and FirstGlance in Jmol allowed the viewer to spin or move the molecule in three-dimensional space. Each participant was asked several warm up questions to help him/her feel comfortable talking with the interviewer, and to turn their attention to proteins. Students were able to use a LiveScribe [7] pen to make drawings. This pen allows researchers to synchronize the students' drawings with what they say while making them.

The interview protocol questions discussed in Part I of this series included asking participants about the features of the protein they could interpret in the ribbon, vines, and hydrophobic/hydrophilic representations. Additionally, the participants were asked about the limitations of the ribbon, vines, and hydrophobic/hydrophilic representations. Before moving on to protocol questions regarding the proteins function (Part II), the participants were asked if they could name the protein. If not, then the name was given to them so they could use that information to respond to questions about the protein's function. During this part of the interview participants could use and move between any representation to help them respond to questions. Students were asked to describe how the protein was oriented, how the potassium ion moves through the channel, and the interactions the potassium ion has with the environment outside or inside the cell. Follow-up probes were used to encourage students to elaborate on their claims, data (evidence from the representations or concepts used), and warrants (which explain why the data supports the claim).

Data Analysis

All interviews were transcribed and grounded theory was used to guide the approach to analysis [8]. This approach emphasizes analyzing what participants say (or in this study draw) to discern what they mean and understand. Thus, what emerges from the analysis of the transcripts is firmly grounded in the data.

The transcripts were coded to identify ways in which the students used external representations to consider the potassium ion channel protein's structure and function. AtlasTi [9], a qualitative data analysis software package, was used to help sort, store, and retrieve codes during analysis. The codes were sorted into categories, which were then examined and compared with determine their relationships to each other. The integration and interrelationships of the categories formed the foundation upon which the assertions presented in the findings are based.

The interrater reliability study described in Part I also carried forward to Part II of the study. Two raters independently coded two transcripts by using the codes and definitions provided by one of the authors (MH). Each rater received directions about the coding process and how to record their responses. When the raters met as a team the percent agreement of codes was 72%, which was above the acceptable limit of 70% [10]. After discussion of our disagreements, the transcripts were individually recoded and the percent agreement increased to 89%.

RESULTS AND DISCUSSION

For the sake of brevity, we focus our discussion on two areas: 1) how the students used the ribbon and hydrophobic/hydrophilic representations to make claims about the orientation of the protein in the cell membrane and 2) the fragmented knowledge structure of the students, which emerged when they described the interactions of the potassium ion outside the cell membrane. Students could use any representation in this portion of the interview to guide and shape their claims and responses. Additionally, some students created their own drawings to support their explanations. The findings are presented as assertions followed by interpretation and discussion of exemplar data quotes.

Assertion One: Students Used the Alpha Helices in the Ribbon Representation to Support Their Claims About the Vertical Orientation of the Protein in the Cell Membrane

Students used the ribbon representation to consider how the protein would be oriented three dimensionally in the cell membrane. The channel running through the center of the protein was made explicit in the ribbon representation and that helped participants determine the vertical orientation of the protein in the membrane. The channel, in combination with the potassium ions dis-

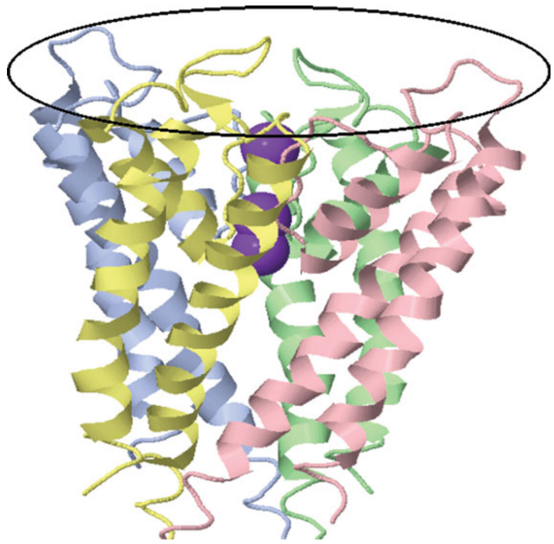

FIG. 1. The potassium ion channel protein. Circled are the "nutrient grabbers" that Abe pointed to.

played inside, was used to make claims that the protein was perpendicular to the membrane as exemplified by Ken and Amber.

Ken-CHM 533: Ok um there seems to be a channel here so maybe ah those potassiums are being pumped either into or out of the cell so it would probably be either straight down like this with the channel . . . the membrane would be perpendicular to this here and then the potassiums would be coming in.

Amber-BCHM 307: Ah because it looks like it could almost form a channel maybe for potassium to go through um and if it is like this the alpha helices are sort of um vertical so it would be able to span the membrane.

While some participants specifically spoke about the channel of the protein, others such as Bethany and Christine referred to the "hole" or "gap" that helped them determine, which way the protein would be oriented.

Bethany-BHCM 100: Because if you look at it this way you can see that they go all the way through . . . like and there is a hole . . . like, if you look at it, there's a hole like right in the middle of it . . . like, if you look at it this way, it does not make much sense to go through like that. Um, and the fact that those, um atoms are lined up like that . . . they are going straight . . . straight through.

Christine-CHM 333: Well it looks like um there is a gap in this protein that the potassium can go through and it kind of looks like what they're doing . . . and since um this end seems more open . . . it might not be letting them in through the other ends.

Abe used the notion that the pieces of random coil, which he called "nutrient grabbers," would guide the orientation of the protein as shown in Fig. 1.

Abe-BCHM 307: Ok ah the top right here, would able to get nutrients from the outside and transport it down into the actual cell or wherever the membrane is, so it would be able to like, transfer any kind of nutrients in between. I don't kn . . . I don't know exactly what these parts are, but to me, they seem like some sort of nutrient grabber.

Students used the protein secondary structure, the channel, hole, or gap, and bits of random coil to help make claims about the vertical orientation of the protein in the cell membrane. Together these data support a central theme in protein biochemistry, which is that structure and function are related. In order for this protein to transport ions across the cell membrane, it must be placed perpendicularly to it, which the students were able to conclude based upon the structure and knowing the protein's name.

Assertion Two: Students Use the Polarity of the Hydrophobic/Hydrophilic Representation to Discuss the Protein's Interaction with the Cell Membrane

Students were able to use the polarity indicated in the hydrophobic/hydrophilic representation to discuss how the protein interacted with the lipid bilayer that composes the membrane. The major affordance of this representation is that it highlights the polar and nonpolar regions of a protein. Students noticed that the protein had hydrophilic regions at the top and bottom that sandwiched a hydrophobic region in the middle. Ken, Kate, and Amy recalled that the lipid bilayer (membrane) contained similar polarity patterns and could provide support for considering the orientation of the protein.

Ken-CHM 533: Yeah you want um . . . same reason I said before you'd want hydrophobic on the sides to keep it stuck in the membrane and you'd want hydrophilic at the top and the bottom because that's where the polar stuff is.

Note that Ken anchors the location of the protein in the membrane with the hydrophobic region. He refers to it as "keep[ing] it stuck in the membrane." Thus, he uses the concept of molecular interactions in his reasoning.

Kate recalls the names of the molecules composing the membrane, "lipids", and orients the protein to match up with the "phospho-groups" at the top and bottom of the membrane as well as the hydrophobic region in the "center" of the membrane where as she recalls "all of the lipid tails are" located.

Kate-CHM 533: Um it actually from this looks like they are on the outside . . . well middle outside I would say, which is I guess another reason why I would again orient it in the same fashion because you would have your phospho groups up here where all of your purple polar groups ah like on the outside of the cell and I guess on the bottom as well . . . um and then you have all of your hydrophobic groups here in the center where I would say all of the lipid tails are.

Amy again uses the features of the membrane to orient the protein, which "spans" the membrane.

Amy-BCHM 307: Yeah because if this was like a tube that was going through if you have your . . . your polar, which are your hydrophilic on both ends then they would sit on the very outside um of this like membrane tube and then everything in the middle has to be hydrophobic. This is a membrane protein so it needs um hydrophobic ah parts that span the membrane and then on the inside since it is dealing with an ion, it needs to have something polar.

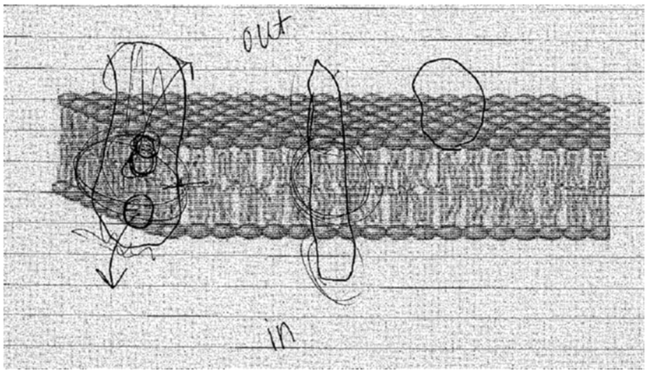

FIG. 2. Abbie's drawing of a protein spanning the lipid bilayer (membrane).

Abbie created a drawing shown in Fig. 2 to explain her understanding of how the protein would interact with the membrane. The membrane had been provided earlier in the interview and in the central portion of her drawing, Abbie drew three circles on it. She then described the circles by stating:

Abbie-BCHM 307: Because I feel like more of the polar is right at the top and there's a lot on the bottom too ... the top and the bottom ... and then it looks like most of the hydrophobic is in the middle so like my one drawing like the ... so that it would fit in the ... the membrane ... it would have the two polars out in the aqueous and then the hydrophobic in the middle of the membrane ... um yeah um ... usually polar are more on the surface of a protein and the hydrophobic are more in the middle of a protein ... basically because of their reactions ... the hydrophobic go together in the middle and then the polar on the outside to react so.

These students used their knowledge of the cell membrane and molecular interactions with their interpretation of the hydrophobic/hydrophilic representation to make claims about the orientation of the protein.

Assertion Three: Students Display Highly Fragmented Knowledge Structures When Describing the Interactions of the Potassium Ion in the Extracellular Environment

Students described a wide array of understandings about the interactions of the potassium ion in environment outside of the cell that represent a highly fragmented knowledge structure. The notion of a fragmented knowledge structure has its roots in physics education research with diSessa [11] and Minstrell [12]. The basic notion is that small grain sized pieces of information or concepts are acquired from everyday experience (p-prims in diSessa's work) or instruction (see Minstrell). Recently Yayon *et al*. noted that the notion of "knowledge in pieces" is appropriate to explore in chemistry (as Taber and Garcia-Franco suggested [13]) and coined the term "elements of knowledge," which are considered to be small grain sized bits of canonical knowledge [14].

Whether one uses "facets of knowledge" [12], "knowledge in pieces" [13], or "elements of knowledge" [14] we believe that we have strong evidence of the fragmented nature of knowledge students possess in biochemistry. Our analysis of student understanding of the interaction of the potassium ion outside the cell membrane has led us to this conclusion. Students possess incomplete and disconnected understandings such that they frequently fail to include interactions with the aqueous solvent as they access and use the concepts that seem appropriate to them. These conceptual bits and pieces and how students choose to apply them are representative of a fragmented knowledge structure.

No interactions, free floating, or didaskalogenic confusion (from the Greek "didaskalos" meaning teacher and "gennan" meaning to produce)—When asked about the nature of the interactions between the potassium ion and the environment inside or outside the cell, some students represented by Brad's quote below, were not able to provide specific details about those interactions.

Brad-BCHM 100: They probably could be interacting with something but I do not know what would be out there.

Karla and Cara referred to "free(ly) floating" potassium ions neglecting the role of water. Cara's response suggests that the potassium ion would be interacting with "something" to promote "work to be done."

Karla-CHM 533: They are probably serving some sort of purpose but there's probably a number of them that are just freely floating as well.

Cara-CHM 333: Um it might be ... I think also depending on like where it's at in the body or where that's using like you may have it free floating but it also might be attached to something like pro ... or promoting the work to be done.

Christine specifically recalled a picture from her biology book that helped her make claims about how the potassium ions interacted with the environment inside the cell.

Christine-CHM 333: Um you know I'm remembering pictures from my biology book and I just remember potassium ions having a little arrow and going into the cell ... so I'm going to say they're just free.

Christine's quote is an example of what Cooper and Klymkowsky have termed "didaskalogenic confusion," which is to say that it is instruction induced [15]. Amber showed signs of the same type of confusion.

Amber-BCHM 100: The only thing I remember from our intro class is that they're just ions floating around.

We found that over one fourth of our participants in the study could have their understanding described in this broad category. Probably more important to notice is that none of these students have included the presence of the aqueous solvent and its' interactions with the potassium ion. It is also difficult to discern in what way, if at all, these students consider the interactions of potassium at a particulate level as other students were able to demonstrate.

Ion-Pairs—Research by Kelly and Jones firmly established that some students believe that soluble ionic compounds form ion pairs in aqueous solution [16, 17]. In describing how the potassium ion interacts with the extracellular environment, Hannah used the idea that an ion pair exists. Note that she does not include water, either as a solvent or on the molecular level, in her explanation.

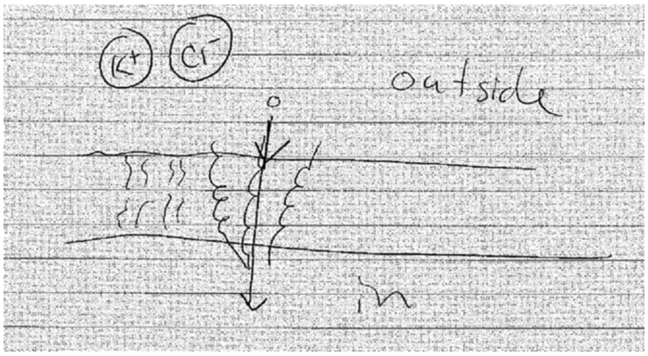

FIG. 3. Hannah's drawing of the interaction between a potassium ion and a chloride ion outside the cell membrane.

Hannah-HIST 151: It might be like interacting with like ionic-ly with something negatively charged or something like that . . . but I don't know.

Interviewer: Could you draw how the potassium ion would interact with something negative?

Hannah-HIST 151: Yeah (begins drawing shown in Fig. 3) so like this would be K plus and then um this . . . you could have something minus like I don't know like Cl minus . . . and then they just stick to each other because plus and minus attract each other.

Hannah reasoned that the potassium ion is positively charged and would interact electrostatically with a negative particle such as the chloride ion. In Fig. 3, Hannah draws them side-by-side in an ion pair with no water molecules present, which is reminiscent of the students in Kelly and Jones' research [16, 17]. Hannah never included the role of water in her verbal description or drawing.

Acid-Base Chemistry—Christine and Abe had notions of the potassium ion interacting with ions depending upon the acidity or basicity of the environment. Here, Christine conceives of KOH being present and she stated this prior to her comments about remembering pictures from her biology book.

Christine-CHM 333: Um it would probably . . . it depends . . . I feel like it depends on the concentration of the extra. . .extracellular fluid because um well if it were originally . . . if it were more in an acidic environment then . . . or if it were more in a basic environment, say if it was originally bound to KOH then it would fully dissociate and just be K plus while there were like OH minuses in solution.

Note that like Hannah, Christine does not include the role of water and in fact never uses the word "water" or "aqueous" in her description of the extracellular environment. She has bits and pieces of the notions we might believe originate from considering the ions to be in an aqueous solution (the words fluid, dissociate, and solution), but she never actually uses the notion of water in her explanation.

Abe also uses potassium interacting with hydroxide ion in his explanation.

Abe-BCHM 307: Um I'm . . . some may be but I think there's also some that are bonded that would be taken off by things happening on the outside of the cell.

Interviewer: Do you know what they would be bonded to . . . any ideas?

Abe-BCHM 307: Um maybe like a hydroxide . . . like potassium hydroxide . . . so KOH . . . or.

Interviewer: KOH and what part of that would be interacting with the . . . the potassium ion?

Abe-BCHM 307: What do you mean . . . the K would be?

Interviewer: Oh I get it . . . I'm sorry

Abe-BCHM 307: On the OH (drawing KOH to clarify what he means)

Here Abe again draws something like an ion-pair and conceives of a bonded KOH units existing outside the cell. Note again that water is not mentioned and does not play a role (Fig. 4).

Water Included in Interactions—Ken, Kyle, and Kate all provided explanations including water molecules interacting with the potassium ion in the environment outside of the cell. They also provided drawings that describe those interactions in Figs. 5–7.

Ken's notion of how the potassium ion interacts with water was the most complete of all the interviews. To further describe his explanation of the interaction he spontaneously began to draw on the cell membrane had been provided in the warm-up. In Fig. 5, he explicitly draws the positive potassium ion interacting with the partial negative charge on the oxygen end of a water molecule using a lowercase delta and negative sign, which is a conventional notation used by chemists and biochemists.

Ken-CHM 533: Um yes there's not just sitting there they're you know orienting the waters around them the . . . the oxygens face the potassium . . . would you like me to draw it?

Interviewer: Would you?

Ken-CHM 533: Yeah so um (begins drawing Fig. 5).

Interviewer: Yeah anywhere is perfect.

Ken-CHM 533: You know just regular water you've got . . . they could be facing pretty much any which direction . . . um but when you put the potassium in there it orients them because the water is . . . the oxygen takes more of the electricity so it . . . its partially negative and the potassium is positive so um I think it interacts with six and all of those waters are . . . and then one on top and one on bottom (he's drawing) and then there's you know even

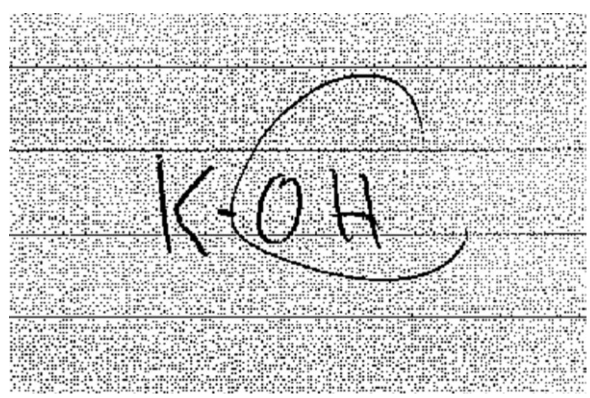

FIG. 4. Abe's drawing of the interaction between a potassium ion and a hydroxide ion.

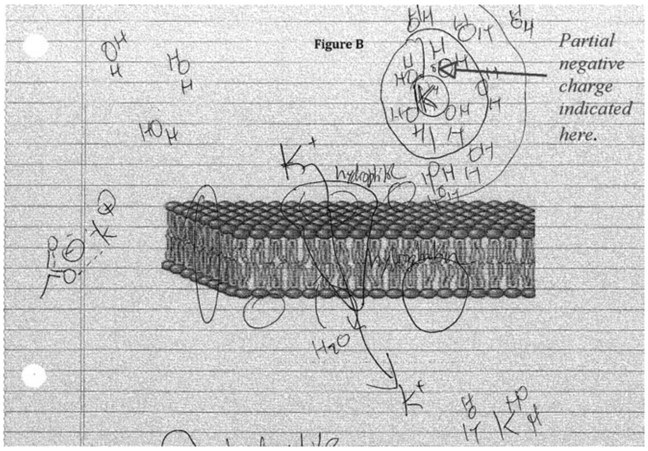

FIG. 5. Ken's drawing of the interactions between a potassium ion and water molecules. He explicitly draws water as a dipole with the negative region interacting with the potassium ion. He also draws a second layer of the hydration shell and discusses the dynamic nature of the shell.

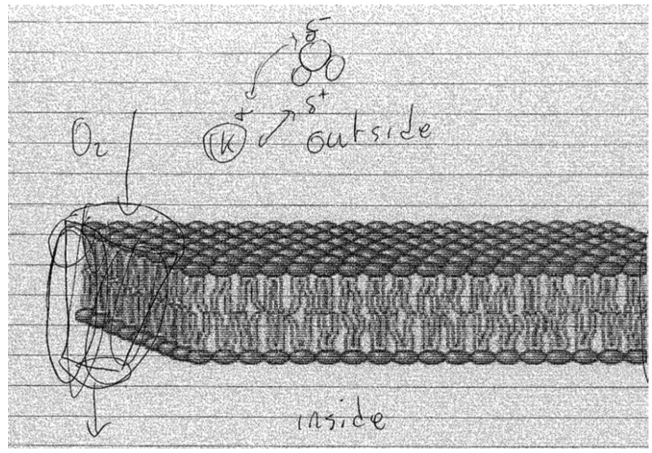

FIG. 6. Kate's drawing of the interactions between a potassium ion and a water molecule. As in Ken's drawing Kate has partial positive and negative charges identified on the water molecule drawn as circles, one larger for the oxygen atom and two smaller for the hydrogen atoms, at the top center of the figure.

more now there's a positive from the hydrogens so you have more waters come in and um ... or I don't need to draw the whole thing but ... so you have the potassium ... you have the primary coordination shell here and then you have a secondary, which has even more waters and probably even a tertiary though these waters are leaving and coming ... new ones coming in all the time ... um so you know just by sitting in water potassium is very you know ordered and it's you know got a lot of waters around it stabilizing it as opposed to just you know sitting in water facing any which way which it wouldn't be as stable it doesn't do that (Fig. 5).

In his drawing, he shows a great degree of sophistication and integration of concepts. He draws the hydration shell with the water molecules oriented properly in the first and second layer. He discussed the dynamic nature of the hydration shell and used stability arguments.

Kate also drew a diagram (see Fig. 6) to support her description of how the potassium ion interacted with a water molecule outside of the cell.

Kate-CHM 533: Yeah so it would be oh sorry I guess I should say water would be the solvent

Interviewer: Ok

Kate-CHM 533: For this

Interviewer: Ok so um does potassium interact with ... with that in any way?

Kate-CHM 533: Um I mean it could there's not going to be a solvent bond obviously but if you have this positive potassium running around you um could have some kind of water over here with his little groups in his partial ... I'm going to do partial negative charge up here and they might kind of notice each other for a little bit (draws one way arrow) or if you have this partial positive over here they might be like I don't like you (draws double ended arrow) ... I don't know it's just various attractions between things (Fig. 6).

Notice that she anthropomorphizes the water molecule ("his little groups" referring to the water molecule) rather than stabilization (lower energy) arguments as Ken did.

Lastly, while Kyle verbally described the interaction between the potassium ion and water at a particulate level, he was not able to show explicitly those interactions in his drawing shown in Fig. 7.

Kyle-CHM 533: ... if this is the outside of the cell then there's presumably some sort of water solution or else I think the cell would like break and so on and so forth ... um so this is probably a water or some other polar solvent.

Interviewer: Do you think those are interacting with the potassium?

Kyle-CHM 533: Yeah I think the potassium is probably dissolved in this ... some sort of solution and those potassiums are of course interacting with the um oxygens and then I think that potassiums are also interacting with the polar end of the phospholipid (Fig. 7).

Kyle demonstrates the least sophistication of the three in his description, but he still has the notion that the potassium ion is interacting with a solvent such as water. He also states that the potassium is interacting with the oxygen atoms on the water molecule.

Ken, Kate, and Kyle were all chemistry majors enrolled in CHM 533 at the time of the study. We speculate that having taken upper division courses in chemistry, such as inorganic chemistry, which Ken mentioned exposed them to the concept of a hydration shell and helped to

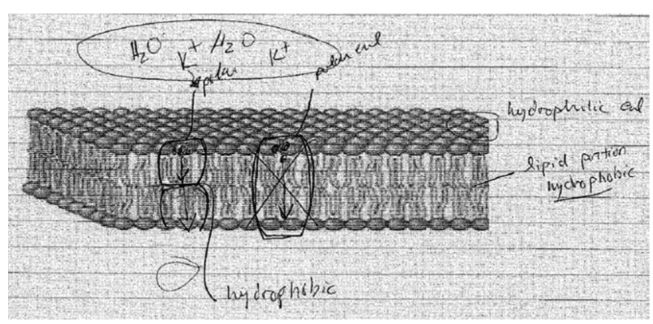

FIG. 7. Kyle's drawing of the interaction between a potassium ion and surrounding water molecules.

develop their particulate level concepts of how ions interact with an aqueous solvent.

CONCLUSIONS

In our research we seek to build the empirical knowledge base in biochemistry education research by investigating student understanding of protein representations. Key to this research is the ability of the students to interpret visualization and to use them with appropriate conceptual knowledge to make claims supported by data.

This portion of our two-part study focused on how students use representations and prior knowledge to consider the potassium ion channel's structure and function. We found that students used the alpha helices in the ribbon representation to support claims about the orientation of the protein in the cell membrane. The fact that the protein had a tube, gap, or channel running through the center helped determine its placement within the cell membrane. Students were able to use the protein's structure and name to think about its function (transporting potassium ions), and that understanding was used to determine that the protein would be placed perpendicular to the cell membrane. The polarity of the hydrophobic/hydrophilic representation was used by students to contemplate the protein's interaction with the cell membrane. This representation provided information about the polar and nonpolar regions of the protein, and participants were able to interpret and use that information, along with their understanding of the structure of the cell membrane, to claim 1) that the polar parts of the protein would interact with the polar parts of the cell membrane and 2) the that the nonpolar parts of the protein would interact with the nonpolar parts of the cell membrane. Thus, they used concepts associated with intermolecular forces.

Nearly all students demonstrated a fragmented understanding of the interactions between the potassium ion and aqueous environment outside and inside of the cell membrane. This finding supports previous research by Kelly and Jones that highlighted the difficulty students have in generating particulate level explanations of the interactions of ions in water [16, 17]. As only three participants were able to use water molecules in their descriptions of the interactions between the potassium ion and aqueous environment, our data indicate that students neglected the role of water. Of the three participants who could correctly describe the interactions between the potassium ion and water, two of them also provided explicit drawings of those interactions.

We believe this research has significant implications for future studies in biochemistry. First, due to the complexity of the field the area is fertile for studies that emphasize the transfer of knowledge. Researchers in physics education have studied transfer for quite some time, but biochemistry and chemistry education research have largely left this area untouched. Second, again due to the complexity of the field, the notion of "knowledge in pieces" should be quite useful. As Schönborn and Anderson [1–3] have previously noted students must make use of appropriate conceptual knowledge to interpret and use representations. Whether those concepts are isolated bits and bobs, or more of a connected meaningful whole is an interesting area of inquiry.

In terms of improving student understanding of representations our findings, support recommendations from Schönborn and Anderson [1–3] about helping students become visually literate. Frighteningly, it also demonstrates that we have much to do in order to optimize those abilities.

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