Faculty of Technology and Science

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3D-dynamic visualization of complex molecular cell biology processes

1-year university students' understanding of visualizations of signal transduction

Chemistry education

D-level thesis

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Abstract

This study deals with the use of 3D-dynamic visualizations for teaching complex molecular cell biology concepts. The focus is on signal transduction, which is a concept that constitutes an important part of biological systems. 3D-dynamic visualizations (animations) were produced and shown for a total of 24 students attending a course in molecular cell biology at Karlstad University, Sweden. Data were collected by questionnaires and interviews which were structured around the understandability and usefulness of the animations. The results indicate that animations are useful for teaching life science concepts and can serve as a complement to lectures. They are useful for visualizing continuous time-dependent processes like signal transduction chains. Several connections between students' issues of understanding and layout-issues of the animations were established. A number of implications follow from the study. Basic understanding of animations is fundamental for understanding of advanced concepts, which should be kept in mind in the design phase of production. The level of realism of different factors in animations, like molecule speed and distances, has to be set to strike a balance between conceptual understanding and scientific correctness. Visualization of 3D-structure of molecules provides an understanding of molecule and systemic function. The study reinforces the need to use visualizations in life science teaching.

Table of contents

Contents

1. Introduction

1.1 Complex cell biology processes

Molecular life science is the term for the interdisciplinary science associated with the development of modern biology, biochemistry, biotechnology, genetic research and proteomics. The area contains many concepts, for example the gene concept, metabolism, translation and the relationship between protein structure and function. Most life science concepts relate to a cluster of processes. This is due to the unique complexity of biological systems, which, in turn, is due to an evolutionary history.

The complexity of life phenomena and the dynamic nature of biomolecular processes may turn plain text into an inadequate learning tool. Images, diagrams and other forms of visualizations are becoming more and more important in molecular life science teaching and research. They can provide information about molecular structure and function as well as biochemical reaction mechanisms. Images have also been used to visualize how molecules cooperate in a larger context by forming biological inter- and intracellular functional and communication networks.

Life processes, in general, are dynamic. Sequences of static images leave it to the observer to figure out what happens in between the images. This can give rise to misconceptions regarding the nature of time-dependent, multi-step, biochemical processes like cell cooperation. Animation, on the other hand, has the potential to show continuous processes. The increasing use of computers in almost all domains of life has also influenced science education. A number of science educators believe that computer animation has great potential for teaching life science concepts (Ellis, 1984; Marks, 1982).

Central to all cell cooperation is signal transduction. The development of signal transduction was necessary for evolution to make the step from uni-cellularity to multi-cellularity. The cells of a multi-cellular organism need to cooperate for the benefit of the organism as a whole. This is achieved by intercellular communication. Cells also have to be able to integrate incoming signals from surrounding cells to achieve a specific cell response which is achieved by intracellular communication. The intercellular communication mechanisms depend on extracellular signal molecules that are produced by cells and sent to other cells. The intracellular communication mechanisms depend on molecules that are located inside or on the surface of cells. The intracellular molecules include cell-surface receptor molecules which bind extracellular signal molecules. There are also intracellular signaling molecules that transmit the signal to appropriate parts of the cell in order to achieve a cell response.

The intracellular signaling molecules form signal transduction pathways. At the end of pathways there are target molecules which are altered by interaction with signal molecules. The target molecules directly change the behavior of cells. Multiple pathways may interact with a single target molecule. This provides a mechanism for the integration of signals to achieve specific cell response. Animals produce hundreds of signal molecules that can be used to create an almost unlimited number of signaling combinations. The use of these combinations enables an animal to control its cells in highly specific ways.

Proteins are the most important class of signal molecules. It has to be kept in mind that the original signal molecule is not physically passed along a signaling pathway; in most cases, it never enters the cell. When we say, that the signal is relayed along a pathway we mean that certain information is passed on. At each step, the signal is transduced into a different form, commonly a conformational change in a protein. The conformational change gives the protein new properties that enable it to accomplish new tasks. Very often the conformational change is brought about by phosphorylation, a form of chemical modification often employed by signal proteins. Phosphorylation is the addition of a phosphate (PO_4) group to a protein molecule. The addition often serves as a chemical tag.

Whatever the mechanism for relaying information is, the final result of signaling is often altered gene expression, either as a direct consequence of target protein action (if the target protein is a gene regulatory protein) or by indirect mechanisms. Since cell signaling affects gene regulation it will affect the amount of specific proteins synthesized. By different mechanisms cell signaling will also affect the localization of proteins and their lifetime. Proteins can be tagged for transport to a specific cell compartment or for chemical degradation.

Individual protein molecules often assemble into macromolecular complexes. Groups of macromolecular complexes assemble into pathways. It has been estimated that the protein molecules in a cell are, on average, separated by a space that would contain a few molecules of water. In this crowded environment, different states of signaling pathways will give rise to differing patterns of protein connectivity and thus, also different 3D-structures of pathways. It is a fundamental axiom of biology that the 3D-structure of proteins determines its function. Thus the 3D-structures of pathways governs cell function, since, the 3D-structures of pathways, governed by cell signaling also govern the localization and life-time of protein molecules in the cell. Cell signaling governs the dynamics of cell structure and thereby its function.

The regulation of protein activity occurs by several different mechanisms but all involve interaction between molecules at some stage. Protein molecules in cells, as a consequence, form signaling pathways as a means of regulating the activity of individual protein molecules for suiting the needs of the cell. The relationships between the protein molecules are of great interest. These relationships can be described as networks, in which the molecules are the vertices (nodes, points) and the relationships are the edges (arcs, lines). But not only protein signaling pathways can be described as networks. Other biological networks at the molecular level are gene regulation and metabolic networks. Gene regulation networks control gene expression in cells. The expression of one gene can be controlled by the gene product of another. A directed graph in which vertices are genes and directed edges represent control can be used to model these networks.

Signal transduction networks can be understood as gene regulation networks extended by signaling chains that contain different kinds of vertices and edges such as protein–protein interaction and phosphorylation. In contrast to protein interaction networks that refer to the association of protein molecules and are undirected, signal transduction networks refer to reactions and are basically directed.

Although signal transduction processes are mediated by protein–protein interactions, by far not all protein-protein interactions in the cell are followed by chemical reactions. Therefore, many interactions are not "mirrored" in signal transduction. On the contrary, many components of signal transduction are not proteins. Protein–DNA interactions between gene regulatory proteins and the regulatory regions of genes must be added to the list as well. All these reactions are not mirrored in the system of protein–protein interactions. Finally, gene regulatory and signal transduction networks have much in common with protein interaction networks. At the same time, they refer to different aspects of cellular activity and display several important differences.

1.2 Visualizations of complex cell biology processes

In living organisms, there is a variety of different cell types responsible for various functions. A number of cells that perform a similar function constitute a tissue, examples for animal cells are epithelium and connective tissue, for plant cells epidermis or vascular tissue. A group of tissues that perform a specific function or a set of functions form an organ. All organs together constitute the entire organism. Signal transduction achieves cooperation between the different components of living organisms, at all levels.

Biological systems display emergent properties that are not readily explainable by the features of their components: a system is more than just a sum of different elements. In life science research, there has been an increasingly strong emphasis placed upon a systems biology approach. This approach takes advantage of the large amount of gene sequence information available and the fast progress of high-throughput molecular technologies that make large-scale analyses of complex biological molecular systems possible. It does not focus on the individual components themselves but rather on the nature of the links that connect them and the functional states of the networks resulting from the assembly of all such links. The ultimate goal of systems biology is to understand entire biological systems by elucidating, modeling, and predicting the behavior of all components and interactions.

Both protein interaction networks and signal transduction networks have been visualized by diagrams. They can provide information about connectivity as well as functional and stoichometric relationship between molecules and reactions. Visualization of pathway diagrams can aid interpretation of chemical data. Pathway visualization has also been proven useful for hypothesis generation of cellular function (Prinz *et al*., 2004). But the connectivity of biological networks undergoes transitions between different states and may be difficult to visualize with single images. Even visualization of a single specific state has been proven difficult; problems in interpreting flow diagrams representing pathways in the immune system and metabolic pathways have been identified (Hull *et al*., 2002). Graphics visualization tools such as molecular modeling and animation can be used to give an accurate and rich picture of the dynamic nature of molecules and molecular interaction, which is often very difficult to understand from text-based information (NSF, 2001).

Signal transduction is central to cell function and constitutes an important part of biological systems. Because of its centrality the concept of signal transduction is related to a complete array of processes. The basic concept may also be divided into many sub-concepts.

Since signal transduction involves so many different concepts and processes, plain text may fail to convey understanding in every aspect. Educators and researchers have been commenting on the potential of using animations to facilitate the visualization of concepts and processes within the field of genetics (Tsui & Treagust, 2004; Wu *et al*., 2001), which is closely related to signal transduction.

1.3 Overview of the thesis

This thesis has been divided into eight sections. Section 1 introduces complex cell biology processes and visualizations of them. Section 2 includes previous research in the use of visualizations for teaching life science and the aim of the study. Section 3 presents the animations used in the study as well as the issues of layout of the animations. Section 4 states the research questions of the study. Section 5 is the method section and deals with the participants of the study, the research instruments (questionnaires, interviews) and data analysis. Sections 6 and 7 are the result sections and include the findings from questionnaire 2 and 1 respectively. Data from the interviews and lay-out related discussion can be found in these sections as well. Section 8 includes general results, discussion and implications.

2. Background

2.1 Previous research in the use of visualizations

The use of images, diagrams and other forms of visualization in order to teach life science has become more common. Kozma concludes that the way we understand chemical phenomena is connected to the external representations we use to present them (Kozma, 2003; Kozma *et al*., 2000). Textbooks dealing with life sciences are rich in illustrations and often include graphical complements. Multimedia, in which several forms of representations are used at the same time, has emerged as a learning tool. However, relatively little is known about conditions that promote the effectiveness of combining illustrations, animations, audio and text. The combination of words and images can enhance understanding and the ability to solve problems (Mayer, 1997). The combination is suitable as an introduction to a new concept or discipline. Learners, who have deficiencies in their prior learning knowledge, can especially benefit from teaching where text is combined with pictures (Mayer, 1989). But if the connection between text and image is not logical and clear, learning can become difficult (Schnotz and Bannert, 2003). There might also be a risk of students being distracted due to the abundance of information.

The way concepts are visualized affects the understandability of complex concepts. Proteins have been called “semi-liquid” because the movements of their atoms are larger than those found in solids, but smaller than those observed in liquids. These atomic rearrangements occur faster than the time required to determine the structure with instruments. Thus, pictures of protein structure emerging from different forms of instrumentation are average structures. It is probable that there is no such thing as a free-floating protein in an eukaryotic cell. Many proteins are constrained, whether in a complex with other macromolecules, within a specific organelle, in a cargo vesicle, by attachment to a membrane or the cytoskeleton. Prokaryotic cells, which lack organelles and cytoskeleton, may be less highly structured.

A cell contains thousands of different protein molecules, which are packed into a small volume. Visualization of the cell might be complicated since the environment in cells is so crowded and structurally complex (Petsko and Ringe, 2004).

Realistic visualization of time-dependent processes in cells may be hard to grasp since events occur on a time scale different from ours. Because of these complications it may be appropriate to use schematic depictions for specific life science concepts. Of course schematics may result in flawed comprehension of biological phenomena for students. But Carl-Johan Rundgren (2006) concludes that the learning of life science concepts is based on piecing together facts from many different sources.

Life science includes numerous concepts, ranging from molecular rearrangements of individual molecules to complex gene regulation pathways. What makes life science unique is that the wide range of concepts within the field is interrelated and includes elements from every major scientific discipline like physics, biology and chemistry. Central to many life science concepts, including signal transduction, is the flow of information from genes to protein molecules. The genetic code is strictly a digital one, consisting of the four variables Adenine, Thymine, Guanine and Cytosine. With the intermediate stages of transcription and translation the sequence of base-pairs in the DNA-double helix is transformed to the sequence of amino-acids constituting protein molecules. This process has been proven suitable for visualization with computer animations. Computer animations were more useful than illustrations when teaching the concept of the relationship between genetic material and its products (Rotbain *et al.*, 2006).

Student learning research has shown that visual perception is the most developed sense in humans and is an important way by which we learn (Sekular and Blake, 1985). Further research has shown that by using visual tools, students can comprehend large amounts of information in a relatively short time and construct their personal visualization of a process (Kraidy, 2002). Motion leads to longer-term memory, an effect not observed with static images (Goldstein *et al*., 1982). This result is most dramatic for individuals who have difficulty in grasping spatial relationships (Blake, 1977). This beneficial effect of animation for long-term memory has also been observed with life science animations (O'Day, 2007).

The use of animation for teaching life science concepts has been studied at several occasions by different research groups. 2D-animations have been found useful for teaching some life science concepts (O'Day, 2006), whereas 3D-animations were found useful for teaching basic life science concepts like transcription, translation and cellular respiration (McClean, 2005). 3D-animation can show molecular structure and interactions and has potential for teaching structure-dependent life science concepts. It has been suggested that structures are often the easiest aspect of a complex system to learn (Hmelo *et al*., 2000). In molecular genetics, understanding structures of molecules such as DNA and RNA is important to comprehend their functions.

O'Day (2006) investigated the use of 2D-animation for teaching signal transduction. But 2D-animations will fail to convey understanding of life science concepts, including signal transduction, in every aspect. Since they are flat, important spatial relationships of the processes are not captured.

The existence of living cells relies on numerous highly interconnected interactions and chemical reactions between various types of molecules such as proteins, DNA, RNA, and small metabolites. Various activities of cells are controlled by the action of molecules upon molecules. 3D-visualizations illustrate how and where molecules interact and provide a spatial representation of the molecules during the process and may be useful for teaching signal transduction.

To my knowledge, no study has investigated so far the use of 3D-animation for teaching complex signal transduction pathways.

2.2 Context and aim of the study

The participants of the study were Bioscience students at university level attending a course in molecular cell biology at Karlstad University. The sample of students was limited by the fact that there were no alternative courses with the same content at Karlstad University. The students had taken introductory courses in various disciplines like organic chemistry, biochemistry, anatomy and physiology. The course in molecular cell biology was on introductory level and signal transduction was one of the more advanced topics in the course.

The aim of this research project was to investigate the understandability and usefulness of 3D-dynamic visualizations (animations) in life science, in general, and signal transduction, in particular, for Bioscience university students.

3. The animations

3.1 Aspects of the animations

Four animations were developed. Animation 1 and 2 were produced before any data had been gathered. The design choices were made based on previous experience with illustrations in textbooks (Alberts *et al*., 2002; Petsko and Ringe, 2004) dealing with life sciences and other graphical complements often included.

Animation 3 and 4 were produced after the conduction of a pilot study (see section 5.2.1). Two major changes in the general layout (text and zoom) of the animations were introduced because of the pilot study. Otherwise the design choices for these animations were also based on previous experiences with textbooks and accompanied graphical complements. The aspects of layout of the animations were classified into issues. The issues are listed in table 1.

Table 1. The aspects of layout of the animations were classified into the following issues:
(a) text labeling of molecules in animations
(b) explaining text in animations
(c) color of molecules in animations
(d) zoom in animations
(e) pace of animations
(f) level of realism in animations
(g) multimedia
(h) protein shape
(i) protein motion
(j) 3D-dynamic visualizations
Each category will be clarified below.

3.1.1 (a) Text labeling of molecules in animations

One of the main differences in design choices between the two sets of animation was the use of text. In animation 1 and 2 the voiceover was not supported by text at any occasion. Animation 3 and 4 included text labels of molecules that indicated either biochemical function or chemical identity. Animation 3 also included temporary labels that indicated chemical reactions (phosphorylations). The text labels of molecules were either temporary (animation 3) or constant (animation 4). Text in the animations was introduced after conduction of the pilot study where students missed labels on molecules.

3.1.2 (b) Explaining text in animations

Explaining text like headings or introductions was left out due to the risk of students being distracted by the abundance of information. Another reason for leaving explaining text out was the aim of the study: to investigate the understandability and usefulness of 3D-dynamic visualizations. Without explaining text it would be easier to evaluate the impact of 3D-dynamic visualizations on students learning of life science concepts.

3.1.3 (c) Color of molecules

The use of color in textbooks dealing with life science is inconsistent and molecules were assigned different colors arbitrarily to achieve visual distinction between molecules. Activation of protein molecules in a signal transduction pathway was visualized by a temporary light effect. The color of the plasma membrane of animation 1 and 2 were gray and then changed to blue in animation 3 to see if any students reacted.

3.1.4 (d) Zoom in and out of the scene

In animation 1 and 2 the camera did not zoom in or out of the scene to a great extent at any occasion. One student in the pilot study requested an aid for identifying the localization of processes visualized in the animations. In animation 3 and 4 the camera zooms out of the scene to provide an overview of the complete process. Another solution to the problem of localization would have been a mini-map of the cell beside the main animation that showed the localization of processes. This would have been more technically demanding to produce than camera zoom effects and were left out due to time constraints. Yet another solution would have been to let explaining text indicate the localization of processes. As mentioned above explaining text was left out due to the risk of students being distracted by the abundance of information.

3.1.5 (e) Pace of the animations

The pace (tempo) of the animations were set arbitrary dependent on the personal preference of the producer.

3.1.6 (f) Level of realism in animations

The animations were produced without background scenery like organelles and the cytoskeleton. Only the molecules most relevant to the current topic were depicted because the producer thought this would increase clarity. The velocities of molecules in the animation were not realistic (much slower) but set arbitrarily by the producer to fit the human level of perception. The distances between molecules were sometimes much larger than the realistic crowded environment in living cells to increase general visual clarity. The structural rearrangements of molecules were exaggerated to increase clarity of reaction mechanisms. The teacher said that the structural rearrangements could have been exaggerated even more. Biochemical reactions were visualized step-by-step to convey understanding of the reaction processes.

3.1.7 (g) Multimedia

Voiceover was added to make use of an additional way to present information. The animation includes two codes; pictorial and verbal. The use of voiceover is also the convention in animation for learning purposes. The images in the animations were timed to fit the voiceover. As mentioned above the use of text in the animations was restricted.

3.1.8 (h) Protein shape

It is the sequence of amino-acids that determines the protein molecules´ 3D-structure and thus its function. There are many levels of protein function, ranging from atomic reorganizations to changes in the development of an organism, but all of them involve binding to other molecules. Sometimes this specific molecular recognition is the sole biochemical function of a protein, but in other cases the protein also promotes a chemical transformation in the molecule that it binds. The structural features of protein molecules govern the ability of proteins to specifically recognize and bind a wide variety of molecules, small and large. These structural features also govern the ability of protein molecules to catalyze the wide variety of chemical transformations on which life depends.

The structure of proteins is important for their function in a cellular context. Many proteins are constructed in a modular fashion from a number of different small domains with distinct binding specificities and functions. Many gene regulatory proteins, for example, are composed of a domain that binds a specific DNA sequence and a protein-binding domain, which may target another gene-regulatory protein. The first protein molecules position the second protein molecule in close proximity to the DNA-molecule, consequently enabling the binding of the second protein molecule to the DNA-molecule. In some enzymes, a catalytic domain is attached to one or more protein-binding domains. The protein-binding domains, or interaction domains, target the attached catalytic domain to a particular multi-protein complex or an appropriate sub-cellular location, such as the nucleus or the plasma membrane.

The protein molecules were visualized as compact bodies with different shape which facilitates visual distinction between molecules. In animation 2 which introduces different classes of signaling proteins, protein shape was sometimes used to illustrate protein function. For example, a protein (anchoring protein) whose primary function is to hold another protein in place was visualized as a tongs-shaped protein. In animation 3, which introduce the concept of interaction domains, the surface structure of proteins was carefully modeled with the aid of molecule structure data in PDB-format (Protein Data Bank) because surface structure is important for the complementary fit between interaction domains and the structures they recognize.

3.1.9 (i) Protein motion

Only the proteins undergoing reactions were visualized moving while the rest remained still in order to help the students focus on the current process. The teacher said this would possibly increase the understandability of the scientific content in the animations.

3.1.10 (j) 3D-dynamic visualizations

All cellular processes are subject to regulation. The cell can be regarded as a dynamic machine, whose function needs to be regulated according to environmental needs. The genome of an organism constitutes the guide for the actions of the molecular machinery. Protein molecules estimate the status of the environment and report back to the genome, which responds by altering its transcriptional activity. Information flows in both directions from the genome and the protein molecules that regulate its transcriptional activity.

In molecular genetics, understanding structures of molecules such as DNA and RNA is important to comprehend their functions. The information flow in cells is often facilitated by conformational changes in protein molecules and is structure dependent.

Individual protein molecules often assemble into macromolecular complexes, which assemble into pathways. Since the 3D-structure or connectivity of pathways regulates various activities in cells, 3D-animation can visualize how cell signaling governs the dynamics of cell structure and thereby its function. These animations were designed to make use of the third dimension, by visualizing continuous time-dependent processes like signal transduction chains. There are many levels of protein function and all of them involve binding to other molecules. 3D-visualizations can include surface structure of proteins and illustrate the concept of protein interaction and its importance for systemic function.

3.2 Description of the animations

3.2.1 Production

3D-dynamic visualizations were produced with the commercial software package 3D Studio Max from Autodesk. 3D Studio Max (3ds max) is commonly used in the 3D-graphics industry for architectural visualization, computer games and special effects in movies. It is not originally intended for visualization of molecular graphics, but plug-ins have been developed in order to import molecular structure data from bioinformatics databases. The data of molecular structures were processed with the software to attain the desired visual appearance. Sequences of images with molecular graphics were rendered with the built-in renderer of the software package; Mental ray. The images were composited to animations with the software package After Effects from Adobe. Voiceover and text were added with the same software package.

3.2.2 Animation 1: Introduction to cell signaling

The first animation serves as a general introduction to signal transduction. The students were introduced to and familiarized with basic terminology in the field. The animation gives an overview over inter- and intracellular signaling and its importance for cell function.

Some concepts introduced:

- Activation of cell-surface receptor molecules in the plasma membrane by extracellular signal molecules.
- Relay of information by intracellular signaling molecules that distribute the signal to appropriate parts of the cell in order to achieve a cell response.
- Molecular structure of large and small signaling molecules.
- The importance of molecular structure for relay of information along a pathway.
- How conformational change gives the protein molecule new properties that enable it to accomplish new tasks.
- Different types of target molecules like gene-regulatory proteins, ion-channels and components of metabolic pathways or the cytoskeleton.

3.2.3 Animation 2: Classes of signaling proteins

Intracellular signaling proteins relay the signal into the cell by either activating the next signaling protein in the chain or generating small intracellular mediators. These proteins can be classified according to their particular function, although many fall into more than one category. The animation introduced the following classes of signaling proteins to the students:

- Relay proteins simply pass the signal to the next component in the signal transduction chain.
- Messenger proteins carry the signal from one part of the cell to another.
- Adaptor proteins link two signaling proteins, without themselves conveying a signal (figure 1).

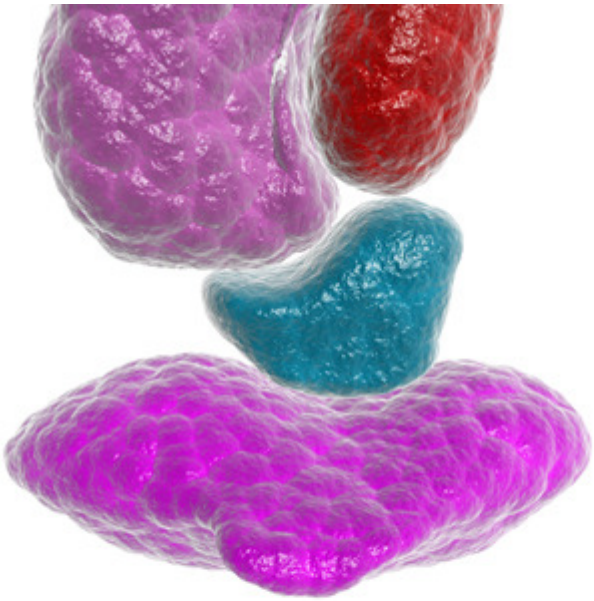

Figure 1. Adaptor protein.

- Amplifier and transducer proteins greatly increase the signal they receive, either by producing large amounts of small intracellular mediators or by activating large numbers of downstream intra-cellular signaling proteins.
- Bifurcation proteins spread the signal from one signaling pathway to another.
- Integrator proteins receive signals from two or more signaling pathways and integrate them before relaying a signal onward.
- Scaffold proteins bind multiple signaling proteins together in a functional complex and often hold them at a specific location.
- Anchoring proteins maintain specific signaling proteins at a precise location in the cell by binding them to a membrane or the cytoskeleton (figure 2).

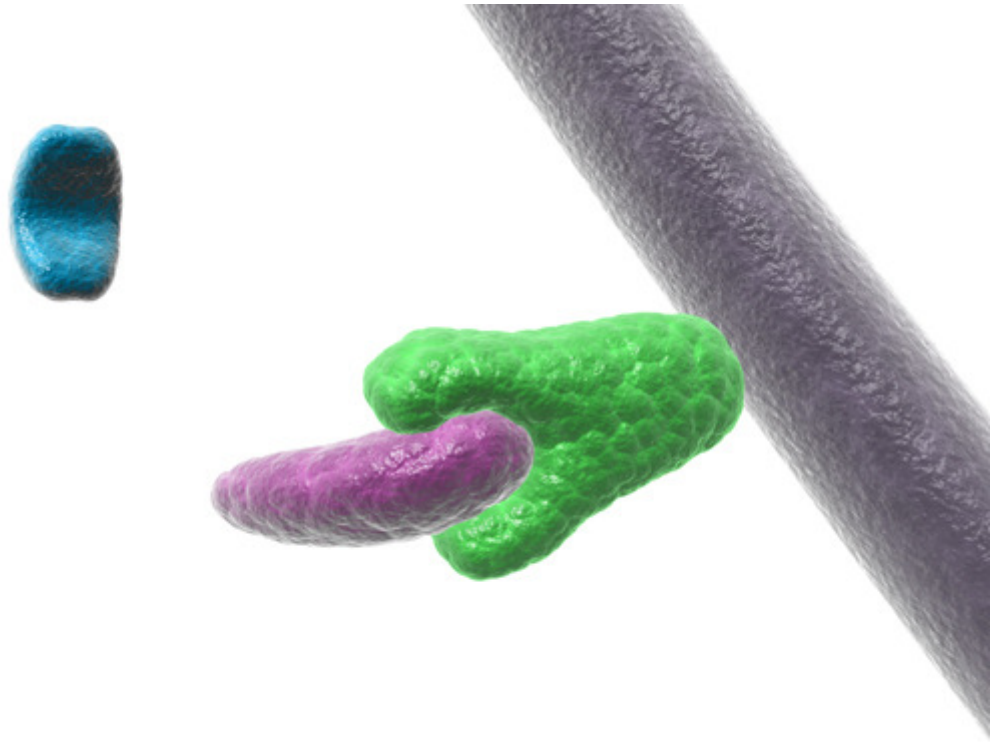

Figure 2. Anchoring protein.

3.2.4 Animation 3: Interaction domains

The assembly of both stable and transient multi-molecular protein complexes depends on the existence of interaction domains. The animation visualizes the assembly of a fictional signaling complex in a multi-step, time-dependent process involving several conformational changes in the participating proteins. The assembly of the signaling complex is initiated by activation of a cell-surface receptor which leads to the creation of various phosphorylated docking sites on the cytosolic face of the membrane.

A signaling protein has an interaction domain that recognizes the docking sites and binds to the membrane, as illustrated in figure 3.

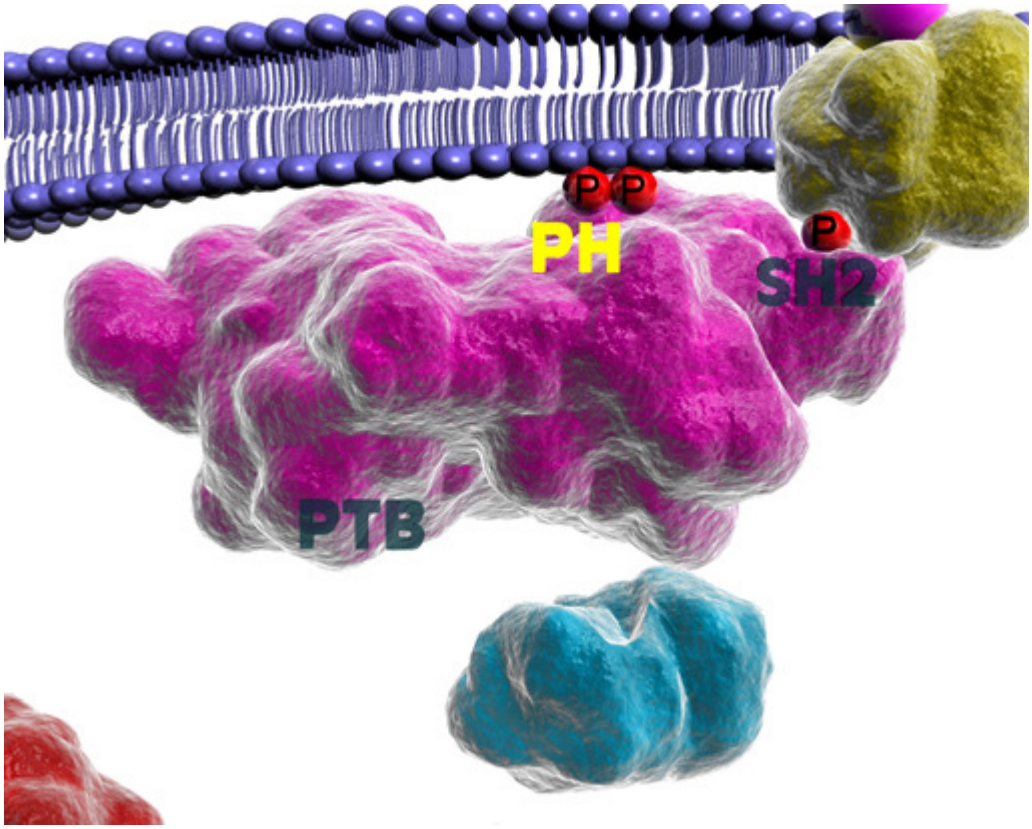

Figure 3. The PH-domain of signaling protein 1 binds to phosphorylated docking sites in the cell membrane.

The second protein to join the complex has an interaction domain that recognizes a specific structure on the first protein. The interaction domain positions the second protein in close proximity to the catalytic domain of the first protein that catalyzes the phosphorylation of the second protein. In some cases the phosphorylation triggers a conformational change in a distant part of a protein. In this case the phosphorylation itself provides the structural change being recognized by the next component to join the signaling complex, since protein three recognizes the phosphate (PO_4) group added to protein two. After a while the camera zooms out of the scene for an overview of the assembled complex.

Finally the camera zooms on two interaction domains of the same family to illustrate how small differences in amino-acid sequence among members of a family makes the binding between individual interaction domains and their targets specific.

The second scene of the animation visualizes how the 3D-structure of proteins is dependent on the sequence of amino-acids. Instead of being visualized as rigid structures the protein molecules in this sequence are flexible structures that bind to their targets with induced fit, as illustrated in figure 4.

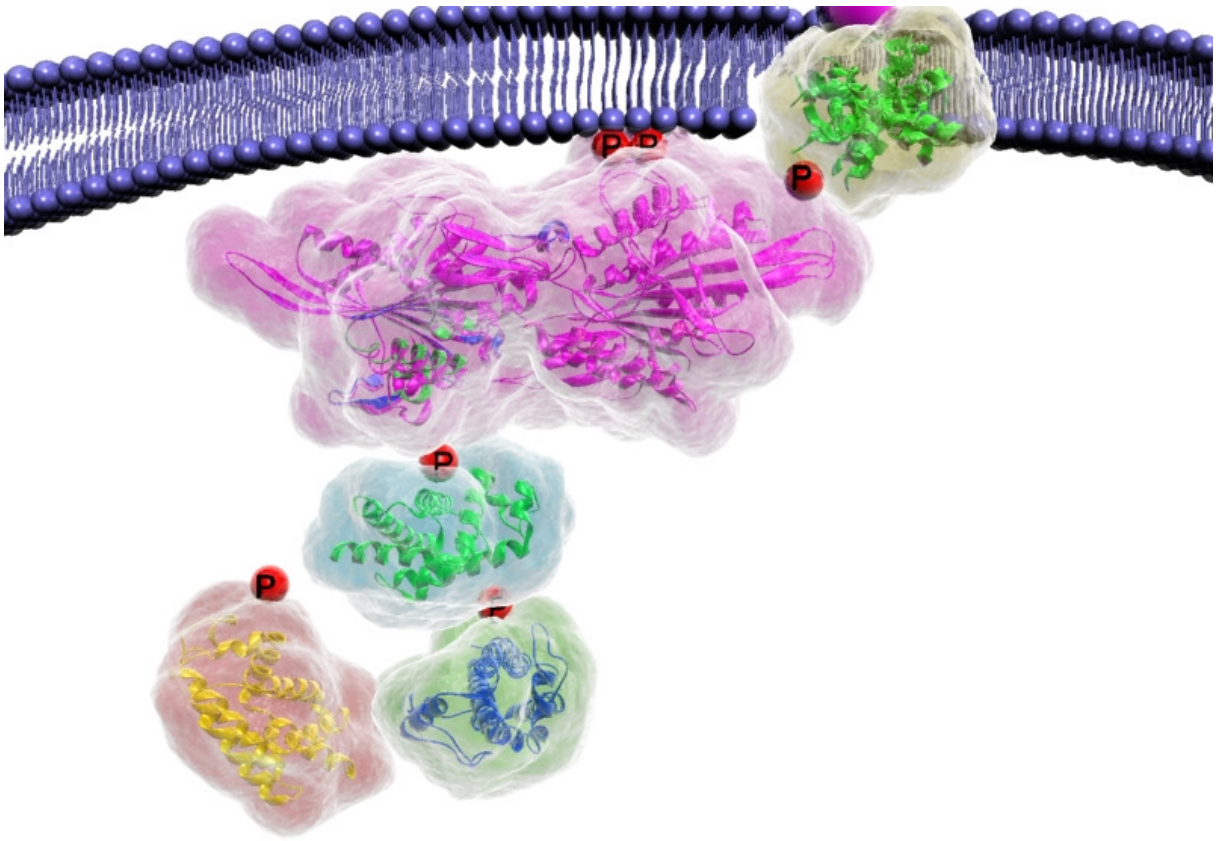

Figure 4. Proteins illustrated as flexible structures.

3.2.5 Animation 4: G protein signaling

The animation introduced the students to a real signaling pathway; G protein signaling. Signal transduction through G protein-linked receptors requires three membrane-bound components: (1) A cell surface receptor that determines to which signal the cell can respond.

(2) A G protein on the intracellular side of the membrane that is stimulated by the activated receptor.

(3) Either an effector enzyme that changes the level of a second messenger or an effector channel that changes ionic fluxes in the cell in response to the activated G protein.

The human genome encodes for more than 600 receptors for extracellular molecules that bind to one or more of the identified G proteins. These, in turn, regulate one or more different effector channels and enzymes. The key feature of this flow of information is the ability of G proteins to detect the presence of activated receptors and to amplify the signal by altering the activity of appropriate effector enzymes and channels.

The animation visualizes the complete chain of events from activation of the G protein-linked receptor by an extracellular signal molecule to the activation of gene transcription by the gene regulatory protein CREB. In the end the camera zooms out of the scene for an overview of the signaling pathway.

4. Research questions

The first research question of the present study was related to the understandability of the animations. This question was formulated as follows:

To what extent do the issues of layout contribute to students' understanding of the animations and the biochemical content?

The second research question of the present study was related to the usefulness of the animations. This question was formulated as follows:

To what extent do these animations contribute to the teaching of life science in general and signal transduction in particular?

Both questions will be answered in terms of the opinions of the bioscience university students and their course teacher.

5. Methods

5.1 Participants

The investigation was performed with a total of 24 students attending a course in molecular cell biology at the faculty of Technology and Science, Karlstad University, Sweden. The group consisted of 6 males, age 21 – 42 and 18 females, age 19 – 41. Most of the students were in their second year of study at the Bioscience program. The students had taken introductory courses in various disciplines like organic chemistry, biochemistry, anatomy and physiology. The course in molecular cell biology was on introductory level and signal transduction was one of the more advanced topics in the course.

5.2 Design

5.2.1 Pilot study

Before the animations were produced a pilot study was conducted with eight Bioscience students from Karlstad University. The participants had attended the course in molecular cell biology the year before the students of the main study. A DVD with two sample animations was distributed together with questionnaires and data were collected by a mailbox within one week. The students' opinions were summarized and categorized. An interview was performed with one of the students to get an in depth understanding of the categories from the questionnaires. The result of the pilot study was evaluated before the animations for the main study were produced.

The results from the pilot study were used to design animation 3 and 4 as well as for the design of questionnaires to the main study. Two major changes in the general layout (text and zoom) of the animations were introduced.

In animation 1 and 2 the camera did not zoom in or out of the scene to a great extent at any occasion. One student in the pilot study requested an aid for identifying the localization of processes visualized in the animations. In animation 3 and 4 the camera zooms out of the scene to provide an overview of the complete process and help with localization.

The other main difference in design choices between the two sets of animation was the use of text. In animation 1 and 2 the voiceover was not supported by text at any occasion. Animation 3 and 4 included text labels of molecules that indicated either biochemical function or chemical identity. Animation 3 also included temporary labels that indicated chemical reactions (phosphorylations). The text labels of molecules were either temporary (animation 3) or constant (animation 4). Text in the animations was introduced after conduction of the pilot study where students missed labels on molecules.

5.2.2 Main study

The lectures in the main study were distributed over three days. The first day had a 2 hours lecture. There was a main 4-hour lecture on the second day. The third day had another 2 hours lecture. The animations were shown at two different occasions, once on day 1 before the first 2 hours lecture and once on day 3 after the second 2 hours lecture. The animations were shown on a big screen with a projector in the classroom. The teacher gave an introductory talk about the study and the animations before the first time they were shown. The complete movie with four parts was 15 minutes long and was shown without breaks. The students could ask questions after the animations.

Data were collected by questionnaires from all students referred to as St. 1 - St. 24 in the result section. Questionnaire 1 was handed out to the students after the first time the animations were shown (day 1). Questionnaire 2 was handed out after the second time the animations were shown (day 3). There were audio-taped whole class discussions and group interviews after each occasion. After each questionnaire the students reported plenary what were unclear to them. The lectures between the two occasions were audio-taped. Interviews with the teacher were conducted before and after the lectures of the study. The student interviews were conducted with two student groups consisting of three students each. Each group was interviewed twice for about 45 minutes each occasion. The interview questions were the same as in the questionnaires. The students had access to their finished questionnaires during the interviews and could elaborate on their responses. The students participating in the interviews were volunteers.

5.3 Instruments

5.3.1 The questionnaires

The results from the pilot study were used for the design of questionnaires to the main study. Otherwise the design choices for the questionnaires were made based on previous experience with illustrations in textbooks dealing with life sciences and other graphical complements often included.

Questionnaire 1

The questionnaire handed out before the lectures (questionnaire 1) was a test of the students' actual understanding of the scientific concepts in the animations and included some pictures. Questionnaire 1 comprised five questions about signal transduction. The questions were designed with the intention to give an estimation of the students' knowledge about signal transduction. This questionnaire was filled in by 24 students.

Questionnaire 2

The questionnaire handed out after the lectures (questionnaire 2) mostly treated the layout of the animations. Questionnaire 2 comprised seven questions and was open-ended. The questions were designed with the intention to give an overview of the student's opinions regarding the animations. This questionnaire was filled in by 12 students (2 males, age 25 – 30 and 10 females, age 20 – 41).

The students had 20 minutes to finish the questionnaire at each occasion. After the questionnaire the students reported plenary what was unclear to them. The response to questionnaires were read several times and categorized. The findings in the rough data were listed and the categories redefined in an iterative process. Issues of students' understanding were identified and categorized in a similar process. Questionnaire 1 was corrected and individual responses were merged to find the overall trend. Each question in the questionnaire is referred to as Q_xY under each finding category in the result section, where x indicates which questionnaire and Y which question. Both questionnaire 1 and 2 are added as appendixes.

5.3.2 The interviews

Student

The interviews were conducted with two student groups consisting of three students each. The students participating in the interviews were: St. 1, 2, 3, 4, 5 and 12. Each group was interviewed twice. One interview was conducted after questionnaire 1 and the other after questionnaire 2. The interview questions were the same as in the questionnaires to probe deeper into individual responses. The students had access to their finished questionnaires during the interviews and could elaborate on their responses. The students participating in the interviews were volunteers. They approved of audio-taping the interviews. Each interview lasted for about 45 minutes.

Teacher

Interviews with the teacher were conducted before and after the lectures of the study. The interviews with the teacher were conducted with the intention of finding out if his expectations would match the results from study. The teacher gave his opinion on the outcome of the questionnaires, in terms of their understanding of the biochemical content in the animations, as well as the layout-aspects. In the second interview the teacher commented on the usefulness of the animations for teaching signal transduction.

The interview transcripts were read several times and categorized. The findings in the rough data were listed and the categories redefined in an iterative process. Issues of students' understanding were identified and categorized in a similar process. The results from the interviews are presented together with the results from the questionnaires.

5.4 Data analysis

The interviews and the questionnaires were analyzed in the following 12 steps:

(1) The interviews were transcribed in full.

(2) The transcripts were read several times to get an overview of the interviews.

(3) The responses to questionnaires were translated and listed in tables.

(4) Questionnaire 1 was corrected and individual responses were merged to find the overall trend.

(5) The responses to questionnaires were read several times and categorized.

(6) The findings in the rough data were listed.

(7) The findings were relisted and the categories redefined in an iterative process.

(8) Issues of students' understanding were identified and categorized.

(9) The results from the interviews were compared with the results from the questionnaires and summarized.

(10) From these summaries main categories and subcategories were identified.

(11) A mind map illustrating the relationship between issues of layout and categories of understanding were produced.

(12) The connection between issues of layout and categories of understanding were listed in a table.

5.5 Issues of layout and categories of students' understanding

Issues of students' understanding were identified and categorized into the following classes:

(1) identification of molecules

(2) distinction between molecules

(3) localization of processes

(4) protein function

(5) systemic function

The connection between issues of layout and categories of students' understanding is summarized in Table 2. A cross indicates a connection between the layout-issue and category of understanding.

Table 2. Connection between layout-issues and categories of students' understanding.

Issues of layout	**Categories of students' understanding**				
	Identification	Distinction	Localization	Protein function	Systemic function
Text: (a) Labeling molecules (b) Explaining text	x		x	x	x
(c) Color		x			
(d) Zoom			x		x
(e) Pace				x	x
(f) Level of realism				x	x
(g) Multimedia				x	x
(h) Protein shape (i) Protein motion	x x	x x		x x	x x
(j) 3D-dynamic viz.				x	x

Categories of students' understanding

(1) Identification of molecules

When more than one molecule is visible, the identification of individual molecules is important for the interpretation of visualizations. The category is related to the layout issues: (a) text labeling of molecules, (h) protein shape, (i) protein motion

(2) Distinction between molecules

Distinction is the issue of observing the physical boundaries of individual molecules. The category is related to the layout issues: (c) color of molecules, (h) protein shape, (i) protein motion

(3) Localization of processes

Specific biochemical processes take place in different and physiologically distinct cellular compartments, for example, the nucleus or the cytoplasm. Knowledge of where specific biochemical processes take place is important for understanding the visualizations. The category is related to the layout issues: (b) explaining text, (d) zoom in and out of the scene

(4) Protein function

Proteins have particular functions in the cell and for that reason affect cell behavior. Knowledge of protein function is important for understanding biochemical reaction mechanisms. The category is related to the layout issues: (b) explaining text, (e) pace, (f) level of realism, (g) multimedia, (h) protein shape, (i) protein motion, (j) 3D-dynamic visualizations

(5) Systemic function

Molecules cooperate by forming biological inter- and intracellular functional and communicational networks. Realization of how emergent properties arise from a multiplicity of individual molecule interactions is important for understanding biological systems. The category is related to the layout issues: (b) explaining text, (d) zoom in and out of the scene, (e) pace, (f) level of realism, (g) multimedia, (h) protein shape, (i) protein motion, (j) 3D-dynamic visualizations

6. Findings and discussion (data from Questionnaire 2 and student interview 2)

The data in this section will be presented according to table 1 in section 3.1, where the issues of layout of the animations were classified into categories. This section also includes data from the teacher interviews.

6.1 (a) Labeling of molecules in animations

Students' ideas in this category are based on their answers to $Q_2$1, $Q_2$2 and $Q_2$5.

6.1.1 Findings:

Individual molecules have to be labeled when there is more than one molecule visible. Otherwise students will not be able to identify the molecule at hand. No student reacted to the fact that different ways to label were used. One student said that arrows could be used to identify molecules.

> *$Q_2$2 Were there unclear parts in the movie?*
>
> *"The proteins should be labeled all the time and light up when mentioned. Otherwise the animations were unclear."* (St. 9)

> *$Q_2$5 Do you want more text in the movie?*
>
> *"Yes on every molecule. Then you know for sure which is which one. A name-tag should be enough, if the voiceover is clear. If something is activated, maybe it should be made visible by text."* (St. 9)

Two students said that they liked when the proteins undergoing reactions light up. It made it easier to identify the protein at hand.

$Q_2$1 Write down 3 things you liked about the movie.

"It was clarifying when the proteins light up, it made it easier to understand which protein was at hand." (St. 9)

6.1.2 Discussion:

No student reacted to the fact that there were different ways to label molecules and processes in the animations, which could mean that they were satisfied with the ways used. In contrast to animation 1 and 2, no students had problems identifying molecules in animation 4. This indicates that constant text labeling facilitates identification of molecules. One student said that arrows could be used to identify molecules. But the use of arrows as a label in animations might be misleading (Hull *et al*., 2002) due to the common use of arrows for other purposes in textbooks and diagrams. Another option would have been to combine arrows with text. But then there might be a risk of students being distracted due to the abundance of information.

The activation of protein molecules in signal transduction chains were visualized by a temporary light effect. Yet another option for labeling would have been to exclusively use light effects on proteins at hand. But this might be problematic in some cases since not all proteins at hand undergo reactions like activation.

6.2 (b) Explaining text in animations

Students' ideas in this category are based on their answers to $Q_2$2, $Q_2$5 and $Q_2$7.

6.2.1 Findings:

Four students missed an introductory text or summary before each scene. The students found the animations unclear and lost track.

$Q_2$5 Do you want more text in the movie?

"Maybe text explaining what will happen, like a heading." (St. 4)

$Q_2$7 What parts of the movie can be improved to make it easier for future students?

"Heading before each scene otherwise one loses track of what´s happening." (St. 3)

"Summary at empty frames and introductory text about the content of the movie." (St. 4)

One student said the use of explaining text would take away the attention from the animation. Another student said that since he had previous experience with the subject no text was needed (he had watched the animation twice). But he said that text might be useful if the animation is an introduction to the concept of signal transduction.

$Q_2$5 Do you want more text in the movie?

"Names on proteins, otherwise too much text. If you have to read text you can´t concentrate on the animation." (St. 2)

"Because you have previous experience with the subject no text is needed. But the first time I watched the animations explaining text might have been useful." (St. 3)

6.2.2 Discussion:

The results from the pilot study indicated that there might be a risk of students being distracted by the abundance of information in case there is too much text. This was supported by literature. There might also be a risk of using text if the connection between text and image is not logical and clear (Schnotz and Bannert, 2003). There was a risk of ruining the animations. The intention of the research project was to investigate the use of 3D dynamic visualizations. If students have prior knowledge of basic biochemical principles, concepts, processes, molecules then probably less explaining text is needed.

6.3 (c) Color of molecules in animations

Students' ideas in this category are based on their answers to $Q_2$1 and $Q_2$3.

6.3.1 Findings:

Five of the students said that they appreciated molecules with different colors. The teacher agreed. No student had problems observing the physical boundaries of molecules. No one reacted to the fact that there was no consistent way of using colors.

$Q_2$1 Write down 3 things you liked about the movie.

"That there were different colors used for the protein and the receptor, for example." (St. 12)

$Q_2$3 What did you think about the appearance of the proteins?

"The proteins had different colors which increased clarity" (St. 10)

6.3.2 Discussion:

Colors in the animation were most important for distinction because the molecules were assigned colors arbitrarily. The color of the cell membrane was changed between the animations without any students reacting. The use of color in textbooks dealing with life science is inconsistent, for that reason, students do not connect a specific color to a specific molecule or process.

The drawback of using color for distinction between molecules is that in more complex animations colors can be needed for other purposes, like for example indicating frequencies or energy levels. Colors have potential to be used for indicating expression frequencies, molecule amounts, energy levels and distribution of particles/charges.

6.4 (d) Zoom in animations

Students' ideas in this category are based on their answers to $Q_2$1 and $Q_2$2.

6.4.1 Findings:

Three students reported that zooming in and out of the scene increased their understanding.

> *$Q_2$1 Write down 3 things you liked about the movie.*
>
> *"Zoom in and out helps to understand the concepts."* (St. 4)
>
> *"Great overview, zoom-out in the end of the movie increases comprehension."* (St. 9)

One student had problems identifying in which cellular compartment a reaction took place. The sequence the student refers to lack of zoom. His statement indicates that zoom can facilitate localization.

> *$Q_2$2 Were there unclear parts in the movie?*
>
> *"Sometimes there were only balls in the scene; you have no idea where they belong to."* (St. 3)

6.4.2 Discussion:

Video-microscopy is the use of a high-quality video camera or other fast camera (such as a charge-coupled device) attached to a research-quality light microscope for the purpose of real-time or high-speed imaging of samples on a microscopic stage. These images are recorded at regular intervals, often at the rate of 30 images per second, and the time-laps sequence can be played back in the form of a movie. In contrast to video-microscopy, computer animation provides almost unlimited resolution and magnification. But unlimited magnification also poses design issues, since the producers of animations have to decide which magnification to use. One option is to let the camera zoom in and out of the scene. This can help with overview and localization of biochemical processes.

Another solution to the problem of localization would have been a mini-map of the cell beside the main animation that showed the localization of processes. Yet another solution would have been to let explaining text indicate the localization of processes. Explaining text was left out due to the risk of students being distracted by the abundance of information.

6.5 (e) Pace of animations

Students' ideas in this category are based on their answers to $Q_2$4, $Q_2$7 and student and teacher interviews.

6.5.1 Findings:

One student said the pace of the movie decreased the second time she watched it. She reported that she understood more of the scientific content the second time.

In the interviews both the teacher and the students said watching the movie twice increased comprehension of the movie content more than being at lectures.

> *$Q_2$4 Would you like to change the voiceover? If so, how?*
>
> "*The first time I watched the movie I thought the voiceover was too fast. The second time the pace was alright.*" (St. 4)

> *$Q_2$7 What parts of the movie can be improved to make it easier for future students?*
>
> "*But the best for future students is to watch the animations twice. You think you recognize a little bit more the second time.*" (St. 4)

6.5.2 Discussion:

The pace of the animations were set arbitrary dependent on the personal preference of the producer. The students reported that watching the movies twice was beneficial. The second time over the students were prepared for the movie content. If the students had any previous problems with interpretation of specific sequences or concepts in the movie, they would be prepared to listen extra carefully at these sections the second time.

6.6 (f) Level of realism in animations

Students' ideas in this category are based on their answers to $Q_2$1, $Q_2$3 and interview with St. 3.

6.6.1 Findings:

Four students reported that the animations were clear. No student reacted to the fact that the animations lacked background scenery or that the velocities of and distances between molecules in the animation were not realistic. One student reported that the exaggerated structural rearrangements of molecules helped to clarify reaction mechanisms. The teacher agreed but said that the rearrangements should have been exaggerated even more to increase clarity. Three students reflected on realistic depictions of proteins. One student reported that the exchange of GDP for GTP in the activation of the G-protein (animation 4) was too slow. In the interview he elaborated on the topic and said that the activation of the G-protein should not be visualized step-by-step but all-at-once.

> *$Q_2$1 Write down 3 things you liked about the movie.*
>
> *"Good/appealing and clear animations, you could easily tell how everything in the cell comes together"* (St. 8)
>
> *"You could easily tell when proteins were changing"* (St. 12)

$Q_2$3 What did you think about the appearance of the proteins?

"Good as far as I know, I don´t really know how they should look like" (St. 6)

"They looked fine; I can imagine that protein molecules may look something like that in reality" (St. 8)

"They looked a bit bombastic, but that is maybe *how they should look like in reality? But they didn´t need to be rose-colored"* (St. 9)

One student had problems to identify in which cellular compartment a reaction took place. The sequence the student referred to lacks background scenery.

$Q_2$2 Were there unclear parts in the movie?

"*Sometimes there were only balls in the scene; you have no idea where they belong to.*" (St. 3)

6.6.2 Discussion:

Since cells and molecules exist on a level of perception utterly different from ours it might be difficult to define criteria for realistic depictions. The movement of atoms in protein molecules is larger than those found in solids, but smaller than those observed in a liquid. These atomic rearrangements occur so fast that pictures of protein structure emerging from different forms of instrumentation are average structures. It would be more realistic to visualize structures for multiple conformational sub-states of molecules and how they might morph between these states. Visualization of the cell might be complicated since the environment in cells is so crowded and structurally complex (Petsko and Ringe, 2004). There is a problem of deciding which molecules and structures to include in the animation.

The animations were produced without background scenery like organelles and the cytoskeleton. The lack of background scenery in the animations seems to have made them clear but was negative for localization. The conceptual understanding of the specific processes depicted in the animations was probably increased by leaving out unnecessary details. Only the molecules most relevant to the current topic were depicted to increase clarity. The velocities of and distances between molecules were set to increase general visual clarity. The structural rearrangements of molecules were exaggerated to increase clarity of reaction mechanisms. One might have to strike a balance between realism and conceptual understanding when deciding what details to incorporate in visualization. It may not always be appropriate to use realistic visualizations exclusively. The learning of life science concepts is based on piecing together facts from many different sources and intuition (c.f. Rundgren).

6.7 (g) Multimedia

Students' ideas in this category are based on their answers to $Q_2$2.

6.7.1 Findings:

Three students reported the combination of images and voiceover was more useful compared to voiceover alone. Visualization increased understanding of the scientific content.

> *$Q_2$2 Were there unclear parts in the movie?*
>
> *"When the screen goes blank and there is only voiceover. Understanding is easier if there is something to rest the eyes on."* (St. 4)

6.7.2 Discussion:

Animation can have advantages over video microscopy, including simplification; unlimited resolution and magnification; ability to highlight certain symbols within a complex background; control of motion, shape, or color changes; and the stepwise fading in and out of symbols. To appreciate multimedia there is a need to be able to connect visualizations with other forms of representation such as text. The combination of words and images can enhance understanding and the ability to solve problems. But there is always the risk that too many ways to present information is distracting. If the connection between text and image is not logical and clear, learning can become difficult (Schnotz and Bannert, 2003). Although this study focuses on the teaching of cell biology, the discussion is readily applicable to the teaching of all fields of science.

6.8 (h) Protein shape

Students' ideas in this category are based on their answers to $Q_2$1 and interview with St. 4.

6.8.1 Findings:

Two students said it was good with protein molecules of different shape. Two students reported that visualization of the 3D-structure of protein molecules facilitated understanding of protein interaction and its importance for systemic function. Two other students reported that illustration of the amino-acid sequence was useful. One of them said that it increased her understanding of how protein molecules can transmit signals. During the interview she elaborated on how the amino-acid sequence constituting proteins achieved specificity of chemical reactions in signaling pathways. One student said it would have been clearer if the amino-acid sequences had not been illustrated. The teacher said that for most signal transduction topics in the course the illustration of amino-acid sequence was unnecessary.

> *$Q_2$1 Write down 3 things you liked about the movie.*
>
> *"You can see how the proteins interact; the chain in the cell goes from A, B, C and so on."* (St. 2)

6.8.2 Discussion:

The protein molecules in the animations were often visualized as compact bodies with different shape. The results from the study indicated that protein molecules with different shape increase distinction between molecules. Protein shape can be used to illustrate protein function. For example, a protein (anchoring protein) whose primary function is to hold another protein in place was visualized as a tongs-shaped protein. But the results from $Q_1$3 indicated that symbols or labels might be clarifying.

In signal transduction pathways, multi-protein complexes are assembled by interaction domains that target the components to the complex. Interaction domains are independently folded modules, which can still bind their target molecules if expressed independently of their host protein. Interaction domains can be divided into distinct families, whose members are related by sequence. Small differences in amino-acid sequence among members of a family make the binding between individual interaction domains and their targets specific. Interaction domains provides the structural basis for complex, biomolecular processes like signal transduction cascades that require specificity of interaction partners.

Visualization of protein structure increases comprehension of the relationship between protein structure and function. In animation 3, which introduce the concept of interaction domains, the surface structure of proteins was carefully modeled. Surface structure is important for the complementary fit between interaction domains and the structures they recognize.

6.9 (i) Protein motion

Students' ideas in this category are based on their answers to $Q_2$1 and interviews with the teacher.

6.9.1 Findings:

The teacher said it was good that the molecules that do not undergo reactions remain still. No students reacted to this.

> *$Q_2$1 Write down 3 things you liked about the movie.*
>
> *"You could easily tell when proteins were changing. A nice and slow pace when the proteins moved. You could tell what was happening."* (st. 12)

6.9.2 Discussion:

The results from the study indicated that most students had no problems interpreting biochemical reaction mechanisms visualized in the animations. This might not have been possible, if I had made all the molecules move about in the cell. Another option would have been to move all unimportant molecules very slow while the ones that undergo reactions move fast. But this might give rise to misconceptions. The voiceover would probably have to comment on the movements of protein molecules for correct interpretation of the animations.

6.10 (j) 3D-dynamic visualizations

Students' ideas in this category are based on their answers to $Q_2$1.

6.10.1 Findings:

Five students reported that visualization of every intermediate reaction provided an overview of signal transduction chains. Two students said that the animations helped understanding the lectures and gave a complementary image. Two other students said that it would have been easier to understand the animations if they had had more lectures before. One student reported that the animations helped memorizing processes. The teacher said that the animations were more useful than he had expected. He was impressed by the students' level of knowledge in the student interviews.

> *$Q_2$1 Write down 3 things you liked about the movie.*
>
> *"You can see how the proteins interact; the chain in the cell goes from A, B, C and so on. It helped understanding the lectures."* (st. 2)
>
> *"Every intermediate reaction was described which support understanding of the whole process."* (st. 9)
>
> *"You get a more real image of all these proteins with strange names"* (st.1)
>
> *"To see what happens – sticks in one´s memory. Increases understanding, gives a complementary image."* (st. 3)
>
> *"Great/visually appealing and clear animations – you get an understanding of how everything works in the cell."* (st. 8)
>
> *"Good illustration with the images and you get an overview of the mechanisms involved."* (st. 10)
>
> *"Great, animation helps with understanding."* (st. 11)

6.10.2 Discussion:

An environmental or internal signal can be multiplied and processed through signal transduction chains. Then, a regulatory action can take place, for example, at the transcriptional level through activation or repression of gene expression or at the translational level through alternative splicing or posttranslational modification. Regulation and information flow, at all levels in the cell, is structure and time-dependent. 3D-animation can show how molecular structure and interactions change over time and has potential for teaching life science concepts. Dynamic visualization makes memorization of biochemical processes easier (O'Day, 2007). The results from the current study indicate that 3D-dynamic visualizations are useful for teaching life science concepts and can serve as a complement to lectures. They are useful for visualizing continuous time-dependent processes like signal transduction chains. This is in line with previous research on the use of computer animation for teaching life science.

7. Findings and discussion (data from Questionnaire 1 and student interview 1)

7.1 Question 1: Describe the steps of the process when a signal is transferred from the outside of the cell until the cell response.

7.1.1 Findings:

The expected answer to the question was to describe the activation of the receptor in the cell membrane and the successive signal transduction chain that ultimately leads to activation of target proteins, which directly mediates the cell response. The students had problems with identifying signaling steps after the receptor's activation. Many students did not mention the signal transduction chain or the activation of target proteins. Most students correctly describe the activation of the receptor in the plasma membrane and some describe how activation of the receptor transmits the signal to the cell interior.

7.1.2 Discussion:

Many students did not identify the signaling steps after activation of the receptor. This may be due to the lack of pictures of intracellular signaling steps in the questionnaire. The questionnaire only included pictures of the receptor in the plasma membrane that gets activated by an extracellular molecule. Many students correctly describe this event but do not make the connection between receptor activation and the consequent signal transduction chain.

With access to the animations during filling out the questionnaire the students might have identified and described signaling events after the receptor's activation. Now the students had to rely on their memory. Some might have forgotten the specific sequences in the animation that illustrated signal transduction steps. Since the students had no lectures in signal transduction before filling out the questionnaires they could not make a connection between the animations and lectures. More zoom effects that provided an overview of the complete process might have been beneficial.

7.2 Question 2: Write down every class of target protein you know.

7.2.1 Findings:

The expected answer is to mention general classes of target proteins like gene regulatory proteins and components in metabolic pathways or the cytoskeleton. The students had problems with naming target proteins. Some students provided specific names of proteins as answers. During the whole class group discussion after the animations one student mentioned DNA-polymerase. This is indeed the ultimate target protein since it directly affects cell behavior.

7.2.2 Discussion:

The students' difficulties with naming target proteins might have had a number of reasons. The questionnaire did not include any pictures for this question.

The target proteins were depicted in animation 1 and were not labeled with text. Some target proteins were only mentioned by the voiceover and not shown in the animation. As concluded in section 6.7 the combination of animations and voiceover has increased value compared to voiceover alone.

7.3 Question 3: Write the correct number beside the pictures (pictures of different classes of proteins). Every picture can only have one number. The proteins (numbered 1 to 3) are arranged according to alphabetical order.

7.3.1 Findings:

To correctly answer the question the students had to combine the pictures of proteins with the appropriate protein names and descriptions of protein function. The students had general problems correctly assigning the right name and description to the right protein picture. The students' problems were general in the sense that no specific categories of incorrect answers could be identified.

7.3.2 Discussion:

Due to the importance of protein function for systemic function and signal transduction this is an essential question. The shapes of individual protein molecules in the animation were designed to facilitate understanding of their function. For example a protein molecule that holds another one in place had a tongs-like shape. But perhaps, it would have been beneficial to use more symbols to indicate functional and structural relationships. The motion of the molecules in the animation may have been clarifying for the students, because motion can illustrate function. But since the students lacked access to the animations when completing the questionnaires, the impact of motion on the students' answers was not completely measured.

Understanding function through structure is a primary goal of structural biology. But this is not always simple, partly because a biologically useful definition of the function of a protein molecule requires a description at several different levels. To the biochemist, function means the biochemical role of an individual protein. If it is an enzyme, function refers to the reaction being catalyzed. If it is a signaling protein, function refers to the interactions of the protein molecule with other molecules in the signaling pathway and the signal transduction reactions that it catalyzes. To cell biologist, function includes these roles, but will also encompass the cellular roles of the protein molecule. Because the function of proteins can be described at different levels the naming and classification of proteins according to function may be complicated and this may have affected the outcome of the question.

7.4 Question 4: What happens to signaling protein 2 that enables it to bind to the PTB-domain of protein 1?

7.4.1 Findings:

The correct answer is that signaling protein 2 is phosphorylated by the catalytic protein kinase domain of protein 1. Most students provided the correct answer.

But the answers indicated that some students may not have realized that the phosphate group itself in this case provides the complementary fit. Some may have only realized that the phosphorylation triggers a conformational change, which is indeed true.

7.4.2 Discussion:

The outcome of this question would probably have been different with a questionnaire including pictures of the phosphorylated molecule interacting with the interaction domain that recognizes the phosphorylated structure. But the high frequency of correct answers indicate that 3D-animations are useful for visualizing time-dependent, multi-step biochemical processes like the assembly of macromolecular complexes.

7.5 Question 5: Interaction domains of the same type, for example, the SH2-domains on signaling protein 1 and the adaptor-protein, seldom bind to the same protein. Explain!

7.5.1 Findings:

The correct answer is that interaction domains of the same type have small differences in amino-acid sequence that makes the surface structure slightly different. This facilitates specificity of interaction partners. The students had general problems answering this question. The students' problems were general in the sense that no specific categories of incorrect answers could be identified. Many students simply did not answer the question at all.

7.5.2 Discussion:

A more detailed visualization specifically illustrating the differences in amino-acid sequence may have helped. Now the difference in amino-acid sequence was only mentioned by the voiceover. The illustration of surface structure differences with proteins depicted as compact bodies was insufficient. It can be concluded that specific concepts require specific illustrations. Access to the animations would probably have been beneficial, since one sequence illustrated how the amino-acid sequence of proteins facilitates flexible binding and induced fit.

General findings, questionnaire 1

The students had problems with concepts which are only mentioned and not shown in animations, like for example target proteins and the impact of differences in amino-acid sequence on protein structure.

One data collection mistake was conducted; the students should have had access to computers with the animations when answering the questionnaires.

8. General discussion and implications

The main results of the study, that is, the contribution of layout-issues to understandability and usefulness of the animations, are summarized in figure 5.

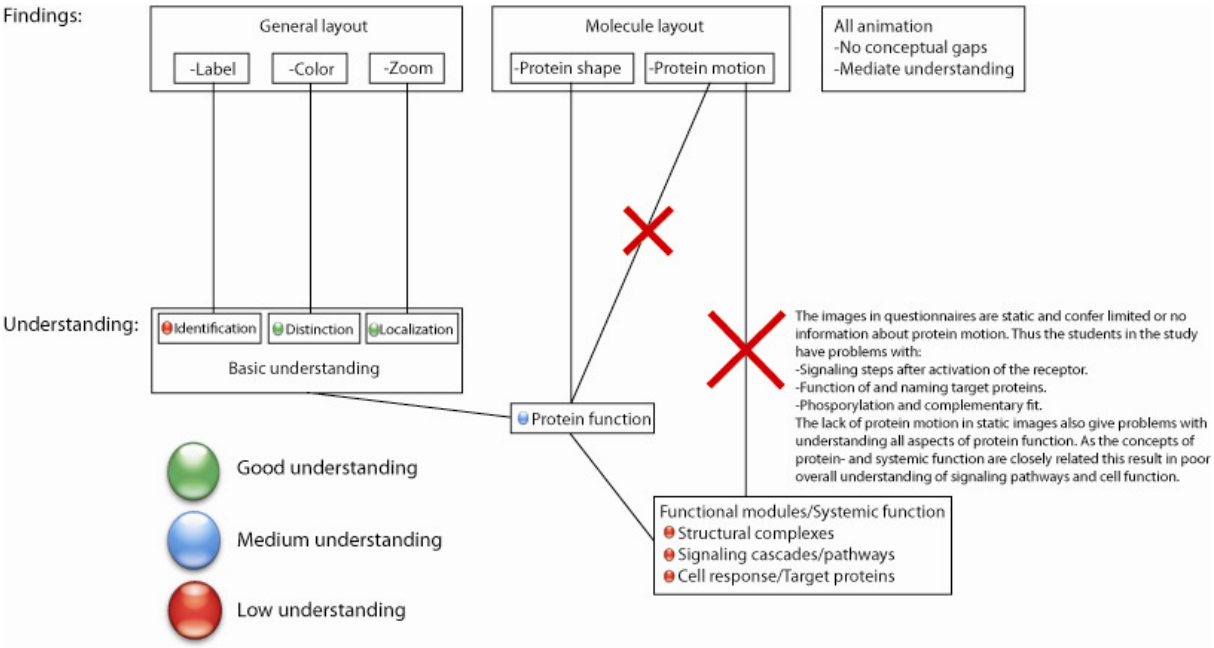

Figure 5. The main results of the study summarized.

Answers to research questions

The connection between a layout-issue and category of understanding is indicated by a line. Crossed over lines indicate that there is a connection between the layout-issue and category of understanding but the current study failed to address this connection completely. In figure 5, this relates to the lack of access to animations when students answered questionnaires, as explained below. The inherent properties of all animations, that is, their capacity to visualize continuous processes and mediate understanding of life science concepts is illustrated in a separate box up to the right in figure 5.

The categories of understanding deals with more complex issues from left to right in figure 5. For example, systemic function is a more complex issue than identification of molecules. Good, medium and low understanding of specific issues is indicated by a green, blue and red light bulb respectively. Basic understanding, which is illustrated by a box to the left in figure 5, in this context means understanding of the animations themselves. Students have to be able to identify molecules at hand, see the physical boundaries between them and realize where specific processes take place. If any of these requirements is missing students will not be able to correctly interpret every aspect of the animations and their understanding of the biochemical content (protein function and systemic function) will be reduced.

The identification of molecules at hand is mostly related to labeling of molecules but to some extent also protein shape and protein motion. The results from questionnaire 2 (layout-issues) indicated that the students missed labels in animation 2 that introduces different classes of signaling proteins. Since they could not always identify the molecule at hand their understanding of the biochemical content, in this case protein function, were lessened. This is indicated by a blue light bulb beside the category protein function in figure 5. This finding is supported by the students' general problems with $Q_1 3$ (students' knowledge).

Knowledge of where in the cell specific biochemical processes are located is another requirement for basic understanding of the animations. Localization was facilitated by zoom effects. Differentially colored and shaped molecules, helped with distinction between molecules. All in all two of the three requirements for basic understanding were present, which in figure 5 is indicated by two green light bulbs and one red in the box of basic understanding. Since the basic understanding was not complete this affected the students understanding of protein function. To fully comprehend these animations students have to climb a step-ladder, where each step represents one level of understanding. Each level is built from prior understanding. Lacking basic understanding due to missing labels (indicated by a red light bulb beside the category identification in figure 5) affected understanding of protein function. Deficient understanding of protein function will affect comprehension of systemic function negatively, which in figure 5 is indicated by red light bulbs beside systemic function concepts. Thus basic understanding has affected understanding of an advanced concept like systemic function.

Protein function is also connected to protein shape (cf. Kozma, 2003). The protein shape was of importance in $Q_1 5$ where students had problems because the proteins were depicted as compact bodies. This is confirmed by results from category (h) protein shape in questionnaire 2 and student interview 2, where it was reported that illustration of the amino-acid sequence facilitated understanding of protein and systemic function.

Both protein function and systemic function is connected to protein motion (NSF, 2001). In figure 5, the lack of access to animations when students answered questionnaires, is indicated by crossing over of the two lines from the layout-issue protein motion. The lack of motion (lack of access to animations) probably had an impact on the students' ability to answer questions regarding protein function and systemic function. This could help explain difficulties with question $Q_1 3$ and $Q_1 1$ which dealt with protein function and systemic function respectively. This is confirmed by results from the categories (i) protein motion and (j) 3D-dynamic visualizations in questionnaire 2.

The first research question was formulated as follows: to what extent do the issues of layout contribute to students' understanding of the animations and the biochemical content? As concluded above comprehension of the animations can be regarded as a step-like process. The basic understanding of both the animations themselves and their biochemical content is facilitated by the layout-issues label, color and zoom. Protein shape and protein motion directly affect the understandability of the biochemical content.

The second research question was related to the usefulness of the animations in a learning environment. Animations have to be understandable to be useful. They might not be useful even if they are understandable, but understandability is a basic requirement that facilitates usefulness. The results from the study indicate that these 3D-animations are useful for teaching life science concepts and can serve as a complement to lectures. They are useful for visualizing continuous time-dependent processes like signal transduction chains. This is in line with previous research on the use of computer animation for teaching life science. As stated in section 6.10, the teacher thought the animations were useful. Results from the study indicated that they could have the function as a memorization aid. This is supported by literature (Goldstein *et al*., 1982; Blake, 1977; O'Day, 2007). They might also be used to provide an overview over biochemical processes (cf. Kraidy, 2002). These animations could be useful for self-studying and for repetition of life science concepts, which is supported by previous research on the usefulness of the animations in life science (O'Day, 2006; McClean, 2005).

I think at least animation 3 or a similar animation should be included in life science teaching in general and signal transduction teaching in particular. In my opinion the most important concept in signal transduction is the relationship between structure and information flow in the cell. 3D-dynamic visualizations may facilitate understanding of this relationship. The usefulness of these animations stem from the fact that the complexity of life phenomena and the dynamic nature of biomolecular processes may turn plain text into an inadequate learning tool.

Revisions

The results from the study indicated that there were difficulties in the animations due to bad design choices. A number of revisions that would probably improve the understandability and usefulness of these and other animations are suggested. This includes revisions for study design. In my opinion the needed revisions are:

- Molecule labels for all important molecules.
- Visualization support to all voiceover.
- More detailed introduction to target proteins.
- The sequence of amino-acids should have been visualized in the scene illustrating differences between interaction domains of the same type.
- The students should have had access to the animations when completing the questionnaires.

The first round of animations and the test of the students' actual knowledge were performed before there had been any lectures. This study design focuses on the usefulness of the animations as an introduction to signal transduction. If no second questionnaire had been distributed, no measure of the value of the animations as a complement to lectures would have been achieved. But since questionnaire 2 did not include a test of the students' knowledge, the measure of the value of the animations as a complement to lectures, is merely based on their opinions.

One possible study revision is thus to include a second test of the students actual knowledge. Another option would have been to separate the students into different groups with different study designs, to facilitate a comparison between the usefulness of the animations as an introduction and complement to lectures.

Implications

A number of implications follow from results of the study. These might be used to aid design of other animations in life science in general and signal transduction in particular.

- The study reinforces the need to use visualizations in life science teaching.
- In the design of animations, care has to be taken with layout-details even though they might seem unimportant.
- Basic understanding of the animations is fundamental for understanding of advanced concepts. In the design phase of producing animations this should be kept in mind.
- A central concept in a field requires a thorough explanation.
- Specific concepts require specific illustrations.
- Labeling of molecules is important to facilitate identification of them.
- Zoom effects can be used as a means to help with localization of processes.
- Different colors can be used to facilitate distinction between molecules.
- The use of multimedia can be restricted. Sometimes more is less. The connection between image and text should be logical and clear.
- Visualization of 3D-structure of molecules provides an understanding of molecule and systemic function.
- Leaving out background scenery makes the animations clear.
- Unrealistic visualizations can be useful for teaching specific concepts.
- The level of realism of different factors in animations, like molecule speed and distances, has to be set to strike a balance between conceptual understanding and scientific correctness.
- The students should have access to animations during the data collection phase of studies, if the purpose is not to study specific aspects of memorization mechanisms.
- The design of the study has to be carefully selected in order to get the sought data. This step requires thorough planning.

Future directions

The evolution of modern cell biology tools, such as advanced electron microscopy methodologies, has allowed for ever improving structural and functional characterizations of the cell.

Biologists are also beginning to probe the dynamics of macromolecules in new ways. It is now possible to examine directly how a protein's conformational flexibility affects its function during catalysis (Henzler-Wildman *et al*., 2007). Moreover, increased processing speed of computers and new algorithms are beginning to address the behavior of larger molecular assemblies over longer timescales (Maragakis *et al*., 2008; Sotomayor and Schulten, 2007; Karplus and Kuriyan, 2005).

The insights emerging from structural cell and molecular biology call for more sophisticated visual renderings. Until now, we have used mostly static representations of proteins outside of their cellular context that lack a critical layer of kinetic information. Proteins are dynamic and shape-shifting and constantly explore their surroundings. Their association and dissociation from each other involve a range of conformational states that are critical to their function. There is a need to incorporate this information into workable models that can be communicated to others.

Because the effect of a single bond rearrangement can influence the structure of an entire protein complex, we face the task of depicting a wide continuum of scale. This property sometimes calls for mixed visual metaphors (such as a sticks and balls model surrounded by a semitransparent surface mesh).

Many people are astounded by the fact that so few genes can build such a complex organism as the human and that there seems to be no relation between genome size and the apparent complexity of an organism in terms of its intelligence. But many might forget that there are many copies of the genomes in a multi-cellular organism, one in each cell. Emergent properties arise when all copies of the genome in an organism interact. It is the level of complexity in the interaction between all copies of the genome that specify the complexity of the organism. Perhaps the greatest challenge so far in life science is the modeling, and prediction of all cell behavior. But the even greater challenge of modeling and predicting the behavior of the whole organism is the next step and 3D-animation might be a valuable tool for this daunting task.

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Appendix

Questionnaire 1

Question 1: Describe the steps of the process when a signal is transferred from the outside of the cell until the cell response.

Question 2: Write down every type of target protein you know.

Question 3: Write the correct number beside the pictures (pictures of different classes of proteins). Every picture can only have one number. The proteins (numbered 1 to 3) are arranged according to alphabetical order.

Question 4: What happens to signaling protein 2 that enables it to bind to the PTB-domain of protein 1?

Question 5: Interaction domains of the same type, for example, the SH2-domains on signaling protein 1 and the adaptor-protein, seldom bind to the same protein. Explain!

Questionnaire 2

Question 1: Write down 3 things you liked about the movie.

Question 2: Were there unclear parts in the movie?

Question 3: What did you think about the appearance of the proteins?

Question 4: Would you like to change the voiceover? If so, how?

Question 5: Do you want more text in the movie?

Question 6: What parts were too fast? Which parts were too slow?

Question 7: What parts of the movie can be improved to make it easier for future students?