Critical Features of Visualizations of Transport through the Cell Membrane: An Empirical Study of Upper Secondary and Tertiary Students' Meaning-Making of a Still Image and an Animation

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CRITICAL FEATURES OF VISUALIZATIONS OF TRANSPORT THROUGH THE CELL MEMBRANE—AN EMPIRICAL STUDY OF UPPER SECONDARY AND TERTIARY STUDENTS' MEANING-MAKING OF A STILL IMAGE AND AN ANIMATION

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ABSTRACT. Images, diagrams, and other forms of visualization are playing increasingly important roles in molecular life science teaching and research, both for conveying information and as conceptual tools, transforming the way we think about the events and processes the subject covers. This study examines how upper secondary and tertiary students interpret visualizations of transport through the cell membrane in the form of a still image and an animation. Twenty upper secondary and five tertiary students were interviewed. In addition, 31 university students participated in a group discussion and answered a questionnaire regarding the animation. A model, based on variation theory, was then tested as a tool for distinguishing between what is expected to be learned, what is present in the visualizations, and what is actually learned by the students. Three critical features of the ability to visualize biomolecular processes were identified from the students' interpretations of the animation: the complexity of biomolecular processes, the dynamic and random nature of biomolecular interactions, and extrapolation between 2D and 3D. The results of this study support the use of multiple representations to achieve different learning goals.

KEY WORDS: life science education, multimodal learning, phenomenography, variation theory, visualization, water transport

INTRODUCTION

In the seventeenth century, Amos Comenius maintained that observations of the real world and pictures could make valuable contributions to education, and accordingly in 1658, he published a textbook for children, *Orbis Sensualium Pictus* (The Visible World in Pictures). However, the use of visual representations in teaching has varied substantially over time. Today, we are surrounded by a plethora of visual information. Some authors, e.g., Kress (2003), have argued that a fundamental shift from a text-based to a more image-based society is currently taking place.

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According to these authors, this shift will also change our conception of literacy.

The Importance of Visualizations in Molecular Life Science

The subject matter of molecular life sciences consists of highly complex, interacting, aperceptual substances and processes. A common way to handle this material is to use visual representations and models. Ever since Watson and Crick published the first image of the double helix of DNA in 1953, the use of visualizations in molecular life science has grown in importance. The subsequent decades have seen rapid developments in the types of images, diagrams, and visual models available and in their use to convey information and aid conceptual understanding in both teaching and research contexts. Visualizations and various modeling systems are used as experimental tools in research. Furthermore, visualizations have proven to be very powerful cognitive aids, enabling better understanding of the cellular and subcellular events. These developments are transforming the way we think about the events and processes the subject covers.

The fundamental importance of visualizations is also mirrored in life science textbooks, which are very rich in illustrations and often include graphic supplements: A multitude of websites display abundant images of atomic structures and animations of molecular and cellular processes.

Despite this development and the high availability of multimedia technology in schools in developed countries in recent decades, awareness of the scope for using visual materials in education could be greatly increased. However, learning with visualizations and interpreting the messages they aim to convey is not without complications. In this paper, we review some aspects of using visualizations in molecular life science education and present the results from a study of upper secondary and tertiary students' learning about transport across the cell membrane using different visual representations.

Learning with Visualizations

In recent years, the use and interpretation of visualizations in science education, especially physics, has received increasing attention. Indeed, new book volumes have further focused the role of visualizations in learning science (e.g., Gilbert, 2005; Gilbert, Reiner & Nakhleh, 2008b).

Students' interpretations of images and other forms of visualizations have been examined in several science education studies, which have shown (inter alia) that ambiguities, simplifications, and potentially misleading elements in the design of visualizations can give rise to unexpected difficulties or alternative interpretations (e.g., Cook, Carter & Wiebe, 2008; Rundgren, 2006; Schönborn, Anderson & Grayson, 2002). Schönborn & Anderson (2008) have developed a model of the factors influencing students' ability to interpret visualizations: the representational mode (i.e., the design of the visualization), the conceptual factor (i.e., the students' prior knowledge), and the reasoning factor (i.e., the students' ability to interact with the representation).

Some findings imply that the use of less stylized, less schematic, and (hence) more realistic images could be beneficial. For example, Menger, Zana & Lindman (1998) have drawn attention to erroneous depictions of the structure of micelles in textbooks. According to the authors, the students take the stylized images of micelles as a realistic depiction of their structures. Indeed, several studies indicate that school students generally see models as realistic reproductions of the phenomena they depict (Harrison & Treagust, 2000). "Naïve" realist conceptions of scientific models are common not only among school pupils but also among university students. After considering such evidence, Schönborn & Anderson (2006) concluded that more attention has to be paid to learning how to read visualizations and that this skill, visual literacy, has to be learned.

A comparative study of the way in which experienced chemists and undergraduate chemistry students use multiple representations found that the latter's conceptions were closely connected to the representations they had experienced in their teaching (Kozma, 2003; Kozma & Russell, 2005). Experienced chemists, however, move freely between different representations of a phenomenon and make connections between them. This was much more difficult for the students, and their reasoning was constrained by superficial features in the representations used (Kozma, 2003). Research on expertise has demonstrated the need for several years of experience to become expert in a certain area (Chi, 2006). This would also apply to skill in using representations, and, if so, early introduction to visual representations and training in visualizing skills could make valuable contributions to science education.

Representation and Visualization

The terms *representation* and *visualization* need some consideration. *Representation* has been applied to both *internal*, mental, representations, and *external* symbolic expressions. Internal representations refer to concepts, principles, or "mental models" that encompass the individual's state of understanding of, for example, the molecular world of life science. As thinking and communication tools, such internal representations are transformed into a range of external representations, including symbolic expressions, chemical formulae, drawings, sketches, schemes, graphs, equations, and verbal expressions. The external representations are tools that shape the way we conceptualize the content (Kozma, Chin, Russell & Marx, 2000). Hence, there is a mutual feedback relationship between our internal and external representations of a scientific content.

There is to date no commonly accepted definition of the term *visualization*, even though the word is used with increasing frequency in science. It is used with two different meanings: as a verb (meaning to visualize something in your mind), which relates to internal representations; and as a noun (a visualization is something in the public realm), that is, relating to external representations (Gilbert et al., 2008a).

However, in addition to those external representations discussed above, there are, for example, photos and outputs from analytical instruments that "depict" measured aspects of the studied objects. We have chosen to use the term in the second meaning, as defined by Kozma & Russell (2005), for all images, symbolic images, graphs, diagrams, pictures, animations, instrumental outputs, and objects in the physical world as well as in virtual reality that are used to represent different aspects of phenomena, much of which cannot be seen, touched, or otherwise sensed.

The terms *animation* and *simulation* refer to dynamic representations of dynamic processes or systems. Simulations allow the user to select values for input variables within suitable ranges and observe the results on output variables. Animations can be interactive and represent outputs of simulations, or noninteractive and used more as movies to illustrate particular events or concepts. Developments in computer technology have greatly increased the possibilities for advanced modeling and interactive visualization and animation, while the production and use of educational multimedia technology has increased substantially in recent years, especially in developed countries.

How can Animations Contribute to Learning?

When learning molecular life science, students often struggle to understand (visualize) the complexities underlying the most essential molecular and cellular processes. It is a major challenge for educators to teach these processes in such a way that students can comprehend, understand, and appreciate their complexity. Visualizations are a valuable tool here for learning (Gordin & Pea, 1995). However, the number of studies that provide experiment-based reports on the effect of using animations in learning molecular biology is limited (Marbach-Ad, Rotbain & Stavy, 2008).

Research on multiple representations indicates that the use of different representations can be benificial to learning, firstly, by mutually complementing each other, secondly that familiar representations can constrain and explain more unfamiliar representations, and thirdly that a combination of representations could provide the learner with a deeper conceptual understanding of the topic (Ainsworth, 1999; 2008).

There has been a long-standing debate in multimedia research on the educational value of animations. Lewalter (2003) states that it has been hard to empirically prove the value of animations for learning. Several authors stress that animations can often cause cognitive overload for learners, due to a surplus of information (e.g., Lowe, 2003; Tversky, Morrison & Betrancourt, 2002). There is also a concern that animations may give rise to new and resistant misconceptions (Tasker & Dalton, 2006).

According to the structure-mapping principle (Schnotz, 2005; Schnotz & Bannert, 2003), pictures and animations only enhance comprehension if the learning content is visualized in a task-appropriate way. Furthermore, Tasker & Dalton (2008) have shown that different people with different prior knowledge perceive different features in an animation, highlighting the need to consider the prior knowledge of the students when using animations. Consequently, it is very important to consider the content, aim, and learning goal of the visualization when investigating the nature of the comprehension of the receiver of the message.

A relatively substantial amount of research has been done which compares the educational effects of animations contra diagrams. An empirical study by Wu, Krajcik & Soloway (2001) shows that computer models can be beneficial in helping students to visualize how to transform a 2D model into 3D, and there are a series of studies which indicate that animations can provide students with a deeper conceptual understanding of the dynamic and stochastic behavior of molecules.

Williamson & Abraham (1995) have studied the learning effect of animations and diagrams in learning of particular models in chemistry. They found that students who had only been exposed to diagrams either formed static mental models or were unable to form any adequate understanding of the process at all. Another study, by Pallant & Tinker (2004), showed that a teaching setup, including animations of molecular dynamics, gives students a better understanding of the states of matter, relating these to interactions between particles. Marbach-Ad et al. (2008) compared the use of an animation or a diagram in learning molecular genetics at upper secondary level and concluded that animations are beneficial for learning dynamic aspects. Finally, Sanger, Brecheisen & Hynek (2001) studied the effect of computer animations depicting the processes of diffusion and osmosis. After being exposed to an animation, the students were less likely to think that particles stopped moving at equilibrium. However, their study also shows that animations could give rise to misconceptions (ibid).

Lewalter (2003) claims that animations are superior for visualizing spatial aspects and dynamic processes, but that series of images and arrows can be sufficient in certain cases. Diagrams can facilitate recognition, identification, and naming of components. They can also provide rough approximations of objects' relative orientations.

Overall, the literature on visualization and animation research shows the need to consider the design of the visualization/animation and the connection between the design and the learning goal and students' prior knowledge when using visualizations in education. In the present study, our aim has mainly been to clarify the first two aspects.

Variation Theory and Critical Features of Learning

In this study, we have used a terminology from *variation theory* (Marton, 2006; Marton & Tsui, 2004) as a framework for our analysis. According to variation theory, which can be characterized as a theoretical development and framework for phenomenographic research (Marton & Booth, 1997), variation in how a phenomenon is experienced by a learner plays a major role in the learning outcome. Discerning different aspects of a phenomenon provides a wider experience-based knowledge about it, which can be connected to and compared with knowledge about other phenomena.

Phenomenography is generally used for studies in which the research focuses on the variation between different conceptions or ways of understanding a phenomenon or concept among learners. The goal is to comprehend peoples' conceptions and experiences of a phenomenon or concept. However, in this study, we focus on the variation of how a specific concept is communicated (visualized) and how the variation of expressions affects the learners' understanding.

Variation theory holds that in order to be able to learn about something, you must discern it from its background and without variation, there can be no discernment. For example, the word "heavy" does not mean anything to us unless we have experienced different weights, i.e., variation in weight. To determine whether a plum is ripe or not, we must have experienced the texture, softness, and color of both ripe and unripe plums: Variation implies both sameness and difference. Marton claims that variation creates new opportunities for learning. We are not merely discerning something not only as it is in itself but also as why it is not anything else. The aspects that determine whether an object is heavy or not, or whether a plum is ripe or not, are using Marton's terminology, the *critical features*.

Critical Features and Variation. Marton & Tsui (2004) introduce the term critical features to describe the features or conditions that are necessary for learning. Whenever someone is concentrating on something, they perceive different aspects of it. The learner's concentration is on some parts of the object, and they pay more attention to those parts than others. This means that learners can discern different aspects of the object and then see the same thing in different ways. The different ways of seeing something depend on the critical features the learner perceives. Marton and Tsui claim that the students' actual attention and focus are of great importance for apprehending the critical features of the object of learning. According to variation theory, students can learn about an object by focusing on the critical features. If the critical features are not discerned by the students, they will be likely to fail to learn the intended content. Marton and Tsui emphasize that in order to develop certain capabilities, a variation must be perceived in every learning situation. By this, they do not mean variation in general or that the more variation there is, the more the students will learn. Instead, they are referring to variation that can contribute to and develop the students' ability to discern critical features of the object of learning.

In this study, we study how the variation in the use of features in the visualizations can contribute to students' learning about transport through the cell membrane. The features that can be discerned depend on the spectrum of variation previously encountered by the student.

The Intended, Enacted, and Lived Object of Learning. Using the terminology developed by Marton & Tsui (2004) to describe the goals of teaching, the achieved teaching, and what has been learned by the students, we have developed a model which aims to describe teaching with visualizations (see Figure 1). We define the meaning of the terms used in the model as follows:

> The *intended object of learning* (IOL) consists of the concepts and their features that the teacher/visualization designer aims to communicate. In this case, the concepts and processes are those involved in transport over a biological membrane.

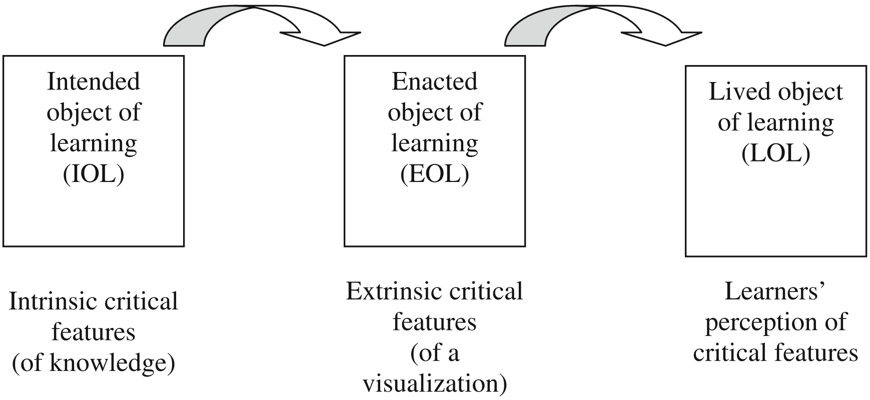

Figure 1. Proposed model for visualizing the different objects of learning, based on the distinctions made in Marton & Tsui (2004)

The *enacted object of learning* (EOL) is the resulting teaching strategy and the choice of features used. In the present case, this is the way this process is expressed in the two visualizations.
The *lived object of learning* (LOL) refers to the learners' meaning-making and understanding of the intended object of learning. Here, this means the way the learners understand and can use their understanding of the concepts and processes involved in transport over a biological membrane while reasoning about the visualizations.
The *intrinsic critical features* (iCF) are the critical aspects required for the learner to understand the intended object of learning. These are always connected to the intended object of learning (see further details under "Method" and "The Scientific Content—The Intended Object of Learning and Intrinsic Critical Features" sections).
The *extrinsic critical features* (eCF) are the critical properties/signs/colors/modes that help the leaner to grasp the critical aspects of the intended object of learning. These are the critical features of knowledge conveyed in a visualization or in a teaching sequence (see details under "Method" and "The Visualizations—The Enacted Object of Learning and Extrinsic Critical Features" sections).

If the enacted object of learning is identical to the intended object of learning, then the teaching is optimal, and if the lived object of learning equals the enacted object of learning, then the learning outcome is completely successful.

Aim

The aim of this study is to obtain a better understanding of how to express different types of content in visualizations. A model, based on variation theory, has been tested as a tool to distinguish between what is expected to be learned, what is present in the visualizations, and what is actually learned by the students.

Research Questions.

- Which iCF of the IOL (transport over a biological membrane) are conveyed in the visualizations studied?
- Which eCF in the diagram and the animation (EOL) corresponds to which iCF of the IOL?
- Does the use of an animation allow students a deeper conceptual understanding of transport through the cell membrane, and if so, what aspects of the scientific content may be better understood?

Method

The Sample Populations

Students from two schools and grades, seven from second year (grade 11) and 13 from third year (grade 12) of upper secondary education and 35 students from years 1 and 3 in a chemical biology program at university level, participated in the study. All of the upper secondary students were enrolled in the natural science program or the combined natural science/ social science program. The university students had a relatively even background knowledge since they were enrolled in the same program, while the upper secondary students had studied various combinations of natural science courses, and consequently, their preknowledge varied to a relatively high degree. The upper secondary students were taught about transport through the cell membrane during the second year of their studies. The tertiary students received teaching about this content during their first year in the biochemistry department. The first-year students received this teaching after their participation in this study. However, the students' actual preknowledge about membrane transport at the time of the study was never investigated.

Three types of data were collected: students' responses and contributions in interviews, written individual exercises, and group discussions. The students' interpretations of the two visualizations were investigated using interviews. At the upper secondary level, four girls and three boys in grade 11 and 12 girls and one boy in grade 12 were interviewed, and at university level, two girls and two boys in the third year of the chemical biology program were interviewed. The students were asked questions (see ESM 1) focusing on the two visualizations, the diagram and the animation described below. In addition, 31 university students (16 girls and 15 boys) in the first year of biochemistry studies took part in an individual exercise answering open-ended questions (see ESM 2), followed by a group discussion—both centered on the animation. The interviews aimed at getting an in-depth understanding of the students' interpretation of the two visualizations. For practical reasons, it was not possible to perform individual interviews with the first-year university students. Instead, the individual written responses aimed to give us an individual insight into the students' interpretations and the group interview an insight into their reasoning. However, group interviews were hampered by the fact that all students were not equally talkative.

The rationale for using students at different level of education was (a) to validate our results by comparing results from different contexts and (b) to gain some insight into the importance of students' preknowledge in relation to their capability in making meaning of the visualizations.

There is an overrepresentation of girls (18 girls and only six boys) among the interviewees. The main reason for this is that girls were in the majority in the classes, but in addition, fewer boys were willing to take part in the interview part of the study. Our original goal was to choose students representing all levels of achievement (based on their grades in natural science subjects). However, since only a small number of students in each class volunteered to be interviewed, high-achieving students came to be overrepresented in the interview material.

Data Collection

Semistructured revised clinical interviews (Kvale, 1996) were used, structured around two visualizations: (a) a diagram of three different types of transport through the cell membrane and (b) an animation showing molecules being transported through a channel in the cell membrane (see ESM 3). The interviews were audio-recorded and transcribed in full. All students are given fictional names in the quotes.

In the interviews, the students were asked to interpret the visual representations using their prior knowledge. All students were shown the same visual representations and the diagram was always shown before the animation. During the interviews, the student was first shown the diagram and asked to describe what it depicted. Later, the student was shown the animation and asked the same question. The students were not given any specific information or clue as to the interpretation of the visualization.

The individual exercise and the group discussions focused solely on the animation. In the individual exercise, the student was asked to consider the animation and provide written responses to a questionnaire (see ESM 2). The questionnaire had two sections: In the first, the student was asked to interpret the animation without any clues, while the second contained information about what process the animation illustrated and questions probing the students' understanding of the process and how they linked this process to other processes. The responses were collected and analyzed. In the group discussion, the same questions were discussed. The group discussions were video- and audio-recorded and transcribed.

The Scientific Content—The Intended Object of Learning and Intrinsic Critical Features

The cell membrane consists of a bilayer of phospholipids, each of which has a polar part (which collectively form the inner and outer surfaces) and a nonpolar part (which constitutes the interior of the bilayer). The phospholipid bilayer also contains other molecules, primarily proteins, such as receptors and transport proteins. The membrane should be considered a dynamic structure, in which molecules are continuously moving, changing places, and sometimes moving into and out of the membrane.

The membrane functions (inter alia) as a barrier that separates the interior of the cell from its surrounding environment. Small uncharged molecules, such as oxygen and carbon dioxide, can readily move through the membrane without aid (via "passive transport"), while charged and large molecules are "locked out". However, appropriate metabolites must be taken into the cell and, for example, waste products must be removed. This transport occurs through an array of different specialized transmembrane proteins. Much of the complex structure of biological membranes is therefore involved in the regulation of transport. The main critical features (iCF) of the scientific content (IOL) are presented in Table 1.

The Visualizations—The Enacted Object of Learning and Extrinsic Critical Features

In the following paragraphs, we identify the extrinsic critical features of visualizations related to transport through the cell membrane conveyed in the two visualizations.

TABLE 1

Critical features of the Intended object of learning (IOL) and the Enacted object of learning (Visualizations)

Intended object of learning (IOL)	*iCF of IOL*	*eCF of Diagram*	*eCF of Animation*
Transport through the cell membrane			
i) The cell membrane consists of a bilayer composed of lipids of which phospholipids are the most abundant. ii) Transmembran proteins are components of the cell membrane iii) Transmembran proteins are contained within the dynamic fluid mosaic of the cell membrane.	A) The structure and composition of the cell membrane.	A) i) ii)	A) ii)
i) The lipids of which phospholipids are the most abundant amphiphilic molecules. These amphiphilic lipids have a polar and a non-polar part. The polar part is turned outwards, while the non-polar parts form the centre of the bilayer. ii) The proteins have a hydrophobic surface and often a hydrophilic center.	B) The chemical properties of the molecules contained in the cell membrane.	B) i)	B) ii)
The lipid bilayer of the cell membrane is semi-permeable and functions as a selective barrier against large molecules and small charged or polar molecules (or more exactly, its permeability is very low for charged and polar particles but high for small, non-polar molecules). The cell membrane is permeable to small non-polar molecules.	C) The selective barrier function of the cell membrane	C)	

TABLE 1 (continued)

Intended object of learning (IOL)	*iCF of IOL*	*eCF of Diagram*	*eCF of Animation*
i) Selected larger, polar or charged molecules can be transported over the cell membrane through different kinds of transport-proteins. Transport occurs through three principles; ii) active, energy-consuming pumps; iii) channels for facilitated passive transport; and iv) proteins that transport uncharged particles passively along their concentration gradient by means of conformational changes.	D) Transport mechanisms of the cell membrane.	D) ii) iii) iv)	D) iv)
The water transport through the cell membrane (D iii)			
The movement of water through the cell membrane occurs via osmosis, i.e. the passive diffusion of water molecules along a concentration gradient that does not require any energy input.	E) Water transport is passive.	E)	E)
Water molecules are polar. This makes it energetically unfavourable for water molecules to travel through the hydrophobic centre of the phospholipid bilayer.	F) The chemical properties of water molecules.		
i) The largest proportion of the water molecules that move into or out of the cell do so through facilitated channel-mediated diffusion. ii) Water molecules move (or diffuse) across the phospholipid bilayer through specific, and specialized transmembrane water-channel proteins (called aquaporins).	G) Water molecules are mainly transported through specialized channels.		G) ii)

The Diagram. The intended meaning of the diagram (see Table 1 and ESM 3) is to illustrate the principles involved in three different types of transport of small molecules across a biological membrane. The diagram shows a cross-section of a cell membrane comprising a bilayer of phospholipids. The membrane is shown in two dimensions as a flat double layer of phospholipids. In the visualization, three proteins acting as channels or pumps are shown in red. These are more or less purely symbolically conveyed. The visualization shows that the transport proteins are transmembranal: Otherwise, there is no relation to the size or structure between the image and the actual proteins. Various substances flow into or out of the cell in a controlled manner through proteins such as these. The substance to be transported is deliberately depicted without any special characteristics, indicating that it could refer to any kind of substance.

The protein to the left illustrates a channel that facilitates transport of a substance (shown in gray) that diffuses in the direction of its concentration gradient. The middle protein illustrates transport of specific molecules, also in the direction of their concentration gradients. The protein to the right is an active transporter which transports molecules against their concentration gradients, in a process coupled to energy generated through the breaking of bonds in ATP molecules ("active transport").

The dynamic character of the transport process is symbolized by the arrows indicating the direction of the transport. Only one molecule is shown in each transportation event. No dynamics for the proteins or the transport event in the membrane is indicated.

The Animation. The animation depicts the facilitated transport of water molecules through an aquaporin (which constitute a specialized water channel in the cell membrane), according to the findings of Agre, who was awarded the Nobel Prize in chemistry year 2003 (Agre, Preston, Smith, Jung, Raina, Moon et al., 1993; De Groot & Grubmüller, 2001). In the animation (which can be viewed at http://nobelprize.org/chemistry/laureates/2003/animations.html; see also ESM 3), a large number of water molecules, diffusing and colliding, are shown. The critical features are indicated in Table 1. The water molecules are indicated according to the chemical conventions, showing the oxygen atom in red and the two hydrogen atoms in white. The transport of one of the water molecules, seen in yellow, through the aquaporin (viewed in cross section) is shown. In the middle of the aquaporin, positively charged side chains that repel positively charged particles, such as hydrogen ions, are highlighted. The membrane itself is not shown at all in the animation. The animation shows the dynamic and stochastic movement of the water molecules and provides an image of the large number of molecules that are constantly interacting, in a way that is different from more simplified and schematic animations.

Analysis of the Transcripts and Questionnaire Responses

Defining the intended, enacted, and lived objects of learning is not always straightforward. Discussion among the authors as to what constituted the intended object of learning and a review of text books and primary literature on the topic (e.g., Agre et al., 1993; Campbell & Heyer, 2007; Fettiplace & Haydon, 1980; King & Agre, 1996; Tieleman, Marrink & Berendsen, 1997) resulted in the dissemination of a number of critical features of the structure of the cell membrane and the movement of water molecules across it, as presented in Table 1. The ELO and eCF are described in "The Visualizations—The Enacted Object of Learning and Extrinsic Critical Features" section. The LOL are defined here as the corresponding explanations and features that could be identified by two researchers, independently, in the students' explanations.

The transcripts from interviews, group discussions, and the responses to the questionnaires were analyzed iteratively according to the method of analytical induction (Abell & Smith, 1994). Identified categories were interpreted and characterized. The material was read several times and categorized independently by the two authors and the analysis aimed to elucidate the relationships between the scientific content, the visualizations, and the understanding and ideas evoked among the students by these visualizations.

The meaning-making, depth, and character of learning through visualizations may be studied from several perspectives. Here, we use the model derived from variation theory to identify the different qualitative categories of critical features. The transcripts were analyzed for content knowledge (lived object of learning, LOL), specifically in correlation to IOL and its iCF and the indentified eCF of visualizations.

Results—The Lived Object of Learning in Relation to Intrinsic and Extrinsic Critical Features

The students in this study are in most cases aware that the cell membrane consists of a bilayer composed of amphiphilic lipids (Table 1) and proteins (Table 1). The fluidity and dynamics of the membrane (Table 1) was generally not well-understood by the upper secondary students and only partially understood by the university students. Most of them, however, appear to know that the cell membrane is a selective barrier (Table 1) and that there are different kinds of transport through the cell membrane—active and passive. They are generally also aware that transport along the concentration gradient does not require energy generated from ATP (Table 1).

However, few of the students appear to envision the cell membrane as a dynamic structure that the interior channel of transport proteins is often hydrophilic and that some of the transport proteins may undergo conformational change upon the transport. Furthermore, most students were not aware that the largest proportions of the water molecules that move into or out of the cell do so by facilitated channel-mediated diffusion through a specialized transmembrane water-channel protein, aquaporins.

Critical Features Identified from Students' Interpretation of the Visualizations

The Diagram. Most students could identify the main message of the diagram (see ESM 3): that it depicted different types of transport over a biological membrane. The categories that can be identified from the students' reasoning with the diagram are mainly: B (the chemical properties of the molecules contained in the cell membrane) and D (transport mechanisms of the cell membrane). However, not all students could clearly differentiate between the three principles of transport. For example, from looking at the diagram, some of them had difficulties distinguishing the two different kinds of transport along the concentration gradient: through open channels and through transport proteins that undergo conformational change. Two of the upper secondary students stated that it is the passive transport that is coupled to ATP breakdown.

The nature of the molecules which are transported was seldom discussed and none of the students discussed the chemical properties of the membrane transport proteins or the stochastic and dynamic nature of the process in association with the diagram. Category A (the structure and composition of the cell membrane) was also present, but less frequently than in the students' explanations of the animation. If it was present, it was clear that the students were aware of the fluid mosaic model of the cell membrane from prior studies (university students; for illustrative citations see ESM 4)

The Animation. Since the animation conveys the transport of water transport through an aquaporin channel in the cell membrane, unsurprisingly the category G (water molecules are mainly transported through specialized channels) could be identified from the animation. However, it was harder for the students to identify category E (water transport is passive), since the concentration gradient is less clearly visible in the animation as compared to the still image. Most of the students were already aware of the driving force of water movement and added reasoning about osmosis and concentration gradients to their explanations, even though this was less clearly visible from the design of the animation. Category F (the chemical properties of water molecules) was not directly distinguishable from the animation. However, the majority of the students both at upper secondary and university level had this prior knowledge.

A few students, mostly at upper secondary but also some at tertiary level, found difficulties in interpreting the animation. In most cases, this was due to the design and use of symbols. One aspect that made the interpretation difficult for these students was the lack of indication of the cell membrane. However, the majority of the students were aware of the implicit presence of the membrane.

Another problem related to what is not clearly apparent in the animation is that the concentration of water molecules is higher outside the cell membrane than inside. This made a number of students doubtful whether the diffusion of water molecules really took place along a concentration gradient, even if they had explicitly said that the passive transport was along a concentration gradient when interpreting the diagram, where this is schematically shown. In some cases, at upper secondary level, the students also lacked the conceptual knowledge of passive diffusion of particles from a higher to lower concentration. Furthermore, about one third of the students, regardless of educational level, misinterpreted the highlighted water molecule in the animation (in yellow) as constituting some kind of another atom, i.e., sulfur. The yellow color led many of the students to associate this with their prior knowledge of the chemical convention depicting sulfur atoms in yellow. The occurrence of this difficulty in all student groups shows that it was more related to the representational design of the animation than to students' prior conceptual knowledge.

What can be Learned from the Animation?. Despite the shortcomings of the animation, many of the students appeared to gain new fundamental insights into the molecular world of life science through it. Interestingly, we were able to identify three additional critical features that relate to molecular interaction in general, rather than just in connection to transport through the cell membrane (see Table 2). These aspects can be categorized as critical features because the students either formulated their responses consciously in

TABLE 2

Fraction of students, in percent of the total number in each group, expressing the three identified additional CFs in their response to the animation

Level	*The complexity of molecular interactions*	*Dynamic and stochastic movement*	*Extrapolation between 2D and 3D*
Upper secondary year 2	14	14	0
Upper secondary year 3	23	38	31
University year 1	29	34	6
University year 3	67	100	33

terms of these and also displayed an awareness that these aspects made them think in a new way about the scientific content or to neglect them completely in their explanations. There are no clear intermediary cases in this study: Either the students was aware of these aspects and took them into account or they were not really aware of them and did not consider them at all.

The critical features identified here were associated with spontaneous insights about the content. In addition, the reasoning of the students and the commentaries they made when watching the animation revealed some aspects that they had been unable to grasp using just the diagram.

1. Features that show processes taking place in 3D: extrapolation between 2D and 3D
2. Features that show the dynamic and stochastic character of movements of the particles, including the unimaginable speed at which reactions occur
3. Features that shows the complexity of molecular interactions arising from the multitude of different molecules that are simultaneously interacting with each other

In the following list, we give brief examples of the different categories from the interview transcripts and the questionnaires.

1. Extrapolation between 2D and 3D

When Kajsa, in her third year at upper secondary level, compared the animation with the still image, she stated that 2D visualizations did not make her think in three dimensions:

> K: Well, the picture that the books give, gives a very flat picture... very simplified, like this, and in many ways [Interviewer (I):Yes] that... it's easy to get the wrong picture... or maybe not wrong but... it's easier to get an overall impression with

pictures like this [the animation] and maybe easier to make progress later [I:Yes] if you want to understand more...

2. Dynamic and stochastic movement

Maria, in her second year at upper secondary level, was surprised by the dynamic nature of molecular interactions. Her statement indicates that her conception of transport through the cell membrane was strongly connected to the type of representation that had been used in her teaching.

M: [...] well, they look as if they're moving more in... They're moving all the time. And maybe you didn't think so. I mean, you probably thought that it was completely still and went straight through and that it went through like a straight line without any motion.

3. The complexity of molecular interactions

When shown the animation, Jonna, who was studying chemical biology in her third year at university level, reflected on the more complex picture of molecular interactions and the electrochemical forces driving them than her university studies had given her:

J: Because it feels like it's to a high degree- this thing about order and lack of order and energy that cause... [I:Mm] what really happens... Because there is a lack of order and the molecules like, bounce around and then they end up where it is most likely that they end up... This, particularly, is something that... I've got... some kind of new insight or deeper understanding of now at university, which I had not been working with at upper secondary level...

Discussion

The role of visualizations in molecular life science is clearly becoming increasingly important and new technologies are assumed to promote more effective learning of visually and spatially complex topics such as molecular life science. In this study, our aim has been to clarify what the students are supposed to learn from a visualization, what aspects are actually possible to be discerned from the visualization, and what the students actually learn from it.

Students Meaning-Making, Difficulties, IOL, and iCF

We have found that the amount of information presented simultaneously in the animation (together with the lack of indication of the cell membrane and the seemingly even concentration of water molecules on both sides of the membrane) gave rise to some difficulties, which is in line with the findings of Tasker & Dalton (2006), Lowe (2003), and others. However, our results also show that a schematic simplified visualization is not necessarily more helpful. The transport through the cell membrane was frequently understood as ordered and simply directed and the students acquired no understanding of the mechanisms or the process from the diagram. Indeed, the simplification can cause misunderstanding, especially if the students interpret a simplified visualization as a more or less realistic depiction of molecular events, in accordance with the findings of Menger et al. (1998). These examples imply the importance of using appropriate eCF for the intended object of learning. If the aim was to communicate the dynamic and stochastic nature of the transport over a cell membrane, the diagram would have been a bad choice.

Influence of Preknowledge

The ability of a learner to interpret visualizations is considered to be strongly related to prior knowledge (Tasker & Dalton, 2008). We never tested the subjects for their preknowledge regarding water transport. However, the use of student groups representing four educational levels gives us some indications of the correlation between background knowledge and ability to interpret the visualizations. The university students were to a higher degree than the upper secondary students, able to “see” the cell membrane in the animation, even though it was not shown. They were also better at interpreting the different modes of transport shown in the diagram and had a slightly better understanding of the dynamics of the cell membrane. This supports the importance of prior conceptual knowledge when making meaning of these visualizations. However, in the case of the highlighted water molecule (in yellow) in the animation, the proportion of students who misinterpreted the highlighted water molecule as constituting some other kind of atom, i.e., sulfur, was the same at all educational levels. This illustrates that there are also problems that relate more to the design of the animation than to prior conceptual knowledge.

The Visual Design and eCF

When designing a visualization, there are choices to be made on which aspects (iCF) of the IOL to emphasize (eCF) in the visualization. However, even if the students have appropriate background knowledge, they may fail to understand a visualization because they cannot interpret its visual design or identify the phenomena it is supposed to depict.

Several conceptual difficulties may, in fact, be coupled to the way the content is represented and the symbolism used. This is in line with the finding of Schönborn & Anderson (2008).

For example, the absence of an indication of the lipid bilayer and that the driving force of the water transport, the concentration gradient, was not easily distinguishable from the animation caused problems for the students in our investigation. The more schematic drawing in the diagram gives a more easily interpreted, but less realistic image of the concentration gradient. Despite these shortcomings, the majority of the students, at both upper secondary and tertiary level, were able to adequately interpret these extrinsic critical features of the animation. In contrary, the yellow color of the tagged water molecules caused many more problems and confusion.

However, our results also showed that using the more complex animation could give students a deeper conceptual understanding of three critical aspects of molecular life science: (1) spatial features and the extrapolation between 2D and 3D, which is in the line with the findings of Wu et al. (2001) and Lewalter (2003); (2) the dynamic and stochastic behavior of molecules, which agrees with the findings of Williamson & Abraham (1995), Marbach-Ad et al. (2008), and Sanger et al. (2001); and (3) the complexity of molecular interaction, which is an aspect that is especially characteristic of molecular life science and needs to be further studied.

These three critical features are all implicit in the knowledge intended to be discerned by the students concerning cell membrane transport (IOL). However, it was not until the students saw the animated movement of water molecules and their interaction with the side chains in the aquaporin (eCF of the animation) that they become conscious of the stochastic and dynamic nature of the process. It is possible to interpret the positive correlation between the educational level and the fraction of awareness of these aspects (Table 2) as an effect of preknowledge. Interestingly, in most cases, the awareness of dynamics and complexity was characterized by sudden insights (according to the students' responses), stimulating new ways to think and to "visualize" abstract concepts. It might, however, be possible that a higher degree of preknowledge make the students better well-prepared to get these new insights from the animation. All these aspects need to be explored by further research.

Implications for Teaching

The results from this study show the need for proper teaching contextualization of the use of diagrams as well as of animations, as they may otherwise give rise to student difficulties or misinterpretations. Our results also show that different visualizations convey different critical features of knowledge. For instance, the diagram of transport through the cell membrane conveys the critical feature of molecules being transported by means of different mechanisms through specialized transport mechanisms. However, this still image failed to convey the critical features of three-dimensionality, dynamics, randomness, and complexity. The critical features of visualizations must always be related to the intended object of learning. Therefore, there is an urgent need to consider the question that the students are supposed to learn from the visualization. No single visualization can convey all the critical aspects of knowledge, and thus, a decision must be made about what kinds of visualizations are appropriate to convey a certain intended object of learning before constructing or using visualizations in education. This study points to the value of using multiple representations in science teaching (Ainsworth, 1999; 2008).

If dynamics, randomness, complexity, and three-dimensionality are regarded as important constituents of the intended object of learning, then teaching using only still images will undoubtedly fall short. The animated visualizations seem to provide a necessary complement.

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