Visualizing the Complexity of the Molecular World: Examining the Role of Animated Representations in the Development of Undergraduate Students' Understanding of Dynamic Cellular Events

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
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Ontario Institute for Studies in Education
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Abstract

The purpose of this study was to examine the relative effectiveness of three-dimensional visualization techniques for learning about protein conformation and molecular motion in association with a ligand and receptor binding event. Increasingly complex versions of the same binding event were depicted in each of four animated treatments. Students (n = 131) were tested at three time points, and over both the short and longer term, the most complex of the four animated treatments was the most successful at fostering students’ understanding of the events depicted. A follow-up study including eight biology students was conducted to gain greater insight into the students’ underlying thought processes and better characterize their understanding of the animated representations. Analysis of verbal reports and eye tracking data suggest that students are able to attend to the same narrative elements regardless of the level of complexity depicted in each animation. Analysis of verbal protocol data revealed a positive correlation between the number of explanatory statements expressed by participants and the complexity of the animation viewed. As well, prior knowledge was positively correlated with
the number of explanatory statements contained in each protocol. Overall, students demonstrated an understanding of protein conformation and molecular crowding. However results suggest that students have difficulty understanding and associating randomness with molecular events. The verbal reports contained several instances of students’ attaching agency to protein and ligand, anthropomorphizing their movements and subsequent binding.

Ordinarily cellular events, owing to their sheer complexity, are depicted in a highly schematized, simplified form. The results of this study would suggest that under select circumstances this may not be the most appropriate approach to depicting dynamic events. However additional attention must be given to exploring techniques that can satisfactorily balance the random nature of molecular events with narrative explanations of these processes.
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Visualization is a powerful integrator; and it's an integrator not just for scientific research, I would say…It must be a powerful tool for education as well. We are reaching the point now where there is no excuse for teaching the way we used to teach. It's just inexcusable to be doing 21st century science with 19th century teaching…I know that with the right visualization tools one could do amazing things in teaching …nowhere is this more needed than in biology…

…If a picture is only worth a thousand words, we're [doomed].

(Eric Lander, 2011)¹

¹ Eric Lander is a Professor of Biology at the Massachusetts Institute of Technology and Co-chair of U.S. President Barack Obama's Council of Advisors on Science and Technology. This excerpt is taken from his opening remarks at the Visualizing Biological Data (VIZBI) Meeting, March 16, 2011, Cambridge, Massachusetts.
1.0 THE PROBLEM

1.1 Overview

An understanding of molecular biology at an undergraduate level is dependent on students’ ability to assimilate dynamic and increasingly complex cellular and molecular processes. It requires an appreciation of interactions between the various cell systems occurring at multiple scales of temporal and spatial magnitude, and an ability to translate readily between these different levels of organization. As well, many of the difficulties associated with teaching and learning molecular life science are linked to the emerging interdisciplinarity of the field, bridging cell biology, chemistry, and mathematics; one which demands a much more integrated understanding of complex systems. Unfortunately, undergraduate learning environments, which are often characterized by lecture-based courses with high enrollment are not always conducive to higher order learning of complex subject matter. Rather, greater emphasis is placed on factual recall rather than depth of understanding (Momsen, Long, Wyse, & Ebert-May, 2010). Moreover, the tools available to instructors to supplement lecture and lab are not necessarily well suited to the learning task at hand. As Chandler (2004) observes, instructors frequently make the mistake of allowing technology to guide the learning experience rather than allowing our understanding of cognition and learning to guide the design and use of technology for instructional purposes. Since much of science education exists at the sub microscopic level, well beyond the level of our experience and senses, we rely heavily on representations of molecular structures to make sense of these environments. Confusion and misconception on the part of the student may arise from misuse of tools that are not designed with the learning objectives in mind, or from tools where the idealized or simplified representation of concepts is interpreted literally by the student.
There has been much discussion around the use of online interactive environments that incorporate visualizations\(^2\). Using tools such as Molecular Workbench (Tinker & Xie, 2008), NetLogo (Wilensky, 1999), or the Web-based Inquiry Science Environment (WISE, Linn & Slotta, 2003), visualizations are integrated with instructional scaffolds. While there has been much research devoted to the successful design of pedagogically impactful learning environments in which to embed visualizations, the body of literature addressing the design and evaluation of didactic visualizations is relatively underdeveloped.

Most biology instructors would agree that visual representations are essential to learning molecular biology. Textbook illustrations, diagrams, animations, and interactive learning tools are commonly used to make sense of cellular and molecular phenomena. These tools are collectively termed external representations, as they depict “knowledge and structure in the environment, as physical symbols, objects, or dimensions” (Zhang, 1997). In this way, they distinguish themselves from internal representations or mental models, which represent knowledge and structure as schemas, propositions, or some other form. External Representations may be examined, analyzed, and processed by the perceptual system, as a means of amplifying cognition (Card, Mackinlay, & Shneiderman, 1999). To facilitate the understanding of biomolecular processes, biologists utilize a visual language comprised of multiple external representations to convey phenomena occurring at multiple levels of organization. Structures may be depicted using imaging techniques, structurally modeled according to a variety of pictorial conventions, or symbolically represented as part of a process. This presents an

\(^2\) There is no commonly accepted definition for the term visualization, although it is used with increasing frequency in science education. For the purpose of this dissertation I have adopted the definition by Kozma and Russell (2005), which includes any image, graph, diagram, animation, instrumental output, real world object and virtual reality object that is used to represent phenomena that cannot otherwise be seen, touched, or otherwise sensed.
additional learning challenge to the student who is, more often than not, unfamiliar with the many symbolic conventions used to depict biological phenomena (Schönborn & Anderson, 2006).

Recently there has been a tremendous growth in the availability of external representations for teaching, learning, and research. The ubiquity of the Web and increasing adoption of portable devices (smart phones, tablet computers, iPads, etc), have contributed to driving the demand for visual tools. As a result, animated representations have become much more widely accessible. Whereas static graphics may depict temporal events as a sequential series of images, highlighting key transitional moments, they do not provide appropriate information about dynamic systems. Animation is used to teach a variety of dynamic concepts across a range of subject matter. For example, animation is frequently used in biology to convey dynamic processes, such as membrane transport, biochemical pathways, etc. As the field of molecular biology continues to evolve, driven by scientific and technical innovation, it becomes critical to understand not only the impact of such visuals on students’ understanding but also to develop a visual language that maximizes pedagogical outcomes and helps animators and media designers develop educationally effective tools. While there has been enthusiastic adoption of animated representations at various levels of education, surprisingly little is known about the efficacy of this visual medium. Whether or not animated representations are successful in supporting understanding is a topic of contentious debate, and much of the development of animated media continues to be guided more by intuition than evidence. Increasingly however, researchers studying the impact of animation are looking at its effects less in terms of the absolute benefits of animation and more in terms of the contextual benefits of animation. The issue is not whether animation is beneficial to learning, but rather when and why animated instruction may be effective (Ainsworth, 2008a; Betrancourt & Chassot, 2008). Addressing this issue requires
further investigation of the features of animated media that render it more or less effective, and the cognitive processing of these dynamic visualizations.

1.2 Study Objectives

In order to understand the role of animation in the development of understanding it is fundamental that we explore the visual affordances of this medium as well as the way in which students process the information contained in animated representations. This study strives to characterize the role of animation by addressing the following three research objectives:

1. To explore the visual features in the animated representation of a dynamic molecular event that inform understanding in a sample population of undergraduate biology students.

2. To examine the role of visual complexity in supporting students’ understanding of dynamic molecular events.

3. To characterize the learning that takes place when students are exposed to animated representations depicting a dynamic process at varying levels of complexity.

Ultimately it is the goal of this study to extract information useful in furthering our understanding of how students learn from animated representations of dynamic subject matter, particularly when the animations themselves are visually complex. As well, in characterizing how students learn from these representations, it is the goal of this study to provide insight into how the design of these educational visualizations may be improved to support learning.
1.3 Personal Interest (Experience and Assumptions)

I have been interested in the visual communication of science and medicine for a number of years. My graduate training, a Master of Science in Biomedical Communications, prepared me for a career as a medical illustrator and instructional media designer. I have continued to contribute to the field of Biomedical Communications on many levels, as a practitioner, researcher, and instructor. The present study draws upon previous knowledge gained in practice and research. As a result these experiences, I have a few preconceptions related to the impact and utility of visual media, and approaches to evaluating media, that I have attempted to outline for the reader here.

My Master’s research project and paper in Biomedical Communications examined the role of visual media in helping patients newly-diagnosed with prostate cancer understand their diagnosis and the treatment options available to them. This research contributed much to my understanding of the potential of visual media in helping patients to understand new information under stressful conditions. The study, which followed an iterative design process, involved patients at each stage of development. Perhaps one of the most important findings of this study was the role that visual media played in supporting the communication process. Images acted as a scaffold for discussing information needs, preferences, and illness-related concerns; topics which would otherwise have been very difficult to articulate (Jenkinson, Wilson-Pauwels, Woolridge, & Jewitt, 1998).

Shortly following the completion of my Masters degree I was invited to co-ordinate a research study examining the role of visual complexity in communicating oncology information to “culturally” diverse audiences (professional and patient). The goal of this 3-year study was to identify subjective differences in the visual preferences of surgical oncologists learning about
sentinel node biopsy (a minimally invasive technique for treating breast cancer), compared with patients of the Ontario Breast Screening Program. The study revealed many differences both within and between the two groups. For example, amongst surgical oncologists, those with the least experience had a preference for highly visually complex, photo realistic representation of surgical procedures, whereas surgeons with many years of experience, preferred more simplified, animated representations of the same procedures. Inexperienced clinicians regarded photo realistic representations as being more accurate and “true to life”, while experienced clinicians recognized greater didactic value in the less complex representations. Patients, on the other hand, were unified in their visual preferences and this was dictated more by comfort level with the subject matter than by didactic value (Jenkinson, Woolridge, Wilson-Pauwels, McCready, & Brierley, 2004). My involvement in this study contributed greatly to my experience as a researcher. In particular, as I familiarized myself with the literature examining the impact of visuals upon learning, I was surprised to note that much of this research treated the visual as an adjunct, examining it in combination with other modalities. There did not appear to be a coherent model describing how visual representations per se impact upon understanding. Some researchers (most notably Dwyer 1972, 1978, 1987) have made attempts to evaluate the efficacy of various styles of visual representation (both static and dynamic). However, these studies are marked by a notable lack of control over the quality of the stimuli, and are often evaluated without insight into the educational objectives that the illustrations were initially created to fulfill. This raises many questions about the appropriateness of comparing different image modalities, the learning context in which these representations are used, and the tools used to assess their impact.

A third experience that informed my attitude toward the assessment of visually rich educational material involved an exploration of the impact of animation and interactivity in fostering
students' understanding of specific concepts in neurobiology (specifically neurotransmitter release). In two related studies first-year biology students were exposed to one of two e-learning modules (described in Jenkinson, 2009). The first study measured differences between e-learning tools containing either animated graphics or static graphics (where direction of motion was indicated using arrows). The second study measured the impact of an interactive version of the program (one demanding active participation on the part of the student to trigger animations) against the animated version. In every other respect the programs were identical. We hypothesized firstly, that by integrating animation and secondly by integrating a level of interactivity, student engagement with the material would increase and that this would be reflected in test scores. This appeared not to be the case. In both experiments we failed to detect a significant difference between treatments. However these findings were not reflected in the qualitative data collected (feedback forms and focus group interviews). Student participants’ reported notable differences in their experience with the learning material. I learned a great deal from this experience, not the least of which was that we were asking the wrong questions. Rather than trying to determine “what” students were learning from animated and interactive media, we might have been more successful had we attempted to examine “how” students were learning from these different media modalities.

1.4 Summary

The success of using animations in instructional settings has met with mixed reviews. In part this may be explained by examining the quality of the stimuli. Perhaps this may also be explained by considering the nature of inquiry guiding the evaluation design. What follows is a discussion of challenges faced by undergraduate biology students grappling to understand the
complexity of the molecular world, and by extension, the challenge this poses to the design and evaluation of visual tools intended to meet the learning needs of these students.
2.0 REVIEW OF THE LITERATURE

2.1 Overview

In this chapter a theoretical foundation for this study is established by reviewing the relevant literature that informs our understanding of the challenges related to undergraduate biology, changes occurring in this field, and the implications of this for teaching and learning. In addition, the ways in which visual media are well suited to molecular biology education are discussed; this includes an in-depth exploration of the perceptual and cognitive affordances of animated media. This chapter is organized in five main sections:

i. Challenges Related to Undergraduate Biology

ii. The Visual Language of Science

iii. Representing Visual Complexity

iv. The Role of Three-Dimensional Visualization in Science Education

v. The Role of Dynamic Visualization – Learning from Animation

2.2 Challenges Related to Undergraduate Biology

2.2.1 Overview

Molecular life science is a rapidly growing field that poses a number of challenges for both teachers and students of this domain. Contemporary biology has experienced a great shift away from fractioned thinking toward a much more integrated study of complex and interconnected systems. Where biology was once more focused on isolated evidence directly related to the field,
it now draws upon findings in disciplines such as chemistry, mathematics, and computer science. A recent report issued by the National Academy of Sciences (2009) called for reform to undergraduate biology education (see Woodin, Carter, & Fletcher, 2010 for a discussion and summary of this report). Its authors described the new biology as more integrative, and reflective of real world research and practice; adopting knowledge from a number of related disciplines (including for example, chemical and computational sciences) in hopes of fostering deeper understanding of biological systems. Addressing this challenge demands a collective effort to change undergraduate biology education; one focused on interdisciplinarity and providing students and teachers with the skills to understand the connections within these disciplines (Labov, Reid, & Yamamoto, 2010).

In a discussion of teaching and research challenges related to the field, Tibell and Rundgren (2010) identify four areas of key importance in addressing the future of education in molecular life science: 1) Challenges relating to content selection; 2) Challenges relating to understanding at multiple levels of abstraction; 3) Challenges relating to domain specific language; and 4) Challenges relating to the use of visualization in molecular life science education. Content selection poses a great challenge to instructors, who are expected to keep pace with the rapid rate at which new information is generated in this field. For the novice student the choice of content to support learning is even more difficult, given the student’s inability to distinguish between key and peripheral learning material. Moreover, students experience difficulty understanding biological concepts as they involve numerous interacting processes occurring at multiple scales of time and space. In order to make sense of these processes “learners must be able to sift through complex information spaces, discriminate between important and unimportant information, and recognize critical patterns and relationships” (Tibell & Rundgren, 2010). Rather, students tend to approach biology as a series of unconnected ideas or theories,
rarely integrating their knowledge and making a connection with real life phenomenon (J.L. Momsen, T.M. Long, S.A. Wyse, & Ebert-May, 2010; Tanner & Allen, 2005). Subject areas of particular difficulty for students include protein conformational change and stability (Robic, 2010), diffusion and random molecular motion (Garvin-Doxas & Klymkowsky, 2008), and molecular crowding, poorly understood in part, because it is often neglected in undergraduate curriculum (Ellis, 2001). Students have trouble reconciling the perceived efficiency of biological systems with the concept of randomness. In addition to the challenge of trying to come to terms with the full complexity of the molecular world, students entering biology are faced with learning a new “visual” language that is used to describe phenomena occurring at multiple orders of magnitude.

2.2.2 Challenges Related to Understanding at Multiple Levels of Abstraction

The challenge of understanding a complex system is by no means limited to biology. In many other academic areas such as mathematics, chemistry, physics, and engineering, students’ success depends upon their ability to envision and manipulate multidimensional information spaces. Many of the difficulties associated with understanding a complex system have to do with the intangibility of domain concepts. Teaching and learning take place at multiple levels, in biology for example, from the nanoscale to the study of entire populations of organisms. Many of these ideas operate at a level far beyond our senses, and students have no experience in constructing meaningful models of these concepts. In addition to conceptualizing entities operating along multiple spatial dimensions, biology students are faced with the added challenge of understanding that these relationships operate across multiple temporal scales as well. Proteins are dynamic entities that are in perpetual motion, both relative to other interaction partners in their environment and also internal to the protein chain itself. The thermal motion of
individual atoms, amino acid side chain motions, diffusional events, transient and large conformational changes all contribute to our understanding of molecular function (illustrated in Figure 1). While these temporal cues are incredibly difficult to convey visually, in the context of designing pedagogically beneficial learning materials for the classroom, it may be misleading to selectively omit these different types of molecular movement. Yet, as is often the case in the development of instructional tools, both spatial and temporal scale are sacrificed for the sake of clarity in the depiction of molecular processes.

Figure 1. Simultaneous planes of molecular motion occur at each level of protein structure (respectively highlighted in red in each successive panel from left to right). The relevant timescale of these events is given below the type of motion. Illustration reproduced with permission of Gaël McGill.

In exploring the ways in which students struggle with some complex dynamic concepts, Chi (2005) offers an explanation that is illustrated by contrasting the differences between “direct” processes and those that are “emergent”. Misconceptions surrounding direct processes (for example the human circulatory system) are much more easily corrected than emergent processes (for example diffusion). Direct processes (using again the example of the human circulatory system) may be explained in terms of the direct influence of aggregate components (eg. heart and veins) and their constituents (eg. heart valves) upon blood flow. By contrast, in a process such as molecular diffusion (the example the author provides is the apparent flow of dye when it is added to a container of water), neither the aggregate components (water and dye), nor their
constituents (molecular water and dye) are directly responsible for the diffusion of molecules (Chi, 2005). Chi further distinguishes between these two types of processes by classifying their ontological attributes. For example the component level interactions of direct processes may be categorized as distinct, constrained, sequential, etc., whereas emergent processes are better described as uniform, unconstrained and simultaneous (Chi, 2005).

Perhaps one of the most relevant observations the author makes is with regard to component-pattern relations within each type of process. Direct processes may be broken down into subgroups or classes. In this way, the concept of spatial or temporal scale much more readily lends itself to systematic, hierarchical organization. Emergent processes, on the other hand, are not as easy to describe in terms of subgroup or hierarchy. They consist of a collection of components contributing equally to a reaction. Chi proposes that it is much easier to identify member of a class, based upon share features and behaviours, than it is to identify members of a collection, identifiable only by shared interactions and interrelationships. The author further suggests that students fail to comprehend complex dynamic events because they seek causality and group entities according to shared perceptual properties. In the case of a student trying to understand diffusion, they might associate water and dye on the basis of colour, as 2 separate classes, rather than thinking about water and dye molecules collectively interacting (one collection).

A Piagetian explanation for this phenomenon would suggest that students who fail to understand the emergent nature of diffusion are assimilating the experience of diffusion into an already existing framework without changing that framework (Piaget, 1972). A student’s understanding of ‘process’ as a concept is informed by prior experience with processes they encounter in the world. The experience of diffusion as an emergent process is without a referent internal
representation. No equivalent schema can be resourced from prior experience that informs or provides an anchor for the integration of these new experiences into long term memory.

Students are quick to propose their own rational explanations for complex biological concepts (from diffusion to natural selection). They will try to find analogies that may be used to explain phenomena, but more often than not this leads to misconception. There is an increasing awareness of the central role visualization plays in scientific research, and it is argued that it should play a correspondingly equivalent role in science education to support students understanding of emergent phenomena (Gilbert, 2005).

2.3 The Visual Language of Science

Biology is an inherently visual domain. Much of what we know about cell and molecular structure is derived from imaging technologies such as x-ray crystallography and electron microscopy. These techniques provide us with a glimpse of the great complexity of the molecular world. In addition we rely upon a range of visual depiction conventions and strategies to describe different aspects of these structures. Molecular structures may be described numerous ways, from atomic models to space-filling representations (See for example Figure 2).

![Figure 2](image_url)

*Figure 2. Five different conventions for visually representing the molecular structure of the protein myoglobin. From left to right: backbone, ball and stick, ribbon, sphere, and surface representations each teach us something different about the structure of myoglobin [PDB ID: 1MBN (Watson, H.C. (1969) Prog. Stereochem. 4: 299)]. Molecular graphics images were produced using the UCSF Chimera package from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco (supported by NIH P41 RR001081).*
Understanding these depictions requires that students familiarize themselves with the visual language used to describe a world operating at multiple levels of organization. Indeed, the visual conventions used to represent molecular structure and function can be very challenging for students to understand and can lead to a number of conceptual and reasoning difficulties (Schönborn & T. R. Anderson, 2006). In part this is owing to a lack of necessary visualization skills on the part of the student who is newly exposed to this material. Undergraduate biology curriculum does not include training in visual literacy. Rather students are expected to “catch on” and acquire these skills as they learn (Flannery, 2006; Schönborn & T. R. Anderson, 2006).

As well, the design and presentation of the visual learning tool greatly impacts upon students’ interpretation of scientific phenomenon. For example, difficulties may arise from visual explanations in which phenomena are represented with “deceptive clarity” (Harris et al., 2009; Linn, Chang, Chiu, Zhang, & McElhaney, 2010; Tasker & Dalton, 2006). This is certainly true of visual representations that offer an oversimplified explanation of scientific concepts for the sake of clarity. In this scenario students may recall a sequence of events but hold only a superficial understanding of the concept overall. Conversely, visual explanations that introduce extraneous complexity that is not relevant to the learning goal, may be equally misleading. Visually rich materials are often borrowed from sources that do not ‘contextualize’ them for classroom use. Since there is little time for teachers to remain up-to-date in rapidly evolving areas of the life sciences, the value of such visuals is diluted and their impact lowered. However, when carefully designed, with a clear learning objective in mind, scientific visualizations are powerful tools for describing the intricacies of cellular and molecular systems.

Perhaps more than in any other area of science, visualization helps us grasp the complexity of biological events that are both too small to see with the naked eye (or microscope in the case of
biomolecules) or too rapid to experience with our own senses. Aside from being the ‘only option’ to represent such complex events in space and time, visual explanations are often more engaging and memorable than other forms of communication.

2.4 Representing Visual Complexity

A century of research in cognitive science has taught us a great deal regarding the nature of visual perception and cognition. However, there are still many areas that demand exploration, such as the relationship between image complexity and understanding. In order to appreciate what makes an image complex we need to consider the way in which we perceive and order the constituent features that comprise the image as a whole. Ware (2004) provides this informative three-stage model of image perception:

At the early stages of feature abstraction, the visual image is analyzed in terms of primitive elements of form, motion, color, and stereoscopic depth. At the next 2D pattern perception stage, the contours are discovered and the visual world is segmented into distinct regions, based on texture, color, motion, and contour. Next, the structures of objects and scenes are discovered, using information about the connections between component parts, shape-from-shading information, and so on. Pattern perception can be thought of as a set of mostly 2D processes occurring between feature analysis and full object perception, although aspects of 3D space perception, such as stereoscopic depth and structure-from-motion, can be considered particular kinds of pattern perception. Finally, objects and significant patterns are pulled out by attentional processes to meet the needs of the task at hand (p. 187)
2.4.1 Preattentive Feature Abstraction

Certain of the visual features that we perceive in the early stage of perception “pop out” in a way that distinguishes them from their surroundings. The mechanism underlying this phenomenon is called preattentive processing, as it occurs prior to conscious attention (Ware, 2004). The features that are preattentively processed can be broadly categorized based upon form, colour, motion, and spatial arrangement. An understanding of preattentive processing can translate into principles for organizing and displaying information in such a way that focuses the viewer’s attention.

2.4.2 Visual Pattern Perception

Pattern perception constitutes the flexible middle ground, where objects are extracted from patterns as we address the cognitive needs of the task at hand. An early attempt to understand pattern perception was undertaken in 1912 by a group of German psychologists, who founded what is known as the Gestalt School of Psychology. Gestalt psychologists proposed that the human brain is particularly adept at pattern recognition. Due to these supposed innate abilities, we are capable of perceptually organizing and structuring individual elements, shapes or forms into a coherent, organized whole. Although the individual elements may contain some meaning, the coherent whole will have a greater meaning than the sum of the parts. This satisfies the human brain’s need to find balance and impose meaning to situations. The theories proposed by this group greatly influenced practitioners at the Bauhaus School who adopted these ideas elaborating them into a set of principles informing Twentieth Century design. The word gestalt essentially means pattern in German. Although the proposed neural mechanisms underlying gestalt theory have since been disproven, it provides a clear description of many basic perceptual phenomena. Psychologists produced a set of Gestalt principles of pattern perception
that, to this day, have tremendous relevance. The Gestalt laws easily translate into a set of design principles for visual displays. Some of the more significant principles include:

- **Proximity** – objects that are situated close to one another are grouped together
- **Similarity** – elements that share similar features are also perceptually grouped
- **Connectedness**\(^3\) – objects that are connected with a line are perceived collectively
- **Continuity** – features that overlap are perceived of as unbroken and smooth
- **Symmetry** – objects that share symmetry are perceived collectively
- **Closure** – contours that contain gaps are perceived of as whole (we mentally close the gaps)
- **Common fate** – objects that appear to be moving in the same direction are associated with one another

The recurring theme in this theory of pattern perception is that we try to resolve what we see in the simplest manner possible. Gestalt psychologists would call this the law of *prägnanz* (translated this means pithiness). The relevance of this to thinking about visual complexity, is that the way in which these features are organized may be effective in either reducing or contributing to the inherent level of complexity in visual imagery.

### 2.4.3 Visual Complexity

The term ‘complexity’ merits some consideration. We can examine visual complexity from at least two perspectives. It can be described as the methods used to represent visual imagery (from the schematic to the photo-realistic); however we can also think of it as the interaction between germane and extraneous information. Tufte (1983) would call this the data-ink ratio. This refers

\(^3\)The principle of Connectedness was not actually proposed by Gestalt theorists, but it has been suggested that it was overlooked. It is considered to be one of the strongest grouping principles; more so than, colour, size, shape, or proximity (Ware, 2004).
to the proportion of “ink” that is used to present information compared to the total amount of “ink” used in the entire visual display. These two perspectives are closely related, for both should be guided by the pedagogical roles that they are intended to fulfill. In other words, the degree of realism with which an image is rendered and the amount of information that it contains together determine the level of visual complexity in a visual display.

The literature examining visual complexity, as it relates to pedagogical impact, is largely informed by Dwyer’s Program of Systematic Evaluation (cf. F.M. Dwyer, 1972, 1978, 1987; Joseph, Francis M Dwyer, & Taylor, 1984). Founded in the 1960’s, Dwyer’s program examined the types of visual materials best suited to facilitating student achievement of specific learning goals when the learning material is presented in different instructional formats. Since its inception, over 50,000 students have been involved in this program making it the most comprehensive of its kind. Dwyer’s findings have been distilled into a set of principles to guide the use of instructional media in teaching. Below are listed select examples:

- Learners with higher I.Q.’s learn more from complex visual displays
- Achievement is enhanced when embedded cueing strategies are integrated into computer-based instruction
- Colour-coding improves attention, learner motivation, and the formation of structure in memory
- An increase in the amount of realistic detail contained in an illustration will not produce a corresponding increase in the amount of information a student will assimilate from it (in some instances hi fidelity representations may overwhelm the student).

Dwyer’s research often made comparisons between didactic images rendered at various levels of complexity. For example, in a study examining students’ understanding of the anatomy of the
heart, the authors uses as stimuli a line drawing and a photograph of the heart (Joseph & Dwyer, 1984), both depicted in cross-section (Figure 3).

![Figure 3](image)

*Figure 3. Sample visuals used by Joseph and Dwyer to assess tenth grade students’ understanding of heart anatomy. Students were exposed to either a schematic illustration (left) or a photograph (right) or a hybrid of both (not shown).*

The authors found that students with a low level of general knowledge of the subject may find all types of visuals equally effective, students with moderate and high levels of knowledge of the subject may benefit from realistic visualizations and/or illustrations that integrate abstract and realistic visualization. This is dependent upon the pacing of the instruction and the type of instructional objective (Joseph & Dwyer, 1984).

The visual representations used by Dwyer and his colleagues are not without problems, which may have influenced the outcome of the author's research. One concern is that the number of
visible features varies dramatically between the representations, with neither the schematic illustration nor the photo realistic representation clearly depicting all of the features. In additional to variability in the information described, there is the issue of visual fidelity and level of abstraction. The line drawing is extremely unrefined and the photograph of the dissected heart does not depict structures clearly (in part this is due to the angle of dissection and in part a reflection of the quality of the photo). The illustrations generally fail to support these learning objectives (which were to highlight heart valves and explain blood flow through the heart). Unfortunately, the quality of the stimuli used in these experiments undermine the sheer magnitude of Dwyer's efforts.

Beyond the work of Dwyer, there have been very few research studies that explore the role of realism or added visual complexity in teaching scientific concepts. In a recent study by Scheiter et al. (Scheiter, Gerjets, Huk, Imhof, & Kammerer, 2009), the authors found that schematic representation was more effective than realistic depiction in teaching mitosis (cell reproduction) to a class of undergraduate students. In the two experiments reported by the authors, students watched either a video of mitosis (shot through a microscope) or a schematic animation of the same process. Both dynamic visualizations were segmented to depict the six stages of mitosis, with short pauses between each stage. The authors found that regardless of learning objective or the task assigned to measure learning outcomes, students exposed to the schematic visualization outperformed the students who watched the realistic version. This contradicts Dwyer’s (eg. Joseph & Dwyer, 1984) findings, which suggest that the relationship between performance and learning objectives is impacted upon by mode of visual representation.

There may be a number of explanations for the differences in the findings of these two groups. To begin with, Dwyer’s research program was primarily concerned with the interrogation of
static imagery, whereas Scheiter’s cell mitosis study examined dynamic media. It is possible that many of Dwyer’s recommendations for the presentation instructional media cannot be extended to animated media. However, a more likely explanation has to do with the disparities between stimuli used in the Scheiter study. The schematic version of the animation is highly refined, and assiduously rendered in line. To use Tufte’s (1983) standard for measuring the effectiveness of a didactic graphic, it contains a very high data-ink ratio (to subtract any of the ink would take meaning from the content). By contrast, the “realistic” treatment has a very low data-ink ratio. As the authors note, the microscope recording contains much extraneous information “many irrelevant static as well as dynamic details were depicted in the animation (e.g., irrelevant particles floating around the nucleus)” (p. 485). As well, the video was of very low contrast, making it difficult to distinguish details. This study does not confirm the superiority of schematic media over photorealistic media so much as it demonstrates the potential of a well designed visualization to foster understanding.

Those who argue in favour of a schematic approach to depicting complex concepts do so on the grounds that it reduces cognitive load. Proponents of a realistic approach to depicting phenomena would argue that it is a more faithful and true-to-life form of representation. It is true that there are advantages and disadvantages associated with either, but it is context dependent. There are many instances of students misinterpreting or interpreting literally schematic representations of scientific phenomenon, just as there are instances of students being overwhelmed by the complexity of visual representations. The question is not whether simple or complex visualizations help learning, but rather it is a question of identifying the specific circumstances under which learning with one or the other is more helpful. The thematically relevant features of visualizations must relate to the learning objectives. In some cases, no single visualization can address all of the learning objectives.
A possible alternative to seeking the single most effective media modality or representational style for students, is to utilize multiple external representations (Ainsworth refers to these with the acronym MERs) for teaching difficult concepts. There has been some research that suggests students might benefit more from access to multiple external representations when learning about complex phenomena (Ainsworth & VanLabeke, 2004; Kozma, 2003; Rundgren & Tibell, 2009; Vandermeij & Dejong, 2006). Figure 2 in this paper is an example of multiple external representations depicting different aspects the same protein structure. More often however, these groupings include representations that combine a wide variety of visual modalities (for example charts, illustrations, diagrams, and animations might all be used to convey different aspects of a phenomenon).

Ainsworth (2006) has proposed a framework for categorizing the effectiveness of different external representations. Ainsworth’s DeFT (Designs, Functions, and Tasks) framework is based on the premise that the effectiveness of multiple representations is best understood when one considers three core aspects of learning: "the design parameters that are unique to learning with multiple representations; the functions that multiple representations serve in supporting learning and the cognitive tasks that must be undertaken by a learner interacting with multiple representations" (p. 184). Multiple external representations have the potential to support deeper understanding by providing multiple complementary perspectives on the same subject matter. However, there is some evidence suggesting that students may have difficulty in relating the different representational formats to one another (Kozma, 2003; W Schnottz, 2003; Schwonke, Berthold, & Renkl, 2009). In part this may be because of the perceptual demands placed upon the learner, who is exposed to multiple different forms of information at once.
2.5 The Role of Three-Dimensional Visualization in Science Education

Visualization involves the use of external representation (for example, interactive visual displays or simulations) in order to clarify abstract concepts (Uttal & Doherty, 2008; Ware, 2004). Simply put, it allows us to think in visual rather than abstract or symbolic terms. Visualizations are particularly effective at highlighting conceptual relationships that would otherwise be difficult to appreciate. In order to accomplish this, these tools often integrate scaffolding cues, such as pre-attentive visual cues, interface, or content-based metaphors to help students develop a deeper understanding of scientific principles (Rapp & Kurby, 2008; Ware, 2004).

Visualization as an emergent field has tremendous potential to contribute to advancing science curricula, and it is a discipline that is rapidly expanding. Three-dimensional visualizations can be powerful tools of intuition, playing a critical role in transforming the way we think about the cellular and molecular world. In particular, they are expected to have an impact on the ability of students to assimilate complex spatial and temporal events, and studies suggest that teachers are increasingly positive in their use, acceptance, and attitudes toward these kinds of visually rich media (Schönborn & T. R. Anderson, 2006; Harris et al., 2009).

To date, however, research examining the educational impact of visualizations is both contradictory and inconclusive (Linn, 2003; Linn et al., 2008; Lowe, 2003; O’Day, 2007; Tversky et al., 2002). In addition, a significant portion of the existing professionally produced 3D cell and molecular visualizations used in the classroom lack the requisite scientific accuracy and mechanism-based design approaches that we believe are critical in the context of education. This is, in part, due to an acknowledged gap between what is known by practicing scientists and what is taught at post-secondary institutions (Howitt, T. Anderson, Costa, Hamilton, & Wright,
However, as discussed, there are difficulties associated with the design of communication tools themselves.

2.6 The Role of Dynamic Visualization – Learning From Animation

Sequential illustration has for years been an invaluable learning resource in science education. It is often used in explaining physiological, chemical, or biological processes. While this has been a helpful tool for communicating change, it relies heavily on the student’s ability to map out the connections between the illustrations. Animation would appear to be a logical means of interpolating between a sequence of static images. Animated media is particularly well suited to demonstrating temporal processes, as it can convey dynamic change over time. As well, it appeals to our natural inclination toward narrative explanations, which has great implications for learning (Bruner, 1990). While we perceive the potential educational value of animations to be great, this is not borne out by the research assessing the impact of animation upon learning. Many studies have demonstrated that animation is equal to (Rieber, 1989; Rieber & Hannafin, 1988; Sanger & Greenbowe, 2000), or in some cases less effective than, static imagery (Lewalter, 2003; Lowe, 2003; Tversky et al., 2002). This is not surprising, since a single form of educational media is not likely to be equally effective across all learning contexts. However, in part, this may also be because animation is a very general term that refers to many different forms of representation. Hence, for the purposes of this discussion, I have adopted Schnottz and Lowe’s (2008) definition of animation as “a pictorial display that changes its structure or other properties over time and which triggers the perception of a continuous change” (p. 304).

Regardless of the negative or neutral effects reported in the literature, our enthusiasm for animated media persists. Recent advances in technology, including the availability of low-cost
consumer-level software applications, have led to a dramatic increase in the development and subsequent use of animated media in the classroom. Although the psychological processes involved in learning with animated visualizations are not yet fully understood, two notable contributions from the domain of educational psychology, have helped to stimulate more informed debate about how animated representations should be used in educational materials. Richard Mayer’s *Cognitive Theory of Multimedia Learning* and John Sweller’s *Cognitive Load Theory* are discussed here.

### 2.6.1 Cognitive Theories of Learning from Animation

Richard Mayer (2005) has contributed extensively to establishing a cognitive theory describing the role of multimedia in education that is often applied to animated media; building upon assumptions of how individuals learn. Expanding upon Paivio’s (1986) theory of dual coding, Mayer firstly asserts that humans possess separate channels for processing visual and auditory information. Secondly, Mayer observes that humans have a limited capacity for the amount of information that each channel can process at one time. Lastly, he suggests that individuals learn by active engagement with cognitive processes, such as the selection, organization and integration of information (sensory memory, working memory, and long-term memory). Mayer’s cognitive theory of multimedia learning addresses both the strengths and limitations of human perception and cognition, and is closely linked to the cognitive load theory proposed by Sweller, van Merriënboer, and Paas (1998).

Sweller *et al.* describe the limitations of working memory and devise instructional techniques to facilitate the acquisition of knowledge in long-term memory. For example, animated media may be considered in terms of three categories of cognitive load. Intrinsic cognitive load would describe the inherent load associated with learning new material. Extraneous cognitive load is
associated with the form in which the instructional media is presented. Germane load too may be impacted upon by instructional design, as it is associated with the degree of working memory devoted to the construction of schemas in long term memory. More recently Paas and Sweller (2011) have expanded upon the framework that informs Cognitive Load Theory by incorporating Geary’s (2008) evolutionary account of educational psychology. Geary suggests that working memory limitations are less critical when acquiring evolutionarily relevant information than when acquiring culturally relevant information. He describes these respectively as biologically primary knowledge (that which enhances our survival and reproductive prospects) and biologically secondary knowledge (that which is explicitly taught within a culture). Paas and Sweller propose that we might leverage the systems by which we acquire primary knowledge to facilitate the acquisition of secondary knowledge. For example, primary knowledge in the form of physical gestures (embodied knowledge) may be supportive in developing secondary knowledge about concepts in math and science.

Both the Cognitive Theory of Multimedia Learning and Cognitive Load Theory have contributed greatly to describing the basic mechanisms of learning in a multimedia environment and to examining general instructional strategies that increase or decrease cognitive load. However much of this work is devoted to the evaluation of animation combined or compared with other modalities. Moreover, while the temporal aspects of animation, as it relates to holding concepts in memory, are a primary consideration in examining its impact, the visual treatment of the animated elements is largely overlooked. Considering that animations are visual descriptions of events more attention should be devoted to understanding the perceptual features embedded within the animations that support or hinder learning. Unfortunately however, the design of instructional animations, due to the lack of research-based principles, is guided primarily by intuition (Lowe & Boucheix, 2008; Ploetzner, 2004).
2.6.2 Criticisms of Animated Media

There may be a number of other explanations that would account for the reported inconsistencies in the effectiveness of animation. For example, Narayanan and Hegarty (1998) propose that animations are a form of external representation that may be poorly matched to the internal representation or mental model of the learner. They argue that animated representations are deconstructed by the viewer into simpler components. How these parts are incorporated into the learner’s mental model is highly dependent upon their prior knowledge. Problems may arise when changes either contradict or place too great a demand on the viewer. This occurs when changes are depicted concurrently rather than serially (mental animations are thought to be performed serially), or when change is depicted at too great a speed, or with too great a level of complexity. Tversky et al. (2002) would refer to this the *Apprehension Principle*. In addition to dynamic representations being overwhelming, Lowe (2004) remarks that they are sometimes “underwhelming” in their depiction of events, failing to engage the viewer. There is always a risk of passive observation on the part of the student, who fails engage in the cognitive processing that might otherwise be required by a static representation.

Animations are also criticized for contributing to misconceptions (Tversky, Heiser, MacKenzie, Lozano, & Morrison, 2008). Particularly with scientific representations students may interpret them too literally. Rather than interpreting colour, shape, and motion as symbolic or idealized depictions of structures, students perceive them as faithful representations. As well, animations are criticized for not conforming to what Tversky et al. (2002) term the *Congruence Principle*. Animations are very successful at depicting temporal processes. However, in our daily lives we often interpret temporal events as a series of discrete steps (Tversky et al., 2008). We tend to think about events hierarchically and in a stepwise fashion. Tversky (2008) proposes that “if animated events are thought of as a series of discrete steps, then the congruent way to present
them is as such” (p. 267). This raises the question of how to approach the depiction of dynamic events that may not be conceived of as a set of discrete steps. It may be misleading to decompose these events into a sequential series. In other words, how can we best represent complex dynamic events that do not conform to our everyday experience and for which there is not a naturally delineated, hierarchical structure?

2.6.3 Emergent Views on Learning from Animation

More recently researchers such as Ainsworth (2008; 2004) and Lowe (2004; 2008; 2008b; 2011) have begun to explore the ways in which we do learn from animation that might in-turn inform how animation should be designed in order to support learning. Ainsworth has closely studied the role of multiple representations in learning dynamic concepts. She argues that animation may be much more effective when combined with other forms of dynamic representation. For example time-persistent representations show a range of values over time; time-implicit representations similarly show value ranges without reference to time; and time-singular representations depict a single point in time. Ainsworth suggests that by using multiple representations to depict dynamic phenomena, the student is provided with the opportunity to integrate and coordinate more than one source of information. She notes however, that multiple representations are only successful insofar as the learner is able to establish relationships between them (Ainsworth & VanLabeke, 2004). In discussing the specific role of animation in supporting learning, Ainsworth (2008) argues that current research “needs to be supplemented by a more integrated theoretical account of role of animation, which acknowledges there are multiple levels of explanation that must be applied” (p. 41). Ainsworth identifies 6 levels of explanation that should inform our understanding of the role of animation in learning, namely a)
expressive, b) cognitive, motor and perceptual, c) affective and motivational, d) strategic, e) metacognitive and f) rhetorical. These are described in greater detail in the following table.

Table 1.
*A Summary of Ainsworth’s Six Explanatory Levels of Learning from Animation.*

<table>
<thead>
<tr>
<th>Explanation</th>
<th>Definition</th>
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</thead>
<tbody>
<tr>
<td><strong>Expressive</strong></td>
<td>Expressive explanations focus on how the inherent properties of a dynamic representation may affect the effort required to make inferences from it. As such, it is independent of the nature of the interpreter.</td>
</tr>
</tbody>
</table>
| **Cognitive, Motor, and Perceptual** | This level of explanation focuses on the interaction between the form of a representation and an individual's capacities, knowledge, and skills:  
  a) Informed by cognitive theories of learning from animation  
  b) Characterized by theoretical views of embodied learning  
  c) Guided by the interaction between the human visual system and the basic perceptual characteristics of the animation |
| **Affective and Motivational** | These explanations address the affective and motivational benefits of animation that may contribute to learning                                    |
| **Metacognitive**            | This level examines the relationship between the form of representation and its impact upon the learner's engagement in metacognitive thinking. It is informed in-part by the cognitive level explanations. |
| **Rhetorical**               | Rhetorical explanations are concerned with how animations may influence learning when people are learning in social situations               |

For the purposes of the present study we will limit our discussion to explanations relating to the perceptual and cognitive affordances of animation that contribute to metacognitive thinking.

Much of the research examining the impact of animation, Ainsworth remarks, is based upon the
cognitive level of explanation. Both Mayer’s Theory of Multimedia Learning and Sweller’s Cognitive Load Theory are examples of this. It is surprising, however, that so little attention is paid to the perceptual aspects of animation. Given that animation is a visual medium and that a very large portion of the brain is devoted to making meaning of visual stimuli\(^4\), it seems reasonable to suggest that the visual representation of dynamic concepts can play a very large role in the development of understanding and by extension metacognitive thinking.

Examining the basic perceptual characteristics of animation has become an area of discussion in recent research by Lowe and Schnotz (2008b). The authors argue for a unified approach to examining both animations and images. The basis for this argument is rooted in the observation that animations and pictures are psychologically more similar than they are different. Both are processed by the same perceptual and cognitive system. If we consider the visual attributes that are pre-attentively processed by the brain, the remaining features (with the exception of motion) are evident in both static imagery and animation. Of course, we mustn’t ignore the fact that motion is a powerful preattentive feature, capable of conveying meaning, intention, and emotion (Ware, 2004). The point here is that we may apply these theories of pattern recognition and preattentive processing, usually reserved for static imagery, to the design and evaluation of animated representations.

Lowe and Boucheix (2008) have proposed a preliminary theoretical framework describing learners’ perceptual and conceptual processing of animation. The model is divided into five

\(^4\) It is estimated that over 40% of the human cortex is devoted to visual processing. The human eye and the visual cortex together form a massive parallel processor that provides the "highest-bandwidth" conduit into human cognitive centres. Perception and cognition are closely interrelated at higher levels of processing. (Ware, 2004).
phases, each characterizing a level of processing involved in creating a dynamic mental model of an event or process (described in Table 2).

Table 2.  
_A Summary of Lowe and Boucheix’s Five Phases of Learning from Animation._

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>1 Localized Perceptual Exploration</td>
<td>Initial perceptual parsing of a complex animation into the individual event units.</td>
</tr>
<tr>
<td>2 Regional Structural Formation</td>
<td>Emergence of broader scale regional structures due to the formation of relationships between the individual event units</td>
</tr>
<tr>
<td>3 Global Characterization</td>
<td>Development of a comprehensive internal characterization of the animation in terms of its component operations across space and time</td>
</tr>
<tr>
<td>4 Functional Differentiation</td>
<td>Interpretation of events in terms of the referent’s central purpose and characterization of events into functionally distinct action sets</td>
</tr>
<tr>
<td>5 Mental Model Consolidation</td>
<td>Formation of a high quality, dynamic mental model that can explain the system’s behavior across a variety of circumstances</td>
</tr>
</tbody>
</table>

The first three phases of this model are concerned with bottom up perceptual processes. During the first phase the viewer experiences the preattentive features of the animation. Due to the particular combinations of visuospatial and temporal properties features exhibit at any instant, they become more or less conspicuous. This informs the second phase during which the viewer perceives similarities and differences among the units, forming related perceptual groupings. During the third phase of processing the viewer begins to form an internal model characterized by integration of the various groupings into a more coherent global system. Phase Three allows
for inclusion of some features that were unattended to in earlier phases because of their relatively low perceptual salience. However prior knowledge and expertise may be essential to extracting information at this level. With Phase four the focus shifts from perceptual processing to the top-down interpretation of events and consolidation into an efficient functional mental model. At this point without prior knowledge the novice is likely to struggle, unable to hierarchically organize events within the system. Understanding the functional purpose of events depicted is essential to building the predictive and flexible mental model that is the hallmark of Phase five. Mental model consolidation involves the transformation of a Phase four level model into one of sufficient quality to cover the full range of performance across a wide range of circumstances.

As Lowe and Boucheix have noted, this model is still very preliminary and not yet fully elaborated. However, it provides a perspective on learning from animation that is conspicuously absent elsewhere in the literature. With the exception of Ainsworth’s “perceptual explanation” of learning from animation, the majority of models describe learning from within a cognitive framework, essentially skipping over the perceptual learning that occurs from this visual medium. Where Lowe and Boucheix’s model needs elaborating however, is in that it is not as well suited to describing natural, highly dynamic systems as it is to characterizing mechanical events. As the authors note, the model is evolved from their detailed studies of animation depicting the functional stages in the operation of an upright piano. In addition to depicting a mechanical cycle, the animation is schematically rendered (it identifies the parts of the piano mechanism with minimal detail, highlighting relationships between the parts). It would be interesting to determine if the model is sustainable when used to measure more visually complex systems (for example those containing greater surface detail, tonal variation, and colour).
Nevertheless, Boucheix and Lowe’s framework is a good starting point for examining the perceptual affordances of animation and how they inform the development of mental models.

2.7 The Present Study

2.7.1 Rationale

The two experiments reported in this dissertation are guided by several questions that, due to a lack of an overarching theory on the role of visual complexity in animation for learning, were inspired by a variety of sources. These include Sweller’s cognitive load theory (Sweller et al., 1998), Mayer’s cognitive theory of multimedia learning (2005), the comprehensive research program headed by Dwyer (F.M. Dwyer, 1972, 1978, 1987; Joseph & Dwyer, 1984), Scheiter’s exploration of realism in dynamic visualization (Scheiter et al., 2009), Lowe’s (1999, 2003, 2004, 2007) numerous investigations of animated representations and complex subject matter, and Lowe and Boucheix’s (2008) theoretical framework for the processing of animation. This study will investigate the role of animation from a number of perspectives. This will focus largely on characterizing the learning that takes place when students interrogate a dynamic representation depicting complex molecular concepts. This will also entail an examination of the perceptual and cognitive affordances of animation in supporting students’ learning of these concepts.

2.7.2 Guiding Questions

This research will attempt to address the following questions:
1. Is the complexity continuum an appropriate predictor of instructional effectiveness, or are there points along the continuum at which further increases of complexity in dynamic representations impact negatively upon student achievement of specific educational objectives?

2. Does a student's prior knowledge in a content area influence ability to benefit from visualized instruction?

3. Is selective use of preattentive variables (namely colour, contrast, and motion) in visuals an important instructional feature in guiding attention toward more thematically relevant structures?

4. To what extent does perceptually salient information contribute to informing the development of a mental model of events depicted?
3.0 METHODS

3.1 Overview

Visually rich learning environments tend to be highly complex, containing a number of interacting variables. This poses a significant challenge when one attempts to assess the impact of visual representations upon learning. The standard approach to managing this complexity is to strictly control the manipulations to the variables being compared (Dwyer, 1978, 1987; Harskamp, Mayer, & Suhre, 2007; Joseph & Dwyer, 1984; Mayer & Moreno, 1998, 2002). While carefully controlled experimental studies can yield statistically reliable results, the picture they paint of the phenomenon being studied is often incomplete. Direct investigations of the cognitive and perceptual processes underlying learning are somewhat rare. More recently however, there has been an increase in approaches to evaluation that combine quantitative techniques with qualitative measurements to better characterize these aspects of the learning process (Jarodzka, Scheiter, Gerjets, & van Gog, 2010; Kriz & Hegarty, 2007; Lowe, 2003, 2004; Lowe & Boucheix, 2011; van Gog, Paas, & Van Merrienboer, 2005).

The research described in this dissertation offers an explanatory mixed methods investigation examining the role of animated representations in the development of understanding dynamic molecular events. The explanatory design model is a two-phase approach to evaluation, in which collection and analysis of quantitative data is followed by subsequent collection and analysis of qualitative data (Creswell & Plano Clark, 2007). The two studies comprising this investigation were undertaken during the Fall term of 2010 and during the Spring term of 2011. The first of these studies examines the role of animation from within an experimental framework. The second study seeks to elaborate upon the findings of the first study by further exploring this
topic from a qualitative perspective, as informed by verbal reports, test scores and physiological measures (tracking eye movements).

3.2 Experiment One

3.2.1 Research Design

This experiment was structured as a repeated measures design with a between-subject factor. At three time points the researcher examined the relative effectiveness of three-dimensional animation for learning about molecular biology, specifically protein conformation and molecular motion in association with a membrane receptor binding event. Student participants were randomly assigned to one of four animated treatments. Increasingly complex versions of the same receptor-ligand binding event were depicted in each of these treatments. It was hypothesized that, as a result of viewing simpler animated treatments (Versions 1 and 2), students would perform better on straightforward questions testing basic concepts such as “A binds to B”. The opposite was expected to be true when students were asked about more difficult, abstract concepts relating to the random nature of molecular binding events (where the simpler versions do not provide any visual cues of such behavior while the more complex ones do). An objective of this study was to examine the role of the more complex animated treatments (Versions 3 and 4) in supporting students learning; whether they helped or hindered understanding (given their high level of visual complexity). A second objective in designing this study was to explore how different visual variables, across the four animated treatments, map to the students’ performance on test questions that ranged from more straightforward fact-based to more abstract intuitions of protein behavior at the molecular scale.
3.2.2 Participants

The participants in this study were 131 undergraduate biology students (aged 18-24; 35 males, 93 females, and 3 students of undeclared gender) from University of Toronto Mississauga (UTM). The distribution of males to females enrolled in this study is representative of the student population in Biology at UTM. While the department does not track the gender of registered students, it is anecdotally reported that the majority of students are female (Yen Du, Biology Undergraduate Advisor, personal communication, May 24, 2011). All participants were English speakers with normal (n=72) or corrected-to-normal vision (n=59), in either the first (n= 19), second (n=52), third (33), or fourth (n=27) year of study. Participants each received a 20 Dollar gift certificate at the University of Toronto Bookstore upon completing the study. All students had a basic understanding of cell biology. A condition of enrollment in the study was the completion of a first year introductory cell biology course. It was important that students have a basic understanding of cell biology in order to benefit from viewing the visualizations.

3.2.3 Recruitment

In order to ensure that an adequate number of students were reached, four different methods of recruitment were utilized in this study. Firstly, an announcement was posted on the University of Toronto Portal to students enrolled in Biology at UTM. As well, the same announcement was posted on the Erindale Biology Society Facebook® page (Appendix A). Secondly, recruitment posters announcing the study were displayed in the Biology department at UTM and throughout the Communication, Culture, and Technology (CCT) Building at UTM (Appendix B). Thirdly a slide presentation (Appendix C) was made at the start of three classes, BIO 206H5F (Introductory Cell and Molecular Biology), BIO315H5F (Human Cell Biology), and BIO
372H5F (Molecular Biology), all of which were offered during the fall semester at UTM. Students interested in participating were encouraged to respond by email to biovis.study@gmail.com. Those students who did respond to recruitment efforts received a detailed email response describing the requirements of the study, location, and timeframe during which the study would be held (Appendix D). Participants were then invited to select a time slot (these were scheduled over a two week period and staggered in five minute intervals to avoid line ups and delays in seating students) and to report to CCT Rm. 3160 at the appointed time to participate in the assessment.

3.2.4 Informed Consent

Informed consent to participate in this study was obtained in writing. Students were assigned a random number and given an information and consent form describing the study (see Ethics Approval and Consent Appendix E), their role in the study and their right to withdraw from the study at any time without notice. Students who agreed to participate in the study were asked to sign the consent form in duplicate, receiving a copy of this form for their own records.

3.2.5 Materials and Measures

3.2.5.1 Stimulus Materials: Animations Used in this Study

In the initial planning stages the researcher and a committee member (Gaël McGill) identified three visual variables (in addition to protein conformation) that pose particular difficulty for students: 1) Brownian (random) motion; 2) Molecular crowding; and 3) Depiction of molecular water. It was determined that these three features would be introduced additively to each of the four animated treatments. The visual treatments were subsequently mocked up in a storyboard (see Appendix F).
3.2.5.1.1 Subject Matter

The animations used in this study were developed in the Center for Molecular and Cellular Dynamics at Harvard Medical School. Stem cell factor (SCF) ligand and the receptor tyrosine kinase (KIT) were used as a classical example of a ligand-induced receptor dimerization and activation event (Figure 4). These animations may be viewed and downloaded at the following website address: http://www.molecularmovies.com/bindingstudy/.

![Static frames from treatment 2 (random protein motion, receptor conformational flexibility and membrane fluidity) that show the different stages of binding and receptor activation.](image)

- a) ligand in the extracellular space, receptor as monomers;
- b) ligand approaches membrane, conformationally flexible receptors diffuse in the plane of membrane;
- c) ligand randomly encounters and binds a receptor monomer, no receptor activation occurs;
- d) ligand-bound and -unbound receptors continue to diffuse, trimer forms, no immediate activation occurs;
- e) ligand-induced tether of receptor extracellular domains allows cytoplasmic tails to make contact; and
- f) contact between cytoplasmic tails leads to cross-phosphorylation and receptor activation.

**Figure 4.**

Stem Cell Factor is a type of cytokine or signaling molecule used extensively in intercellular communication. Each cytokine has a matching cell-surface receptor. KIT is expressed on the surface of hematopoietic stem cells (stem cells that give rise to different blood cell types) and is the cell-surface receptor for SCF. Altered forms of KIT are associated with some types of
cancer. When SCF binds to the ectodomain of KIT it drives the dimerization of KIT, activating signal transduction within the cell. This plays a role in cell survival, proliferation, and differentiation.

3.2.5.1.2 Subject Matter Accuracy

The overall shapes of KIT and SCF proteins are relatively simple (in comparison to other more complex receptors), making them suitable teaching examples. As well, a recent crystallographic study provided us with the accurate conformation of both the ligand-bound and -unbound states of KIT (Yuzawa et al., 2007).

3.2.5.1.3 Technical Properties

Using the 3D software Autodesk® Maya® in combination with the Molecular Maya (mMaya) toolkit, the animator was able to: 1) Import the Protein Data Bank (PDB) crystallographic datasets for both unbound and ligand-bound states of the receptor; 2) Create a surface mesh representation of SCF; 3) Rig the receptor to convey the conformational changes to occur; and 4) Animate not only the proteins moving relative to one another in the scene, but also the motion of individual domains within KIT (as it undergoes ligand-induced conformational changes). The animations were rendered and then imported to Adobe® After Effects® where colour and additional lighting effects were composited. Animated movies were exported from After Effects® as QuickTime® .MOV formatted videos at a screen resolution of 720 x 540 pixels and a play rate of 30 frames per second. The timing and framing of the event were treated consistently across the four animations. Each of the four animations was 20 seconds in duration. With each successive treatment, additive layers of visual complexity were integrated (shown in Figure 5).
3.2.5.1.4 Perceptual Properties

A series of test renders was completed in order to determine the final rendering. Given the increasing level of complexity depicted in each of the representations, it was decided to reduce the level of visual complexity (without compromising the detail) by rendering the environment using a lighting effect called ambient occlusion. This technique produces soft diffuse lighting in open spaces, giving uniform lighting to the scene and reducing the overall contrast. It was also decided to focus the viewers’ attention on the primary narrative (the binding of SCF to KIT and
subsequent dimerization of KIT) by limiting the use of colour to the protein receptor and ligand, thus heightening their perceptual salience.

3.2.5.2 Assessment Materials

3.2.5.2.1 Participant Survey

A participant survey (Appendix G) containing six questions was used to collect background information from participants regarding their age, sex, visual acuity, year of study, major area of study, and specific details about courses completed.

3.2.5.2.2 Knowledge Assessments

The three test instruments used in this study were developed based on a review of popular North American textbooks, and in particular, those texts used at University of Toronto (Alberts et al., 2009, 2002; Lodish, 2007). Moreover, questions were reviewed by a member of the researcher’s supervisory committee, Gaël McGill, who holds a PhD in Cell and Molecular Biology. Each of three tests (Pre-test, Post-test, and Delayed Post-test) used in this study, were comprised of 10 short answer questions. This testing format was used to discourage students from guessing at the right answer. The test instruments were piloted on four students and questions were modified based upon student feedback. The tests assessed students’ understanding in three areas: 1) protein conformation; 2) molecular motion; and 3) molecular crowding (including the role of molecular water). Each test included questions to measure both students’ surface level understanding and their deep level understanding. A subset of questions across the instruments were isomorphinc, while others were designed to begin assessing students’ ability to infer broader concepts from the content of the animations. Indeed, both the Post-test, and Delayed Post-test included questions that were more predictive in nature and intended to measure students near
transfer of knowledge. Examples of each question type are included in Table 3 (The complete test instruments are included in Appendices H - J).

Table 3.
Examples of Assessment Questions Included in Each of Three Test Instruments.

<table>
<thead>
<tr>
<th>Level of Measurement</th>
<th>Question</th>
<th>Test Instrument</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surface</strong></td>
<td>Proteins are inherently rigid structures. Is this statement true or false? Please explain. Pre-test</td>
<td></td>
</tr>
<tr>
<td></td>
<td>How many functional units or domains would you say [KIT] has? Post-test</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Would you describe the movement of molecules through the extracellular space as random or directed? Please explain. Delayed</td>
<td></td>
</tr>
<tr>
<td><strong>Deep</strong></td>
<td>What are the forces that contribute to the conformation of a protein? Pre-test</td>
<td></td>
</tr>
<tr>
<td></td>
<td>How is the binding of the ligand SCF to the receptor KIT mediated? Please explain. Post-test</td>
<td></td>
</tr>
<tr>
<td></td>
<td>How does SCF drive the dimerization of KIT? Please explain. Delayed</td>
<td></td>
</tr>
<tr>
<td><strong>Transfer</strong></td>
<td>What do you think would happen if the temperature were increased in this environment? Please explain. Post-test</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Knowing that temperature impacts upon water vibration what do you think would have happened in the animation you viewed if the temperature had been decreased? Please explain. Delayed</td>
<td></td>
</tr>
</tbody>
</table>

3.2.5.2.3 Assessment Equipment

The study was held in a computer lab on the third floor of the Communication Culture and Information Technology (CCT) Building at University of Toronto Mississauga. The lab was equipped with 40 - 21.5-inch display Apple® iMac® computers, with a screen resolution of 1920 x 1080 pixels (see Figure 6). Each CPU was equipped with 4 GB RAM and a 3.06GHz Intel Core 2 Duo processor. Animations were played using QuickTime® and displayed at their native resolution of 720 x 540 pixels.
3.2.6 Procedure

The design comprised four instructional conditions, each described in Figure 7. Students were randomly assigned to one of four treatments (Group one = 35, Group two = 31, Group three = 33, Group four = 32). Each participant was assigned a random number and received an instructional package explaining the study, a request for written consent, and a Background Information form. Students were assessed individually and each assessment took approximately 40 minutes. There were no time limits imposed for either completing tests or viewing the animation. Each participant completed a pre-test, for which no feedback was provided. The Pre-test served as a baseline measure for ensuring equivalent prior knowledge in each of the four groups.
Participants then viewed one of four variants of the animation. Students were given a brief description of the subject matter (Appendix K), informed that the animation did not include any identifying labels or audio and that this was deliberate. Students were instructed to view the animation as many times as they desired. Upon viewing the animation, students were asked to raise their hands, and the animation file was closed. They were then given a Post-test assessing their factual and conceptual understanding of molecular binding events. Two weeks following the assessment students were sent an email (Appendix L) providing them with login information and instructing them to complete an online Delayed Post-test (in Blackboard®, consisting also of ten short-answer questions. Upon completion of the study students were invited to collect a 20 Dollar University of Toronto Bookstore gift certificate.
3.2.7 Analysis

Students’ answers at all three time points were scored as correct (1 point) or incorrect (0 points) out of a possible 10 points for each test. The researcher and a committee member developed a preliminary Answer Key for grading tests. We then graded 10 copies each of the Pre-test, Post-test, and Delayed Post-test. Test results were compared for consistency in grading. Any cases of disagreement were resolved by negotiation and the Answer Key was revised accordingly (see Appendix M). Background information and test results were entered into a spreadsheet and imported to IBM® SPSS® Statistics for analysis. A repeated-measures ANOVA with time as the within-subjects factor, test scores as the dependent variable, and group assignment as the between-subjects factor, was conducted to measure the impact of treatment over time. To fulfill the assumptions of the repeated-measures ANOVA, Levene's Test for homogeneity of variance and Mauchly's Test for the compound symmetry of the variance-covariance matrix were also conducted. In addition, follow-up pairwise comparisons were conducted to determine which of the means differed significantly from one another.

3.3 Experiment Two

3.3.1 Research Design

The second experiment was intended to elaborate upon the findings of the first experiment. Whereas the aim of experiment one was to quantify learning in relation to visual complexity, the aim of the second experiment was to characterize the learning that occurred across each of the four animations by collecting data on thought process (as captured by verbal reports), and perceptual processing (eye tracking measurements). In this scenario student participants viewed
each of the four animations either in sequential order from least to most complex, or in reverse sequence. The rationale in establishing two methods for viewing the animation was to ensure that the viewing order did not bias qualitative data collection measures (verbal reports and eye tracking measurements). The multiple data sources (test scores, verbal reports, and eye tracking measurements) are analysed in combination to increase their validity, thus aiming at methodological triangulation (Denzin, 1970). In this way, eye tracking records can be matched to the verbal reports and compared against responses to specific test questions.

3.3.1.1 Verbal Protocols as a Method of Assessment

Using verbal reports as data follows the basic assumption that an individual’s thought process can be verbalized as they move through the problem space. Verbal reports, such as concurrent (‘think aloud’) and retrospective reporting are among the most widely used techniques for eliciting and describing cognitive process (Ericsson & Simon, 1993). With the method of concurrent reporting, participants are instructed to ‘think aloud’, that is, verbalize everything that comes to mind, while they are working on a task, problem solving, or responding to a prompt. Verbal protocols allow for making inferences about cognitive processes, but to ensure validity of those inferences, the wording of verbalization instructions and prompts is crucial (Ericsson & Simon, 1993). Verbal reports are used increasingly in educational research examining the role of visual representations, in particular animation, upon learning (Haak Van Den & Jong De, 2003; Jarodzka et al., 2010; Kriz & Hegarty, 2007; Lowe, 1999; de Koning, Tabbers, Rikers, & Paas, 2010; van Gog & Scheiter, 2010; van Gog et al., 2005). For example, it is used by Jarodzka et al. (2010) to analyse expert and novice strategies in interpreting videos of fish locomotion. Kriz and Hegarty (2007) also use verbal reports to investigate top-down and bottom-up processes contribute to learning from animated displays of mechanical processes.
3.3.1.2  Eye Tracking as a Method of Assessment

Eye tracking is used to record eye movements that occur when an individual is exposed to a visual environment. Eye movement is usually divided into fixations (maintaining gaze on a single location) and saccades (continuous movement of the eye). Eye tracking measurement is often used in combination with concurrent or retrospective verbal reports, and it is well suited to providing a detailed account of attentional processes. It offers a unique opportunity to contribute to understanding a subject’s perceptual processing during learning. Eye tracking measurements have been incorporated more and more into the educational assessment of external visualizations upon learning (For a review of the uses of eye tracking in educational research see Duchowski, 2007; for commentaries specific to visualization see Hyönä, 2010; Mayer, 2010; Scheiter & Van Gog, 2009). For example, eye tracking has been successful in measuring the use of visual cues in animation (Boucheix & Lowe, 2010; Fischer & Schwan, 2009; Lowe & Boucheix, 2011; de Koning et al., 2010); or evaluating the impact of multiple representations upon learning (Schwonke et al., 2009). As well it has been effective in assessing the attentional biases of experts and novices (van Gog et al., 2005; Jarodzka et al., 2010).

3.3.2  Participants

The participants in this second study were 8 undergraduate biology students (aged 18-24; 2 males and 6 females) from University of Toronto Mississauga (UTM). All were English speakers with normal (n=5) or corrected-to-normal vision with contact lenses (n=3), in either the first (n=1), second (n=3), third (2), or fourth (n=2) year of study. Participants each received a 20 Dollar gift certificate at the University of Toronto Bookstore upon completing the study. As with the first study, all students had a basic understanding of cell biology.
3.3.3 Recruitment

Students who expressed an interest in participating in the first study, but who could not because of scheduling constraints, were contacted by email and asked if they would be interested in participating in this second study. Since the study sample was small (eight participants in total) students were recruited according to year of study, in order to ensure representation across all four years. Since the assessment protocol included measurement of eye movements, an additional consideration in recruiting participants was whether or not a student wore corrective lenses (eyeglasses). Eyeglasses are known to disturb eye tracking systems, by producing reflections or “glare” which interfere with the detection of the pupil by the system.

3.3.4 Informed Consent

As with Experiment 1, informed consent was obtained in writing. Students were assigned a random number and given an information and consent form describing the study, their role in the study, and their right to withdraw from the study at any time without notice. Students who agreed to participate in the study were asked to sign the consent form in duplicate, receiving a copy of this form for their own records.

3.3.5 Materials and Measures

3.3.5.1 Stimulus Materials

The animations used in Experiment One were used again in this second experiment. The files were combined into one file using QuickTime® Pro, in order from the least complex to the most complex (Versions 1 through 4). A second file was compiled containing the same four animations, ordered from the most complex to the least complex representation (Versions 4
through 1). Students assigned to Group A viewed the animations in order, while students assigned to Group B viewed the animations in reverse order.

3.3.5.2 Assessment Materials

3.3.5.2.1 Background Survey and Knowledge Assessments
The Background Information Form (Appendix G) used in Experiment One was used again in this assessment, as were the Pre-test (Appendix H), and Post-test (Appendix I).

3.3.5.2.2 Verbal Reports
Verbal reports were elicited concurrent with viewing the animations (Ericsson, & Simon, 1993). The goal of the think-aloud procedure was to document thought processes as they occurred and thus capture the learners' thoughts as they engaged with the animations. Verbal reports were recorded with an iPhone® 4 and an attached microphone.

3.3.5.2.3 Eye Tracking Apparatus and Measures
Participants’ eye movements were recorded with a 60Hz video-based remote eye-tracking device from Mirametrix with an angular resolution of less than 0.5 degrees (see Appendix P for a description of this system). The infrared camera was placed below the level of the monitor of the PC. The stimulus PC was a 27-inch Apple® iMac®, equipped with 4 GB RAM and a 2.7 GHz Quad Core Intel Core Processor, running Windows® XP SP2 in Boot Camp®. Two additional Sony® HVLIRM infrared light sources were placed at the level of the camera to heighten the reflective patterns on the cornea and pupil. The resolution of the unit running Windows® was set at 1920 x 1200 pixels. The size of the recording window on the screen was 720 x 580 pixels (see Figure 8).
The researcher sits opposite the participant and from a second monitor controls the eye tracking camera and configures the recording using Mirametrix Eye Tracking Viewer Software. The software records the screen activity (in this case the animation) and an overlay fixation map is drawn with fixations connected in sequence, and the duration of the fixation proportional to the size of the fixation circle drawn. The eye gaze overlay corresponds to fixation gaze, identifying fixations and saccades (rather than unfiltered raw fixations). Screen recordings of the stimulus are output at 30 frames per second as .AVI formatted files. In order to assess visual attention, corresponding dynamic areas of interest may be established within each of the four animations and data is collected on the number of fixations within each area of interest (Boucheix & Lowe, 2010; Meyer, Rasch, & Schnitz, 2010; de Koning et al., 2010).

3.3.6 Procedure

The study was held in a research office on the third floor of the Communication Culture and Information Technology (CCT) Building at University of Toronto Mississauga. The design comprised two instructional conditions, each described in Figure 9.
Figure 9. Experimental protocol for assessing how students attend to and interpret an animated depiction of a receptor ligand binding event rendered at varying levels of visual complexity.

Students were randomly assigned to one of two instructional conditions (four students in each group). Depending on the group assignment, participants either viewed the animations in order of complexity or in reverse order. Students were assessed individually and each assessment took approximately one hour. Each participant was assigned a random number and received an instructional package explaining the study and briefly describing the animation, a request for written consent, and a Background Information form. Each participant completed a pre-test, for which no feedback was provided. The Pre-test served as a baseline measure for ensuring equivalent prior knowledge in each of the two groups. Upon completion of the pre-test, Participants were seated approximately 60 centimetres in front of the display and were verbally introduced to the nature of the experiment.

Before the data collection could start a calibration procedure was completed to determine the correspondence between pupil position in the eye-camera image and the gaze position on the display shown to the participants. The researcher instructed the participants, “I am going to
record your eye movements while you view the animation on the screen, so I’ll calibrate the eye tracking system before we start. In a moment you’ll see a large red dot appear on the screen. Please follow it with your eyes as it appears at different locations on the screen”. The calibration procedure was repeated for validation.

Once the calibration was complete participants were asked to view the animations once through and instructed to, “…think-aloud your thought process without censoring anything.” Recording of eye movements was initiated. At the same time participants’ verbalizations were recorded with a microphone attached to an iPhone. The iPhone was also used to make a video recording from the screen, both for the purpose of back-up and also as a means of registering the timecode on the audio recording with the eye tracking recording (see eye tracking station Figure 8). If participants grew silent, after five seconds they were prompted to verbalize their thoughts with reminders such as “Don’t forget to think aloud” or “Tell me what you’re thinking”. Once the participants had completed viewing the animations, six\(^5\) were asked to view the animations again, only this time to verbalize their thought process in relation to “the factors that influence the binding event”. The researcher prompted the student by saying, “I’d like you to view the animations again. Only this time I want you to tell me how it is this binding event is coming about. What are the contributing factors?” After viewing the animations for the second time, participants were asked, “which of the four animations do you think is most representative of the actual binding event?” Responses to this question were recorded and then participants were given the post-test to complete. Upon completing the post-test, student participants were

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\(^5\) It became obvious after the first two interviews that students needed an additional prompt for eliciting meaningful verbalizations. For consistency sake, the remaining six participants watched the animations without additional prompt and subsequently with prompt.
remunerated for their time with a 20 Dollar gift certificate at the University of Toronto Bookstore.

3.3.7 Analysis
The pre-test and post-test were graded using the answer key provided in experiment one (see Appendix M). Test answers were scored as either correct (1 point) or incorrect (0 points) out of a possible total of ten points on each test. Background information and test results were entered into a spreadsheet and imported to IBM® SPSS® Statistics for analysis. Descriptive statistics on test scores were generated to measure the difference in mean scores between the pre-test and post-test and to provide a comparison with mean scores obtained on the pre-test by participants in experiment one. Given the small sample size, nonparametric testing (Wilcoxon) was used to evaluate the difference between pre-test and post-test scores.

The audio track from the iPhone® recording was synchronized with the video recording of eye movements by registering the time stamp on each. For all but one participant, for whom the video file was corrupted when saved, the two media were edited together in Final Cut Pro® and exported as a single video file. For the remaining participant the back-up iPhone® video served as both an audio and visual record of the session. Verbal reports were transcribed verbatim in a three-column table, with records divided into individual units of analysis, flanked by the time stamp recorded beside each statement in the left-hand column, and comments regarding the corresponding video noted in the right-hand column.

Idea units were coded in keeping with Lowe’s (1999) distinction between descriptive statements and explanatory statements (Lowe classifies these as non-causal and causal respectively). Descriptive statements make reference to activity by observing perceptually salient features
(either visuospatial or temporal changes) without attempting to account for effects observed. Explanatory statements attempt to identify causal agents or establish linkages between cause and effect. In order to ensure the validity of the coding scheme two independent raters, unfamiliar with this study, were enlisted to code four of the eight verbal reports. The raters were given an instruction form describing the coding schema (Appendix N), with definitions for “descriptive” and “explanatory” as coding categories. As well examples unrelated to the study were provided for each of the two categories. Raters were also given a coding form to complete for each of the four interviews (Appendix O). An interrater reliability analysis using the Kappa statistic was performed to determine consistency among raters.

Since an important aspect of this study involved understanding the role of visual complexity in the development of understanding, eye tracking also served as a measure of perceptual salience. In all, six areas of interest (see Figure 10) were identified, each of which corresponded to functionally relevant aspect of the narrative (Boucheix & Lowe, 2010; Kriz & Hegarty, 2007; de Koning et al., 2010). For each participant, a count was made of the number of fixations that occurred within each area of interest.

Data collected throughout this experiment (post-test responses, verbal reports, and eye tracking videos) were analyzed in relation to each other; this served to triangulate the data and to help enhance the credibility of the findings and assertions made (Lincoln & Guba, 1985)
Figure 10. Areas of Interest identified for the assessment of visual attention.
4.0 RESULTS

4.1 Results of Experiment One

4.1.1 Repeated Measures Assessment

A repeated-measures ANOVA with time as the within-subjects factor, test scores as the dependent variable, and group assignment as the between-subjects factor, was conducted to measure the impact of treatment over time. The means and standard deviations for test scores at 3 time intervals are illustrated in Figure 11 and presented in Table 4.

*Figure 11. Distributions of test scores for groups at 3 time points*
Table 4.

Means and Standard Deviations for Test Scores.

<table>
<thead>
<tr>
<th>Group Assignment</th>
<th>Mean Test Scores (and SD)</th>
<th>Pre-test</th>
<th>Post-test</th>
<th>Delayed Post-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n = 35)</td>
<td></td>
<td>3.60 (1.97)</td>
<td>3.34 (1.57)</td>
<td>3.51 (1.67)</td>
</tr>
<tr>
<td>Group 2 (n = 31)</td>
<td></td>
<td>2.97 (2.10)</td>
<td>4.23 (1.98)</td>
<td>4.61 (1.69)</td>
</tr>
<tr>
<td>Group 3 (n = 33)</td>
<td></td>
<td>3.24 (1.64)</td>
<td>5.00 (2.12)</td>
<td>4.91 (2.08)</td>
</tr>
<tr>
<td>Group 4 (n = 32)</td>
<td></td>
<td>3.06 (1.66)</td>
<td>4.88 (2.07)</td>
<td>5.00 (2.38)</td>
</tr>
</tbody>
</table>

The result of Levene’s test at Pretest (F(3,127) = 1.13, p = .339), Post-test (F(3,127) = 1.24, p = .296), and Delayed Post-test (F(3,127) = 2.58, p = .056), indicate that homogeneity of variance was not violated. As well, Mauchly's criterion for the data (0.0085, P = .096) indicates that the assumption of sphericity was not violated. The results of the repeated-measures ANOVA, summarized in Table 5, show that test scores varied significantly over time (Wilk’s Λ = .665, F(2,126) = 31.74, p < .001, multivariate η2 = .33).

Table 5.

Results of Repeated Measures ANOVA

<table>
<thead>
<tr>
<th>Effect</th>
<th>Value</th>
<th>F</th>
<th>Hypothesis df</th>
<th>Error df</th>
<th>Sig.</th>
<th>Partial Eta Squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Wilks’ Lambda</td>
<td>.665</td>
<td>31.740</td>
<td>2.000</td>
<td>126.000</td>
<td>.000</td>
</tr>
<tr>
<td>Time*Group</td>
<td>Wilks’ Lambda</td>
<td>.795</td>
<td>5.092</td>
<td>6.000</td>
<td>252</td>
<td>.000</td>
</tr>
</tbody>
</table>
4.1.2 Post Hoc Comparison of Group Performance

Post Hoc analyses (one-way ANOVA) with a Bonferroni correction were conducted to identify differences between treatment groups at each time point (Table 6 and Figure 12).

Table 6.
Post Hoc Comparisons of Post-test and Delayed Post-test Results

<table>
<thead>
<tr>
<th></th>
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<tbody>
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<td>1.88</td>
<td>.479</td>
<td>.405</td>
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<td>.154</td>
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<td>4</td>
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<td>3</td>
<td>3</td>
<td>.09</td>
<td>.490</td>
<td>1.00</td>
</tr>
</tbody>
</table>
The results showed no significant differences between groups at pre-test. There were significant differences between groups at Post-test \([F(3,127) = 5.19, p = .002, \text{partial } \eta^2 = .11]\) and Delayed Post-test \([F(3,127) = 4.10, p = .008, \text{partial } \eta^2 = .10]\). There were notable differences between treatments 1 and 2 at both Post-test and Delayed Post-test, however these results failed to reach significance. Smaller differences were also found between groups 2, 3, and 4. There were significant differences between group 1 and groups 3 and 4 at both Post-test (G1 and G3 \(p < .01\); G1 and G4 \(p < .05\)) and Delayed Post-test (G1 and G3 \(p < .05\) ; G1 and G4 \(p < .05\)).

![Figure 12. Plot comparing mean test scores over 3 time intervals](image)
4.1.3 Post Hoc Comparison of Performance on Basic and Advanced Questions

A one-way ANOVA of Post-test results by question type revealed insignificant differences in student performance on surface level (basic) questions \[F(3, 127) = 0.539, p = 0.657\]. Student performance on questions measuring depth of understanding (advanced) however, differed significantly \[F(3, 127) = 10.935, p > 0.001, \text{partial } \eta^2 = .21\]. Further post hoc analysis (Bonferroni) identified significant differences in scores on advanced questions between Groups 1 and 3 \((p < .001)\) and Groups 1 and 4 \((p < .001)\). A more detailed breakdown of these results is reported in Table 7.

Table 7.
Post Hoc Comparisons of Post-test Results by Question Type.

<table>
<thead>
<tr>
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<td>-1.51</td>
<td>.347</td>
<td>.000</td>
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</tbody>
</table>

A comparison of Delayed Post-test results by question type showed significant differences in group performance on basic questions \[F(3, 127) = 10.199, p < 0.001, \text{partial } \eta^2 = .19\]. Further post hoc analysis (Bonferroni) revealed significant differences between Groups 1 and 2 \((p = .025)\) and Groups 1 and 3 \((p < .001)\); and Groups 1 and 4 \((p < .001)\). However differences in
group performance on questions testing depth of understanding (advanced) were not significant.

A more detailed breakdown of these results is reported in Table 8.

Table 8.
*Post Hoc Comparisons of Delayed Post-test Results by Question Type.*

<table>
<thead>
<tr>
<th></th>
<th>Delayed Post-test Basic Questions</th>
<th>Delayed Post-test Advanced Questions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (I-J)</td>
<td>Std. Error</td>
</tr>
<tr>
<td>Group (I)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group (J)</td>
<td></td>
<td></td>
</tr>
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<td>1</td>
<td>.61</td>
<td>.210</td>
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<tr>
<td>3</td>
<td>-.94</td>
<td>.208</td>
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</tbody>
</table>

Test scores by year of study and group assignment were also compared. They showed a Group 1 trend toward higher scores among Year 4 students at each time point among when compared with Year 1 students. Although students in their fourth year of study achieved a higher score on these tests the results failed to reach significance.

4.1.4 Summary of Results from Experiment 1

The results of this first experiment show that the two most visually complex representations of the binding event (Versions 3 and 4) were the most successful overall in fostering students’ understanding of this process both over the short term and the long term. Test scores at Posttest and Delayed Posttest were significantly higher than those achieved by students who viewed the least complex representation of the event (Version 1). Closer examination of Posttest results by question type suggest that students in all groups performed equally on questions testing basic
concepts, but that students in Groups 3 and 4 performed significantly better than students in Group 1 on questions testing more abstract concepts. Further examination of Delayed Posttest results showed that while students in Groups 2, 3, and 4 performed significantly better than Group 1 students on basic questions, this was not the case with more complex questions. Students in all groups performed equally on these questions. In summary, increasingly complex animated representations of a molecular binding event were the most effective at informing students understanding of complex concepts over the short term and basic concepts over the long term.

### 4.2 Results of Experiment Two

#### 4.2.1 Overview

The results of the second experiment were analyzed in three phases. Firstly pretest and posttest scores were analyzed to create a baseline measure for students’ prior knowledge and level of expertise. As well pretest means were compared with experiment one to ensure equivalency in the two populations. Secondly, the eye tracking videos were analyzed for frequency of fixation in perceptually salient areas (areas of interest) within the display. Thirdly, the verbalizations were analyzed for their descriptive and explanatory content. These three phases combined served to characterize students’ interactions with the learning material.
4.2.2 Performance on Pretest and Posttest

The results of statistical analysis showed a mean score of 4.0 (SD=2.39) on the pretest and a mean score of 5.13(SD=2.99) on the posttest. These results are presented in Table 9 and illustrated in Figure 13.

Table 9.  
*Pretest and Posttest Descriptive Statistics*

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>SD</th>
<th>Variance</th>
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<td>7</td>
<td>4.00</td>
<td>2.39</td>
<td>5.71</td>
</tr>
<tr>
<td>Posttest</td>
<td>8</td>
<td>2</td>
<td>9</td>
<td>5.13</td>
<td>2.99</td>
<td>8.98</td>
</tr>
</tbody>
</table>

*Figure 13. Distribution of test scores for pretest and posttest.*

Although posttest performance was demonstrably superior to pretest performance, a nonparametric assessment (Wilcoxon Signed Rank Test) comparing performance at both time
points indicate that the differences between the pretest and posttest scores were not significant (p = .102). Finally, in comparing the mean scores of the pretest in experiment 1 (M=3.23, SD=1.85) with pretest scores in experiment 2 (M=4.0, SD=2.39), the means of the two experiments were not significantly different from one another.

### 4.2.3 Results of Concurrent Verbal Reports

#### 4.2.3.1 Results of Quantitative Assessment

A quantitative assessment of the verbal protocol data examined: 1) total verbalizations (words); 2) total idea units (statements); 3) total explanatory statements; and 4) total descriptive statements. These results are summarized in Table 10.

<table>
<thead>
<tr>
<th>Participant</th>
<th>Pretest Score /10</th>
<th>Year of Study</th>
<th>Total Words</th>
<th>Total Statements</th>
<th>Descriptive Statements</th>
<th>Explanatory Statements</th>
</tr>
</thead>
<tbody>
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<td>18</td>
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<td>3</td>
<td>123</td>
<td>12</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

*Participants 1 and 2 viewed the animations one time, while the remaining participants viewed the animations twice.*
To ensure the reliability of the coding scheme, verbal protocols were given to two independent raters, who coded a sample of four protocols each (Participants 3 through 6), on the basis of whether statements were descriptive or explanatory. There was 89% agreement between the raters. To estimate inter-rater reliability, Cohen’s Kappa was calculated at .679 (p < .001).

There was no correlation between prior knowledge (as determined by year of study and number of courses completed) and the total number of statements contained in participants’ verbal reports. Nor were the number of descriptive statements correlated with prior knowledge. However prior knowledge was positively correlated with the number of explanatory statements contained in each protocol ($r = .81$, $p < .05$). With the exception of one student (Participant 8), a third year student whose report comprised primarily explanatory statements, each verbal report contained many more descriptive statements than explanatory statements (see Figure 14).

Figure 14. Proportion of descriptive and explanatory statements contained in the verbal report of each participant.
Further investigation of explanatory statements contained within the verbal reports revealed a positive correlation between complexity of representation and frequency of explanatory statements ($r = .98$, $p < .05$). In other words, the more visually complex the animation was, the more explanatory statements it elicited (illustrated in figure 15).

![Figure 15](image)

*Figure 15.* Total frequency of descriptive and explanatory statements referencing each version of the animation.

### 4.2.3.2 Results of Qualitative Assessment

A qualitative interpretation of the verbal protocol data included an examination of both descriptive and explanatory statements. Descriptive statements were evaluated for evidence of thematic relevance and perceptual salience. Explanatory statements were assessed for evidence
of understanding in three areas: 1) conformational change; 2) random molecular motion; 3) molecular crowding.

4.2.3.2.1 Interpretation of Descriptive Statements

4.2.3.2.1.1 Verbalizations related to Perceptual Salience

Of the many descriptive statements contained within the verbal protocols, fewer than one half related to the perceptual salience of features. These statements, while descriptive in nature, did not necessarily relate to the narrative structure of the animations. Rather they were focused more on describing the visually distinct features of the animations. Many of these statements related to describing fluidity of the cell membrane, the motion of the ligand, the “background”, or the colour of the activated KIT endodomain. For example, the first four participants, who were exposed to the animations in order (1 to 4), remarked on the fluidity of the cell membrane:

P1: Cell membrane moving
P2: It seems…um…the membrane is a bit more fluid
P3: And the cell membrane is moving…it’s moving a lot
P4: Then…the layer moves the two receptors

Participant 4, who is a first year student, several times referred to “the layer”, when her gaze was fixated on the cell membrane.

Participants also made many references to the colour of the activated KIT dimer. Depending on the student’s prior knowledge these statements were elaborated in greater depth.

P4(Yr 1): The yellow thing glows
P3(Yr 2): And something lights up on the other side of the cell membrane
P2(Yr 3): And wondering what the bright light at the bottom is

P8(Yr 3): What happens at the bottom?...the yellow stuff...when it binds to the receptor?

P5(Yr 4): And again, activation of the inside as you can see by the yellow colour

A number of descriptive statements relating to the presence of other molecules outside the cell treated them as though they were some kind of “background” to the main narrative.

P2: Mmmm…I’m wondering what everything in the background is…it looks…a bit too much…my focus is more on the background than on the interaction between protein and ligand

P4: Uh…there’s a lot of things in the background

These molecules have salience due to their stochastic behavior, but they are monochromatic (unlike the KIT monomers and the ligand which have salience due to their colour coding).

In select cases statements evoked by perceptually salient features contributed to the development of explanatory statements. For example, Participant 2, who commented on the background as a detracting factor (see previous example), continued to focus on the background as her understanding about its role in the binding process evolves.

P2: My focus is more on the background than on the interaction between protein and ligand…Mmm…although the background doesn’t seem to have any influence…Mmm…wondering how the background is affecting the binding process.
Another example of this can be found in a descriptive statement by Participant 3, who remarked upon a change between Versions 1 and 2 of the animations. She noticed changes in the cell membrane and in trying to understand the nature of these visual changes, extended the idea of fluidity to the remaining extracellular space, attributing this as causing binding difficulties.

P3: And now they’re showing that the membrane is moving…I guess it’s showing inside a fluid…before it was still so now there is fluid in the cell or around the cell…so it’s having trouble to bind.

Even some of the more advanced students depended upon perceptual cues in the formulation of explanatory statements. For example Participant 5, who is advanced in terms of her prior knowledge (she is a fourth year student, and achieved the second highest pre-test score on this assessment), used perceptual cues to inform her understanding of activation.

P5: The two proteins bind together and they turn one solid colour, and the inside of the protein membrane protein changes colour…So it might be activating something on the inside.

4.2.3.2.1.2 Verbalizations related to Thematic Relevance

The majority of descriptive statements that were identified as thematically related, contributed to a description of the narrative, and did so in a stepwise fashion. The following is an example of a verbal report in reference to the second animation (random motion, no molecular crowding):

P4: The layer moves the two receptors…and the ligand joins to one of the receptors…and then goes away…and then comes towards the middle…and attaches to one of them…and the second one unites and then the yellow thing glows.
This type of verbal description is not unusual, and appears to occur more frequently in the reports of students with low prior knowledge. The participant breaks down the narrative into a sequence of events, without attaching causality to the actions depicted.

A second example of this can be found in the verbal report of Participant 1, whose statements were comprised entirely of thematically descriptive statements.

P1: Protein moving towards the cell membrane… binding…
    diffusion… cell membrane moving… not stable… protein attaching
to the ligand… moving towards the cell membrane… finally
attaching…

At times thematically related statements anthropomorphize actions in describing the narrative by attaching a sense of agency to the actions of the ligand or protein. We see this demonstrated in the choice of words such as “trying”, “see”, or “need”.

P5: It’s trying to bind to its membrane protein

P5: Can’t see any of the other molecules that are in its way

P7: And now it needs to dimerize

These examples are taken from the reports of more advanced students who, like their less experience peers, applied teleological reasoning to explaining the events depicted.

4.2.3.2.2 Interpretation of Explanatory Statements

The explanatory verbalizations of participants were examined in relationship to three problem areas identified in the literature review (protein conformation, random motion and molecular
crowding). Results of this assessment are examined in relationship to participant responses to the corresponding posttest questions.

4.2.3.2.2.1 Evidence of Understanding Protein Conformation

There are many instances of students referring to the conformation of the receptor as it relates to the binding process. Participant 5 suggested that conformation is involved in binding when she noticed that the ligand had detached from the receptor

P5: Uh… maybe the membrane protein isn’t in the right conformation

This proposition is supported by the participant’s response to Questions 3 and 4 in the posttest:

Q3: What factors influence contact and successful binding of the ligand SCF with the receptor KIT?

A3: The ligand must come into close proximity of the receptor. Also, the receptor must have the correct confirmation \([sic]\) for the ligand to bind

Q4: After viewing the animation, how would you describe the structure of the receptor KIT? Would you say that it is inherently flexible or inflexible?

A4: Flexible. The receptor must change it \([sic]\) shape to be able to bind the ligand.

Participant 5 has a well-developed understanding of protein conformation that is clearly articulated in her written responses to posttest questions.
Participant 8 makes several implicit references to the conformation of KIT as she attempted to conceptualize the binding process. She began her interrogation of the animations by suggesting that there is a “right way” or optimal fit between ligand and receptor.

P8: I think it has to attach the right way…and then it gets accepted by the receptor.

The student next tried to rationalize the binding process by suggesting that SCF won’t properly bind with KIT unless it is held by the two monomers at once.

P8: If it’s only attached to one receptor then it gets detached… unless it’s squeezed between the two of them…then it remains intact

Finally, Participant 8 arrived at an understanding of binding that is related to the conformation of the receptor.

P8: It depends on how it binds to the ligand…if it binds properly to the ligand then it will stick on to it…otherwise it wouldn’t…the receptor has to be in a certain direction…certain position to-uh-hold on to the ligand.

The understanding that she has arrived at is demonstrated in her responses to posttest questions as well.

Q3: What factors influence contact and successful binding of the ligand SCF with the receptor KIT?

A3: The positioning of both receptor [sic] influence the control & binding of the ligand

Q4: After viewing the animation, how would you describe the structure of the receptor KIT? Would you say that it is inherently flexible or inflexible?
A4: It is flexible because it had to move around to properly bind to the ligand & had to be in the right position.

References to protein conformation are absent from the remaining verbal reports. However, a review of student responses in both pretest and posttest illustrates a shift in understanding (accommodation) present in the responses of participants 1 and 3.

Pretest Misconceptions

Q1: Proteins are inherently rigid structures. Is this statement true or false?

P1-A1: I don’t know

P3-A1: True – proteins have 3 diff [sic] types of structures & they’re not easily degraded

Posttest Adjustment of Mental Model

Q4: After viewing the animation, how would you describe the structure of the receptor KIT? Would you say that it is inherently flexible or inflexible?

P1-A4: Structure is flexible able to twist and attach

P3-A4: Inherently flexible – flexible because the force of the liquid forces KIT to bend. Plus the movement of the entire cell membrane causes it to move around.

There is also evidence of robust misconception that is resistant to change in the responses of participant 6 who attempted to rationalize the binding process by assimilating it into his current understanding (as demonstrated in his responses to pretest questions) of proteins as inflexible
structures. The following excerpt from his verbal report and response to posttest questions are examples of this.

P6: K-well…stem cell factor binds to the KIT and then it binds to the other KIT…but what are the white things …there’s a force that attracts the two…

P6: Um, I see as soon as this monomer binds to the KIT structure, It binds the two KIT structures together…It probably has some kind of interactions that cause it to…the two KIT structures to bind together.

This excerpt from the participant’s verbal report, suggests that he is trying to understand what the forces or interactions are responsible for enabling the binding process. However, when we examine his responses to the corresponding posttest questions, he remains firm in his conviction that proteins are inflexible and attributes this interaction to the forces of the ligand.

Q3: What factors influence contact and successful binding of the ligand SCF with the receptor KIT?

A3: Intermolecular forces of the ligand SCF that bring together the two receptor KITs

Q4: After viewing the animation, how would you describe the structure of the receptor KIT? Would you say that it is inherently flexible or inflexible?

A4: The receptor kit [sic] is inherently inflexible because no rotation has been viewed yet it binds to the other receptor kit as the ligand comes near.

In general, participants demonstrated an existing or emergent understanding the relevance of protein conformation to the binding process.
4.2.3.2.2  Evidence of Understanding Random (Brownian) Motion

There were very few instances of explanatory statements relating to random motion in the participants’ protocols. Students who appeared to have a more elaborated mental model of the binding process as a whole (namely Participants 5 and 8) included statements about random motion in their verbal reports. Participant 5 made four separate mentions of diffusion (the gradual random movement of molecules, from regions of higher concentration to regions of lower concentration) in discussing the movement of the ligand throughout the extracellular space. Two examples are shown here:

   P5: The ligand’s diffusing toward the membrane protein

   P5: The ligand looks like it’s maybe moving through maybe because of diffusion, changes in temperature, pH.

Each of these five references to random motion are made during the interrogation of Versions 3 and 4 of the animations.

Participant 8 frames many of her explanatory statements in the form of questions. In observing the ligand colliding with other molecules (Version 4) she makes the following observation:

   P8: How does the ligand know where it has to go?

   P8: Well, basically the other proteins and extracellular…are pushing it towards it…towards the receptor

The verbalizations of both students are supported by their responses to posttest questions.

   Q7: How is the binding of the ligand SCF to the receptor KIT mediated?
P5-A7: Other molecules in the extracellular space impact the movement of the ligand

P8-A7: It is mediated by the molecules colliding which mediates the movement of the ligand

Although Participant 8 understands the general concept of random motion, she attaches agency to the actions of the molecules in relation to the ligand. The molecules are “pushing” the ligand. This language appears throughout the students’ protocols in their verbal descriptions of both random motion and molecular crowding. For example, the actions of the ligand and other molecules are described using terms such as “trying to bind” (P5), “in search of” (P5), “it needs to dimerize” (P7), “it needs to get back” (P7 – in reference to the detachment of the ligand).

4.2.3.2.2.3 Evidence of Understanding Molecular Crowding

Participants make a number of explanatory statements associating molecular crowding with the binding event, but more often than not crowding is perceived of as getting in the way of binding rather than enabling it. For example, in two instances Participant 3 makes reference to the interference of other molecules in blocking the access of SCF to KIT.

P3: So, now it’s harder for it to come across and bind to the uh…factor, because there’s a lot more um…a lot more molecules…things around the cell.

P3: But it’s the attraction of other molecules and other things in and around that cell that’s kind of getting in the way and letting it come close and bind ASAP.

This student has a basic grasp of the concept of molecular crowding, but has difficulty appreciating the randomness of the movements of SCF in this crowded environment. This is further demonstrated in her responses to posttest questions.
Q3: What factors influence contact and successful binding of the ligand SCF with the receptor KIT?

A3: The molecules / fluid crowed SCF – pushes SCF closer to KIT.

Q7: How is the binding of the ligand SCF to the receptor KIT mediated?

A7: Mediated through the medium (water) & with the presence of other substances present in & around the cell.

Q10: How would you say crowding impacts upon the rate of this reaction?

A10: Crowding slows down the rate of reaction; harder for SCF to get through to KIT.

Participant 3 understands that the binding process is mediated by the presence of other molecules (A7). However her language suggests that she perceives the mediation to be teleologically oriented (A3 and A10).

A similar interpretation of molecular crowding appears in many of the verbal protocol of participant 7.

P7: It’s not easy for the ligand to bind, but it’s got it…

P7: There’s still lots of other molecules but it is able to get to the receptor and activate it

Conversely, Participant 5, who has a relatively high level of prior knowledge, but who was not familiar with the concept of molecular crowding before viewing the animations, offers a well-elaborated description of its mediating role in her posttest responses.
**Pretest**

Q8: What is molecular crowding?

A8: I don’t know.

**Posttest**

Q10: How would you say crowding impacts upon the rate of this reaction?

A10: Crowding seems to slow the rate of this reaction because the ligand undergoes more collisions and therefore takes longer to get to its receptor

In general, participants understood aspects of molecular binding such as protein conformation and molecular crowding. However their understanding of random motion was very limited.

### 4.2.3.2.2.4 Evidence of Perceived Understanding

Following the end of each session, students were asked which of the four animated representations they felt most accurately represented a receptor-ligand binding event. Six of eight participants selected Version 4 (the most complex representation) as the most accurate. Participant 8 chose Version 3 as the most accurate and added additional comment:

P8: Number 3 is the most accurate depiction because it shows how the ligand is jostled around and makes its way through space

This is an interesting response as it suggests that this version held greater perceptual salience for this student.
The remaining student, Participant 6 identified the first animation as being the most accurate. This student, as noted earlier, also maintains misconceptions related to the “forces” governing the binding process, and these are verbalized when he is viewing the more complex versions (3 and 4) of the animation.

Although, in reporting the results of Experiment 2, understanding has been examined in relationship to three conceptual features of the narrative, these three factors (conformation, random motion, and crowding) are by no means separate entities. Rather they are intrinsically linked, and impact upon one another.

### 4.2.4 Results of Eye Tracking Measures

Eye tracking measures served a primary purpose of informing the findings of the verbal reports. However they were also analyzed to gauge the comparative distribution of fixations across each version of the animation. The purpose of this was to determine the general allocation of attention in each animation and also to identify the more perceptually salient features of each version of the animation.

The assessment of eye tracking measures involved counting the number of fixations occurring in each video recording. The participant’s first exposure to the stimuli (verbalization without additional prompts) was used for this purpose. Recordings were 20 seconds in duration each. This process was completed for seven of the eight recordings. Since the eye tracking data for Participant 2 did not appear to calibrate properly this protocol was not included in the assessment.
The mean number of total fixations occurring in each animation was 44. Comparatively speaking, the greatest number of fixations occurred in Version 2 (329, M=47), followed by Version 1 (317, M=45), Version 3 (313, M=45), and Version 4 (269, M=38). A breakdown of fixations by animated version and area of interest is presented in Table 11.

Table 11.

*Mean Distribution of Fixations Across Each Version of Animation*

<table>
<thead>
<tr>
<th>Ver.</th>
<th>Mean Total Fixations</th>
<th>Mean Areas of Interest (AOI)</th>
<th>Mean AOI</th>
<th>Mean Non-AOI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SCF</td>
<td>KIT Ect. (left)</td>
<td>KIT Ect. (right)</td>
<td>KIT End. (left)</td>
</tr>
<tr>
<td>1</td>
<td>45</td>
<td>4</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>47</td>
<td>6</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>6</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>5</td>
<td>9</td>
<td>3</td>
</tr>
</tbody>
</table>


In addition to containing the highest mean number of fixations, Version 2 also contained the highest mean number of fixations within areas of interest. In comparing the average count of fixations within and outside of areas of interest in each version of the animation, in every version the number of fixations occurring in areas of interest was greater than the number of fixations occurring outside areas of interest. This suggests that the most thematically relevant material (material linked directly to the narrative) attracted the greatest amount of visual attention. In Versions 1 and 2, the area of interest with the largest number of fixations in each
version of the animation was the cell membrane. In Versions 3 and 4 the ectodomain of the KIT monomer on the left-hand side was attended to most.

In examining the distribution of fixations outside the areas of interest across the four treatments, the animation containing the greatest mean number of fixations distributed throughout the extracellular or intracellular domain but not contained within a specific area of interest was Version 3. By contrast, Version 4 comprised the fewest number of fixations outside the areas of interest. Figure 16 illustrates the general distribution of fixation patterns for seven participants across the four animations.

In exploring the overall distribution of fixations for each participant illustrated in Figure 16 it would appear that the viewing pattern is dictated more by the individual student’s viewing characteristics than it is by the relative complexity of the animations. For example, if we compare the viewing patterns of Participant 4 (Year 1 student) with those of Participant 5 (Year 4 student), they do not appear to be similarly distributed. Participant 4’s viewing pattern across each of the four animations appears to be more randomly distributed, as there are many more fixations located outside of the AOIs. Conversely, Participant 5’s pattern of fixations across the four animations share a resemblance to one another and appear to be located primarily within areas of interest.
Figure 16. Comparative distribution of fixation patterns across four versions of the animation (V = Version; P = Participant). Fixations falling within areas of interest were positioned with respect to their location within the specific structure. Fixations in regions outside defined areas of interest were positioned relative to their location within the display.
An independent-samples t test was conducted to measure the differences in the total fixations of students according to their level of prior knowledge (low vs. high, as determined by year of study and number of courses completed). Although there was a trend toward increased total fixations among participants with low prior knowledge ($M = 217.67$, $SD = 39.55$) when compared with high prior knowledge participants ($M = 143.75$, $SD = 39.04$), this proved not to be significant, $t(5) = 2.46$, $p = .057$. Figure 17 shows the distributions for the two groups and Figure 18 illustrates the relative percentage of fixations within and outside the defined areas of interest for each participant.

![Figure 17. Error bars (two standard deviations above and below the mean) for the mean number of fixations among participants with low and high prior knowledge](image-url)
Figure 18. Average distribution of fixations within areas of interest and outside areas of interest. Fixations are converted to a percentage of the total number of fixations for each participant.

4.2.5 Summary of Results from Experiment 2

A quantitative assessment of the verbal protocol data showed that the majority of protocols contained a greater number of descriptive statements than explanatory statements and that there was no correlation between the total number of statements and the student’s prior knowledge. However the number of explanatory statements was positively correlated with prior knowledge and also with complexity of representation.

Qualitative assessment of verbal protocols further categorized descriptive statements as either perceptually salient or thematically related. Perceptually salient statements made reference to visual features such as movement of the cell membrane, stochastic behavior of the molecules in the environment, and the colour changes that occur during activation. In select cases perceptually salient statements gave rise to explanatory statements as students searched for
causal explanations of the perceptual features. The majority of descriptive statements were identified as thematically related. Often students would describe the narrative in a linear stepwise fashion. This was particularly true among students with low prior knowledge. At times participant’s reports contained language suggesting an anthropomorphic account of events. Regardless of students’ prior knowledge they frequently attached agency to molecular actions.

An examination of explanatory statements showed that in general, participants understood aspects of molecular binding such as protein conformation and molecular crowding. However their understanding of random motion was very limited. The majority of participants demonstrated an existing or emergent understanding of the relevance of protein conformation to the binding process. This finding was supported by posttest results. The concepts of random motion and molecular crowding, both of which are closely linked, were not as well understood by participants. There were very few instances of explanatory statements relating to random motion except among students with a more elaborated mental model of the binding process as a whole. Even among these students, while they understood the general concept of random motion, they attached agency to the actions of the molecules in relation to the ligand. Similarly, students had an adequate understanding of crowding, as demonstrated in posttest responses, but not as it related specifically to the binding event. Molecular crowding was treated more as a background feature, one that prevented binding rather than enabling it.

Results of the eye tracking measures showed that the greatest number of overall fixations occurred in Version 2 of the animation, and the fewest number of fixations occurred in Version 4. In every version of the animation the number of fixations within areas of interest was greater than the number of fixations outside of these defined areas, suggesting that the most thematically relevant material (material linked directly to the narrative) attracted the greatest
amount of visual attention. Version 3 contained the greatest mean number of fixations
distributed throughout the extracellular or intracellular domain but not contained within a
specific area of interest, while Version 4 comprised the fewest number of fixations outside areas
of interest. An examination of viewing patterns among seven participants suggest that fixation
patterns related more closely to the individual student’s viewing characteristics than to the visual
complexity of the animation. However, the relationship between fixation patterns and prior
knowledge was not significant.
5.0 GENERAL DISCUSSION AND CONCLUSIONS

5.1 Overview

The present study aimed at identifying the role of visual complexity within animated representations in the development of students’ understanding of dynamic molecular events. The formulation of this problem was derived from a review of the literature identifying problem areas of learning in molecular biology and the literature examining the role of visualization, in particular animation, in teaching complex dynamic concepts. The primary focus of the two experiments reported here was to examine the role of key visual variables, rendered at increasing levels of complexity, in supporting students’ understanding of corresponding features of the molecular environment that are not well understood at this level of study. These features include: protein conformational changes, random molecular movement, and molecular crowding (including molecular water). This problem was explored quantitatively (Experiment 1) and qualitatively (Experiment 2) in order to better capture the interactions that occur between the student and the visualization media, and to describe the learning that occurs under these conditions.

The guiding questions introduced in the opening section of this dissertation provide the structure for the following discussion and are restated below:

1. Is the complexity continuum an appropriate predictor of instructional effectiveness, or are there points along the continuum at which further increases of complexity in dynamic representations impact negatively upon student achievement of specific educational objectives?
2. Does a student's prior knowledge in a content area influence ability to benefit from animated instruction?

3. Is selective use of preattentive variables (namely colour, contrast, and motion) in visuals an important instructional variable in guiding attention toward more thematically relevant structures?

4. To what extent does perceptually salient information contribute to informing the development of a mental model of events depicted?

5.2 Performance Along the Complexity Continuum

The results of the first experiment show that with increasing levels of visual complexity students’ overall performance improved significantly. In assessing student understanding of surface level information, while it was hypothesized that the simpler animations might be more effective at conveying basic concepts this was not the case. Regardless of whether students were exposed to the most simple or the most complex visual treatment, their ability to explain basic concepts on the Post-test was comparable. Furthermore, participants’ assigned to the more complex treatments (Groups 3 and 4) scored significantly higher on basic questions in the Delayed Post-test than did students in Group 1.

In accordance with the second hypothesis, it was observed that increasingly complex animations (3 and 4) fostered greater understanding of abstract concepts related to molecular binding events. Students assigned to these groups scored significantly higher on advanced questions in the Post-test than their counterparts in Group 1. However, students’ scores on more advanced questions in the Delayed Post-test were comparable across all four groups. In other words, the
learning effects of the more complex visualizations were lasting, but only with regard to the more basic concepts.

An exploration of verbal protocol data in the second experiment supports the finding that the most complex animation was the most effective. Version 4 of the animation was positively correlated with the highest number of explanatory statements, suggesting that students drew greater perceived understanding from this representation of the binding event.

While the results between pretest and posttest in the second experiment were not significantly different there may be explanations that would account for this. In this treatment, students were exposed to all four versions of the animation. It is possible that the multiple levels of representation confused two students in particular, who scored lower on the posttest than on the pretest. Both students had a lower level of prior knowledge. This is supported by the research literature on multiple external representations, which suggests that multiple representations can be confusing for students with lower levels of domain specific prior knowledge (Ainsworth, 2006). Perhaps these students require instructional scaffolds that help them bridge the various representations in their understanding.

5.3 Influence of Prior Knowledge

There is increasing evidence that learning from visualizations is moderated by students’ level of domain- specific prior knowledge. For instance, learners with a high level of prior knowledge are more skilled at directing their visual attention towards relevant information (Lowe, 2003; Scheiter, Wiebe, & Holsanova, 2008). Although the findings of Experiment 1 did not point to significant differences in learning amongst students in different years of study, there was a trend
toward improved learning amongst senior students, and this was more deeply explored in Experiment 2.

An examination of the verbalizations of students participating in Experiment 2, showed that students with a high level of prior knowledge (in their fourth year of study) made a greater number of explanatory statements in their verbal protocols. These students had well elaborated statements that demonstrated depth of understanding in key areas such as protein conformation and molecular diffusion. Students with lower levels of prior knowledge used much more descriptive terms in the animation. Their verbal reports were focused predominately on the narrative explanations of the function of the event, rather than wondering which physical mechanisms might explain the phenomenon.

Prior knowledge did not guide students’ understanding of the random nature of molecular events. Unilaterally, students described random motion and molecular crowding as though they were impediments to the ligand reaching its target protein. It is well established that students have great difficulty reconciling the random nature of molecular events with the efficient nature of biological systems. In a study by Tasker (cited in Tversky et al., 2008) students watching the random movements of molecules colliding into one another, interpreted them as “pushing” one another so that they would join. Tversky (2008) also remarks that people are known to interpret movements of geometric forms as having causality, agency, and even intention. These forms when moving in a sparse environment may be seen as chasing, bullying, hiding, or talking. Similarly in the present study, students attached agency to both the ligand and the other molecular structures occupying the extracellular space. Students interpreted molecular movement as either “pushing” the ligand or “getting in the way”, and described the ligand as though it had a mission or goal to fulfill.
An assumption of this study was that, in part, this interpretation of molecular motion was due to the way in which these events are traditionally portrayed. Typically molecular events are depicted as schematized static illustrations, or as highly simplified animations. These may be interpreted literally by students. However, even in the context of this study, these misconceptions proved to be robust and resistant to change. In part this may have to do with basic human nature. We have a great propensity toward narrative structure, and this proves to be very useful in navigating our surroundings, and rationalizing our experiences (Scholl & Tremoulet, 2000). So, it is natural for us to organize new information in this way; to seek causality and order events in chronological sequence. This makes it very difficult for students to appreciate the emergent nature of molecular phenomenon.

This sentiment is echoed by Chi (2005), who, as noted previously, proposes that students seek causality according to shared perceptual properties among entities within a visual display. In seeking causality, students will separate elements of a display into classes of objects rather than treating them as members of a collective (recall the example of diffusion in which students associate water and dye on the basis of colour, as two separate classes, rather than thinking about water and dye molecules collectively interacting). Chi’s explanation of students’ concepts of emergent processes helps to inform our understanding of how the perceptual features of a visual display can be manipulated (for better or for worse) to impact upon students’ understanding.
5.4 The Role of Visual Perception

5.4.1 Perceptually Salient Features

The development of the stimuli used in this study involved a number of design decisions aimed at creating representations that would focus viewer’s attention on thematically relevant aspects of the animations by increasing their perceptual salience. Drawing on theories of pattern recognition and preattentive processing, usually reserved for static imagery, Schnotz and Lowe’s unified view was adopted in the approach to the design and qualitative evaluation of these animated representations. Overall, it would appear to have been successful as students were able to attend to the important features of the more complex animations without getting lost in the complexity. What follows is a discussion of colour, contrast, and motion and their respective roles in informing students’ interpretation of the learning material.

5.4.1.1 The Role of Colour

In designing the animations we made the decision to use colour selectively and applied it to only the most thematically relevant areas of interest, namely stem cell factor (the ligand), and the two KIT monomers (the receptor). The ligand was coloured green and the receptor was rendered with its complementary colour, red. The remaining features in the display were shaded with tone (the cell membrane and all other intracellular and extracellular structures). Selective use of colour in both the ligand and protein receptor proved successful as a means of helping the viewer focus on the main narrative (obviously motion plays a key role in this as well). As well, explicit color cues were used successfully as a means of helping the viewer focus on changes in the main narrative. This finding is consistent with Lowe and Boucheix’s (2011) study of the role of colour cues in directing attention while viewing a complex animation. The authors found that in initial exposure to an animation demonstrating piano mechanism, viewer’s attention was
successfully guided by colour cues. However it should be noted that over successive viewing of the learning material this proved to be an ineffective feature in Lowe and Boucheix’s study. In the present study, embedded colour cues alerted students to important changes that are triggered when the KIT monomers dimerize. A yellow glow was added to the endodomain of KIT to indicate that the dimer had activated and was signaling to the cell. At the same time the combined KIT/SCF structure transforms to a green hue. The intent in doing this was to delineate the steps in binding and activation of the KIT structure: First the ligand triggers dimerization of the receptor and then the dimerization process induces activation of the intracellular domain. The selective use of colour was successful in focusing participants’ attention on significant aspects of the animation. This was also demonstrated in the eye tracking data, which pointed to a greater number of fixations occurring in areas of interest than in other areas of the display.

While the use of selective colour may have been successful in focusing viewer’s attention, it may also have inadvertently contributed to student’s perceptions of the environment as one containing classes of entities rather than a collective of interacting entities. Students tended to classify other molecules in the environment as “background” elements rather than considering them as part of a collective whole. As in Chi’s (2005) diffusion example students may have classified molecules based on colour coding (green, red, or white). In this respect colour may have detracted from understanding and contributed to students’ classifying objects as background rather than treating them as a collective whole. This phenomenon certainly merits further investigation.

5.4.1.2 The Role of Contrast

In designing this study the expectation was that given the high level of detail and motion contained in Version 4, that it would be the most distracting of the four treatments. In other
words it was anticipated that the greatest number of fixations outside areas of interest would occur while students were watching this version of the animation. However, this expectation was not borne out in the results. Rather, the results of Experiment 2 revealed quite the opposite effect. The lowest number of fixations outside areas of interest was recorded in Version 4, and the highest number of these fixations was recorded in Version 3, followed by Versions 1 and 2 respectively. A possible explanation that might account for this, involves viewers’ preattentive processing of feature contrast in each of the representations. While Version 4 contains the greatest level of visual detail, the figure ground contrast (the relationship of the figure, KIT, to the ground, or surrounding environment) is low when compared to the same elements in Version 3. Figure 19 illustrates the comparative figure ground relationships in each of the four animated treatments.

Figure 19. A comparison of contrast levels in the figure-ground relationship of KIT to the extracellular space. This is shown across three versions of the animation. Each represents a corresponding region outside the areas of interest. a) Versions 1 and 2; b) Version 3; and c) Version 4.

If we examine Version 3 more closely (Figure 17-b), the absence of molecular water serves to heighten the contrast between molecules in the extracellular space and the background of the animation, thus heightening the perceptual salience of the individual molecules. In Version 4, the movements of these molecules are less detectable on a preattentive level, as they are crowded by similarly coloured water molecules. This finding is supported by Parkhurst and
Niebur’s (2003) findings that report higher levels of fixation upon areas with greater contrast. Another possible explanation for Version 4 containing the fewest fixations outside the areas of interest might have to do with the amount of effort required to view that animation. Version 4 would have demanded more effort on the part of the students in order to focus on the narrative. Perhaps the increased investment of mental effort helped to focus viewers’ attention.

That Versions 1 and 2 also contained a greater number of fixations outside the areas of interest, cannot be explained by contrast alone. It is more likely that they did not contain enough visual information to maintain the viewer’s attention. Indeed, while students were viewing Version 1 their fixation paths often tracked to the edge of the screen or to the video controller at the bottom of the screen.

5.4.1.3 The Role of Motion
Motion was used in the design of the animated treatments primarily for the purpose of guiding the narrative. It also served the purpose of emphasizing the randomness of molecular movements throughout the extracellular space. While it was not entirely successful in fostering students understanding of randomness it was successful in conveying various aspects of the binding process. One notable motion-related feature was very effective in directing students’ attention and eliciting questions that helped them conceptualize protein conformation in relation to a binding event. At one point during the animation the ligand makes contact with the receptor and then detaches before making contact for a second time and subsequently binding to the protein. This feature was incorporated into the animations to enforce two concepts: 1) that binding occurs quite randomly; and 2) that the protein receptor must be in the proper conformation in order to bind with the ligand. This proved to be a highly successful feature, as it elicited many statements from students participating in Experiment 2. Students first questioned
the detachment of the ligand from the receptor and then attempted to rationalize this event. The success of this feature owes more to the “surprise factor” of viewing the unexpected than it does to the perceptual salience of the motion cues. However, it suggests that perhaps by incorporating these unexpected events into the narrative that we can encourage students to interrogate the narrative more thoughtfully.

5.4.2 Summarizing the Learning Affordances of Animated Visualizations

In summarizing the role of visual complexity in fostering understanding of animated visualizations, it is worth considering how preattentive cues might be manipulated to best serve the needs of viewers with different levels of prior knowledge. Findings from the present study suggest that students benefit from visual complexity regardless of their level of prior knowledge. As well these students benefit from the selective highlighting of preattentive features. This technique draws attention to thematically important features of the display (drawing them to the foreground) and suppresses remaining features of the display (in effect, sending them to the background). However, there is a point at which students cease to make necessary connections between foreground and background features, associating them by shared visual features rather than by shared behaviors. This is particularly problematic when learning about complex dynamic systems. One possible way to avoid the development of misconceptions might involve treating perceptual cueing as form of a visual scaffold that may be faded as the learner’s understanding increases. In this way, students may be introduced to subject matter in a guided way; one that directs their focus first to specific details and ultimately to the whole of the display.
5.5 Limitations of this Research

There are certain limitations to this research that need to be addressed in future studies. In designing this experiment, the focus of attention was solely on the impact of visual variables in fostering understanding. Given these parameters it was important not to introduce confounding factors such as narration in the form of audio or text. While this was a necessary omission in the design of the stimuli, it detracted from the overall enjoyment and educational benefit of the animations for the participants. While there was improvement in students’ scores, the mean test scores in the higher achieving groups was still a modest five out of a possible ten points. In this way, the visualizations were not ideally suited to teaching, nor were they representative of animated media that students typically encounter in an educational setting or online environment. For visualizations to be maximally effective, according to the theory of dual-processing (Paivio, 1986), they should leverage both the viewer’s visual and verbal cognitive processing skills.

A second potential limitation of the study concerns a possible lack of equivalency across the three test instruments. While an attempt was made to isomorphically match questions in each of the Pre, Post, and Delayed Posttest, this was not always possible. This was because it was important to ask students questions pertaining specifically to the events depicted in the animated treatments; abstract, predictive questions that would test near transfer of knowledge. The largely unchanging scores of Group 1 (the least educationally impactful treatment) in Experiment 1, across three time points suggests that the tests were indeed equivalent. However, the lack of a negative control in this study limits the ability to generalize from the results.

A third possible limitation of the study relates to the performance of students assigned to the more complex visual treatment. That students in Groups 3 and 4 significantly outperformed
Group 1 participants raises the question, did the more complex representations foster deeper understanding by merely providing these students with more information? Certainly the increasing level of detail depicted in each version of the animation likely provided the student with greater overall context for the environment in which the event was occurring. Without more in-depth analysis of student responses this is a difficult question to answer. However, it is worth noting here that the performance level of Group 2 was much closer to that of Groups 3 and 4 than it was to Group 1, even though Treatment 2 contained the same level of visual detail as Group 1. The single feature that distinguished Treatment 2 from Treatment 1 was the addition of random motion and conformational flexibility of the membrane receptors. Those small changes did not provide entirely new information, but rather encouraged students to 1) question the behavior and motion of the ligand in relation to the membrane receptors and 2) observe the structural flexibility of the membrane receptors in relation to ligand-induced activation. The data also show that performance in Group 1 decreased slightly between Pretest and Post-test suggesting that this treatment might actually have been harmful to some students. It should be noted that the majority of student performed similarly in this group across each of the three time points and that there was no correlation between level of expertise (year, number of courses completed) and a decrease in test score between the first and second tests.

A fourth potential limitation of this study concerns the use of eye tracking to measure visual attention. While eye tracking measures are a strong indicator of foveal vision they do not account for peripheral vision (Duchowski, 2007). Hence, in tracking fixations we are not “seeing” the entire picture that the viewer is observing. However we are certainly able to gauge which features within the visual display occupy foveal attention. This in itself is very revealing, as it tells us which features are attended to most and which are suppressed and attended to only in peripheral vision.
5.6 Research Implications

5.6.1 Direct Implications of this Research

Despite the limitations of the present study, it suggests some interesting implications for the use of animated visualizations in undergraduate molecular biology. The purpose of the study was to examine whether or not more complex representation of a receptor and ligand binding event would impact positively or negatively upon students’ understanding of molecular environments. It would appear that with exposure to greater visual complexity, students were able to focus on the more thematically relevant aspects of the animation regardless of the level of detail. Indeed, the more complex versions of the animations contributed the greatest level of insight into the events depicted. This contradicts what we have come to understand about cognitive load and its implications for instruction. Rather, these findings suggest that students are more capable of processing visual complexity than the literature on cognitive load would suggest. This raises questions about prevalent attitudes toward depicting complexity (that complex content must be simplified in order for students to understand it). Linn et. al (2010) distinguish between complexity that is extraneous (tangential to the learning goal) and complexity that is “desirable”. The authors remark that visualizations often depict concepts with a deceptive level of clarity. Indeed, visualizations can be very persuasive. Students may become convinced that they understand a complex concept simply because a visualization depicts it clearly when, in fact, their understanding is superficial. The goal of complexity should be to “encourage students to recognize, revisit, and repair gaps in their knowledge” (p. 242). To avoid the pitfalls associated with extraneous complexity, one must give careful consideration to the design of the visualization such that it focuses the viewer’s attention on relevant information.
In the design of the stimuli used throughout this study, perceptual cues such as colour, contrast, and motion were leveraged with the intent of guiding attention, reducing visual search and extraneous cognitive load, and enhance learning. They were successful in guiding viewers attention and clearly depicting a dynamic process, as evidenced by both experiments. By integrating these preattentive features in the design of animated visualizations, one can communicate clearly without the risk of sacrificing complexity. This assumes, as Schnotz and Lowe (2008b) suggest that the way in which we process animation is closely related to the way in which we process static imagery (or at least that the two share more similarities than differences). The research examining the role of images and text in learning is well developed, however, there is a need for additional inquiry specific to the role of visual perception in learning from visualizations.

Another contribution of this research is in the methodological approach to studying the role of dynamic representation in learning. In our efforts to measure the efficacy of educational technology it would appear as though we are at times sacrificing an opportunity to explore understanding in a more meaningful way, in favor of more replicable, generalizable results. While this model of evaluation may tell us what new knowledge is learned by students, it fails to describe the transformative process by which new knowledge develops, and the factors involved in supporting and sustaining this change. Researchers in education, in particular Ainsworth (2008b), have begun to identify a need for more fine-grained research studies that capture the subtleties of learners’ interactions with dynamic visualizations and interactive tools. By combining multiple methods of assessment (quantitative, qualitative, and physiological), this dissertation goes well beyond examination of isolated factors to provide a richer, descriptive understanding of the interactions between the visual design features of animated representations.
and learning process of the viewer. The research findings underscore the importance of interacting factors, such as prior knowledge, visual complexity, and saliency.

5.6.2 Broader Implications of this Research

The research presented here deals with the visual treatment of very specific subject matter and for that reason the findings cannot be generalized to the broader educational context. However, this dissertation addresses some fundamental issues relating to the importance of role of dynamic visualization in science education. This study highlights the interaction between factors such as media modality, visual treatment, and learning objectives; factors that should be considered together when selecting visualization tools for use in science education. Firstly, if dynamics, randomness, and varying spatial and temporal scales are important constituents in understanding cellular phenomena, then media modality plays a key role in communicating these concepts. Successful teaching of complex dynamic events demands much more than static imagery affords. Animated visualizations seem to provide a necessary complement to teaching this subject matter. However, as this study shows, animation alone was not enough to successfully support students’ understanding of randomness.

Secondly, in considering the appropriate visual treatment for teaching these concepts, the learning objectives should play a key role in determining how much context is necessary in order to convey the various interactions that occur within that environment. In the context of designing pedagogically-impactful tools for learning, it may be misleading to selectively omit features that contribute to the overall understanding of phenomena. The results from this study show the importance of contextualizing subject matter in the use of animated representations. In order for students to understand dynamic events, the narrative must not be devoid of context, as
this may otherwise give rise to learning difficulties or misinterpretations (as demonstrated in
Version 1 of the animated treatments).

5.7 Concluding Remarks

Ordinarily cellular events, owing to their sheer complexity, are depicted in a highly schematized,
simplified form for the sake of clarity. As educators we tend to rely largely on readily available
static and highly schematized representations of proteins outside of their cellular context.
Unfortunately these representations lack critical structural and kinetic information, thus
conveying misconceptions about the nature of molecular world. The insights emerging from this
study demonstrate that in some learning contexts, complexity may well be what is needed to
convey the truly dynamic nature of the molecular realm, and to accurately reflect recent
advances in the study of structural cell and molecular biology. This is true not only for purposes
of scientific communication among scientists, but also for undergraduate and graduate students
who are expected to develop an understanding of the cellular and molecular realms as they
proceed with their studies.

By examining the role of animated representations in effectively representing protein structure
and function, this research has drawn attention to the importance of studying the way in which
the representational features may inform students’ understanding of these complex and dynamic
processes. However, additional attention must be given to exploring techniques that can
satisfactorily balance the random nature of molecular events with narrative explanations of these
processes.
5.7.1 Areas for Future Research

In the present study, of primary interest was exploring the impact of visual complexity upon students’ understanding of molecular environments. As a result, attention was directed at manipulating the visual variables that impact upon complexity and how that relates to students’ understanding of random motion, conformational flexibility of proteins and molecular crowding. The representations used in this study exemplified a very small fraction of the many different variations that can be employed in the design of dynamic representations. Given that these dynamic events operate within multiple spatial and temporal scales, there are likely many additional variables worth exploring that might communicate the ways in which the properties identified above contribute to dynamic events.

Two potentially important factors that were not explored in this study are the possible effect of sound design and cinematic design upon learning. This could include, for example, pitch variation in correspondence with speed of motion, and visual techniques for manipulating temporal scale in order to appreciate events that are happening simultaneously, but at vastly different speeds (an effect known as bullet time cinematography popularized in films like the Matrix). These techniques are used effectively to engage viewers in mainstream media. However, whether or not engagement results in learning is unclear. An important aspect of this research would necessarily involve assessing the relationship between engagement and pedagogical value.

Another factor not explored in this study but of potential relevance is the targeted use of interactivity. How might interactive features of visualizations be designed to effectively further students’ consolidation of a dynamic mental model; one that enables them to engage in more distant transfer of their understanding? Examining ways in which interactivity and user control
over visual variables may further help students to reflect upon their emergent understanding and thus extend it by making predictions related to their manipulations of key variables (rather than thinking of these molecular concepts as isolated factors). Offering students control over a series of key parameters (for example, crowding or temperature) may help to emphasize the interdependence of the molecular concepts, provide an environment in which these concepts (in particular randomness) may be explored further.

A third area of research that merits additional exploration is the role that prior knowledge plays in learning from visualizations. This study demonstrated that prior knowledge did influence students’ ability to benefit from visualized instructional material. A more thorough understanding of the relationship between prior knowledge and learning from visualizations would help to inform the design of visualizations that benefit different learners with varying levels of understanding. These findings could be applied to the development and categorization of design principles that might be applied under different learning contexts and with learners at varying levels of prior knowledge.

Research examining the role of visualization in science education is in its infancy and there remains much to explore in this domain. Visual complexity, media modality, and prior knowledge each play a key role the development of understanding. This dissertation will hopefully prompt readers to ask how we can better design molecular visualizations to address students’ misconceptions and foster depth of understanding over the long-term.
REFERENCES


Recruitment email for undergraduate UTM Biology students

Hi, my name is Jodie Jenkinson and I am a PhD student in Curriculum, Teaching, and Learning at the Ontario Institute for Studies in Education. I am also a graduate of Biomedical Communications here at UTM. I am conducting a study examining students’ understanding of cell biology. I’m interested in how animation may help or hinder your understanding of cellular events.

I would like to invite you to participate in this study to observe how animation impacts upon your understanding. Participation in the study involves a test of your prior knowledge, viewing one or more short animations, and test of your newly acquired knowledge with a follow up 1 week later. The study should take about 45 minutes of your time and it will take place in the CCT Building - so it won’t involve any travel.

You’re under no obligation to participate, but you’d be helping me out tremendously by volunteering... and if that isn’t enough, I’m offering students $20 gift certificates at the U of T bookstore as an incentive to participate.

Let me know if you’d like to get involved in this study or if you have any questions.

I look forward to hearing from you.

Sincerely,

Jodie Jenkinson
PhD Candidate,
Curriculum, Teaching & Learning
Ontario Institute for Studies in Education

biovis.study@gmail.com
Appendix B: Recruitment Poster

HOW DOES ANIMATION HELP YOU LEARN?

What: An invitation to undergraduate UTM Biology students to participate in a study
When: October 12 - 22, 2010
Where: Biomedical Communications - CCT Building
How long will it take? About 30 minutes
Interested? Contact: biovis.study@gmail.com

You will receive a $20 UofT Bookstore Gift Card for participating in this study.
Appendix C: Recruitment Slideshow

Slide Presentation and Recruitment Script

How does animation help you learn?

Molecular visualization study
Today 4 pm - 6 pm
Tuesday 9 am - 1 pm
Friday 9 am - Noon
☞ 30 minutes of your time...

$20 U of T Bookstore gift card

Thanks!
biovis.study@gmail.com
Recruitment script for undergraduate UTM Biology students

Hi, my name is Jodie Jenkinson and I am a PhD student in Curriculum, Teaching, and Learning at the Ontario Institute for Studies in Education. I am also a graduate of Biomedical Communications here at UTM.

In Biomedical Communications we develop interactive tools many of which are geared toward undergraduate students. Here are a few examples... [a slide showing screen captures of a few programs]

I’m here because I am conducting a study examining students’ understanding of cell biology. I’m interested in how animation may help or hinder your understanding of cellular events. I’d like to learn more about how this material might be better designed to support your learning.

Over the next few weeks I’ll be inviting students to complete the study, which involves a test of your prior knowledge, viewing one or more short animations, and test of your newly acquired knowledge with a follow up 1 week later. The study should take about 45 minutes of your time and it will take place in the CCT Building - so it won't involve any travel.

No, doubt some of you are wondering why on earth you would want to participate in this study. Well, for starters, you’ll have greater exposure to the learning material and hopefully you’ll find this helpful in supporting your continued studies. In the grand scheme of things though, you’ll be contributing to our understanding of your needs as a student... and making the world a much better place! At the very least, by participating in this study, you'll receive a $20 gift certificate for the bookstore.

Let me know if you’d like to participate or would like more information about the study or if you have any other questions.

Here’s my email address: [slide: jodie.jenkinson@gmail.com]

Thanks very much for your time!
Appendix D: Email Response to Volunteers

Dear [Student Name]
Thanks very much for your interest in participating in the animation study.

Currently the available dates and times are displayed below. Please choose any time frame that will best suit your schedule:

Tuesday, October 12 --> anytime between 9:00am - 1:00pm
Friday, October 15 --> anytime between 9:00am - 12:00pm
Monday, October 18 --> anytime between 4:00pm - 6:00pm
Tuesday, October 19 --> anytime between 9:00am - 1:00pm
Friday, October 22 --> anytime between 9:00am - 12:00pm

*The study is to be held in the CCT Building, Room 3160. We are asking volunteers to come at 5 minute intervals so that we can get each person set up in the testing venue with no waiting. Please let me know by return e-mail which of these dates work best for you and I will reply with a suggested time.

When you arrive be assigned to one of four groups. We will ask you to sign a copy of the consent form and you will be given a copy for your records. We will ask you to write a short answer pretest to assess your current state of knowledge and then will ask you to watch a short animation. We will then ask you to write a short answer post-test. Finally, we will ask you to complete an online follow-up test 1-2 weeks following the evaluation of the chapter and we will give you a $20 gift card for the University of Toronto Bookstore. We do not anticipate your participation to require more than 30 minutes.

My sincere thanks for your interest. I very much appreciate your help and look forward to hearing from you soon.

Jodie Jenkinson
Appendix E: Ethics Approval and Consent Form

PROTOCOL REFERENCE # 25420

July 12, 2010

Dr. Earl Woodruff
Human Development and Applied Psychology
OISE/University of Toronto
252 Bloor St. W.
Toronto, ON M5S 1V6

Ms. Jodie Jenkinson
Human Development and Applied Psychology
OISE/University of Toronto
252 Bloor St. W.
Toronto, ON M5S 1V6

Dear Dr. Woodruff and Ms. Jenkinson:

Re: Your research protocol entitled, “Visualizing protein interactions & dynamics: How do students learn from animated representations of complex scientific concepts?”

ETHICS APPROVAL

Original Approval Date: July 12, 2010
Expiry Date: July 11, 2011
Continuing Review Level: 1

We are writing to advise you that a member of the Social Sciences, Humanities and Education Research Ethics Board has granted approval to the above-named research study, for a period of one year. Ongoing projects must be renewed prior to the expiry date.

All your most recently submitted documents have been approved for use in this study.

Any changes to the approved protocol or consent materials must be reviewed and approved through the amendment process prior to its implementation. Any adverse or unanticipated events should be reported to the Office of Research Ethics as soon as possible.

Please ensure that you submit an Annual Renewal Form or a Study Completion Report 15 to 30 days prior to the expiry date of your study. Note that annual renewals for studies cannot be accepted more than 30 days prior to the date of expiry, as per federal and international policies.

If your research has funding attached, please contact the relevant Research Funding Officer in Research Services to ensure that your funds are released.

Best wishes for the successful completion of your project.

Yours sincerely,

S. Lanthier
Research Ethics Coordinator

OFFICE OF RESEARCH ETHICS
McMurrich Building, 12 Queen’s Park Crescent West, 2nd Floor, Toronto, ON M5S 1S8 Canada
Tel: +1 416 946-3273 • Fax: +1 416 946-5763 • ethics.review@utoronto.ca • http://www.research.utoronto.ca/for-researchers-administrators/ethics/
**INFORMED CONSENT**

**MOLECULAR VISUALIZATION STUDY**

**Invitation**

You have been invited to participate in a research study designed to evaluate your responses to an animation that describes a basic cellular event. The animation was created for undergraduate students who are studying cell biology and we are interested in how you learn from this material. The information we gain from this study will be valuable in gaining a better understanding of how animation may be effectively used in teaching cell biology. When the study is complete, a summary of the cumulated results and conclusions will be made available to you through the University of Toronto student portal, and the research, will be submitted for publication in a peer-reviewed journal.

**Your Role:**

This study is open to English-speaking UTM students who are over 18 years of age. I would like to gain a better understanding of how you use animation to learn about cellular events. This will involve testing your knowledge both before and after you view the animation. By consenting to participate in this study, you will be asked to:

- Come to room 3160 in the CCT building on the UTM campus at the assigned time.
- Complete a pre-test that will measure your current level of understanding of particular concepts.
- Spend a limited amount of time viewing animation that addresses some of these concepts.
- Record your eye movements as you watch the animation
- Verbalize your thought process (tell me what you are thinking) as you watch the animation
- Complete a post-test that is different from the pretest, but that will test your new level of understanding of these concepts.
- Participate in a brief follow-up assessment, 1 week following your initial participation in the study.
Risks/Benefits

Participating in this study will involve approximately 30 minutes of your time. To compensate you for your time, you will be given a small gift certificate redeemable at the University of Toronto Bookstore at the end of the follow-up assessment. Your participation in this study will have no impact whatsoever on your grade in any course at the University of Toronto.

Your participation is completely voluntary. You are free to withdraw from the study at any time without explanation. You are free to contact the Ethics Review Office at ethics.review@utoronto.ca or 416-946-3273 if you have any questions about your rights as a participant.

Confidentiality

All the information you provide during the session will remain strictly confidential. The pre- and post-tests will be identified by number only. Your name will not be associated in any way with the data analysis. The research team plans to publish the results in an appropriate peer-reviewed journal at the completion of the study. Volunteers will be described in the manuscript as “undergraduate biology students at the University of Toronto Mississauga”. If you have any questions or concerns about participating in this study, please feel free to contact Jodie Jenkinson or her supervisor, Professor Earl Woodruff (contact information below).

Jodie Jenkinson,  
PhD Candidate,  
Curriculum, Teaching & Learning  
Ontario Institute for Studies in Education  
University of Toronto  
Email: j.jenkinson@utoronto.ca

Earl Woodruff,  
Associate Chair,  
Human Development and Applied Psychology  
Ontario Institute for Studies in Education  
University of Toronto  
Email: e.woodruff@oise.utoronto.ca

I __________________________ (please print) agree to participate in this study as described above.

I understand that I can withdraw at any time without reason.
I understand that all information gathered will remain confidential.

My participation will not affect my grade in any course, in any way.

I have had this study and the consent form explained to me, and have been given a copy to keep.

Participant’s signature: ___________________________

Researcher’s signature: ___________________________

Date: ______________________

Thank you for your participation.
Appendix F: Storyboard

STORYBOARD - 2/2/10

Version 1
- concerted binding of ligand with both monomers (i.e. all meet 'at once')
- motion of all proteins is smooth and directed (ease in/ease out-type animation curves)
- no conformational change in cKit
- no fluid membrane motion
- no molecular crowding

Version 2
- ligand encounters one monomer and complex then encounters the other
- motion of all proteins is random/Brownian in nature
- dimerization results in cKit conformational change
- fluid membrane motion
- no molecular crowding

Version 3
- binding, motion and conformational change is same as in v2
- fluid membrane motion as in v2
- molecular crowding - show additional cKit (and other receptors) in the plane of the membrane, and additional proteins in the extracellular space

Version 4
- everything identical to v3, except...
- show molecular water

LEGEND:
- solid arrows depict protein motion
- red lines suggest internal motion (i.e. conformational change)
- blue dots depict water molecules
Appendix G: Background Form

Molecular Visualization Study
BACKGROUND FORM

Sex: □ Male □ Female

Your age:

□ 18-24 □ 25-30 □ 31-35 □ 36+

Do you wear corrective lenses for reading?

□ Yes □ No

What year are you currently enrolled in?

□ 1st □ 2nd □ 3rd □ 4th □ Other – please specify

What is your program of study (major + minor / specialist)?

__________________________________________________________

Which of the following courses have you completed? (check all that apply)

□ BIO152F □ BIO204F □ BIO210Y
□ BIO153S □ BIO206F □ BIO315F

Other related courses

__________________________________________________________

Anything else you’d like us to know about you?

__________________________________________________________

__________________________________________________________
Appendix H: Pretest

Molecular Visualization Study
PRETEST

Instructions: For each of the questions below provide a brief answer. If you do not know the answer please do not guess. Simply respond “I don’t know”.

1. **Proteins are inherently rigid structures.** Is this statement true or false? Please explain.

2. **Proteins fold into a conformation of lowest energy.** Is this statement true or false? Please explain.

3. **What are the forces that determine the conformation of a protein?** Please explain.
4. Individual lipid molecules are capable of rapid lateral diffusion, flexion, and rotation. Is this statement true or false? Please explain.

5. What determines the fluidity of a cell membrane? Please explain.

6. What 3 types of molecular motion exist?

8. **What is molecular crowding?** Please explain.

---

9. **How do the interactions between water molecules impact upon covalent and non-covalent attractions?** Please explain.

---

10. **What role does collision play in the movement of molecules throughout the cell?** Please explain.

---

*When you have finished the test, please raise your hand to attract the attention of the invigilator.*
Appendix I: Posttest

Molecular Visualization Study
POSTTEST

Instructions: For each of the questions below provide a brief answer. If you do not know the answer please do not guess. Simply respond “I don’t know”.

The following questions relate to the animation you viewed depicting the binding of the ligand stem cell factor (SCF) with the protein receptor KIT.

1. What factors impact upon the movement of the ligand SCF across the extracellular space? Please explain.

2. What forces are involved in the movement of the receptor KIT within the cell membrane? Please explain.

3. What factors influence contact of the ligand SCF with the receptor KIT? Please explain.
4. After viewing the animation, how would you describe the structure of the receptor KIT? Would you say that it is inherently flexible or inflexible? Please explain.

5. How many functional units or domains would you say it has?

6. What is the role or job of the ligand SCF in binding to the ectodomain of the receptor KIT? Please explain.

7. How is the binding of the ligand SCF to the receptor KIT mediated? Please explain.
8. **The binding of the ligand SCF with the receptor KIT activates the signaling process?** Is this statement true or false? Please explain.

9. **What do you think would happen if the temperature were increased in this environment?** Please explain.

10. **How would you say crowding impacts upon the rate of this reaction?** Please explain.

---

*When you have finished the test, please raise your hand to attract the attention of the invigilator.*
Appendix J: Delayed Posttest

Random Number

Molecular Visualization Study
FOLLOW-UP assessment

Instructions: For each of the questions below provide a brief answer. If you do not know the answer please do not guess. Simply respond “I don’t know”.

This is the final follow-up assessment to the animation study. Thanks very much for your continued participation!

Recently you viewed an animation depicting the binding of the ligand Stem Cell Factor (SCF) with the protein receptor KIT. The following assessment contains 10 questions and addresses subject matter related to this animation.

1. Would you describe the movement of molecules through the extracellular space as random or directed? Please explain.

2. Does collision play a part in the movement of molecules throughout the cell? Yes or No? Please explain.
3. Explain what is meant by the statement that macromolecules within cells are crowded but seldom concentrated.

4. How does molecular crowding within the cell affect biochemical rates? Please explain.

5. Are all reactions affected equally by crowding?

6. Knowing that temperature impacts upon water vibration what do you think would have happened in the animation you viewed if the temperature had been decreased? Please explain.
7. **Would you describe water as a facilitator of molecular reactions? Yes? No?**
   Please explain.

8. **What is free energy and why is it important to the binding of SCF with KIT?**
   Please explain.

9. **How does SCF drive the dimerization of KIT?** Please explain.

10. **What is steric hindrance and what role does it play in molecular motion?**
    Please explain.
Appendix K: Instructions to Participants

**Instructions**

1. Read and sign the consent form. You may keep a copy for your files. Leave the signed copy on your desk.

2. Complete the pre-test. This will tell us about the knowledge base you are starting out with. Please do not guess at the answers. If you do not know, check the “I don’t know” option. When you are finished the pre-test, put up your hand and the invigilator will collect your paper and start the animation.

3. The animation depicts the binding of the ligand Stem Cell Factor (SCF) with the protein receptor KIT. We have intentionally removed labels and sound for this study. You may watch the animation as many times as you like. Once you have finished watching the animation please raise your hand and the invigilator will give you the post-test. Again, do not guess. If you do not know the answer, select the “I don’t know” option.

4. When you have finished the post test, put up your hand. The invigilator will collect your post test and provide you with a link to a short follow-up test that you can complete one week later. Once the follow-up test is complete you will receive a $20 gift card from the U of T Bookstore.

**PLEASE do not discuss this study with other volunteers who have not yet come to take the test. Knowing about the test in advance will alter their performance and could make the experiment inconclusive.**

*Thank you so much for your help.*

When the study is completed, we will compile the results and send you a summary.
Appendix L: Participant Email for Delayed Posttest

Dear [Student Name]

Thank you for your ongoing participation in the UTM animation study.

The follow-up assessment will be made available to you between October 29 at 9 am and November 6 at 11:59 pm. It must be completed in order to qualify for a $20 U of T gift card.

To access the follow-up assessment you must log in to the portal and join the organization "UTM Animation Study"

Click the "Community" tab at the top right
Browse to organization search.
Enter "animation"
When organization appears, select 'enroll' from the context menu
Enter study password 'anim8' in order to join the organization

The following video will help you if you have trouble navigating this page:
http://www.bmc.med.utoronto.ca/cellstudy/Instructions.mov

Once you have joined this group it will appear in under the "My Organizations" tab of your portal home page. You may complete the assessment once it becomes available.

You must enter your random number assignment before completing the assessment.
Your random number is: [random number]

Once you have completed the assessment you may pick up your gift card at my office (CCT 3065). If you are picking up for another student that student must email me in advance and you must provide me with that student's random number assignment.

Thanks again for your participation in this study.

Best,

Jodie Jenkinson
Appendix M: Assessment Answer Keys

PRETEST Answer Key

1. Proteins are inherently rigid structures. Is this statement true or false?

Please explain.

False. Although proteins typically fold into a stable 3-dimensional structure, changes in protein conformation are common and critical to their function.

2. In most cases, proteins fold into a native conformation of lowest energy. Is this statement true or false? Please explain.

True – often with the help of auxiliary “chaperones,” proteins fold into a native conformation.

3. What are the forces that contribute to the conformation of a protein? Please explain.

There are many – both 1) internal and 2) external. 1) includes salt bridges, disulfide bonds, hydrophobic side chains and 2) includes the pH of the environment (and therefore protonation of different side chains), post-translational modifications such as phosphorylation and association with partner proteins.

4. Individual lipid molecules are capable of rapid lateral diffusion, flexion, and rotation. Is this statement true or false? Please explain.

True – in the context of the lipid bilayer such as the cell’s plasma membrane, the movement of individual lipids is fluid. A lipid’s long hydrocarbon/aliphatic chains are also flexible.

5. What determines the fluidity of a cell membrane? Please explain.

Many things – it’s composition (for example, membrane cholesterol content is known to influence membrane rigidity and fluidity), temperature, associated proteins (like BAR-domain proteins for example).

6. What 3 types of molecular motion exist?

Among others (probably not just 3!), there is thermal vibration of atoms, molecular breathing
of secondary structure domains and Brownian motion of the whole protein.

7. **What factors influence the successful binding of a membrane protein with its ligand?**
   Please explain.

   The concentration of both, molecular crowding of other proteins in the environment (and the size of those proteins), water. Also the conformation of the membrane protein.

8. **What is molecular crowding?**
   Please explain.

   Molecular crowding describes the fact that in a typical cellular environment (or even in an extracellular space), the concentration is so high that...

9. **How do the interactions with water molecules impact upon covalent and non-covalent attractions?**
   Please explain.

   Water molecules have positive and negative dipoles. In solution, covalent attractions are separated and surrounded by water.

10. **What role does collision play in the movement of molecules throughout the cell?**
    Please explain.

   Collisions with other molecules facilitate the movement of molecules through diffusion. Molecules will move from areas of high concentration to areas of lower concentration.
POSTTEST Answer Key

1. What factors impact upon the movement of the ligand SCF through the extracellular space? Please explain.

Water, other proteins and temperature.

2. What forces are involved in the movement of the receptor KIT within the cell membrane? Please explain.

Hydrophobic/hydrophilic regions (in terms of vertical positioning). Laterally, the motion of neighboring lipids, transmembrane proteins and partners (as well, perhaps, as underlying cytoskeletal interactions).

3. What factors influence contact and successful binding of the ligand SCF with the receptor KIT? Please explain.

The concentration of both, molecular crowding and water/temperature and permissive conformation of the proteins (in this case, especially Kit).

4. After viewing the animation, how would you describe the structure of the receptor KIT? Would you say that it is inherently flexible or inflexible? Please explain.

Inherently extended and flexible.

5. How many functional units or domains would you say it has?

Based on the visuals presented, 5 or 4: the intracellular regions (that lights up), the transmembrane (TM) region, D5+D4 (could be seen as 1 flexible hinge) and D1+D2+D3 (the SCF binding region). The answer 3 could also be accepted if the student thinks of units as ecto, trans, and endo.

6. What is the role or function of the ligand SCF in binding to the ectodomain of the receptor KIT? Please explain.

Its role is to bring together 2 cKit monomeric receptors and thereby keep them in proximity
long enough for their cytoplasmic regions to interact and cross-activate.

7. How is the binding of the ligand SCF to the receptor KIT mediated? Please explain.

It is mediated by collisions with other molecules and by the permissive conformation of the receptor.

8. The binding of the ligand SCF with the receptor KIT directly activates the signaling process. Is this statement true or false? Please explain.

False – Binding SCF keeps cKit monomers in proximity so that their cytoplasmic regions may cross-phosphorylate. Once the monomers form a dimer then the signaling process occurs.

9. What do you think would happen if the temperature were increased in this environment? Please explain.

Molecular motion of all kinds would increase (and the speed of interactions along with it) up to the denaturation temperature (upon which proteins would unfold and specific interactions would stop).

10. How would you say crowding impacts upon the rate of this reaction? Please explain.

Crowding is a major factor in interactions and generally slows interactions.
FOLLOWUP ASSESSMENT Answer Key

1. Would you describe the movement of molecules through the extracellular space as random or directed? Please explain.

Random, molecules don’t seem to inherently “know where to go”

2. Does collision play a part in the movement of molecules throughout the cell? Yes or No? Please explain.

Yes – collisions with other molecules facilitate the movement of molecules

3. Explain what is meant by the statement that macromolecules within cells are crowded but seldom concentrated.

They are surrounded with other proteins and molecules, but concentration of a given protein species may not be high locally.

4. How does molecular crowding within the cell affect biochemical rates? Please explain.

There is a general reduction in the rate of diffusion.

5. Are all reactions affected equally by crowding?

No, larger molecules are affected more greatly by crowding than small molecules

6. Knowing that temperature impacts upon water vibration what do you think would have happened in the animation you viewed if the temperature had been decreased? Please explain.

Interactions may have slowed, but the effect of crowding would have probably remained unchanged.

In terms of crowding, water does not ‘facilitate’ – yet water is a facilitator in terms of hydration and stabilization of protein structure and critical for chemical reactions as well.

8. What is free energy and why is it important to the binding of SCF with KIT?

Please explain.

Free energy is the amount of energy available for reactions to occur. Energy is necessary for binding but is released once the bound state occurs.


SCF serves as an extracellular tether that is able to bond two extracellular domains of cKit at once, thereby bringing into proximity their cytoplasmic regions.

10. What is steric hindrance and what role does it play in molecular motion?

Please explain.

Steric hindrance refers to the space occupied by each atom within a molecule. If atoms are brought too close together there is an associated “cost” of energy due to overlapping electron clouds. This may impact upon conformation and reactivity.
Appendix N: Inter-rater Coding Instructions

Coding Instructions

You have been asked to review and code verbal reports from a study examining the role of animation in the development of understanding.

Each of the 4 transcripts that you’ll read have been divided into idea units or statements.

As you read each statement please designate them as either an Explanatory Statement (E) or a Descriptive Statement (D) by entering the appropriate code in the column next to the statement.

Definitions:

Explanatory statements: For a statement to be considered explanatory it must identify either directly or by implication (a) a causal agent, (b) a result or effect (c) an indicator of linkage between cause and effect.

Descriptive statements: For a statement to be considered descriptive it contains purely descriptive terms and it does not show an attempt to account for events that have been observed.

Examples:

Descriptive:

1) The heart consists of four chambers
2) The molecule lights up
3) The tide is high
4) The cell membrane is moving a lot

Explanatory:

1) The electrical system causes the ventricles to contract
2) When the binding event occurs the molecule lights up
3) I noticed the tide is high when the moon is overhead
4) The cell membrane moves because it is fluid
Appendix O: Coding Form

Rater Coding Form
Date: 
Rater ID: 
Participant 3 (v1>4) ID# 990447

Coding Key:
Enter “E” for explanatory
Enter “D” for descriptive

<table>
<thead>
<tr>
<th>Version</th>
<th>Code</th>
<th>Statement</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>V1</strong></td>
<td></td>
<td>Ok...so the factor is moving toward the cell membrane</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>It attaches to the...uh... binding factor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>And something lights up on the other side of the cell membrane</td>
<td></td>
</tr>
<tr>
<td><strong>V2</strong></td>
<td></td>
<td>Ah...now it's slowly moving towards...the cell membrane again</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Or it bind to the factor ...it comes off</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>And the cell membrane is moving</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>It's...moving alot</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>And that happens again...the binding...</td>
<td></td>
</tr>
<tr>
<td><strong>V3</strong></td>
<td></td>
<td>Now there's other molecules around the cell</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>And it comes again</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Binds to the factor ...or the binding factor, K.I.T or Kit</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>And it's binding again, but there's other forces</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>And then it lights up again...so...</td>
<td></td>
</tr>
<tr>
<td><strong>V4</strong></td>
<td></td>
<td>So, now it’s harder for it to come across and bind to the uh... factor, because there’s a lot more um...a lot more molecules...things around the cell</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>And then it binds and something else lights up</td>
<td></td>
</tr>
</tbody>
</table>

Phase II

<table>
<thead>
<tr>
<th>Version</th>
<th>Code</th>
<th>Statement</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>V1</strong></td>
<td></td>
<td>Ok...so...ah ...The background is clear...they don't show anything...</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>This is inside...no...this is showing the cell membrane</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>So the stem cell factor comes and binds to the K.I.T. and something lights up across the membrane</td>
<td></td>
</tr>
<tr>
<td><strong>V2</strong></td>
<td></td>
<td>And now they’re showing that the membrane is moving</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Like it is usually in the cell</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>An its ...now its...</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>I guess it’s showing inside a fluid...before it was still so now there is fluid in the cell or around the cell</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>So it’s having trouble to bind</td>
<td></td>
</tr>
<tr>
<td><strong>V3</strong></td>
<td></td>
<td>Now there’s other molecules in and around the cell so it’s gonna take longer to bind to the KIT</td>
<td></td>
</tr>
<tr>
<td><strong>V3</strong></td>
<td></td>
<td>And it binds...not yet</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>And that lights up</td>
<td></td>
</tr>
<tr>
<td><strong>V4</strong></td>
<td></td>
<td>And so the factor is again, outside of the cell</td>
<td></td>
</tr>
<tr>
<td><strong>V3</strong></td>
<td></td>
<td>It’s binding to the KIT</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>but it’s the attraction of other molecules and other things in and around that cell that’s kind of getting in the way and letting it come close and bind ASAP [laughs]</td>
<td></td>
</tr>
</tbody>
</table>
Appendix P: Eyetracking Product Sheet

Easy, Affordable Eye Tracking for on-screen research

Easy to setup and use, be ready in just minutes. Calibration is fast and holds very well. Accurate and affordable, the Mirametrix S1 Eye Tracker is the ideal solution for on-screen research.

Too often, eye tracking is complicated, time consuming and expensive. You can eliminate all three barriers and start benefiting from eye tracking research immediately.

Eye tracking applications
The S1 Eye Tracker is ideal in many circumstances including academic research, market research, software usability, web usability, education and game design. You can now add eye tracking information to your existing research and analysis into human behavior.

Portable and unobtrusive
Lightweight and easily transportable, you can implement eye tracking studies in a variety of ways. The device is designed to sit just below the computer screen, out of the way and quickly forgotten by test subjects, which means you get objective, quality results.

Painless calibration
The S1 Eye Tracker calibrates very quickly and painlessly. The calibration holds during long tests. A quick, simple calibration that works means your subjects will be at ease and you can get to your research faster.

Mirametrix Viewer Software
Viewer collects eye gaze and other data in real time and provides important analysis tools. Viewer records a video of on-screen activity as subjects are interacting and having their eye movements tracked. A second monitor can be used to show you what people are doing on-the-fly.

Mirametrix Software API
Our API allows you to integrate the S1 Eye Tracker with any other analysis or software tools. The API uses standard TCP/IP for communication and provides XML data output.

Technical Specifications

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>0.5-1 degree range</td>
</tr>
<tr>
<td>Drift</td>
<td>&lt; 0.3 degrees</td>
</tr>
<tr>
<td>Data rate</td>
<td>60 Hz</td>
</tr>
<tr>
<td>Freedom of head movement</td>
<td>25 x 11 x 30 cm (Width x Height x Depth)</td>
</tr>
<tr>
<td>Calibration</td>
<td>9 point (~ 15 seconds to complete)</td>
</tr>
<tr>
<td>Binocular tracking</td>
<td>Yes</td>
</tr>
<tr>
<td>Tracking type</td>
<td>Bright pupil</td>
</tr>
<tr>
<td>Physical dimensions</td>
<td>36 x 4 x 4 cm (Width x Height x Depth)</td>
</tr>
<tr>
<td>Weight</td>
<td>~3 kg (7 lbs)</td>
</tr>
</tbody>
</table>

Call to learn more: 1 (888) 698-6472
S1 Eye Tracker
Quick Start Guide

1) Unpack equipment

Parts included:
1) Mirametrix S1 eye-tracker
2) USB cable
3) Tripod stand
4) 12V power supply

2) Download & install software

1) Download software from Support page at:
   http://www.mirametrix.com/
2) Tracker package includes tracker and data recording software
3) Example package includes test tracker server and example source code

3) Connect hardware & install driver

1) Connect 12V power
2) Connect to USB 2.0 port*
3) Place eye-tracker near the lower edge of the screen

* Avoid using an external USB hub and other high bandwidth devices like hard drives on the same hub

4) Calibrate and operate eye-tracker

1) Eye-tracker software runs in the system tray
2) For quick operation click Calibrate and enable Move Cursor
3) See examples for developing custom applications

Mirametrix Research Inc.
Tel: 1 (888) 698-6472   Web: www.mirametrix.com