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# Learning and Teaching the Molecular Basis of Life

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**Marc van Mil**

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# Learning and Teaching the Molecular Basis of Life

Het leren en onderwijzen van de moleculaire basis van levensprocessen

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 2 juli 2013 des middags te 4.15 uur

door

Marc Hubertus Wilhelmus van Mil

geboren op 19 mei 1978 te Heerlen

**Promotor:** **Prof.dr. A.J. Waarlo**

**Co-promotor:** **Dr. D.J. Boerwinkel**

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# List of papers

The thesis builds on the following papers, which are referred to using Roman numerals.

## Paper I:

**Genomics education in practice: evaluation of a mobile lab design.**
Van Mil, M. H. W., Boerwinkel, D. J., Buizer-Voskamp, J. E., Speksnijder, A. & Waarlo, A. J. (2010). Published in *Biochemistry and Molecular Biology Education*, 38(4), 224–229.

## Paper II:

**Modelling molecular mechanisms: a framework of scientific reasoning to construct molecular-level explanations for cellular behaviour.**
Van Mil, M. H. W., Boerwinkel, D. J. & Waarlo, A. J. (2013).
Published in *Science & Education*, 22(1), 93–118.

## Paper III:

**Molecular mechanistic reasoning: towards bridging the gap between the molecular and cellular level in life science education.**
Van Mil, M.H.W., Postma, P. A., Boerwinkel, D. J., Waarlo, A.J.
Submitted to *Science Education.*

# Structure of this thesis

This thesis reports my PhD study in genomics education. I chose to report about the findings of my study by publishing journal articles. The articles can be found as appendices in this thesis. In the thesis I complement the articles with a summarizing discussion, in the Swedish tradition called the 'kappa', which literally means 'coat'. Although not yet very common in Dutch science education theses, this way of reporting my findings allows me to sketch how the study developed and what the position of the papers is within the study as a whole.

In Chapter 1 of this summarizing discussion, the starting point and the motive for the study is introduced. This is where Paper I is discussed, because it shows how the DNA lab activity was the starting point for identifying and taking up the educational challenge that I worked on in the rest of my PhD study. In Chapter 2, the identified problem is specified and discussed in the light of previous findings in science education research. This leads to formulating the research focus and aims in Chapter 3. Chapter 4 summarizes the theoretical framework based on expert reasoning that I constructed and presented in Paper II. In Chapter 5, I clarify the educational potential of this framework and I show how it helped me to identify the conceptual understandings and reasoning that I consider worth pursuing for pre-university students to get a grip on the molecular basis of life. Chapter 6 summarizes Paper III, in which the construction and testing of an educational design is presented, with the aim of exploring in practice the potential of the developed approach. In Chapter 7, I discuss the study in retrospect and I suggest directions for further research. Chapter 8 reflects on the implications of the study for educational theory and practice.

# 1. Introduction

## 1.1 How I became involved in genomics education

In 2005 several genomics centres, funded by the Netherlands Genomics Initiative, decided to start an educational outreach project for students in upper-secondary education called 'DNA labs on the road'. The format was adopted from the mobile laboratories that were developed in the late 1990s at Wageningen University where I studied biotechnology. Two of my classmates in the biotechnology programme came up with the idea to develop a mobile practical course on DNA technology, with which biotechnology students would travel to upper-secondary schools to teach about the new developments in biotechnology. At that time, there was much societal debate about the application of gene technologies in food production (Hanssen, 2009) and they felt there was a need to inform upper-secondary students about these scientific advances and show them what the public debate was about. This students' initiative was adopted by the university and more mobile laboratories and other activities for upper-secondary students were developed. The mobile laboratories became a great success because teachers experienced it as a chance to demonstrate advanced techniques in the classroom and they appreciated the university students as role models for their students. As many of my classmates, I also got involved in these outreach activities during my studies and after finishing my first internship in a research lab I decided to shift my focus from doing research in the lab to science education and communication. I took education and communication courses and I started an educational research project in which I compared learning styles of Dutch and Chinese students in the biotechnology programme at Wageningen University (Biemans & Van Mil, 2008). This was my first introduction to educational research, although at that time I did not consider starting a PhD project in educational research. When I was looking for a final internship in the field of science communication and education, my experiences with outreach activities for upper-secondary students in Wageningen appeared to be of unexpected value. By coincidence, I came in contact with the Cancer Genomics Centre (CGC) in Utrecht making plans for a mobile laboratory comparable to the practical courses offered by Wageningen University. The genomics research consortia under the umbrella of the Netherlands Genomics Initiative had decided to adopt the mobile laboratory concept developed years ago by my classmates as a way to offer up-to-date genomics education and discuss societal implications of genomics research in upper-secondary science education. This is how I became involved in genomics education: my internship project was to develop and test a mobile practical course on cancer genomics research, which could be taught in upper-secondary schools by university students of the biomedical sciences programme of Utrecht University. At the end of my internship in September 2005, the DNA lab on the road called 'Read the language of the tumour', was ready to be launched. I graduated from the biotechnology programme and the Cancer Genomics Centre offered me a job as an education officer. For the next 3 years, I coordinated the DNA lab, prepared students in the biomedical programme to teach the DNA lab in schools and developed and implemented in-service training for teachers in secondary schools about new developments in cancer research.

## 1.2. Educational research supporting DNA labs on the roads

In the DNA lab project, the Cancer Genomics Centre collaborated closely with the biology education group of the Freudenthal Institute for Science and Mathematics Education (FIsme) at Utrecht University. This collaboration resulted in a joint CGC-FIsme research programme on genomics education (Verhoeff, Boerwinkel & Waarlo, 2009), which was financially supported by CSG Centre for Society and the Life Sciences. I started contributing to this research programme with an evaluation of the design and outcomes of the DNA lab 'Read the language of the tumour'. This work resulted in Paper I in this thesis.

## 1.3. Position of Paper I in this study

Paper I is entitled: 'Genomics education in practice: evaluation of a mobile lab design' (Van Mil, Boerwinkel, Buizer-Voskamp, Speksnijder & Waarlo, 2010). The paper describes the design and evaluation of the DNA lab 'Read the language of the tumour' and it discusses the general goals of the DNA labs on the road project and how these goals have been translated into a lesson module about cancer research. An earlier, more general evaluation of all the DNA labs (Knippels, Van der Rijst & Severiens, 2006) had pointed towards the educational challenge to better include personal and societal implications of genomics in the DNA labs. This resulted in the focus area 'genomics education for citizenship' in the genomics education research programme. The more in-depth evaluation of the mobile lab design presented in Paper I reveals additional challenges that concern students' conceptual understanding of the relationship between genes and the effects of genes, for instance in the human body. These findings are the basis for the focus I chose for the rest my PhD study. Paper I thus sketches the starting point of my thinking about conceptual understanding in genomics education that I elaborate on in the rest of the thesis.

## 1.4. Summary of Paper I

Rapid advances in molecular biology widen the gap between research practice and school science. Applications of genomics research are rapidly finding their way into society and impact everyday life. Major breakthroughs range from medicine to forensics, biofuels and the mitigation of pollution (NGI, 2006). These scientific advances each bring their own choices and dilemmas. To empower future citizens to deal with these personal and societal issues, science education based on relevant and up-to-date science is needed.

Many advances in molecular life sciences are not yet represented in science curricula (Moore, 2007; Verhoeff et al., 2009). However, simply adding new content without rethinking the curriculum is not a viable strategy. In several countries, new curricula concerning molecular life sciences have been proposed or introduced (Boerwinkel & Waarlo, 2009; Cohen & Yarden, 2009; Moore, 2007; Voet, Bell, Boyer, Boyle, O'Leary & Zimmerman, 2003). Advances in genomics research have caused fundamental changes in the scientific view on the inner working of the living cell, while secondary school students still have problems grasping the basic concepts of DNA and proteins (Gericke & Hagberg, 2007; Lewis & Kattman, 2004; Marbach-Ad & Stavy, 2000).

Dutch genomics research centres have developed the DNA labs on the road project to bridge the gap between modern genomics research practice and the secondary-school curriculum in the Netherlands. These mobile DNA labs offer upper-secondary students the opportunity to experience genomics research through experiments with laboratory equipment that is not available in schools and to place genomics research in a relevant societal context

In summary, the DNA labs aim to:
- enhance up-to-date genomics knowledge
- improve the image of and attitude towards genomics topics[1]
- increase the notion of societal implications of genomics research (place genomics in a societal context)
- invoke enthusiasm and interest in genomics research.

These goals have been the starting-point for designing the DNA labs. In each of the DNA labs, these general goals were further specified and translated into an instructional design. Paper I answers the following questions for the DNA lab 'Read the language of the tumour':
*1. How are the general goals of the DNA labs translated into an instructional design?*
*2. To what extent have the educational goals been reached by this design?*

### 1.4.1. The translation of the general goals of the DNA labs into an instructional design

The general learning goal formulated for the DNA lab 'Read the language of the tumour' is: *After performing the DNA lab, students are able to explain what modern DNA research related to cancer entails and how this research is applied.*

To reach this goal, three more specific learning goals have been determined:
- After performing the DNA lab, students know that cancer is 'a disease of the genes' and they are able to explain how one can minimize the risk of getting cancer.

- After performing the DNA lab, students are able to perform practical steps in DNA analysis (DNA isolation, PCR and gel electrophoresis) and they can explain the purpose of each step.

- After performing the DNA lab students, are able to explain that DNA-research is important to improve diagnosis and treatment of cancer.

1. In the original outreach plan the formulation 'improving the image of and attitude towards genomics topics' was used. In later stages this goal was specified as 'promoting informed opinions on genomics-related personal and societal issues.'

To translate these goals into an instructional design, choices were made regarding context, techniques, genes to be investigated and format of the lessons.

*Context:* The DNA lab 'Read the language of the tumour' uses the context of a diagnostic DNA test on tumour tissue to determine the best treatment for a fictitious cancer patient.

*Techniques:* Techniques to illustrate this practice were chosen with the following criteria in mind:
- *Authenticity:* techniques must be used in real practice
- *Comprehension:* techniques must be understood by students of the age of 16–18 years
- *Complexity:* techniques that can be performed by inexperienced students
- *Transportability:* techniques that can easily be transported to and set up at schools
- *Time:* techniques that offer results within the time constraints of the module
- *Cost:* techniques that rely on equipment and materials that fit within the set budget
- *Safety:* techniques that rely on equipment and materials that can be safely used in a school environment

*Genes:* Gene mutations were selected based on the following criteria:
- The mutated genes must represent different steps in the transformation process from a normal cell to a tumour cell to demonstrate that cancer is caused by multiple mutations
- The mutated genes must have implications for the choice of therapy to demonstrate that current therapies are based on specific mutations in tumour cells
- The gene mutations must be diagnosable by gel electrophoresis following PCR amplification
- The function of the mutated genes must be comprehendible for students in upper-secondary education

*Format:* The practical work is guided by university bachelor students that visit the school with the necessary equipment. Introductory and concluding lessons are taught by the teacher. In this way the teacher participates actively in the lessons, thereby linking the lab to regular biology education.

### 1.4.2. Effects of the DNA lab 'Read the language of the tumour'

Results show that most goals of the DNA lab are reached, namely: enhancing up-to-date genomics knowledge; improving the image and attitude towards genomics topics; increasing the notion of societal implications of genomics research; and invoking enthusiasm and interest in genomics research. The context of cancer research is very much appreciated by the students and teachers. Students can perform and understand the techniques and the materials and equipment are interesting and appealing to them. Most of the cognitive and affective goals are reached but some points for improvement remain. More attention should be paid to students' opinion forming on personal and societal implications of genomics research. Also, the coherence between different biological concepts and biological levels of organization could be made more explicit. Another finding is that the importance of the introductory lesson and final lesson is being underrated by teachers. Teachers indicated that they wish to have more background on current genomics research and support on the content and pedagogy in the module.

## 1.5. The contribution of Paper I to this study

Paper I presents an evaluation of the design and effects of one of the DNA labs and suggests some directions for improvements, and indeed this evaluation was the starting point for research-informed improvements in the DNA labs over the next years. However, the intentions of the joint CGC-FIsme research programme on genomics education reach beyond improving the DNA labs. It aims at rethinking how new developments and insights in the molecular life sciences impact science education. In this respect, Paper I highlights educational challenges for life science education into two directions:

1. *How to empower students in their opinion forming on personal and societal implications of genomics?*
2. *How to foster students in their connecting the gene as a molecular-level concept to phenomena at higher levels of biological organization?*

In the joint CGC-FIsme research programme the two questions mentioned above have been dealt with in somewhat separate projects. In this thesis, I focus on the second question, whereas colleagues have reported on the first (Boerwinkel, Swierstra & Waarlo, 2012). I will here clarify why, in my view, the first question fits best in a project aiming at 'genomics education for all', while the second question fits best in a project for advanced-level science students.

Education about developments in genomics can be considered relevant for everyone since these technologies can have major personal and societal implications. From this point of view, an important goal for genomics education is to empower students to make informed decisions, for instance when confronted with the choice whether or not to have a genetic test. This goal is referred to as science education for citizenship (Ryder, 2002) and it can be argued that all students regardless of their level of education or their choice for science of non-science majors should benefit from education that is concerned with these kinds of socio-scientific issues (Boerwinkel & Waarlo, 2011). In such a genomics-for-all curriculum, the conceptual knowledge will be limited and a prominent and challenging question is: What minimal conceptual understanding is required when genomics education for citizenship is the primary goal? Some studies have addressed this issue, but the debate is still in an early stage (Boerwinkel & Waarlo, 2009).

A different educational challenge occurs in the more specialized life science curricula in upper-secondary and undergraduate education that deal with detailed conceptual knowledge about DNA, RNA and proteins. It is reported that these concepts at the molecular level often remain isolated facts and that molecular knowledge hardly contributes to more sophisticated explanations of biological phenomena (Duncan & Reiser, 2007; Marbach-Ad & Stavy, 2000). As a result, many students use memorization and rote learning as a coping strategy when presented with molecular-level concepts (Anderson & Schonborn, 2008; Momsen, Long, Wyse & Ebert-May, 2010; Stanger-Hall, 2012). For instance, in genetics education, it appears that the molecular details of DNA and proteins add very little to students' understanding of genetic phenomena. Lewis and Kattman (2004) report that in introductory genetics education, students treat genes as small particles containing a trait or characteristic and they discuss that many students take this notion with them in further study. Marbach-Ad and Stavy (2000) report similar findings. In their study, many students used concepts and terms from the molecular level such as gene and DNA, but they were unable to explain mechanisms and intermediate stages that link genes to the biological phenomena they are involved in. Furthermore, less than half of the 12th graders in their study were able to explain the function of RNA. These and many other studies (e.g. Duncan & Reiser, 2007; Venville & Treagust, 1998) show that students have difficulty understanding how genes determine traits, even after they have been taught how genes code for proteins via RNA.

# 2. A specification to the problem: Connecting the molecular and the cellular Level



One of the problems in upper-secondary genetics education is that the message about gene function appears to be twofold: classical genetics education emphasizes that genes determine hereditary traits, whereas in molecular genetics education the message is that genes code for proteins. Although these two accounts of gene function are related, it appears to be very difficult for students to combine the two messages into one overall framework that makes gene function intelligible (Lewis & Kattman, 2004). Duncan and Tseng (2011) stress that to understand the relationship between genotype and phenotype it is critical to understand that the genetic code does not directly specify observable effects, but that these effects are driven by interactions at lower organizational levels. They identify that many students lack a robust understanding of the functioning of proteins as parts in complex systems when reasoning about genetic phenomena and they suggest that a framework for reasoning about complex systems might help students to understand how cellular phenomena can emerge from the interactions of molecules in general and proteins in particular. The study presented in this thesis takes up this challenge with the aim to contribute to learning and teaching the molecular basis of life in pre-university life science education.

## 2.1. The cellular and molecular levels in life science education

Concepts in biology education are often grouped and presented based on historical traditions in biological research. The traditional research fields that are concerned with molecular and cellular level concepts are cell biology, molecular biology, biochemistry and (molecular) genetics. I will discuss here how the concepts most central in each of these disciplines are introduced and developed in current biology education and how educational research about these concepts informs the educational challenge of connecting the cellular and molecular levels that I focus on in this study.

*Cells* are the central concept in *cell biology education*. The idea that all living beings are made up of cells is introduced in a very early stage in most biology curricula (Cohen & Yarden, 2009; Duncan, Rogat & Yarden, 2009). However, at that stage, cells are primarily introduced as building blocks of an organism and it remains out of sight for students how the activities of cells are essential for the 'phenomenon of life' at the organism level. It appears that this view persists also in later stages in biology education when details of subcellular structures and processes are introduced. Different studies show that many students fail to acquire a coherent view of the cell as a basic unit of the organism, despite the many subcellular and molecular details that are added in later stages of cell biology education (Dreyfus & Jungwirth, 1988, 1989; Flores, 2003; Verhoeff, Waarlo & Boersma, 2008).

The *molecular constituents of the cell* are the central concepts in *biochemistry* and *molecular biology education*. The main players are DNA, RNA, proteins and small metabolites. It appears to be difficult for students to relate the properties of these molecules in the cell to the activities that cells display. Many students in cell biology education explain cellular function only by referring to

organelle function and are satisfied with this explanation (Barak, Sheva, Gorodetsky & Gurion, 1999). It is suggested that the focus on functional 'why?' or 'what is it for?' questions in biology education (Abrams & Southerland, 2001) contributes to this tendency.

*Genes* are the central concept in *genetics education*. During different stages in genetics education, the gene concept develops basically along the same lines as the model of gene function developed over the course of history (Gericke & Hagberg, 2007). Usually, genetics education start with discussing inheritance and the study of patterns of inheritance, referred to as Mendelian genetics. In these first steps in genetics education, genes are presented as the 'units of inheritance' that contain 'information about a trait' and can be transmitted in sexual and asexual reproduction. At the same time (usually in the same or following book chapter), genes get their physical appearance as 'being made up of DNA'. The DNA molecule is presented as a code and the effect that the genes have in the body is encrypted in this code. How the translation between code and effect in the body works, usually remains unclear at this first stage in genetics education. In later stages, the link is established between the genetic code and the properties of gene products (proteins and RNAs). However, what is effectively missing is an intelligible account for how gene products (proteins and RNAs) contribute to the functioning of an organism, via their role in cellular processes.

This view is endorsed by many studies on genetics education (e.g. Duncan & Reiser, 2007; Knippels, 2002; Lewis & Kattman, 2004; Marbach-Ad & Stavy, 2000; Martins & Ogborn, 1997; Smith & Williams, 2007; Venville & Treagust, 1998). In Gerickes' overview of these studies (Gericke, 2009, p. 39) he concludes that the most frequently reported view on genes seems to be 'particles that determine characteristics' but that genes are rarely linked to protein synthesis. Overall, these studies suggest that students' ideas on the role of genes hardly stem from process thinking, but are mainly based on rules and patterns of inheritance (Gericke, 2009). Among science education researchers, a widespread view can be found that students should be better able to integrate the biochemical processes that mediate gene function with the message in classical genetics that genes determine traits.

Duncan and colleagues (Duncan, 2007; Duncan & Reiser, 2007; Duncan et al., 2009; Duncan & Tseng, 2011) specify the problem by stressing that students need to connect ontologically distinct levels when reasoning about genetic phenomena: on the one hand an informational level (containing the genetic information) and on the other hand a hierarchically organized physical level (proteins, cells, tissues, etc.) (Duncan & Reiser, 2007). They state that: *'… the understandings involved in generating explanations that bridge the information and physical levels are at the crux of students' difficulties in learning molecular genetics.'* (Duncan & Reiser, 2007, p. 941).

Although I find it confusing to use the term 'level' to indicate that the gene concept has an informational as well as a physical component, especially because they use the same term to refer to hierarchically ordered physical entities, I share their conclusion: it is essential when reasoning about genetic phenomena to distinguish between the 'information' component (genes as informational units or 'DNA as a code') and the effects that this information has in the physical world. In my opinion, this is from where much confusion in genetics education originates. A very central question here is: how do students translate the influence of 'genetic information' or 'genetic code' into effects in the physical world? One could argue that students learn about the physical *appearance* of the information, by learning the structure of the DNA molecule and the way variation in nucleotides can be considered a code. It is to be expected that it is obvious to students that information can be captured in physical appearances, for instance if they think

about computer memory, such as a hard disk or memory stick. This will not be the problem. However, the direct effect of genetic information is on the molecular level and, as Duncan and Reiser (2007, p. 939) rightly suggest, *'understanding genetic phenomena entails understanding how mechanisms and interactions at the molecular (genes, proteins) and microlevels (cells) bring about effects at the macrolevel (organism, population).'*

Fig. 1 illustrates this statement by stressing that to understand 'how genes determine traits', one needs to understand 'how genes code for proteins', 'how proteins are involved in cellular processes' and 'how these processes contribute to the effect in the organism that we call trait'. The second how-question stresses the need to connect the molecular and cellular level and the third question connects the cellular level to higher-level phenomena. I conclude from the studies mentioned that, for many students, changes at the molecular level (for instance, proteins that catalyse chemical reactions) and observable phenomena at higher levels (for instance, hereditary traits) remain unconnected even after having been taught the many details in the biology curriculum about cells and their molecular constituents DNA, RNA and proteins.

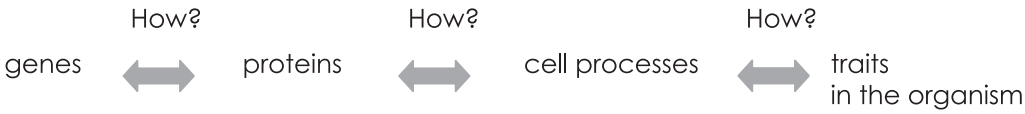



*Fig. 1: Three how-questions to link genes to traits in the organism*

Despite their accurate analyses, the approach presented by Duncan and Tseng (2011) does not sufficiently tackle this problem. They show increased understanding that genes code for proteins via RNA (the first how-question in Fig. 1), but the central role that proteins have in all cell processes is not sufficiently developed in their design (the second how-question in Fig. 1). They use the examples of a receptor, an enzyme, a transporter channel and a structural protein and they hope these examples would help students to build a conceptual toolkit of possible protein functions. However, they conclude that *'the examples provided might not have been enough to support the construction of robust understanding of protein functions that would be sufficiently generative'* (p. 29) and they wonder how many more examples of protein functions would be needed to establish this generative understanding. At the end of their paper, they make an interesting suggestion that a framework for reasoning about complex systems may help students to develop a mechanistic- and systems-oriented understanding of genetic phenomena. Their emphasis on this mechanistic- and systems-oriented understanding relates closely to the central theme in this thesis: to make intelligible how genes contribute to biological phenomena, students should be able to meaningfully connect the molecular level with the cellular level and higher levels of biological organization. However, the question remains what it exactly is that we aim for when we claim that students should be able to connect levels of biological organization.

## 2.2. Connecting levels: what do we aim for?

### 2.2.1. Connecting levels cannot be adequately grasped using the traditional structural levels

A very prominent characteristic of biology as a scientific discipline is that it is concerned with studying phenomena at wide rage of organizational levels. These 'levels of biological organization' are traditionally designated based on general types of hierarchically ordered entities that can be distinguished in living systems, from atoms all the way up to the biosphere. The distinction between levels may seem obvious, but much variation can be found in how these levels are distinguished. For instance, for the levels below the organism level, very often only the most obvious structurally recognizable entities in the human body are used, resulting in a distinction between organisms, organ, tissue, cell, organelle, molecule and atom. However, these structural levels are insufficient to grasp the essence of how biologists 'think in levels' to understand biological systems. For instance, to describe how the human body works, the level of 'organ systems' should also be included as an intermediate level between the organisms and the individual organs. This intermediate level helps, for instance, to explain how the organs of the digestive system are intimately connected with the circulatory system, which in turn connects to the respiratory system. Confusingly, from the point of view of the body and its constitutive systems, we could place the immune system at the same intermediate level of organization as the digestive, circulatory and respiratory systems (Verhoeff, 2003). However, the immune system cannot be described as a system of structurally recognizable organs and tissues. The immune system is traditionally explained directly from the function of different types of cells involved in immune responses, without using the terms tissue or organ. It can be concluded from this example that to describe the body as a system with interconnected and nested functional subsystems, the traditional levels of biological organization are insufficient and even misleading. Another example that illustrates the difficulty in distinguishing between levels of biological organization based on structures can be seen when studying bacteria. In unicellular organisms, obviously the organism level and the cellular are one and the same, and the levels of organs and tissues do not apply. Furthermore, bacteria do not have organelles such as a nucleus and mitochondria, which traditionally form an underlying organizational level in eukaryotic cells. So using the traditional organizational levels, it seems as if there are no intermediate organizational levels between the organism (the bacterium as a whole) and the molecules that constitute the bacterium. However, in bacteria many underlying systems can be identified and described in a functional way, similar to the role of organ systems in higher eukaryotes. For instance, many bacteria have a chemotaxis system that accounts for the ability to sense and respond to attractants and repellants. This intermediate level of 'molecular systems' appears to be indispensable when explaining the behaviour of the bacterium as a whole (Baker, Wolanin & Stock, 2006).

From both examples, we can conclude that relying on structural features to distinguish between levels has serious limitations. This is clarified by Craver (2002), who offers a sharper view on what can be considered a level. He describes three decomposition strategies that offer a rationale for distinguishing between three types of levels.

1. *Structural* levels based on structural (or as Craver calls it aggregative) decomposition: structural decomposition involves dividing some entity into smaller pieces. The question of interest in structural decomposition is 'which is part of what?', thereby neglecting the organization,

interactions and activities of the parts. Note that applying structural decomposition to an entity does not imply that the entity only shows aggregative properties (Wimsatt, 2000).

2. *Functional* levels based on functional (or process) decomposition: in functional decomposition, some event, role, task, activity or process is divided into subtasks, partial activities or subprocesses. A purely functional decomposition identifies underlying events but it does not provide answers to the question how these events come to be (Craver calls this 'how-possibly' descriptions, Craver, 2002, p. S88). It can be compared to dividing a 'black box' into smaller 'black boxes'[2].

3. *Mechanistic* levels based on mechanistic decomposition: a mechanistic decomposition describes the entities and activities organized in the performance of a higher-level role. The activities and properties of the entities in the lower level mechanism may themselves be subject to mechanistic decomposition. Levels of mechanisms combine structural and functional decomposition in the sense that the levels are composed of hierarchically ordered mechanisms:

*'Because mechanisms are organized collections of components and their activities, no component can be larger than the mechanism as a whole, and so levels of mechanisms are ordered by size. For analogous reasons, higher-level behaviours act over longer time-periods than lower-level activities.'* (Craver & Bechtel, 2007, p. 550).

The distinction between these different accounts for levels allows for a reinterpretation of the use of levels in biology education: although many of the phenomena of interest in biology education are discussed with the aim to provide a mechanistic explanation, for instance 'how is food digested in the body' or 'what is the role of haemoglobin in the blood', the traditional levels of biological organization are mainly derived from structural decomposition. This traditional emphasis on structural decomposition in biology education has resulted in the conventional educational approach of identifying underlying structures and specifying their function. Although there is nothing wrong with this approach as such, it often provides only a partial and incoherent explanation for the higher-level phenomenon under study. Moreover, the question whether a plausible and intelligible account for a higher-level phenomenon has been provided with this approach is often not even raised in the biology classroom. From a historical point of view, it is not surprising that the traditional levels of biological organization have been classified based on the most obviously identifiable underlying structures. Structural analyses by means of dissection and decomposition have greatly contributed to our biological understanding. However, Craver's specification of the different types of levels clarifies the problems of applying organizational levels to activities or roles that are not clearly linked to obvious structures, described in this section. It can be concluded that relying on structural features to distinguish between 'levels of biological organization' seriously limits students understanding of the relationships between hierarchically organized biological systems.

2. Note that what Cummins (1975) describes when he talks about functional analysis entails much more than Cravers narrow definition of functional decomposition. It is in my opinion almost identical to what Craver calls mechanistic decomposition, because they both provide an account for analysing how a system works that goes beyond mere structural or functional decompositions.

### 2.2.2. Connecting levels implies more than indicating activities at different levels

Although many studies in science education in general, and biology education in particular, have touched upon the question of what we aim for when we claim that students should be able to connect levels, many questions and educational challenges remain, as will become clear in the next paragraphs. I will first discuss how the topic of 'connecting levels' has been touched upon by other researchers of biology education and I will link this to other discussions in science and science education concerned with 'thinking in levels'.

Knippels (2002) developed a learning and teaching strategy for genetics education called the yo-yo strategy, in which the relevant concepts are positioned at the different levels of biological organization. Reflection activities make sure that during the whole lesson series students are actively thinking backward and forward (or as I would say upward and downward) between the organizational levels that have been explored. The power of this approach is that it uses one central steering question to integrate concepts at different organizational levels in one coherent learning trajectory. For instance, Knippels' approach integrates the concepts variability (population level) hereditary traits (organism level), mitosis/meiosis (cellular level) and DNA (molecular level). However, I discuss this approach so as to better specify what we mean when we say that students should be able to connect organizational levels meaningfully. I think this entails more than being aware of the level of biological organization that a biological concepts is 'assigned' to (which is in fact very often a disputable endeavour, especially for the concept 'gene') (see for instance Gericke and Hagberg, 2007). In my opinion, Knippels' approach contributes little to students' understanding of how the discussed phenomenon at one level comes to be from underlying structures and processes and therefore can be accounted for by describing the phenomenon in terms that belong to lower organizational levels. In other words, the relationships between levels are not discussed in the context of providing explanations, while this is typically the relationship that is of interest to biologists. Although in her lesson series, many of the concepts are explained using lower-level structures and processes (for instance, the effects of mitosis and meiosis are accounted for by describing the different processes in the sorting of chromosomes), this is not the innovation in the approach and it does not differ significantly from traditional teaching approaches. The yo-yo strategy points towards a very important aspect of biology teaching, namely that students in biology education should constantly be aware of the levels of biological organization that are relevant to the concepts discussed. However, it leaves open the question what it actually means to meaningfully connect levels of biological organization and how to promote this competence in students.

Verhoeff developed an approach to improve both horizontal and vertical coherence in biology education (Verhoeff, 2003; Verhoeff et al., 2008). Horizontal coherence refers to the ability to connect concepts that are at the same level of biological organization, for instance how the activity of one organ depends on other organs. Vertical coherence refers to the ability to relate a concept to concepts at higher en lower organizational levels, for instance how an organ plays an essential role in the body (upward) and organs' function depends of the activity different cell types (downward).Verhoeff's approach aims at developing 'systems thinking' as a domain-specific competence that allows students to study biological phenomena from a systems perspective. He combines general systems theory, dynamic systems theory and cybernetics to identify aspects of systems thinking competence and apply these to the domain of cell biology.

The four aspects identified are:

- being able to distinguish between the different levels of organization (i.e. cell, organ, and organism), and to match biological concepts with specific levels of biological organization.
- being able to interrelate concepts at the cellular level of organization (horizontal coherence).
- being able to link cell biology concepts to concepts at higher levels of organization (vertical coherence).
- being able to think back and forth between cell representations ranging from abstract cell models to real cells seen under a microscope.

Here I discuss the systems thinking competence to determine how it relates to 'connecting levels'. Because of the separation between horizontal and vertical coherence, it seems as if connecting levels could be limited to what Verhoeff specifies as vertical coherence. This is somewhat misleading and not what actually happens in his approach. In Verhoeff's systems thinking approach, it is stressed that cooperation between systems at one level is essential to establish an effect at a higher level. This is how horizontal and vertical coherence are connected: the behaviour of the system can only be accounted for by including the connections between underlying systems. In Verhoeff's approach, these connections between systems at the same level are characterized as the exchange of matter, energy and/or information. Verhoeff thus translates connecting levels as the 'understanding the behaviour of the system as a whole in terms of the behaviour of its constituent subsystems and their exchange of materials, energy and information' and in his systems thinking approach he puts processes instead of structures at the centre of the stage. Students are encouraged to build a systems model of the cell by connecting the role of cell organelles to cellular processes and explaining the interrelations between the cell organelles in terms of exchange of matter and information. Typical conclusions that the approach aims for are 'the nucleus receives the hormone and translates the message into mRNA' and 'the nucleus sends mRNA to the ribosomes' and the 'ribosomes need the messenger RNA to produce proteins'. Verhoeff's approach builds mainly on what Craver (2002) calls functional decomposition and the critique that Craver formulated applies here as well: it can be compared to dividing a 'black box' into smaller 'black boxes' and it hardly provides answers to the question how these events come to be in terms of concrete physical objects and events. I would therefore suggest that this approach, although very valuable from the point of view of encouraging process-oriented systems thinking, contributes little to solving the persistent problem in cell biology education that many students accept functional roles of organelles as given facts and rely on intentional accounts without questioning how activities of organelles and other (sub)cellular events can be understood in terms of concrete and intelligible changes in the physical world. In Chapter 7, I will further reflect on this, with specific attention to the educational challenges related to using the concept of energy and information in (cell) biology.

### 2.2.3. Organization is what distinguishes a system from a collection of parts

Studying relationships between phenomena at different 'organizational levels' or 'levels of complexity' is not exclusive for the biological sciences; it is a prominent scientific practice in all natural sciences and it is recognized as a relevant and important goal of science education in general (Lijnse, Licht, de Vos & Waarlo, 1990). Confusing in this respect is that in science education research many terms are used that are all somehow related to the competence of

relating phenomena at different organizational levels. Examples are: systems thinking (Boersma, Waarlo & Klaassen, 2011; Penner, 2000; Verhoeff et al., 2008; Wilensky & Resnick, 1999), yo-yoing (Knippels, 2002), macro-(meso)-micro thinking (Lijnse et al., 1990; Meijer, Bulte & Pilot, 2009; Talanquer, 2011) , mechanistic reasoning (Russ, Scherr, Hammer & Mikeska, 2008; Van Mil, Boerwinkel & Waarlo, 2013), emergent perspective (Rappoport & Ashkenazi, 2008) and structure-(behaviour)-function perspective (Boerwinkel, Waarlo & Boersma, 2009; Hmelo-Silver & Pfeffer, 2004). A general theme in all these studies is that they describe educational challenges and confusion in students concerning the relationships that do or do not apply between phenomena at different organizational levels. Some of the reported problems are:

- Students might attribute emergent or system-level properties at higher levels directly to the individual lower-level constituents, for instance, a molecule has a colour (Wiser & Smith, 2008)
- Students might expect one causal factor to be responsible for the systems property. For instance, it is very difficult for students to believe that the behaviour of a flock of starlings is not guided by something like a leading bird (Penner, 2000).

Emergence is a widely discussed topic in the philosophy of science (e.g. Mayr, 1996; Wimsatt, 2000). It is beyond the scope of this thesis to give an extensive review of the different viewpoint on emergence. What I aim for by discussing this topic here is to show that the notion of emergence is central in explaining biological phenomena (Boogerd, Bruggeman, Richardson, Stephan & Westerhoff, 2005) and that it relates closely to what educators in biology advocate as promoting a systems view or systems thinking competence in biology education[3] . Powell and Dupré (2009) discuss the topic of emergence when discussing the relationship between individual molecules and systems made up from molecules. One of the central aspects they highlight is that the behaviour of the individual molecules is highly influenced by the fact these molecules are part of a system in which they interact with other molecules in the system. This relates to the notion of emergence I want to discuss here, amongst others put forward by Ernst Mayr: '*Systems almost always have the peculiarity that the characteristics of the whole cannot (even in theory) be deduced from the most complete knowledge of the components, taken separately or in other partial combinations. This appearance of new characteristics in wholes has been designated as emergence.'* (Mayr, 1982, p. 63).

The focus I choose here for the term 'emergence' is comparable to the focus that others in science education research have chosen to investigate students understanding of emergent phenomena (Penner, 2000; Wilensky & Resnick, 1999). Wimsatt (2000) describes the account of emergence that I will build on in this study. He describes emergence as the failure of aggregativity, in which 'the whole is nothing more than the sum of its parts'. He states: *'emergence of a system property relative to the properties of the parts of that system indicates its dependence on their mode of organization. It thus presupposes the system's decomposition into parts and their properties, and its dependence is explicated via a mechanistic explanation.'* Wimsatt, 2000, p. 271).

3. I do not claim that developing systems thinking competence can be narrowed down to articulating the notion of emergence. However, I think that systems thinking builds on the notion of emergence, and therefore to promote systems thinking, it is indispensible to articulate how a system can have properties that cannot be reduced to its components in isolation.

This characterization of emergence stresses the importance of getting a grip on how systems behaviour depends on the organization of its components and it points towards the difference between mere decomposition resulting in a 'parts list' and a mechanistic explanation in which the systems' dependence on organization and interaction of its parts is integrated.
To summarize, 'connecting levels' refers to some sort of reasoning that is relevant in the context of explaining a phenomenon in terms of underlying objects and events. This implies the construction of a mechanistic explanation in which these objects and events are organized in such a way that the mechanism provides a reasonable account for the phenomenon. Students' difficulties in connecting levels may be explained by a lack of attention in biology education (both from students and teachers) to evaluate if presented underlying structures and their activities indeed provide a reasonable *explanation* for some higher-level phenomenon. In other words: the explanatory context that makes connecting levels relevant gets little attention and, as a result, presented biological objects and events remain unconnected. Explaining in terms of underlying mechanisms is the central theme in this study. It explores how explicit mechanistic reasoning can form the basis for an educational approach by which students explore meaningfully how to connect cellular behaviour to molecular interactions.

# 3. Research focus and aims

## 3.1. Aims and approaches in the study

This study focuses on the educational challenges of connecting the cellular and molecular levels in life science education. Many students explain cellular function only by referring to organelle function and are satisfied with this explanation (Barak et al., 1999). Here we want to explore the educational potential of studying cellular phenomena from a different perspective: all cellular phenomena emerge from molecular interactions. Although this might be interpreted as a strong reductionist approach, we claim the opposite: only with a systems view on molecular events (Nurse, 2003; Powell & Dupré, 2009; von Wulfingen, 2009) will life science education succeed in making intelligible to students that very complex cellular behaviour can emerge from simple molecular interactions. The aim of this study can thus be specified as an exploration how to equip and encourage students to use molecular interactions as a basis for reasoning about cellular behaviour. In other words, this study aims to inform learning and teaching the *molecular basis of life*.

As a starting point, I need to clarify what characterizes the relationship in general between cell processes and molecular interactions to get a better grip on why this relationship is not obvious to students from current instruction. I choose to approach this by looking at how experts in life science research connect the molecular and cellular level. This entails specifying both the structure of the explanations they consider relevant in their work, as well as the heuristics they use to build these explanations. In Paper II, I construct a framework that characterizes the structure of these explanations and the heuristics used by experts based on the philosophy of science. The analysis of both the structure of the explanations and the scientific heuristics to construct them might provide clues about why connecting the molecular and cellular level is not self-evident for students in upper-secondary education, even after they have been taught the molecular details of protein structure and function.

The next step is to find out how this analysis of scientific explanations and heuristics could inform the learning and teaching of the molecular basis of life. In Paper II, already some of the implications are described and translated into design criteria that need to be taken into account when designing learning trajectories.

Chapter 5 builds on Paper II (summarized in Chapter 4) by clarifying the perceived educational potential of the framework and highlighting how the framework provides inspiration for specific pedagogical choices in an educational strategy that aims at bridging the gap between the molecular and cellular level.

The final step in this study is the design of a learning trajectory that should equip and encourage students to use molecular interactions as a basis for reasoning about processes in the cell. In Paper III (summarized in Chapter 6), I elaborate, justify and pilot such a trajectory in an exploratory case study.

## 3.2. Research questions

The progression in the study as a whole can thus be characterized using the following three research questions:

1. *What characterizes scientific explanations that aim at understanding cellular behaviour in terms of molecular interactions and how do scientists construct these explanations?*
   (answered in Chapter 4, which summarizes Paper II)

2. *How could this characterization inform the learning and teaching of the molecular basis of life?*
   (answered in Chapter 4 and 5)

3. *Can we design and effectuate a learning trajectory by which students meaningfully connect cellular behaviour to molecular interactions?*
   (answered in Chapter 6, which summarizes Paper III)

# 4. Mechanistic explanations in the life sciences

## 4.1. The position of Paper II in this study

Paper II is entitled: 'Modelling molecular mechanisms: a framework of scientific reasoning to construct molecular-level explanations for cellular behaviour' (Van Mil, Boerwinkel & Waarlo, 2013). The paper offers a characterization of how experts in the field of the molecular life sciences explain cellular behaviour in terms of molecular interactions, based on philosophical and historical reflections on their scientific aims and practices.

This characterization offers a sharper perspective on what biology educators call 'connecting levels of biological organization', in particular the connection between cellular behaviour and molecular interactions. The framework presented in Paper II allows me to clarify why the gap between the cellular and molecular levels in life science education is so persistent, despite the increasing molecular details added to biology curricula in the past decades and it offers guidelines for designing education which attempts to bridge this gap. Paper II thus answers research question 1 and partly answers question 2.



## 4.2. Summary of Paper II

Molecular and cell biologists study the behaviour of macromolecules within the context of a living cell in order to discover the relationships between these levels of biological organization. Mental models and heuristics that experts use can be informative for designing education (Glaser, 1999). Therefore, we suggest that if we characterize more precisely what these scientists present as explanations and how they construct these explanations, this might help educators to better design education that links molecular interactions to cellular processes. In this paper, we thus seek to formulate educational design criteria based on the analysis of the goals and strategies in molecular and cell biology research.

The research questions in Paper II are:

1. *What characterizes scientific explanations that aim at understanding cellular processes in terms of molecular interactions?*
2. *Which heuristics are used to construct these explanations?*
3. *What educational design criteria can be derived from the analysis of these scientific explanations and heuristics?*

The paper presents a literature review on the philosophical foundations of molecular biology and the closely related fields of molecular cell biology and molecular systems biology. The philosophy of science is concerned with both the nature of scientific explanations and the strategies scientists use to construct these explanations. Based on the philosophy of molecular systems biology, I propose a framework representing the characteristics of molecular explanations of cellular processes. Heuristics used to construct these explanations can also be represented in this framework. Research on the process of bacterial chemotaxis serves as an example to show that our findings reflect scientific practice and that historical and contemporary scientific explanations of chemotaxis fit in our framework. The framework aims to provide criteria for the design of educational activities that help students to connect cellular-level phenomena to the molecules that constitute the cell.

## 4.2.1. Mechanistic explanations in molecular and cellular biology

The analysis shows that explanations in the molecular life sciences are typically mechanistic explanations that attempt to provide a causal account for a systemic property at the (sub)cellular levels by describing the organization and interactions of underlying entities and activities. The terms 'entities' and 'activities' are used to refer to objects and events that are used in the description of a mechanism. Activities at the cellular level are often described with the cell as the subject: for example, the cell divides, the cell metabolizes glucose, the cell moves. At the molecular level, the entities typically engaging in mechanisms are the gene products (proteins, RNAs) interacting with each other, with DNA and with all sorts of small metabolites. Molecular activities are typically ascribed to gene products. For instance, if the interaction of ATP with a specific protein results in hydrolysis of ATP, this protein is assigned ATPase activity. Between the interactions of individual molecules (the molecular level) and the activities assigned to the cell as whole (the cellular level), many intermediate levels can be distinguished, joint activities can be assigned to groups of cell components that work together to accomplish a relatively autonomous function in the cell. These ensembles of groups of proteins, protein complexes or (parts of) organelles are called functional modules. Examples are signal transduction pathways, the citric acid cycle and the transcription machinery.

Fig. 2 presents how mechanistic explanations for cell activities make use of entities and activities at multiple underlying levels. The level of detail at which the explanation is worked out depends on the research question, the interest of the researcher, pre-existing knowledge, the technical limitations, etc. The schema proved useful and adequate to analyse expert reasoning cell biology and related scientific fields, as is shown in the paper using the example of research on bacterial chemotaxis.



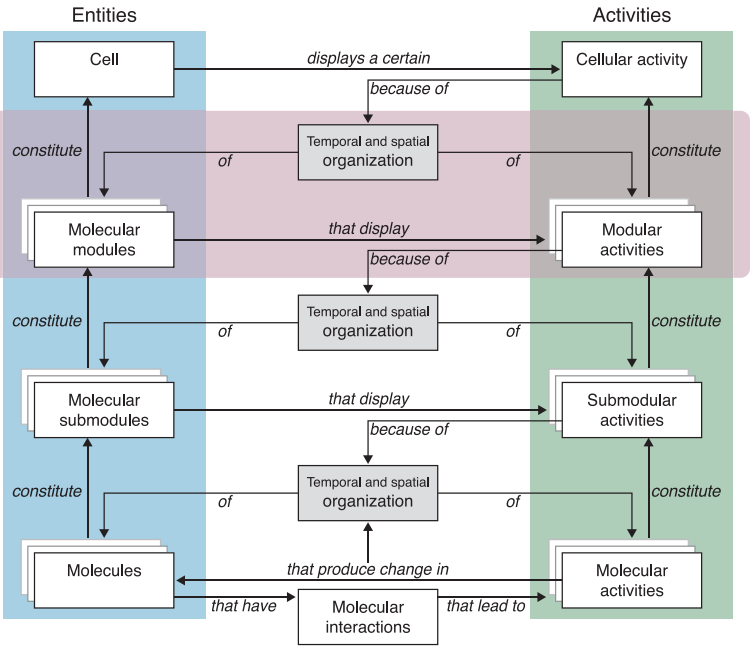



*Fig. 2: A multi-level mechanistic explanation describes a cellular activity in terms of the properties, activities and organization of interacting modular, submodular and/or molecular entities*

## 4.2.2. Heuristics to construct molecular mechanistic explanations

In Paper II five heuristics are identified that scientists use to construct mechanistic explanations:

*1. asking how-questions*
*2. functionally subdividing activities*
*3. hypothesizing mechanistic schemas*
*4. predicting molecular properties from activities and vice versa*
*5. hypothesizing and predicting organization in the mechanism.*

All these heuristics use existing knowledge about activities, entities or organizational aspects to predict and test for unknown activities, entities or organization. The activities, entities and organizational aspects sought can be at the same level, for instance if the chemical properties of a protein predicts a molecular activity in the mechanism. This type of reasoning within one level is called 'forward and backward chaining' or 'causal chaining'. Typical questions are: 'what preceding entities and activities caused the current state of the mechanism' or 'what subsequent entities and activities will be the result of the current state of the mechanism?' The knowledge at one level can also be used to predict and test activities, entities and organization at higher levels (bottom-up) or lower levels (top-down). Typical questions are 'what higher-level activity does this mechanism contribute to?' and 'what underlying entities and activities contribute to this activity?'

With the goal to fill the gaps and to solve inconsistencies in the model of a multi-level mechanism in the cell, scientists reason back and forth between entities, activities and their organization, and up and down between different levels.



## 4.2.3. Mechanistic reasoning to fill the gap between cellular and molecular-level phenomena in life science education

The analysis in this paper shows that scientists in molecular biology model molecular mechanisms to explain cellular processes. The first two research questions of this paper are answered as follows:

1. *Biological explanations of cellular processes are typically mechanistic explanations:* Models of the molecular mechanism explain how a cellular process works by showing how the relationships between the consisting molecular entities, their activities and their spatial and temporal organization together bring about the process. Often intermediate levels are used to show how interacting groups of molecules, called molecular modules, have their own level of organization and fulfil specific functions in the overall process.
2. *Scientists formulate mechanistic research questions and model molecular mechanisms to answer these how-questions*: With the goal being to fill the gaps and to solve inconsistencies in the model, they reason back and forth between molecules, molecular activities and their organization, and they reason up and down between different functional levels between cells and molecules.

In the paper, I describe how relating the cellular level to the molecular level in biology is a crucial but very difficult step for students. In Chapter 2, I concluded that mechanistic reasoning might provide a direction for overcoming these difficulties. The analysis in Paper II shows that connecting the molecular and cellular levels indeed entails a form of mechanistic reasoning, because it requires relating the behaviour of wholes at multiple levels to the properties, activities and organization of their parts and vice versa. As hoped, the characterization of molecular mechanistic explanations and the heuristics that scientists use makes it possible to reinterpret the problem in terms of students' difficulties in reasoning about molecular mechanistic explanations and thus providing criteria to address them. This leads to the answer to research question 3:

3. *What educational design criteria can be derived from the analysis of these scientific explanations and heuristics?* This question is answered by first reinterpreting learning difficulties in terms of the knowledge and reasoning skills needed for reasoning about molecular mechanistic explanations. Then, these needs are further specified into educational design criteria by adapting the identified scientific heuristics for educational use.

Mechanistic reasoning means reasoning about mechanistic explanations (Russ et al., 2008). The term 'molecular mechanistic reasoning' will be used to refer to the reasoning skills needed to construct and to understand mechanistic explanations that concern activities between the molecular and cellular level. Reasoning about mechanisms seems to be quite intuitive; it relates to the question 'How does it work?' and it is abundantly present in student reasoning, even in very young students. But although molecular mechanistic reasoning builds on general mechanistic reasoning, our analysis points towards characteristics of molecular mechanistic explanations that complicate students reasoning. The following obstacles are identified that may prevent students from using this intuitive notion of mechanism to explain phenomena in the cell.

- *Mechanistic explanations*: 'How does it work?' is not an obvious question in cell biology education.
- *Molecular interactions*: Students do not consider protein interactions as basic causal events in the cell.
- *Functional levels*: Students are unfamiliar with the multiple functional levels in between cells and molecules
- *Molecular modules*: The abstract, dynamic and transient nature of molecular modules complicates students' reasoning.
- *Temporal and spatial organization*: Students are unfamiliar with many organizational aspects of proteins and protein activities.

Based on these obstacles, we suggest incorporating the following aspects in a design that aims using molecular mechanistic reasoning to bridging the gap between the cellular and molecular levels:

- *Raising how-questions about cellular activities*: Students are guided towards causal-mechanistic instead of functional explanations for (sub)cellular behaviour.
- *Explaining protein activities from molecular interactions*: Students are provided an intelligible causal explanation for the machine-like behaviour of proteins.
- *Functionally subdividing cellular activities*: Students explore the multiple functional levels in between cells and molecules using by subdividing cellular activities into functional modules, without detailed molecular knowledge.
- *Hypothesizing mechanistic schemas*: Students hypothesize about the characteristics of the mechanism by which certain modules function. These hypotheses can be inspired by mechanistic analogies from daily life.
- *Articulating the role of organization in protein-based mechanisms*: Students explore that not only is the presence of specific proteins required to establish specific activities in the cell, but also that these proteins have to be organized in a way that makes it possible for the mechanism to function.

Mechanistic models and images are not completely unknown in cell biology education. Most upper-secondary curricula already present mechanisms in the cell, mostly by means of cartoon-like models. However, based on the identification of what is needed for molecular mechanistic reasoning, it is concluded that students lack the knowledge base to interpret these models correctly, and that therefore presenting these models does not contribute to

understanding how molecular interactions explain cellular processes. For instance, arrows in cartoon-like models indicate the activities in the mechanism. Without knowledge of protein interactions, these arrows remain meaningless. Molecular mechanistic reasoning thus allows more adequate interpretations of the molecular graphics and animations already used in education. Furthermore, students may use molecular mechanistic reasoning to generate ideas and hypotheses about the mechanisms underlying biological phenomena that have not yet been explored down to the molecular level. Molecular mechanistic reasoning thus offers students the cognitive tools to fill the gap between the molecular level and higher levels of biological organization.

## 4.3. The contribution of Paper II to this study

Going back to the overall research questions in this study, we can conclude that Paper II contributes significantly to answering them. The research questions were:

1. *What characterizes scientific explanations that aim at understanding cellular behaviour in terms of molecular interactions and how do scientists construct these explanations?*
2. *How could this characterization inform the learning and teaching of the molecular basis of life?*
3. *Can we design and effectuate a learning trajectory by which students meaningfully connect cellular behaviour to molecular interactions?*

Paper II answers the first research question fully and it forms the framework for thinking about learning and teaching the molecular basis of life, thereby contributing significantly to research question 2. The starting point is that biological explanations for cellular behaviour are typically mechanistic explanations worked out at different levels, with the level of molecular interactions generally accepted as a basic level. Based on this analysis, the connection between molecular and cellular events can be considered a multi-level mechanistic relationship. Paper II provides suggestions and inspiration for the design of a learning trajectory aimed at connecting molecular and cellular level events. In the following chapter, I discuss the educational potential of tapping mechanistic reasoning and explore how this potential can be made concrete and productive in a lesson series, thereby complementing the answer to research question 2.

# 5. The educational potential of mechanistic reasoning to bridge the gap

As discussed in Paper II, mechanistic reasoning is intuitive. It builds on experiences and expectations of how the natural world works. Obviously for many phenomena, people do not have specific knowledge about underlying entities and activities and as a result they cannot explain it in terms of mechanisms, but this does not mean that they are not aware of the fact that there are underlying mechanisms that can account for the phenomenon. In this thesis, I argue that this notion of mechanism to explain events or changes can help to explore the connection between the molecular and cellular level. This chapter discusses four elements that might be powerful in an educational strategy that aims at exploring the mechanistic relationship between the molecular and cellular level:

1. *Using an explanatory context to explore mechanistic levels.*
2. *Connecting mechanistic activities to intelligible physical changes.*
3. *Defining bottom-level changes that are intelligible, plausible and widely applicable.*
4. *Using visual models to explain and practise the mechanistic reasoning that is needed to interpret mechanistic models meaningfully.*



## 5.1. An explanatory context to explore coherence between levels

Scientists construct and refine molecular mechanistic models to *provide an explanation* for the phenomenon they study. The question they are interested in is 'how does it come to be?' and it is in this explanatory context that the model has a clear function: it is a partial and provisional answer to the how-question that is of interest to scientists.

I aim at using molecular mechanistic models in an educational context as a means for students to get a grip on the complexity of molecular events in the cell. These models describe (sub)cellular activities as chains of molecular interactions, thereby providing a concrete conceptualization of a process in terms of physical changes. This seems to be most powerful if it is clear to students that modelling cellular behaviour as a molecular mechanism is a way to provide an explanation for a phenomenon that until that moment was unexplained. This is what I mean with using an explanatory context: making clear to students that the central activity in the lessons is constructing and interpreting explanations for (sub)cellular behaviour.

## 5.2. An intelligible connection between mechanistic activities and physical changes

### 5.2.1. Biological activities as umbrella terms

In essence, almost all mechanistic explanations are generalized and idealized models. Activities that serve in a mechanistic explanation can be considered umbrella terms that provide a 'causal shortcut' for all the underlying events down to the level of the most fundamental changes. For instance, by referring to the activity 'the heart pumps blood', one takes together all the lower-level changes in the heart that occur during such a 'pumping event', such as the firing of the SA node, the contraction of muscle cells and the opening and closing of the valves. Umbrella

terms are useful and relatively unproblematic for a specific scientific field and it is unnecessary and for most phenomena impossible to provide an exact description of all the changes down to the level of the most fundament particles. Physicists apply the fundamental laws of nature to calculate precisely how particles behave in very simple systems, but the phenomena that are of interest in biology (and chemistry) are so complex that scientists rely on approximations and generalizations about the behaviour of entities under the conditions they study. These approximations and generalizations are implicitly included in the scientific models that present a mechanism at a certain level of interest. If these models are to be interpreted by someone who is unfamiliar with these implicit generalizations and assumptions, meaning-making about the activities in the mechanism will remain superficial or flawed. Take, for instance, a mechanistic activity for the chemotaxis example in Paper II: 'the receptor senses nutrients, which causes the cell to start tumbling'. The activity 'sensing' is an umbrella term that is used by experts to refer to a group of changes that occur when nutrients bind to receptor molecules. Someone who is not familiar to these underlying changes will try to make sense of the term 'sensing' with the knowledge and associations that he or she is familiar with. In this case, it should not come as a surprise if a student interprets the 'sensing activity' of the receptor by referring to the human senses.



### 5.2.2. Biological activities should be explained in terms of intelligible changes in the physical world

In biology education in general, many activities at many levels are presented because explaining by presenting mechanisms is a very prominent and substantial effort in biology education. However, the case of the receptor is one of the many examples in biology education in which activities used to characterize the mechanism are not intelligible and therefore hardly contribute to explaining the phenomenon, although it seems as if an explanation is presented. Note that this does not mean that all events in biological systems need to be worked out to the molecular level, but it implies that every activity in a presented mechanism should be intelligible to students in terms of plausible changes and their effects in the natural world. I will illustrate this with a very basic explanation for the 'pumping' activity of the human heart: 'the heart *pumps* blood because it *contracts'*. To provide meaning to the activity 'contract', students make use of their knowledge (from daily-life experience) that if the volume of a liquid-containing system is reduced, pressure increases and if the system is an open, liquid will leave the system. This link between volume, pressure and liquid transport is essential to understand what is meant by 'the heart pumps blood because it contracts'. Because even young children are aware that reducing the volume provides a mechanism for transporting liquids (or gasses), no extra educational effort is needed to explain that this is plausible mechanism for how the heart works. Although this explanation provides no information on 'how' the heart contracts, students can understand that the activity 'contracting' accounts for transport of blood and that it makes sense to call this 'pumping', analogous to other types of pumps. This elaboration shows that it highly depends on unambiguous nature of the term that is used to characterize activities in a mechanism, whether students interpret a mechanistic model in a way that supports scientific understanding of the phenomenon. In this respect, characterizing activities in biological mechanisms with terms that refer strongly to human abilities or intentions, such as sensing, reacting or knowing, appears to be tricky because it is difficult for students to interpret these activities as physical changes, which is conditional for mechanistic reasoning.

### 5.2.3. 'Internal' and 'external' causes to distinguish between activities and other changes

Using the term 'activity' to characterize the changes in the mechanisms appears to be somewhat limited, because in addition to entities that display activities (the heart contracts), many other changes for which the term 'activity' is not suitable are included in a mechanistic model as well. For instance, the flow of blood through the veins, in a model of the circulatory system, is a change but not an activity of the blood; it is the effect of the activity of the heart. Some changes in a mechanism can be attributed to an internal cause or, in other words, to internal mechanisms in the entities displayed (for instance, the contraction of the heart). These are the changes that can be characterized as an activity. Other changes will be the result of an external cause (for instance, the movement of an entity is caused by natural forces such as gravity that act directly on the entity). These changes cannot be characterized as an activity, but still need to be included in the explanation to account for the phenomenon as a whole. This distinction between internal and external causes is indispensable to meaningfully interpret mechanistic models in general. In the case of mechanistic models of (sub)cellular events, this means that changes in the cell that students perceive or infer from a visual model can either have an internal or an external cause. 'Internal' means that an entity displays an activity that can in principle be accounted for with an internal mechanistic description of how the activity of the entity comes to be. This is the case for organelle activities, modular activities and protein activities, as characterized in Paper II. 'External' means that the change that an entity undergoes can be explained from external physical effects that act on the entity involved. I do not claim that this distinction is philosophically unproblematic, but I argue that it helps students to make productive use of the term 'activity' when reasoning through multi-level mechanistic explanations. To illustrate my argumentations, I use the animation 'The inner life of the cell'[4] as an example of a visual mechanistic model in which multiple mechanistic levels are mixed. According to our framework, many of the events that are visually perceived in this animation can be characterized as activities. Some are protein activities (an enzyme *cuts* an actin filament) and some are higher-level subcellular activities (the nucleus *produces* and *releases* mRNA). At the same time, many of the spatial and temporal changes in the animation are more fundamental than what a biologist would call an 'activity'. For instance, the movement of the enzyme towards the actin filament is explained by the physics principle of Brownian motion. Although the phenomenon of Brownian motion can in principle be explained as a physical mechanism of colliding particles as well, biologists would not call this an activity. This illustrates that, for molecular mechanisms in biology, some changes at the bottom level are considered fundamental and are usually not called an 'activity'.

### 5.2.4. The connection between biological activities and chemical and physical changes

In Paper II, I characterize the level of 'protein activities' as the lowest level at which a series of events is characterized as an 'activity'. However, the educational goal is to make intelligible how molecular interactions can result in higher-level activities. This means that events characterized as 'protein activities' do not suffice as bottom-level events; the connection between protein activities and molecular interactions need to be established as well. The main point I want to

4. http://multimedia.mcb.harvard.edu/anim_innerlife.html

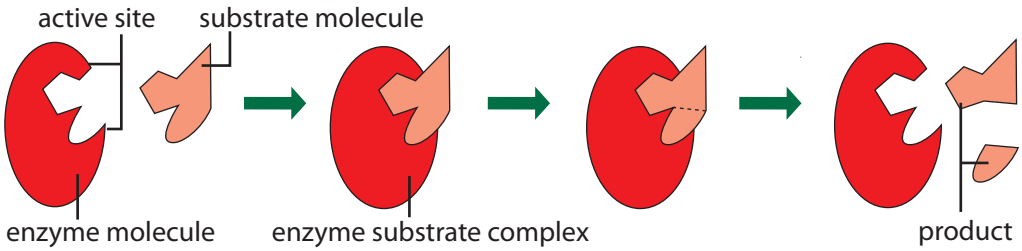
make here is that students are not sufficiently equipped and encouraged in current molecular biology education to connect what is called a 'protein activity' to the more fundamental chemical and physical changes that occur when molecules interact. Even if students learn to identify an entity in a mechanistic model as a protein and the displayed event as a protein activity (the enzyme cuts the filament), it can be expected that they do not relate this to their general chemistry and physics knowledge about the behaviour of molecules.

A few reasons for this can be found in literature. For instance, the fact that biology and chemistry are still organized and approached as separate domains in many upper-secondary science curricula does not encourage students to search for commonalities and overlap in conceptual understanding. Although proteins are discussed in both the biology and the chemistry classrooms, traditionally biology education emphasizes the biological activity or role that the protein has in the cell (the protein is a receptor that *senses* hormones, a transporter that *translocates* ions or an enzyme that *cuts* an actin filament), whereas the chemistry teacher stressed the chemical composition and properties of proteins (the chemical interactions between amino acids and the influence of pH or temperature on the structure). What is lacking in both approaches is the bridge between the biological activities and the physical changes that can explain biological activities.

This explanatory gap raises the question whether the molecular world, as for instance displayed in 'The inner life of the cell', will be perceived by students as actually being 'molecular'. I do not and cannot claim that students do not know that cells consist of molecules and I have no reason to assume that students are not aware of molecular basis of animate objects. But the consequence of the gap I describe here is that the changes students see in 'The inner life of the cell' do not trigger them to make sense of these changes in terms of their knowledge about the behaviour of molecules. Therefore, in order to design a strategy that connects the molecular and the cellular level, I need to answer the question how to trigger students to use their understanding of physical and chemical changes when they interpret mechanistic models of (sub)cellular events.

I suggest that providing students with an intelligible account for 'protein activities' that is grounded in physical and chemical changes can help them to relate higher-level activities to their knowledge about molecules. Although one could argue that this account is already provided in most curricula by the key-lock principle that applies to most proteins, a critical view on how this principle is presented (Fig. 3) shows that this model hardly provides an intelligible account for the changes that are presented in the model.

The key-lock model accounts for the selectivity of the proteins (the activity only occurs, if the substrate fits the protein) but most of the changes that occur are not explained by the model. Most problematic in my opinion is the effect that binding to the enzyme has on the substrate. In the left model in Fig. 3, the substrate breaks in two pieces and in the right model the substrate changes into two differently shaped products. How could students link these changes to their knowledge about molecules? It can be expected that students relate this to breaking chemical bonds in the substrate. But why would chemical bonds in the substrate change when it enters an enzyme? From the model, it seems as if nothing changes in the enzyme and this is strengthened by the statements in most textbooks that enzymes remain 'unchanged' after the reaction. The changes that occur during the reaction are hardly discussed or accounted for. I suggest that it could make a great difference if students see that the effect of binding of the substrate to the protein results in a change in shape (conformational change) of both the substrate and the



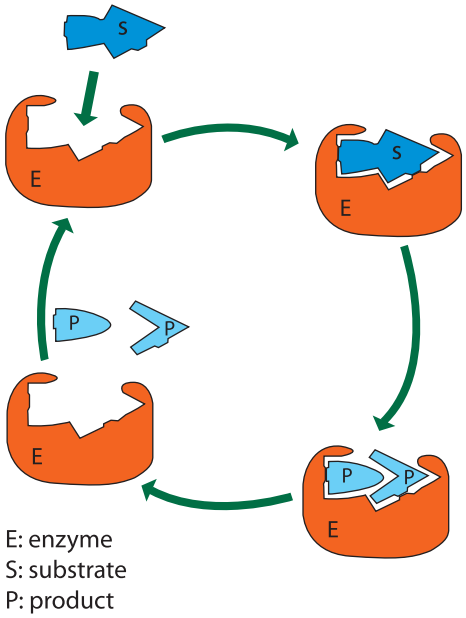




*Fig. 3: The lock and key principle as presented in the two most-used biology textbooks in the Netherlands. The upper model is adapted from Biologie voor jou (2008, Utrecht: Malmberg); the right model is adapted from Nectar (2006, Groningen: Wolters-Noordhof)*

protein. The key-lock model focuses on the effect on the substrate, whereas the conformational change of the protein itself is at least as important to understand how molecular mechanisms work.

From the above, I suggest that a (simplified) intelligible account for the cause and effects of molecular interactions in the cell is indispensable for students to connect the molecular and cellular level. It can form the basis for interpreting higher-level activities in the cell in terms of changes in the physical world and can provide a concrete physical account for the interconnectedness between higher-level activities which, in Verhoeff's approach, is referred to by the abstract statement 'systems exchange matters, information and energy'.

## 5.3. A simplified bottom-level account for the cause and effect of molecular interactions

The study aims at making productive use of mechanistic reasoning to connect the molecular and cellular level, but the question remains what conceptual understanding about proteins and other molecules in the cell is needed to be able to reason about their role in (sub)cellular mechanisms. In this section, I describe how I think students can be offered an intelligible, plausible and generally applicable account for bottom-level molecular changes without using too much detailed knowledge about (bio)chemistry. I build on the idea that protein activities only occur when proteins interact (with proteins or other molecules). The general conditions that determine whether or not molecules interact are not new for students in upper-secondary science education, but it might be that these are not very often explained. First, the appropriate molecules need to collide. The principle of thermal energy resulting in molecular collisions is covered in early chemistry classes. Effects that build on this principle such Brownian motion and diffusion play a role in biology classes as well, although maybe not readily available. Therefore, I suggest raising awareness of the principles of molecular collisions and Brownian motion somewhere in the design, for instance by means of visualizations or simulations. The second condition is that the chemical properties are such that chemical interactions can be formed between atoms or groups of atoms in the colliding molecules. Without the right atoms in the right place, no molecular interaction will occur. In the case of proteins, the spatial distribution of the atoms (the 3-D structure of the protein) is a very prominent feature that determines whether the protein 'fits' another molecule or not. The selectivity of proteins by means of their shape is commonly illustrated with the key-lock principle (Fig. 3) and we can assume that 'selective binding' is not a problematic aspect of the cause of molecular interactions.

It can be expected that students are less familiar with the events that take place once the protein binds another molecule. Although in some cases the overall effects (the protein activity) are presented, for instance in the case of an enzyme that cuts a substrate, the underlying chain of events that can explain such an overall effect are hardly discussed. One central step in understanding how a binding event between proteins can lead to certain outcomes is the principle of conformational change. In simple terms this means that the binding leads to a rearrangement of chemical bonds not only between the molecules involved, but also in the molecules. As a result, proteins can undergo dramatic changes in shape, not only at the site of binding, but in principle in any part of the protein. Obviously, this change in shape allows for new interactions with molecules that did not 'fit' before the conformational change. Although students might not be familiar with this principle, it is not difficult to understand that a change in shape opens up new possibilities for interactions that could not take place beforehand. With the availability of detailed models of 3D structures of proteins in bound and unbound states, I expect it to be unproblematic for students in upper-secondary education to visualize proteins as large but dynamic molecules that can change shape after interaction with other molecules. The principles I identified here form together the basis for a simplified account for cause and effect of molecular interactions. I collectively refer to these principles as the 'molecular dynamics' principles and I summarize the account as the 'colliding, binding, changing shape' account.

It summarizes the following line of reasoning that builds on the molecular dynamics principles:

1. *Because of thermal energy, proteins and other molecules move frantically through the cytoplasm (if not bound to other structures).*
2. *Because of these movements, proteins and other molecules collide constantly.*
3. *If proteins or other molecules with the right shape and chemical properties collide, binding can take place.*
4. *If proteins bind (with each other or with other molecules), the proteins change shape.*
5. *This change in shape creates new interaction possibilities in and between molecules that were not possible before, which leads to subsequent molecular events such as the forming or breaking of chemical bonds and the resulting binding or release of molecules.*

This 'colliding, binding, changing shape' account provides an important knowledge base for interpreting models of molecular or subcellular mechanisms. Because this account forms the basis for all higher-level mechanisms in the cell, all the events or activities that can be perceived or inferred in mechanistic models must somehow relate to this account. Take, for instance, the statement 'the nucleus produces RNA and sends it to the ER' that a student might have inferred from a visual model. If a student is aware of the molecular dynamics principles, this can help to provide meaning to this event because 'sending' can be reinterpreted as 'the RNA probably moves randomly through the cytoplasm, and it collides and binds to the ER because of a part of the RNA fits in some part of the ER'. The same holds true for other events. I do not claim that students can *explain* all events in the cell with the 'colliding, binding, changing shape' account. Obviously, they often lack insight in the specific underlying mechanisms that are not explained in the models. But I suggest that providing students with this account as a basis for reasoning about all cell activities helps them to provide meaning to the changes they perceive and infer from mechanistic models in molecular and cell biology. It enables them to distinguish between a higher-level activity that can be accounted for by an internal mechanism (that is not displayed but in principle is based on colliding, binding, changing shape of molecules) and the more fundamental changes such as the movement of molecules that can be accounted for by referring to external physical causes, which I call molecular dynamics principles.



## 5.4. Mechanistic reasoning to interpret mechanistic models

In molecular and cell biology, visual models are often used as a simplified way to display mechanisms in the cell. If we want our students to reason mechanistically about (sub)cellular activities, this entails being able to read and use the visual language that is used to communicate about these mechanisms, at least to a certain extent. According to the Machamer, Darden and Craver framework presented in Paper II, mechanistic models contain reference to entities, activities and their organization. Therefore, it would make sense to base an educational strategy that promotes the meaningful interpretation of visual mechanistic models (either being static graphics or dynamic animations) on the identification of the entities, activities and organizational aspects that are displayed. In respect to entities in the model, this means that the interpreter should know how the *objects* displayed relate to entities in the real world. For instance, in the case of molecular mechanistic models, proteins are often displayed in very abstract shapes (Fig. 3) and students should be able to recognize these shapes as proteins. In respect to activities in the model, this means that interpreter should also be able to relate the *events* displayed in the model

to changes in the real world and these changes should be intelligible in order to be helpful in explaining the phenomenon. In my opinion, familiarizing students with the type of entities and the way these are displayed as objects in visual models is a matter of good but straightforward instruction. More challenging is the question how to foster students to identify and account for the events displayed in the model. In the previous sections, I clarified that the changes that students perceive or identify in the visual models can differ in their nature and I argued that being aware of the molecular dynamics principles and the 'colliding, binding, changing shape' account derived therefrom could make a great difference in the meaningful interpretation of the events displayed in visual mechanistic models of (sub)cellular activities.

# 6. An educational design based on molecular mechanistic reasoning

## 6.1. The position of Paper III in this study

Paper III is entitled: 'Molecular mechanistic reasoning: towards bridging the gap between the molecular and cellular level in life science education' (van Mil, Postma, Boerwinkel & Waarlo, submitted). It addresses the challenge to *design and effectuate a learning trajectory by which students explore meaningfully how to connect cellular behaviour to molecular interactions.* The paper reports on the design, execution and study of such a learning trajectory. The design is an educational elaboration of the abstract framework of reasoning about multi-level mechanisms in the cell established in Paper II and the ideas that originated from that framework, presented in Chapter 5. The resulting series of lessons were taught by myself and investigated in an exploratory case-study format. The case study was meant as a proof-of-principle, i.e. finding out the feasibility of molecular mechanistic reasoning as a learning goal.

## 6.2. Summary of Paper III

Paper III starts with clarifying the gap between the molecular and cellular level in life science education. I discuss that expert reasoning about molecules in the cell takes place in the context of providing mechanistic explanations for (sub)cellular phenomena and I refer to Paper II for the philosophical underpinning of this claim. The educational design that is discussed in the paper aims at enabling students to explore meaningfully the multi-level mechanistic relationship between molecules and cells. Based on the analysis of experts' thinking and acting, we put 'explaining (sub)cellular activities' central in the lessons and we use 'colliding, binding and changing shape' as a mechanistic account for changes at the bottom level of molecular interactions. The characterization of the reasoning that we aim for in this explanatory context is called 'molecular mechanistic reasoning'. It entails: *hypothesizing, constructing and interpreting mechanistic explanations for (sub)cellular phenomena, while taking into account the physical and chemical principles that drive changes at the bottom level of molecular interactions.*

Paper III seeks to answer the following research questions:

1. *Can we design and effectuate a learning trajectory that guides students meaningfully through the multi-level mechanistic relationship between cell activities and molecular interactions?*
2. *Does the learning trajectory stimulate students to use molecular mechanistic reasoning when they interpret and construct explanations for (sub)cellular activities?*
3. *Do students experience molecular mechanistic reasoning as helpful to connect the molecular and cellular level concepts?*

As demonstrated in Paper II, molecular mechanistic reasoning uses the same reasoning steps as mechanistic reasoning in general; top-down, bottom-up and chaining approaches are distinguishable here as well. In Paper III, I specify what characterizes molecular mechanistic reasoning by combining the general mechanistic reasoning approaches with the domain-specific characteristics of mechanisms in the cell. This leads to a set of domain-specific reasoning strategies that are helpful to explore mechanistic explanations for cellular behaviour.

Top-down
- Identify a (sub)cellular phenomenon to be explained and ask relevant how-questions about it.
- Subdivide a (sub)cellular phenomenon functionally to identify underlying activities.
- Hypothesize relevant mechanistic schemas, for instance by using metaphors or comparisons.

Causal chaining
- Identify/hypothesize the involvement of proteins or protein-based modules.
- Identify/hypothesize activities of proteins or protein-based modules.
- Link protein or module activities into causal chains or recognize gaps in the causal chain.
- Apply the physical and chemical principles of molecular interactions as a basis for causality in the mechanisms.
- Apply the physical and chemical principles of molecules as a basis for organization in the mechanisms.

Bottom-up
- Combine entities, activities, organization and causality into a mechanistic model that accounts for a (sub)cellular phenomena.

Mechanistic reasoning in general will not be the main problem since it is rather intuitive, but students lack an intelligible account for proteins interactions, molecular modules and molecular organization. If they are equipped to recognize and use these domain-specific notions in their reasoning, they are likely capable of constructing meaningful mechanistic explanations for cellular phenomena through applying the above-mentioned general mechanistic reasoning strategies.
To examine whether molecular mechanistic reasoning is within reach for upper-secondary science students, we developed a series of lessons that guide students through the construction and interpretation of molecular-level explanations for cell activities. In the paper, I first describe the guidelines for designing such learning trajectory. The guidelines concern:
- using mechanistic reasoning strategies to guide students
- the role of the teacher
- the role of visual literacy in the lesson series
- the examples used in the lesson series.



### 6.2.1. Using mechanistic reasoning strategies to guide students

The design contains the following three phases based on general mechanistic reasoning strategies: top-down, exploring the bottom, and bottom-up. Together they form a learning path in which students explore the construction of molecular mechanistic explanations for cell activities. In this path, the domain-specific characteristics of mechanisms in the cell are introduced only at moments that students encounter that their existing knowledge is insufficient to construct meaningful mechanistic explanations (Klaassen, 1995).

**Phase 1: Top-down**

In the top-down phase, students descend from the organism level to identify cell activities in the human body. The next step in the top-down phase invites students to descend further, using two top-down strategies: subdividing and hypothesizing. The expected result of this step is that students realize that many cell activities identified in the first step cannot be explained using their prior knowledge about the parts in the cell. It is thus an explicit goal in this phase to confront students with the fact that the knowledge and explanations about (sub)cellular activities they have relied on so far are not sufficient to provide an intelligible explanation for most of the cell activities they came up with.

**Phase 2: Exploring the bottom level**

Since the students experience the limitations of the downward reasoning strategy at the end of phase 1, the beginning of the second phase is to offer them an alternative approach, i.e. determine a bottom level that can be used as a starting point for bottom-up reasoning to fill the gap. In this phase, students are familiarized with the molecular dynamics principles and we introduce 'colliding, binding and changing shape' as an account for cause and effect of proteins interactions. This means: proteins and other molecules move and collide randomly (if not attached to other structures). If the molecules fit (which is determined by their shape and the spatial distribution of chemical groups) they bind, and when they bind a reshuffling of chemical bonds takes place, which changes the shape and thus the binding properties of the molecules involved. This change in shape allows for new interactions that were not possible before. To understand this account, students need to be aware of a number of physical and chemical principles that we call 'molecular dynamics principles'. The principles that we consider to be essential are:

- Brownian motion
- random walk
- molecular collisions
- molecular recognition
- conformational change
- (self-)assembly.

After introducing these principles by using visual models and simulations, we use causal chaining approaches to chain molecular interactions into activities of proteins. In this way, students experience how subsequent causal changes in molecules form the basis of activities that are commonly described as a protein activity. By using the same causal chaining approaches, interdependency between proteins is explored. In this way, the concept of protein-based modules is established and students see that with the same causal chaining approach the interdependency between modules can be explained. We suggest that the 'colliding, binding, changing shape' account can make intelligible to students how temporal and spatial orders of activities can emerge from random collisions of molecules, which provides the fundament for reasoning about the organization in molecular mechanisms. As a result of phase 2, students may understand how complex activities can emerge from the exact same basis of colliding, binding and changing shape of proteins and other molecules.

**Phase 3: Bottom-up**

In phase 3, bottom-up reasoning is used to explain cell activities, thereby closing the gap between molecular interactions and cell activities. We choose three levels of increasing complexity to show that, in all cases, despite increasing complexity, the same bottom-level principles apply. In addition to an example in which the activity of one protein can explain the cell activity, we show how in some cases the cell activity can be explained from the activity of a multi-protein module and how in the third case the cell activity can be explained by combining the activities of multiple protein-based modules. By using these three complexity levels, we expect to make intelligible to students that interactions between proteins are the basis for cell activities and that entities and activities at intermediate levels, such as protein-based modules, are used to handle complexity.

Fig. 4 presents an overview of the phases that we identified to allow students to experience step-by-step how to use molecular mechanistic reasoning to bridge the gap between cell activities and molecular interactions. It displays for every phase the connection to be sought for and the reasoning strategies used to explore these connections.

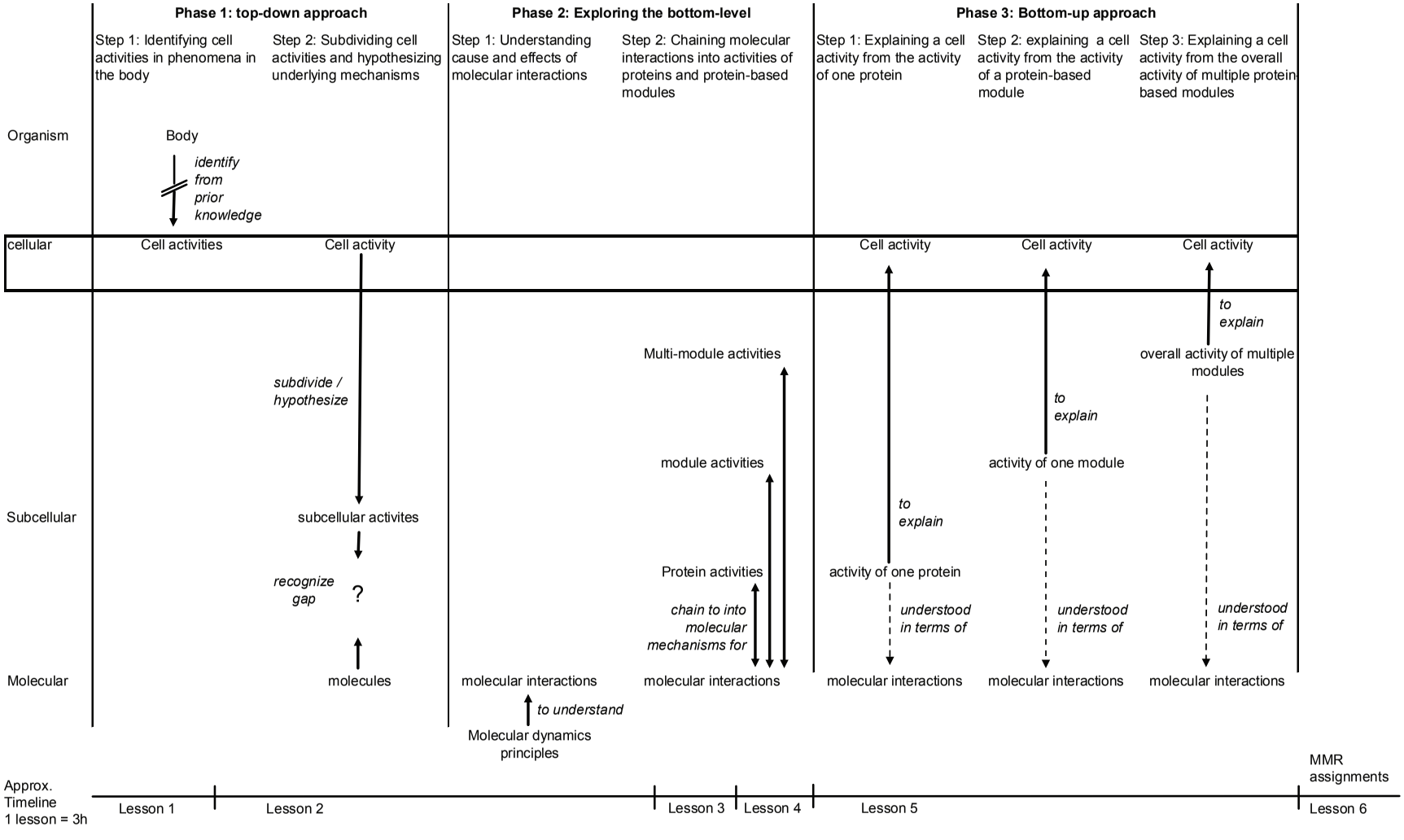



*Fig. 4: Schematic overview of the reasoning strategies and connections between levels sought for in each phase in the design*

### 6.2.2. The role of the teacher

Each of the phases in the design consists of four subsequent roles of the teacher and associated teaching activities, based on the cognitive apprenticeship approach (Collins, Brown & Newman, 1989):

- *Orientation*: the teacher offers a perspective on the progression that students will make in this phase. She emphasizes the endpoint of the previous step and helps students to formulate the question that they will work on to make the next step.
- *Modelling*: the teacher demonstrates the reasoning strategies needed to make this step and offers the content knowledge needed to handle these strategies. In doing so, she explains her thinking and encourages the students to join him in his reasoning.
- *Scaffolding*: the teacher guides the students in practising the reasoning strategies and applying the content knowledge needed, either by verbal instructions and questions or by hints and guiding questions in assignments
- *Articulation, reflection and exploration towards the next step:* the students express in their own words the reasoning strategies they used to make the step. The teacher helps the students to look back on the starting point of this step and to reflect on how the reasoning strategies contributed to the progression they made. Then the teacher helps students to identify the questions that remain to be explored in the next step.

### 6.2.3. The role of visual literacy in the lesson series

As discussed in a previous section, visual models are often used as a simplified way to display mechanisms in the cell. If we want our students to reason mechanistically about (sub)cellular activities, this entails being able to interpret these models meaningfully, at least to a certain extent. We suggest that, to encourage mechanistic reasoning about (sub)cellular activities, modelling and scaffolding the interpretation of molecular mechanistic models is an indispensible element in the lessons. By encouraging molecular mechanistic reasoning, we hope that students start to recognize these gaps in visual models of molecular mechanistic explanations. As concluded in the previous chapter, working with the models should show students that molecular mechanistic reasoning is a crucial competence to give meaning to these types of molecular-level representations. In other words, molecular mechanistic reasoning contributes to a domain-specific visual literacy by providing a framework that applies to all models of protein-based mechanisms, regardless of whether the representation is static and schematic or dynamic, three-dimensional and highly stylized.

### 6.2.4. The examples used in the lesson series

The relationship between cell activities and molecular interactions is illustrated using examples at three levels of complexity. The three examples represent cases in which the cell activity can be explained from the activity of:

- one type of protein
- one molecular module
- multiple interdependent molecular modules.

The three examples are cystic fibrosis, familial hypercholesteraemia and wound healing. Table 1 shows how the cell activities to be identified in these three examples represent three levels of complexity in mechanistic explanations.

*Table 1: The exemplary cell activities that students identify from cystic fibrosis, familial hypercholesteraemia and wound healing represent three different levels of complexity in mechanistic explanations*

| *Phenomenon* | *Cell activity in healthy individuals* | *Complexity level* |
|---|---|---|
| Cystic fibrosis | Mucous cells excrete chloride ions | One protein explains the cell activity |
| Familial hypercholesterolemia | Liver cells take up LDL-cholesterol | The cell activity can be explained from the activity of a multi-protein module |
| Wound healing | Fibroblasts secrete collagen when stimulated with the hormone TGF-β | The cell activity can be explained from the combined activities of multiple protein-based modules. |

LDL = low-density lipoprotein; TGF = transforming growth factor.

Fig. 5 shows the general scheme that is used in phase 3 to identify the gap to be filled in the explanation of the three phenomena. In the lessons, the three examples serve as a context to explore the more general question 'how do cells work?' and we expect that phase 2 in particular makes clear that the principles and concepts are more widely applicable than just these three disease-related phenomena.

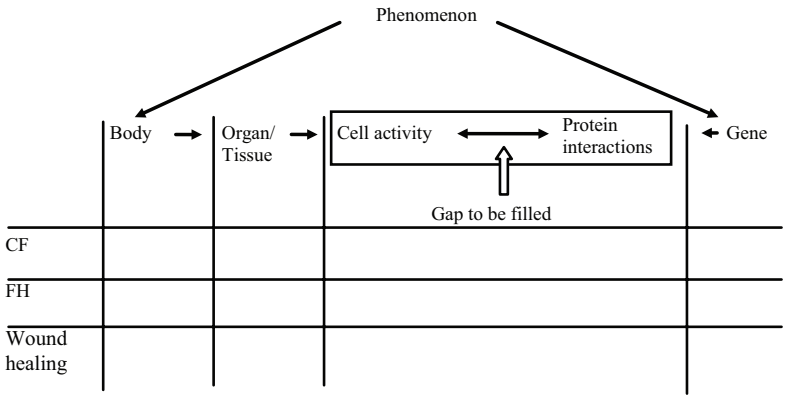



*Fig. 5: General scheme used in the lessons and the gap that is filled in the three examples*

## 6.2.5. Outline of the activities in the lesson series

In the results section, we describe for each phase the rationale in the intended trajectory and we indicate crucial learning activities in the design. A detailed description of all activities, including an overview of how the activities are sequenced in the modelling, scaffolding and reflection phases of each step, is available on request.

## 6.2.6. Proof-of-principle

The theoretically informed lesson series was subjected to an exploratory empirical test. Details of the set up of the lessons, the participants, the data collection and analysis can be found in Paper III. The reported case study was performed with 12 students (nine girls and three boys) from five different schools who chose to participate in the lessons as part of their 'Nature, Life and Technology' curriculum. For 6 weeks, the students came to Utrecht once a week for a 3-hour lesson. Because in our design the teacher has a specific role in modelling explicitly the questions and reasoning central in each step, we decided to first test the design in a somewhat artificial educational setting in which the teaching was done by me. Student interviews and classroom observations were done by a second researcher, who was not involved in the design and teaching of the lessons.

In Paper III, the findings are reported using the three perspectives reflected in the research questions.

**Perspective 1: The learning trajectory**

This section in the paper answers the question: *Can we design and effectuate a learning trajectory that guides students meaningfully through the multi-level mechanistic relationship between cell activities and molecular interactions?* It reports about the intended path and outcomes in each step in the lesson series. For each step, two questions are answered:

A: *How was the intended path in this phase designed and executed?*

B: *How did students progress through this phase?*

**Perspective 2: Students' use of molecular mechanistic reasoning**

This section in the paper answers the question: *Do students use molecular mechanistic reasoning when they interpret and construct explanations for (sub)cellular activities?* To answer this question, the assignments that students worked on at the start and at the end of the lesson are analysed on the different aspects of molecular mechanistic reasoning. The following questions that represent these aspects are used for the analysis:

- *How-questions*: Does the student identify a (sub)cellular phenomenon to be explained and ask relevant how-questions about it?
- *Subdividing*: Does the student subdivide a (sub)cellular phenomenon functionally to identify underlying activities?
- *Hypothesizing*: Does the student hypothesize mechanistic schemas, for instance by using metaphors or comparisons?
- *Entities*: Does the student identify/hypothesize the involvement of proteins or protein-based modules?
- *Activities*: Does the student identify/hypothesize activities of proteins or protein-based modules?
- *Chaining*: Does the student link protein or module activities into causal chains or recognize gaps in the causal chain?
- *Causality*: Does the student apply 'colliding, binding, changing shape' as a basis for causality in the mechanisms?
- *Organization*: Does the student apply the molecular dynamics principles of molecular interactions as a basis for organization in the mechanisms?

- *Model*: Does the student combine entities, activities, organization and causality into a mechanistic model that accounts for a (sub)cellular phenomena?

**Perspective 3: Students' metacognition on molecular mechanisticreasoning**
This section answers the question: *Do students experience molecular mechanistic reasoning to be helpful to connect the molecular- and cellular-level concepts?* Data for this part were mainly based on students' reflections in the interviews at the end of the lesson series and a group discussion with all the students at the end of the last lesson. The analysis focused on students' ideas about cells and how their views changed during the lessons. In addition, students' ideas, comments and questions about cells or the role of molecules in the cells that came forward during the lessons were used.

## 6.2.7. Summary of results

The three perspectives show that the approach guides students meaningfully through the multi-level mechanistic relationship between molecular interactions and cell activities, although some questions and bottlenecks remain. In Table 2, the findings from the result section are summarized in an overview of the achieved effects as well as the remaining questions and bottlenecks that we have identified.
Perspective 1 sheds light on the achieved effects during the learning trajectory and the questions and bottlenecks that remain. In general, we see that after identifying cell activities as partial activities in the body, students find it self-evident that explaining these activities entails 'descending deeper into the cell' and they regard 'descending' as a strategy towards better understanding. This is the core intuition for mechanistic reasoning: changes, in this case 'cell activities', have a cause, and in many cases this cause can be better understood by descending to underlying mechanisms and explore the more fundamental causal relationships that drive these mechanisms (Cummins, 1975). In the learning trajectory, this intuition is made productive, by first confronting the students with the limitations of their ideas about how changes in the cell are caused. They can subdivide activities and use analogies to reason about underlying mechanisms, but they indicate themselves that this does not lead to better understanding and as a consequence they do not experience these activities as informative. At the same time, students are aware that somehow molecules must be involved in all these activities, but they indicate that they have no idea how molecules can cause these activities. Here, the explanatory gap reveals itself and students express a need to better understand how molecules are involved. In fact, these students regard molecules as the logical candidates to form the bottom-level entities when explaining cell activities, but they lack an intelligible account for causality and organization at this bottom level to be able to use molecules to understand the mechanisms that constitute 'higher-level' activities in the cell. In the lessons, we provide such a basic account by using 'colliding, binding and changing shape' to describe cause and effect of protein interactions. This account is easily grasped by the students and it appears to be useful as a basis to understand increasingly complex mechanisms, from the activity of individual proteins to the joint activity of multiple protein-based modules. Some limitations of the 'colliding, binding and changing shape' account appear. For instance, some students interpreted 'changing shape' as mere deformation due to the collision, without including binding as the cause for a rearrangement of chemical bonds. Despite the limitations of the simplification, the 'colliding, binding, changing shape' account made intelligible to these students how protein interactions can be the basis of complex molecular mechanisms. In the

*Table 2: Overview of the achieved effects, strong points, remaining questions and bottlenecks in the design*

| *Aims in each phase in the design* | *Achieved effects* | *Remaining questions and bottlenecks in the design* |
|---|---|---|
| Phase 1: top-down approach | | |
| Step 1: Identifying cell activities in phenomena in the body | The term 'cell activity' makes sense to students and helps to define 'the cellular level'<br>Students produce a list of general cell activities easily<br>Descending from the organism level helps to relate cell activities to phenomena in the body (e.g. diseases) | Sometimes, confusion about what activity can or cannot be assigned to individual cells (e.g. producing blood) |
| Step 2: Subdividing cell activities and hypothesizing underlying mechanisms | Subdividing helps to elicit and integrate knowledge about cell processes<br>Subdividing helps to realize that the 'How?' question can be asked each time again at an underlying level of causation<br>Subdividing and hypothesizing evoke the need for a 'bottom level' | Learning activities not experienced as informative and essential in the design by students |
| Phase 2: Exploring the bottom level | | |
| Understanding cause and effects of molecular interactions | 'Colliding, binding, changing shape' is experienced as logical and fundamental | Makes great demands on students' visual processing; more practice required |
| Chaining molecular interactions into activities of proteins and protein-based modules | The terms protein activity and modular activity make sense to students and help to recognize 'intermediate levels' based on activities instead of structures | Makes great demands on students' abstract reasoning: more practice required<br>Some organelles are difficult to place in a hierarchy of activities |
| Phase 3: Bottom-up | | |
| Explaining cell activities of increasing complexity | Students can explain examples of cell activities at different complexity levels, by applying the terms protein activity and modular activity. | With increasingly complex activities students stick to less detailed explanations, hardly using 'colliding, binding changing'. It remains unclear whether they are nonetheless aware of the physical and chemical basis when reasoning about these activities |
| Overall | Students experience molecular mechanistic reasoning as 'learning how it really works' and 'going deeper into the things we already knew'<br>Students report that the lessons focused on 'learning to think' and 'logical reasoning' and that this also helps to remember the content | Students report that they did not learn much because they do not experience acquiring molecular mechanistic reasoning as 'learning new things' |
| Molecular mechanistic reasoning assignments | Students demonstrate all the elements of molecular mechanistic reasoning<br>Students pose more how-questions after the lessons<br>All students provide mechanistic accounts for the phenomena under study, but the level of detail differs widely<br>Students hardly refer to directed movement and intentional behaviour of the cell or its constituents | Difference between students in the level of detail in their explanations; how to challenge students not to be too easy-going<br>Difficult to judge whether students are indeed aware of underlying physical and chemical principles when they use higher-level activities such as 'activate', 'respond', 'sense', 'produce' |

final step of the trajectory, students used these molecular mechanisms at different complexity levels to explain the cell activities that were identified at the start and as a result they indicated that they found it logical that the same 'bottom-level' principles of molecular interactions apply to all cell activities. However, when explaining more complex activities, students tend to rely on higher-level causal terms such as 'produce, respond, activate' and it remains difficult to judge whether they are aware of the physical and chemical principles when using these terms. Students obviously reason mechanistically when they use these terms. However, our account for *molecular* mechanistic reasoning includes that students are aware of the physical and chemical principles that drive changes at the bottom level of molecular interactions. It is questionable if these six 3-hour lessons provided students with enough examples and practice to transform their tendency to accept and subscribe all kind of (sub)cellular activities without questioning the physical and chemical principles that underlie these activities.

Perspective 2 addresses the question whether indeed students are stimulated to use molecular mechanistic reasoning when they interpret and construct explanations for (sub)cellular activities. The analysis of the assignments shows that, before the lessons, students pose some mechanistic how-questions, but their reasoning in answering these questions is very superficial. After the lessons, much more mechanistic questions are posed and from these questions we can see that students better subdivide cellular phenomena into (hypothetical) underlying activities. Furthermore, when interpreting graphical representations of molecular mechanisms students search for causality, they recognize gaps and they use molecular dynamics principles in their reasoning about causality and organization. They hardly refer to directed movement and intentional behaviour in their explanations. However, many students are not very precise and consequent in applying these principles, and we suggest that much more molecular mechanistic reasoning practice is needed in interpreting, constructing and hypothesizing explanations for (sub)cellular activities.

Perspective 3 shows that students experience this way of reasoning about cells as a new perspective. One aspect they mention as being new is the focus on 'explaining' in contrast to 'just being told how it is'. Another remarkable observation is that, before the lessons started, students have not experienced an explanatory gap between 'what cells do' and what 'molecules do'. Although they knew that cells consist of molecules, they report never having thought about how cells do things. This relates to students' responses about the traditional biology lessons. Students experience the traditional cell biology and molecular biology lessons as being told 'what happens', without questioning 'how it happens'. However, it seems that most of them do not see this as a problem, although they report memorization and rote learning as strategies they use when learning about (sub)cellular and molecular activities.

In general, we can conclude that an intelligible account for the cause and effect of molecular interactions is indispensable for bridging the gap between the molecular and cellular level. We show that this account can be introduced in a meaningful way, which means that it is used to construct mechanistic explanations for cell activities that in the perception of students cannot be explained satisfactorily without this account. Students experience this as a new, useful and generally applicable perspective on how cells work. For students to use molecular mechanistic reasoning consequently and precisely when reasoning about (sub)cellular activities, much more practice is needed, but this study shows that applying molecular mechanistic reasoning strategies meaningfully in the domain of cell biology is within reach for students in upper-secondary life science education.

## 6.3. The contribution of Paper III to this study

Paper III presents a detailed description of the designed and effectuated learning trajectory. It shows that molecular mechanistic reasoning can be initiated in students when they are challenged to interpret, reconstruct and hypothesize explanatory models for (sub)cellular activities and it clarifies that students perceive an added value compared to traditional biology lessons.

# 7. Critical retrospective views

In this chapter, I reflect on the contribution and limitations of the study and directions for future research. I start by summarizing how this study articulates the central problem that students hardly connect the molecular and cellular level in life science education. Then I develop the retrospect of the study along three lines:

*1. The intended conceptual understanding and its relevance, opportunities and limitations for science education.*
*2. The pedagogical approaches and possible alternatives in relation the desired effect in the design.*
*3. The research design and methods used in this study and their limitations.*

## 7.1. Addressing the educational challenge of bridging the gap

In this study, I identified and articulated an explanatory gap between the cellular and molecular levels in pre-university life science education and concluded that students are not sufficiently equipped and encouraged to connect activities of cells to the behaviour of molecules in the cell. I considered the gap to be bridged if students appreciate that complex cell activities can emerge from molecular interactions and that, in the case of relatively simple cell activities, models of molecular mechanisms provide an intelligible account for the cell activity.

To elaborate the relationship between the cellular and molecular levels, I specified in Paper II how experts in the molecular life sciences reason between molecules and cells. They do this in an explanatory context with the aim to provide an (partial) account for a cellular activity in terms of a mechanism of interacting molecules, and they have a particular interest in the role of proteins. To hypothesize, construct and interpret these types of explanations they combine general mechanistic reasoning strategies and heuristics with domain-specific knowledge about cells and their constituents. This theoretical framework of 'modelling molecular mechanisms' highlights that the multi-level mechanistic relationship between molecular interactions and cell activities is the basis for cell biologists to construct explanations for cellular behaviour.

To tackle the educational challenge of bridging the gap, the framework of molecular mechanisms for explaining cell activities, proved valuable. The framework indicated that connecting the molecular and cellular level meaningfully can only be done in an *explanatory* context, which means that students experience learning about molecules in the cell as an essential step towards explaining cell activities. To achieve this, the lessons moved from the traditional focus on the functional role of (sub)cellular activities in higher-level activities in the body ('upward' or 'why' focus) towards explaining cell activities in terms of underlying entities and activities ('downward' or 'how' focus). The lessons were designed to meaningfully guide students through the multi-level mechanistic relationship between cells and molecules in general and proteins in particular. From literature about general mechanistic reasoning, it was concluded that reasoning about multi-level mechanisms as such is not the problem. It is the limited domain-specific conceptual understanding of how mechanisms in the cell can be explained by protein interactions that makes it difficult, if not impossible, for students to explain the behaviour of cells meaningfully in terms of underlying mechanisms. To enable them to meaningfully connect the molecular and cellular level, the study thus sought to identify gaps in students' conceptual understanding that need be closed.

I showed that the different 'upward' and 'downward' questions can be made explicit, that students understand the difference and that they can be stimulated to focus more on the downward 'how-question' when discussing cell activities. Most students value the focus on explaining in terms of underlying mechanisms as being stimulated to think about how it really works, instead of being told and accept it as a given fact. With respect to tapping mechanistic reasoning, I concluded that the use of the term 'activity' is very helpful to distinguish between levels. The terms cell activities, organelle activities, molecular module activities and protein activities all specify an overall change that can be accounted in terms of underlying entities, activities and their organization. It turned out that general mechanistic reasoning strategies such as upward reasoning, downward reasoning, forward and backward reasoning are meaningful to students. They can explicate the general type of questions and answers sought for with these strategies.
Furthermore, we know that conceptual knowledge needed to reason meaningfully about mechanisms in the cell includes an account for 'bottom-level' changes that is plausible, intelligible and generally applicable to a wide variety of mechanisms in the cell. Although with limitations, the colliding, binding, changing shape account appears to provide such a bottom level. More practice is needed for students to consistently question and explore the mechanistic relationship between higher-level activities such as protein activities, module activities, organelle activities and cell activities and the colliding, binding and changing shape of proteins and other molecules. This kind of reasoning will not be relevant in all educational activities, but molecular mechanistic reasoning in general seems to match the view on science education underlying the Next Generation Science Standards (NGSS, www.nextgenscience.org). Based on this study, I consider it a way for students to get grip on complex 'behaviour' that so far remained mysteriously inexplicable. In my opinion, this is a goal worth pursuing in pre-university science education.
A detailed educational strategy to reach this goal has not yet been fully developed. The educational design I used in this study aimed at finding out whether the conceptual understanding was, in principle, within reach of students and whether the general reasoning strategies were meaningful and productive in this domain. Further design research is needed to elaborate and field-test an effective and efficient learning and teaching strategy. The learning objectives articulated in this study and the accompanying tentative learning and teaching activities provide a useful educational framework to build on.



## 7.2. The intended conceptual understanding

In the following section, I discuss if and how my choices for the intended conceptual understanding in the learning trajectory are productive for getting a grip on the complexity that characterizes (sub)cellular processes.

### 7.2.1. Colliding, binding, changing shape is a productive simplification

The 'colliding, binding, changing shape' account can be viewed as an example of a simplified stepping-stone understanding that fosters (future) progression towards deeper scientific understanding. These stepping-stone understandings are important 'intermediate' goals in science learning progressions (Duncan & Rivet, 2013). With this study, I show that 'colliding, binding and changing shape' is an intelligible, plausible and widely applicable account for causality

(Grotzer & Mittlefehldt, 2012) at the molecular level, although very simplified. This simplification is acceptable if the account helps students in their understanding, and when it paves the road for more sophisticated scientific models for molecular changes. Both conditions appear to be met. Students can use the account in their explanations and they indicate that it helps to better understand 'how it happens' in addition to 'why it happens'. Furthermore, more sophisticated explanatory models of molecular changes are not in conflict with 'colliding, binding, changing shape'. In general, more detailed (biochemical) explanations focus on translocation of electrons in and between molecules to explain the binding and changing shape of molecules. This account is a mechanistic explanation as well, because the overall change that happens when molecules interact is described in terms of spatial and temporal organization of lower level chains of events, namely the translocation of electrons. These types of biochemical explanations can therefore be considered a more sophisticated mechanistic account for the binding and changing shape of proteins and other molecules.

Many educational challenges of using a molecular mechanistic perspective have not been addressed in this study. For instance, although many molecular mechanistic models suggest that the overall activity comes to be from discrete step-wise interactions between molecular components, these interactions and their effects (in this study simplified as colliding, binding and changing shape) can only be accounted for by relying on additional (statistical) approximations. For example, given that the components are present in large enough amounts, it can be assumed with reasonable certainty that interaction events between these components will occur within the timescale that is required for this biological process. These statistics-based approximations and assumptions in molecular mechanistic models remain implicit in the qualitative models and it was beyond the scope of this study to address these issues. However, at some point in advanced science education, these approximations and assumptions should be made explicit, for instance when discussing topics that obviously rely on statistical calculations such as reaction rates or diffusion.

### 7.2.2. Mechanistic reasoning is a fundamental reasoning skill in science education

Mechanistic reasoning can be helpful not only in the domain of molecular biology, but also in many other domains of the natural sciences. Recently the NGSS have been formulated as a standard for future science education in the USA. Based on the framework for K-12 science education (National Research Council, 2012) that sketches the science education standards up to the end of secondary education grade 12 (age 18), the NGSS identify concepts such as 'cause and effect', 'structure and function' and 'systems and system models' as cross-cutting concepts that are meant to give students an organizational structure to understand the world and to help them to make sense of and connect core ideas across disciplines. Mechanistic reasoning contributes to the understanding of the cross-cutting concepts 'cause and effect', 'structure and function' and it can be considered a basic aspect of reasoning about 'systems and system models'. Mechanistic reasoning can therefore be considered as a fundamental reasoning skill in science education to aim at. The NGSS promotes a progression in these concepts from grade K-2 (primary school, age 6) up to grade K-12 (end of secondary school, age 18) and it specifies performance expectations for different grades. With regard to 'cause and effect' the expectations for K-9–12 state *'cause and effect relationships can be suggested [by the student] and predicted for complex natural and*

*human designed systems by examining what is known about smaller scale mechanisms within the system.'* For the concept 'systems and system models' the expectations for K-9–12 state: *'While students can use models to predict the behaviour of a system, they understand that these predictions have limited precision and reliability due to assumptions and approximations inherent in models.'* The learning trajectory that I have designed in this study can be considered as an example of how these generally formulated performance expectations can be specified and made operational in science education.

## 7.3. The pedagogical approaches

### 7.3.1. Problem-posing

The problem-posing approach provides inspiration for the design by stressing the need for a local, content-related motive, formulated by students as questions that need to be answered (Klaassen, 1995). This content-related motive provides students a perspective on why and in which direction they are going to extend their conceptual understanding. In this study, an 'explanatory context' is central, which means that learning about cellular constituents serves the construction of intelligible and plausible explanations for cell activities. It is obvious to students that explaining a cell activity entails descending to underlying levels so as to identify how changes in underlying entities contribute to the overall activity. It is this mechanistic intuition that provides students a sense of direction of what they need to know in order to explain cell activities. In other words: they are aware that they can only provide a plausible explanation for the identified cell activity if they know more about 'the things in the cell that make the activity happen'. The explanatory context that called upon the mechanistic intuition thus,puts students in the position that they experience 'a need to know'. This component of 'problem posing' is effectively exploited in the design.

As the next step I chose to simply provide in an ongoing discussion with students the knowledge that could serve as building blocks at the bottom level (i.e. proteins and the molecular dynamics principles that explain their interactions). As an alternative, it would be worthwhile to see if it is possible to actively engage students in the process of identifying and developing missing knowledge themselves. In this study, this would have taken too much time, but to use the full potential of a problem-posing approach the design should be extended with these types of activities.

### 7.3.2. Cognitive apprenticeship

Cognitive apprenticeship is another educational strategy used in the design of the series of lessons (Collins et al., 1989). It concerns the explicit modelling, scaffolding of and reflection on reasoning strategies that students are encouraged to use. The use of cognitive apprenticeship seems at first sight in conflict with the intuitive character of mechanistic reasoning which would make an explicit demonstration unnecessary. However, as indicated in Paper II, students are not sufficiently equipped and encouraged to use mechanistic reasoning explicitly in the domain of cell biology. Therefore cognitive apprenticeship seemed to be a promising educational strategy. The major role of cognitive apprenticeship-based activities was to facilitate and encourage students to recognize and practice how new or activated domain-specific (prior) knowledge can be used in mechanistic reasoning strategies known from other domains. In this study, the reasoning to be encouraged is aimed at constructing and evaluating intelligible and plausible mechanistic

explanations for (sub)cellular events. This is in line with Grotzer and Mittlefehldt (2012) who stress that it is important for students to evaluate provided or self-constructed explanations in the science classroom in terms of intelligibility, plausibility and wide-applicability. Stimulating students to explicate these metacognitive aspects helps to structure their ideas and to progress towards more sophisticated explanatory structures.

Although the problem-posing and cognitive apprenticeship approaches in this study might differ in some aspects from the original ideas (Collins et al., 1989; Klaassen, 1995), the basic functions of both do not. The problem-posing characteristic is that the search for intelligible and meaningful explanations for (sub)cellular events evokes a need to know more about the cells constituents. The cognitive apprenticeship characteristic is mirrored in promoting the explication, application and reflection on the general reasoning strategies that are useful in that search.

## 7.4. Research design

From identifying the problem that students hardly connect the cellular and molecular levels meaningfully, it was not immediately clear how to address this problem in terms of specifying educational objectives and an accompanying learning trajectory. An extensive theoretical reflection was needed to specify why molecular details are hardly used by pre-university students when they reason about biological phenomena. I used philosophical literature that aimed at characterizing the work of cell biologists and related disciplines to further specify the problem and find a direction for overcoming it. This was a fruitful scientific endeavour: an important theoretical outcome of this study is an articulation of the educational problem and a definition of a solution to this problem. Through designing, executing and studying a series of lessons, I provided a proof-of-principle and gained insight in promising learning and teaching activities.

### 7.4.1. Design-based research

The study can be characterized as the initial step in a design-based research approach (Lijnse, 1995; Van den Akker, Gravemeijer, McKenney & Nieveen, 2006). The domain-specific educational problem and the direction for overcoming it are thoroughly substantiated. The exploratory case study indicates that learning how cell activities can be explained mechanistically in terms of interacting molecules helps students to get grip on cellular complexity. The development and testing of this learning trajectory deepens our understanding of how molecular mechanistic reasoning can help students to get a grip on the complexity in the cell and what domain-specific knowledge could form a basis for intelligible mechanistic explanations of (sub)cellular behaviour. From the exploratory case study, I conclude that most of the activities in the design worked as intended and I use this as a proof-of-principle that shows that it is possible to guide students meaningfully through the multi-level mechanistic relationship between molecular interactions and cellular behaviour. However, many questions remain unanswered. For instance, the empirical part of the study provides little insight in the differences between students. Is it mainly a motivational component that explains differences in students' tendency to question the intelligibility and plausibility of provided and self-constructed explanations or do differences in cognitive and metacognitive abilities play a role as well?

## 7.5. Future research

### 7.5.1. The role of the teacher

A major question left open for research concerns the role of the teacher. In the test, I chose to be the teacher for reasons discussed in Paper III. Questions remain on how to convince and motivate teachers to include molecular mechanistic reasoning in their teaching. Teachers' conceptual and epistemological understanding will need attention, because promoting molecular mechanistic reasoning requires an integrated teaching approach in which general heuristics and reasoning strategies support the development of a domain-specific understanding of mechanisms in the cell. The section 'implications for educational theory and practice' provides some suggestions that might help to develop such a teaching approach.

### 7.5.2. Starting systems thinking from the molecular level

Another line of research could be to test the assumption that molecular mechanistic reasoning might form a good fundament for 'systems thinking' that also applies to systems at higher levels. Although reasoning about molecular systems is abstract, the basic events in these systems can be viewed as very concrete physical changes that can be characterized with the understandable causal terms 'colliding, binding and changing shape'. By using these terms to build a mechanistic explanation, there is no need to include teleological reasoning to explain events in the system. In general, it is more difficult to characterize higher-level events (or activities) with very concrete physical terms. This can be seen in Verhoeff's work, where he uses the characterization that systems 'exchange materials, energy and information' (Verhoeff, 2003). As I will elaborate in the next section, the question comes up what it means for a physical system to exchange information or energy. In my opinion, this always includes physical interactions between entities and it indicates that if changes in a system cannot be interpreted meaningfully as changes in the physical world there is a pitfall of relying on teleological or anthropomorphic reasoning. In this respect it should become clear to students that a focus on physical mechanisms to get a grip on complex systems only works in the domain of 'explaining the world as physical'. If mental events, such as human decisions and intentions, are part of an explanation (for instance, to clarify to how social systems work) this focus on physical mechanisms is not suitable[5]. Here lies a challenge for biology educators because both explanations are based on physical events and explanations that rely on mental of social events play a role. Further research could clarify how these types of events are intertwined in biology education and how a clear distinction can be provided to students.



5. See Klaassen (1995) for a reflection in the context of science education on Davidson's distinction between mental and physical event (i.e. Davidson, 1980).

### 7.5.3. The concept of energy and information in biology education

How students interpret the concept of 'exchange of energy' in the cell leaves many interesting questions. As can be see in the work of Verhoeff (2003), for example, students consider ATP as a physical appearance of 'a unit of energy' in the cell. It is produced by mitochondria and it is used by many other organelles, which allow them to function properly. This is analogous to the idea that many organisms use food as an energy source and that muscles, brains and other organs use glucose as an energy source. From the point of view of mechanistic reasoning, this seems to be unproblematic. It is taken for granted that activities of systems or systems components require energy. The energy is provided in the form of physical 'units of energy' and the 'consumption' of these energy units is prerequisite for the system or its components to display productive activities. However, this black-boxing treats the 'use' of energy as if it is separated from the actual activity, whereas in a mechanistic account energy conversions are an integral part of the mechanism and indispensable for understanding why one mechanisms can work while another one cannot. For instance, the mechanism of a combustion engine can only be explained causally if the changes that take place when the fuel ignites are integrated in the explanation. In principle, the same holds for ATP. It is as an entity that is physically integrated in all kinds of mechanisms in the cell and the hydrolysis of ATP into ADP and Pi is one of the causal changes within these mechanisms. In my opinion, rethinking the energy concept in biology education and its similarities and differences with the energy concept in physics and chemistry deserves much more attention. A mechanistic perspective might be valuable in disclosing these.

For the concept of information in biological systems, the mechanistic perspective might be valuable as well. One very prominent 'information-containing' unit in biology is the gene. As described already, I expect it to be of no surprise to students that information can be captured in a physical code and consequently a physical mechanism is needed to 'read' this code and 'translate' it into actions. Comparisons with devices such as a mechanical player piano (Duncan & Reiser, 2007) can be very useful in this respect. For some forms of information, it is more complicated to see the physical appearance of information. For instance, sound, light or other signals based on waves are more difficult to perceive. However, also in these cases, it will not come as a surprise to students that for this information to have an effect, there should be mechanisms that respond in a physical way to the signal. This means that the exchange of information between systems takes place via physical mechanisms and I suggest that students will perfectly understand this. Also, for reasoning about the exchange of information in and between systems, the mechanistic perspective can be useful. It helps to clarify that the exchange of information can in principle be explained in terms of physical mechanisms that respond to certain physical forms of information[6]. Further research on the use of 'information' in biology education could explore the potential and pitfalls of explaining the effect of 'information' in terms of concrete physical changes in biological mechanisms.



6. Note that the distinguishing between physical events and mental events that I mentioned in the previous section is extremely important when discussing the exchange of information. The mechanistic perspective I describe here refers only to physical events.

# 8. Implications for educational theory and practice

## 8.1. Fostering teachers' competency to promote (molecular) mechanistic reasoning

Most teachers in pre-university biology education are used to a curriculum that is structured along the lines of traditional biology domains. Consequently, the domains genetics, cell biology and molecular biology are taught as rather separate topics in traditional biology curricula. Furthermore, the (bio)chemistry and physics content relevant to this study is often only touched on in the biology classroom. Promoting molecular mechanistic reasoning offers a way to increase coherence between these domains. Question remains if teachers are sufficiently equipped to include molecular mechanistic reasoning in their teaching. From my theoretical analysis and empirical findings, I conclude that this mechanistic reasoning with proteins is within reach for students in pre-university education and that the conceptual understanding required is not too complicated. This means that including molecular mechanistic reasoning in teaching is more about familiarizing teachers with why and how to do it. The good news is that current teaching materials already contain many examples of molecular mechanisms that can serve for explicating molecular mechanistic reasoning. The general mechanistic view on proteins might be new to many teachers, but the cellular processes they have been teaching for years can be used to show the opportunities that the current curriculum offers to connect the molecular and cellular level via proteins. The citric acid cycle, the sodium-potassium pump, actin-myosin contraction and DNA replication are examples of cellular processes dealt with. In pre-service and in-service teacher training, teachers can be stimulated to explore for themselves how they would account for the events that are explicitly displayed in the textbook models of these processes and design classroom activities in which interpreting these models are central.

Limitations in teachers' epistemological background might need attention in teacher training as well. For instance, it appears of great importance in a molecular mechanistic reasoning-based educational approach that the teacher can judge and explicate the value and limitations of mechanistic models. After all, a reasonable explanation at one level evokes many new questions about underlying levels. Many teachers might feel uneasy because many of these new questions cannot be answered, not only because of limited knowledge of the students and the teacher but more importantly because no satisfactory scientific answers are currently available. But engaging students in scientific practice includes asking questions, constructing explanations, modelling, etc. The NGSS in the USA emphasize this. Consequently, this puts high demands on teachers' epistemological understanding. 'Providing the correct answers' will no longer be satisfactory and teachers should become less dependent on the textbooks. Less focus on 'providing the correct answer' also asks for a change in assessment strategies. However, it is very difficult to assess scientific practices such as asking relevant questions, formulating hypotheses, criticizing scientific models in a standardized way. Consequently, reproducing or reconstructing the 'correct answers' from the lessons and textbooks is still a major part of the assessment, especially in biology education (Momsen et al., 2010).

The NGSS aim at a shift from an overload of factual knowledge in the science curriculum to a focus on disciplinary core ideas. The same applies to the new biology curricula in the

Netherlands (Boersma, Kamp, Oever & Schalk, 2010). The implementation of a curriculum based on disciplinary core ideas, practices and cross-cutting concepts in textbooks and classroom practices is no sinecure. One aspect that will have great influence on the concrete educational practices will be the way that performance expectations, for instance as formulated in the NGSS, will be assessed. The main challenge for teachers will be how to balance 'providing the correct answers' and challenging students to reason, question, hypothesize, critique and discuss in a scientific way. The more educators and curriculum innovators appreciate and facilitate the latter and search for strategies to adequately assess these aspects of science education, the more these performance expectations will be fulfilled.

## 8.2. Timing the introduction of molecular mechanistic reasoning

Molecular mechanistic reasoning entails a way of thinking about cellular and subcellular phenomena. Obviously, some notion of cellular activities is necessary as a starting point for exploring mechanistic explanations for these activities. The students in the case study had considerable prior knowledge about cells. Identifying cell activities appeared to be a relatively easy task for them. However, an interesting question here is what basic notions about cells students need (or need to develop) to serve as a starting point for top-down and bottom-up reasoning in our strategy. I took very generally formulated cell activities, such as 'transporting, producing, dividing' as the starting point for exploring the question 'how do cells do this?' To get to this point, no detailed knowledge about cellular structures and processes was needed. What seems to be more important is what Verhoeff (2003) calls 'seeing the cell as a system' that needs to be able to fulfil certain functions in order to contribute to the larger system it is part of (e.g. the healthy body).

One could even argue that the introduction of organelles as functional units in the cell before familiarizing students with the molecular mechanistic reasoning approach complicates bridging the gap between the cellular and molecular levels. If we present the mitochondria to provide energy, the nucleus to regulate cell processes and the endoplasmic reticulum to take care of transport in the cell, we should not be surprised that students expect that, in general, cell activities are enabled by individual structural units without further questioning their interrelatedness and their relationship with the molecular constituents of the cell.

Janssen (1999) explored an educational approach towards cells and their function in the body. He describes the development and testing of a 'learning by designing approach' in which students 'design' the immune system. He shows that students (aged 16) can reason meaningfully about the activities that somehow should be carried out by components in the immune system to meet the 'design criteria' of the immune system as a whole. If students would be introduced to the cellular level as the organizational level at which (different types of) cells are the entities and cell activities are the 'things that these cells do', this would suffice as a starting point for exploring mechanistic explanations for these activities down to the level of molecular interactions. I would even suggest that, at least from viewpoint of coherence between organizational levels, organelles could better be introduced after familiarizing students with the molecular level via molecular mechanistic reasoning.

I am aware that this suggestion does not take into account that the molecular mechanistic reasoning approach requires chemistry and physics knowledge. Furthermore, the abstract and

dynamic nature of molecular mechanisms might be too demanding for students to master in grade 10. However, the chemical and physical details needed to understand the cause and effects of molecular interactions are not as abundant as might be expected at first glance. Students need to be familiar with the forces of attraction and repulsion between (groups of) atoms. The explanation of these principles in chemistry class approximately coincides with covering cell biology in biology class. Furthermore, the physical principles of Brownian motion and collisions between molecules are dealt with in physics and chemistry class early in the science curriculum. This study showed that with these basic principles a meaningful account for cause and effects of molecular interactions can be introduced, using the terms 'colliding, binding and changing shape'. In advanced courses, adding biochemistry and biophysics details will enable to refine the 'colliding, binding, changing shape' account. Another factor that might hinder the introduction of molecular mechanistic reasoning in an early stage in life science education is the abstract and dynamic nature of molecular mechanisms. This study provides strong indications that analysing visual representations (graphics, animations and simulations) in a mechanistic way helps students make the abstract molecular world more concrete. This is in line with other studies that stress the importance of visual literacy in getting a grip on abstract molecular and cellular processes (Gilbert, Reiner & Nakhleh, 2008; Rundgren & Tibell, 2010; Schönborn & Anderson, 2006)
To conclude, I expect that introducing the molecular mechanistic reasoning approach in an early stage of life science education will provide students with a valuable basis for the enduring and insightful exploration of the complexity of the cells' architecture and organization.

## 8.3. Wider applicability of mechanistic reasoning

### 8.3.1. Biology education

From the start of science education in primary school, discussing mechanistic models is a central activity. For instance, discussing the working of the human body in ever-greater detail when passing along the biology curriculum is essentially a progressive refinement of the mechanistic model that pupils start to build from their first thoughts about the human body. This is one of the reasons why promoting coherence is such a main effort in biology teaching. Almost every next step in learning biology builds on existing mechanistic models of how the living world works. Based on the theoretical framework presented in Paper II, I chose to build an educational approach in which mechanisms are explored with the explicit use of the term 'activity'. The term 'activity' was not only used to characterize events between the molecular and cellular level but also for descending from the organism level to cell activities. When testing the approach I noticed the educational potential of dividing a higher-level activity in underlying activities, as an alternative to first structurally decomposing the entities involved and then assigning functions to their parts. Consider, for example, the statement 'the lungs are for breathing'. Instead of decomposing the lung and discussing the working and role of all these parts, one could start subdividing the activity 'breathing'. For instance, breathing entails: air flows via the nose or mouth into the lungs, oxygen diffuses from the lung into the blood, penetrates a red blood cell and gets bound to a haemoglobin molecule. All these activities can be further explored mechanistically, by continuously asking the how-question. Although one might argue that this is not very different from the traditional approach of structurally decomposing the lung followed by discussing the role and function of all its parts, the difference is that by using an 'activity-

based explanatory context' students also identify activities that cannot easily be assigned to individual underlying components. For instance, in the example of 'breathing', the influx of air can best be accounted for by describing in mechanistic terms how intercostal muscles and thoracic diaphragm together enlarge the volume of the thoracic cavity. However, in turn, the respiratory centre in the brain is essential for this activity. This activity of the brain is part of 'breathing' as well, but will not automatically appear in a structural decomposition account. Another difference is that traditional structural decomposition does not take place in the context of 'explaining'. Students often have to reproduce names of parts without having to explain the role of the part in the whole or the way the structure of the part relates to its role in the whole. In a mechanistic account, these relations are central. In my opinion, this activity-based explanatory context helps to raise awareness of interrelatedness and emergence, which are central in understanding biological systems. Using this 'activity-based' approach for mechanistic reasoning will contribute to coherence in biology teaching.

This study also points towards the importance of stressing that many of the mechanisms discussed in biology education are to be understood in terms of changes in the physical world. Human capacities such as thoughts, feelings and intentions cannot be used to construct or interpret mechanistic models when these are meant to explain changes in the physical world. I suspect that this does not come as a surprise to students. Although many studies in the cognitive sciences show that people infer intentional and animate behaviour from specific patterns of moving objects (Scholl & Tremoulet, 2000), this does not imply that people conclude that the objects are really alive (in these studies people are looking at a screen with moving squares and circles). I would interpret students relying on intentional and anthropomorphic explanations as using an 'explanatory escape' because no further intelligible and plausible underlying mechanism is available. Therefore educators should be very consistent in the terms they use or approve when describing activities in biological mechanisms. Not only should they avoid activities that suggest a need to include human capacities to understand the activity, they should only use activities that are intelligible in terms of changes in the physical world. A few examples of activities that are unproblematic in my opinion are: growing (in the sense of becoming more), dividing (in the sense of splitting in pieces), contracting, transporting and capturing. More explicit reflection is needed on terms such as: sensing, responding, regulating. These terms can apply in a physical mechanism, but if used to characterize activities of biological systems, a tendency to interpret these activities as human-like capacities can be expected. Most problematic are activities that have no meaning in physical mechanisms such as knowing, feeling, wanting, deciding. In my opinion, these words should be avoided when discussing physical mechanisms in the biology classroom.

### 8.3.2. Science education

In this study, I scratch the surface of the opportunities that mechanistic reasoning in general and molecular mechanistic reasoning in particular offers to science education. The scope of this study is limited to reasoning about mechanisms in the domain of molecular cell biology but I think it offers new perspectives for science education in a broader sense. Constructing, interpreting and hypothesizing mechanistic explanations is a scientific endeavour that is not restricted to the domain of biology. Only a few properties of systems in the natural world are truly aggregative (Wimsatt, 2000), which means that for most natural phenomena we want to understand, providing an explanation entails describing a mechanism in which the organization

of and interactions between the parts are taken into account. As a result mechanistic explanations can be recognized in almost all domains of the natural sciences. This raises the question how these types of explanations are dealt with in the different disciples in science education. The educational potential I see in stimulating the explicit use of mechanistic reasoning in different scientific disciplines relates to what among others diSessa (1993), Klaassen (1995) and Brown (1993) advocate as making productive use of intuitive notions in science education. People make use of intuitive notions constantly to make sense of the world around them. A very basic notion is that 'changes are caused' and we interpret many of the changes we perceive as the effects of underlying mechanisms at work. This does not mean that there is nothing to be learned anymore. For many phenomena, the mechanisms at work are not readily accessible or understandable, for instance because the entities or activities involved are unfamiliar or difficult to perceive. The point is that the notion of 'explaining means searching for underlying mechanisms' can be evoked at the start of exploring underlying entities, activities and their organization that are yet unfamiliar, whether in the domain of biology, chemistry of physics. Students should be well aware that they are constructing an intelligible and plausible explanation for a phenomenon that has been taken for granted so far. This can serve as a clear content-related motive and a sense of direction for extending their knowledge.

In other words, science education is about the construction of meaningful explanations for phenomena in the natural world and therefore new entities or activities should only be introduced if these fit in an account for a certain phenomenon that makes sense to the students.

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# Paper I:
# Genomics education in practice: evaluation of a mobile lab design

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## Abstract

Dutch genomics research centers have developed the 'DNA labs on the road' to bridge the gap between modern genomics research practice and secondary-school curriculum in the Netherlands. These mobile DNA labs offer upper-secondary students the opportunity to experience genomics research through experiments with laboratory equipment that is not available in schools and place genomics research in a relevant societal context. The design of the DNA lab 'read the language of the tumor' is evaluated, by clarifying the goals and choices in the design, and the effects of the DNA lab are presented. Based on the analysis of the design of the DNA lab and supported by the results of the evaluating studies we consider this module to be a good example of relevant and up-to-date genomics education.

# 1. Introduction

Rapid advances in molecular biology increase the gap between research practice and school science. Implications of genomics research are rapidly finding their way to everyday practice. Major breakthroughs range from medicine to forensics, biofuels, vaccine research and the mitigation of pollution (NGI, 2006). These scientific advances each bring their own choices and dilemmas. To empower future citizens to deal with these personal and societal decisions science education based on relevant and up-to-date science is needed.

Many advances in molecular life sciences are not yet represented in science curricula (Moore, 2007; Verhoeff, Boerwinkel, & Waarlo, 2009). However, simply adding new content without rethinking the curriculum is not a viable strategy. In several countries new curricula concerning molecular life sciences have been proposed or introduced (Boerwinkel & Waarlo, 2009; Cohen & Yarden, 2009; Moore, 2007; Voet et al., 2003). Advances in genomics research have caused fundamental changes in the scientific view on the inner working of the living cell, while secondary school students still have problems grasping the basic concepts of DNA and proteins (Gericke & Hagberg, 2007; Lewis & Kattman, 2004; Marbach-Ad & Stavy, 2000).

Since 2006, an extracurricular development in the Netherlands aims to bridge the gap between school science and molecular biology research practice. The Dutch Genomics Centres of Excellence and the Centre for Society & Genomics, which are part of the Netherlands Genomics Initiative (NGI), developed the 'DNA labs on the road'. These mobile DNA labs offer students the opportunity to experience genomics research through experiments with laboratory equipment that is not available in schools. Five different 'DNA labs on the road' were developed in collaboration with Dutch universities. The four-hour educational modules for secondary-school students (ages 16-18) include an introductory lesson, a two-hour practical taught at school by university students and a final lesson. Teacher and student manuals have been developed for each lab and are made available in advance of the introductory lesson. The labs are offered free of charge to all secondary schools in the Netherlands since January 2006. Costs for equipment, transport and training the students are covered by the genomics research centers which are funded in part by the Netherlands Genomics Initiative (NGI: www.genomics.nl). Teachers can obtain additional information and register for the labs on the DNA lab website (www.DNAlabs.eu). The practical work of the lab takes two hours. Taking travel time into account, the university students can teach the lab to a maximum of two different classes within the same school in one day.

## 1.1. Goals of the 'DNA labs on the road'

The DNA labs are an important instrument for the genomics research centers in their communication with the general public. Furthermore, the DNA labs aim at improving and implementing of genomics education in Dutch upper-secondary education. The specific experiments performed in the DNA labs differ, but in each case the students perform hands-on laboratory activities such as DNA isolation, analyzing DNA using Polymerase Chain Reaction (PCR) and bioinformatics tools, in their own classroom. The 'DNA-labs on the road' thus offer genomics techniques in the classroom. More importantly, the DNA labs provide students a context in which new insights in genomics are used to solve everyday problems. The DNA labs deal with different contexts: producing bio-fuels, plant breeding, forensics and the use of

bioinformatics in crime scene investigations and improving the understanding and treatment of diseases such as Alzheimer's and cancer. These topics all rely on genomics research and they reflect the research activities of the genomics centers in the Netherlands. The DNA labs show that genomics research plays an important role in society and they encourage reflection on the personal and societal implications of genomics research. These different aspects are summarized in the goals of the DNA labs formulated at the start of the project.

The DNA labs aim at:

- Enhancing up-to-date genomics knowledge
- Improving the image and attitude towards genomics topics[1]
- Increasing the notion of societal implications of genomics research (place genomics in a societal context)
- Invoking enthusiasm and interest in genomics research

These goals have been the starting-point for designing the DNA labs. In each of the DNA labs, these general goals were further specified and translated into an instructional design. Several studies were performed to test whether the formulated goals had been reached.
In this article, we focus on just one of the DNA labs named 'read the language of the tumor'. This module on cancer research was developed by the Cancer Genomics Centre (CGC) in collaboration with the Freudenthal Institute for Science and Mathematics Education (FIsme) of Utrecht University.
The questions addressed in this paper are:
*1. How are the general goals of the DNA labs translated into an instructional design?*
*2. To what extent have the educational goals been reached by this design?*

1. In the original outreach plan the formulation 'improving the image of and attitude towards genomics topics' was used. In later stages this goal was specified as 'promoting informed opinions on genomics-related personal and societal issues.'

# 2. Materials and Methods

We analyzed the instructional design, the classroom practice, the results in student and teacher appreciation and learning outcomes, and the contribution both to the goals of the NGI and the innovation of biology education.

We made use of study of the scientific literature, analysis of the educational materials of the module and of the student-assistant training course, interviews with people involved in the project, classroom observations, focus group interviews, questionnaires and analysis of results of the assignments in the module.

Parallel to this study an evaluation of five of the DNA labs was performed regarding their quality, their learning outcomes and their effect on the attitude of the students toward genomics applications. This evaluation was based on questionnaires returned by 1824 students of which 436 performed the DNA lab 'read the language of the tumor' (Knippels, Van der Rijst, & Severiens, 2006).

Finally, a study on the effect of the DNA lab 'read the language of the tumor' on the attitude of the students towards biotechnology was published by Klop, Severiens, Knippels, van Mil, and Ten Dam (2010). This study was based on questionnaires filled out by 365 students who did not participate in any of the studies mentioned above. The results of these three studies are combined to come to an overview of the impact of the module.

# 3. Results

## 3.1. The translation of the general goals of the DNA labs into an instructional design

The general learning goal formulated for the DNA lab 'read the language of the tumor' is:

- After performing the DNA lab, students are able to explain what modern DNA research related to cancer entails and how this research is used.

To reach this goal three more specific learning goals have been determined:

- After performing the DNA lab, students know that cancer is 'a disease of the genes' and they are able to explain how one can minimize the risk of getting cancer.
- After performing the DNA lab, students are able to perform practical steps in DNA analysis (DNA isolation, PCR and gel-electrophoresis) and they can explain the purpose of each step.
- After performing the DNA lab students, are able to explain that DNA-research is important to improve diagnosis and treatment of cancer.

These more specific goals offer design criteria for the DNA lab. Other design criteria are derived from the context-concept approach, which is broadly accepted in science education innovation in the Netherlands (Boersma et al., 2005). This approach implies that students learn new concepts and practices in the societal or professional context in which these concepts are used. The advantage of such an approach is that students rapidly understand the value of this knowledge and can relate it to their personal experiences and/or what they observe in the media. Furthermore, they are thus taught concepts in a meaningful setting, which improves retention. A possible disadvantage is that students may find it difficult to apply the concepts learned in one context to another.

To translate these goals into an instructional design, choices were made regarding context, techniques, genes to be investigated and format of the lessons.

### 3.1.1. Context

The DNA lab 'read the language of the tumor' uses the context of a diagnostic DNA test on tumor tissue to determine the best treatment for a fictitious cancer patient. This patient is diagnosed with breast cancer and the physician asks for an analysis of the mutations in the DNA of the tumor. The students are asked to carry out this task and advise the physician on the optimal treatment for this particular patient.

This context was chosen because it illustrates the three different ways knowledge about specific gene mutations can be used: first to understand the genetic changes that lead to cancer, second, to properly diagnose tumors with different genetic make-ups and third, to design tailored treatment strategies based on the genetic make-up of a tumor. Through this context students learn that cancer is caused by multiple gene mutations, that cancer patients can be further diagnosed by gene analysis, and that knowledge about the specific genes that are mutated in a tumor and the biological effects such mutations have can provide a basis for personalized treatment.

### 3.1.2. Techniques

Within this context, steps in the diagnostic practice should include isolating DNA from tumor and normal cells, and comparing selected genes in tumor cells with those in normal cells.

Techniques to illustrate this practice were chosen with the following criteria in mind:

- *Authenticity*: techniques must be used in real practice
- *Comprehension*: techniques must be understood by students of the age of 16-18 years
- *Complexity*: techniques that can be performed by inexperienced students
- *Transportability*: techniques that can easily be transported to and set-up at schools
- *Time*: techniques that offer results within the time constraints of the module
- *Cost*: techniques that rely on equipment and materials that fit within the set budget
- *Safety*: techniques that rely on equipment and materials that can be safely used in a school environment

In authentic clinical practice, gene mutation analysis on tumor tissue is performed by sequencing the region of interest and using bioinformatics tools to detect mutations. However, using such sequencing techniques in a mobile DNA lab would be too complex, too time consuming and too expensive. Therefore, the techniques selected for the mobile lab are a simple version of DNA isolation, amplification by PCR and analysis by gel-electrophoresis on agarose gel. Due to technical and legal limitations, it was decided to have students isolate DNA from calf thymus to illustrate the principles of DNA isolation and to use plasmid based fragments to obtain the PCR-products that simulate the fictitious results we want the students to analyze. The PCR is performed with PCR tubes containing ready-to-go™ beads that include nucleotides and Taq polymerase. Students only have to add DNA and primers to the tubes and the small inserts used in these plasmids make it possible to use a very short PCR protocol that fits within two hour module.
The three techniques performed in the classroom are illustrated in Fig. 1.

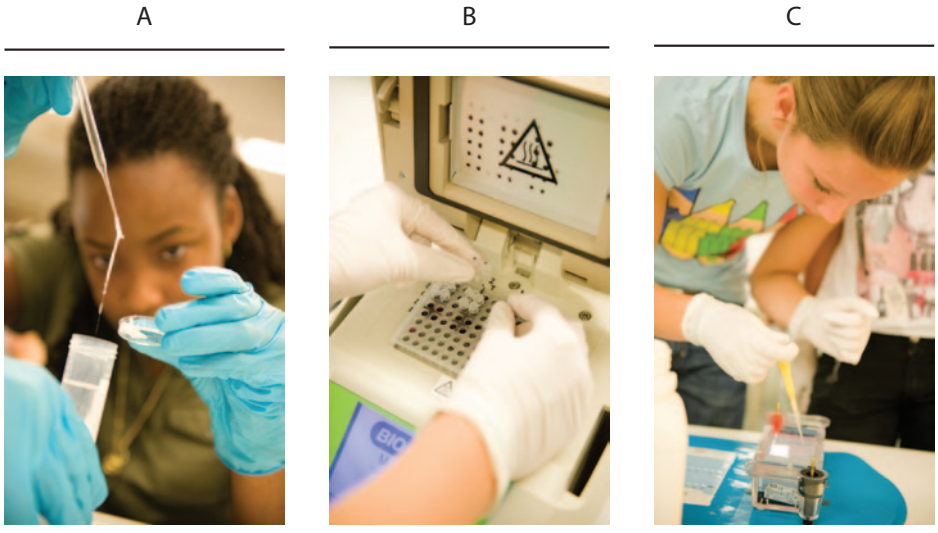


*Fig. 1 The three techniques in the practical: (A) student isolating DNA from calf thymus (B) PCR tubes placed in the thermocycler (C) student using a simple micropipette to load the PCR products on the agarose gel.*

### 3.1.3. Genes

Gene mutations were selected based on the following criteria:

- The mutated genes must represent different steps in the process from a normal cell to a tumor cell to demonstrate that cancer is caused by multiple mutations.
- The mutated genes must have implications for the choice of therapy to demonstrate that current therapies are based on specific mutations in tumor cells.
- The gene mutations must be diagnosable by gel electrophoresis following PCR-amplification.
- The function of the mutated genes must be comprehendible for students in upper-secondary education.

We chose a combination of three mutations that fulfill these criteria: a p53 deletion, HER2 amplification and a CDH1 truncation. Students identify mutations in these genes by comparing PCR fragments obtained from DNA from in healthy cells and tumor cells. An example of the result of the gel-electrophoresis is shown in Fig. 2.

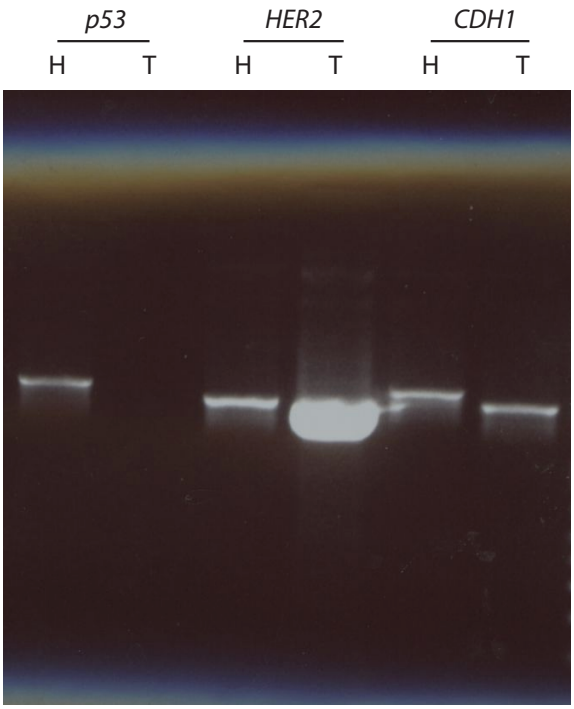


*Fig. 2: Differences in PCR fragments analyzed using gel-electrophoresis. H p53, HER2 and CDH1: represent the three genes in healthy cells. T p53, HER2 and CDH1: represent the three genes in tumor cells*

- A deletion of the p53 gene results in the absence of a p53 fragment in the tumor cell PCR product. Students can conclude that the absence of p53 will lead to the loss of apoptosis (programmed cell death), which is one of the characteristic features of cancer cells. Their conclusion concerning the treatment can be: the tumor cells are not able to destroy themselves, thus therapy must be aimed at removing or destroying cancer cells. Options are surgery, chemotherapy and radiotherapy.

- A HER2 gene amplification leads to an over-expression and thereby auto-activation of HER2 receptors at the plasma membrane. A HER2 gene amplification is illustrated by an increased quantity of HER2 PCR product from tumor cells compared to healthy cells. Students conclude that an amplification of this gene results in the presence of an excess of growth receptors causing the cell to be continually stimulated to divide. Students are told that screening for HER2 positive breast cancer is common clinical practice. They are asked to think of a treatment that will stop overstimulation ofHER2 positive cells. In our experience, almost every group of students comes up with the idea of blocking the receptor. This is exactly the mechanism by which Trastuzumab (Herceptin®) blocks growth of HER2 positive tumors. So the advice to the physician will be Trastuzumab treatment.

- Due to its role in cell junctions, truncation of the CDH1 (e-cadherin) gene may result in incorrect cell adhesion. From the differences in the size of the PCR fragments, students conclude that part of the e-cadherin protein is missing, which may lead to tumor cells not adhering correctly. Students hypothesize that this mutation increases the risk of metastasis and they advise the physician to check for secondary tumors and use chemotherapy as a treatment.

### 3.1.4. Format

The practical work is guided by university bachelor students that visit the school with the necessary equipment. Introductory and concluding lessons are taught by the teacher. In this way the teacher participates actively in the lessons, thereby linking the lab to regular biology education.

The aim of the introductory lesson is to activate prior knowledge about cancer and to relate known molecular concepts such as DNA, gene and protein to cancer. Students formulate their own questions about cancer, for example, 'is cancer age-dependent?' 'What is the role of heredity in cancer?' 'What is the difference between benign and malignant tumors?' During the module students try to find answers to these questions, thereby relating biological knowledge to real-world questions and problems.

The final lesson is used to look back at the results of the experiment and to stimulate the students to think of personal and societal implications of cancer genomics research.

## 3.2. Effects of the DNA lab 'read the language of the tumor'

The effect of the DNA lab can be specified in the implementation of the instructional design of the entire module, the outreach volume and appreciation of the lab, the learning outcomes and the effects on student attitude.

### 3.2.1. Implementation

Interviews with teachers indicate that not all teachers perform the introductory and final lesson. Reasons differ, from lack of time to the conviction that the students already have enough prior knowledge to carry out the practical. The exact percentage of teachers that perform these lessons is at present unknown. However, from the number of students that answered questions about these lessons in the questionnaires, it is estimated that from 325 students about 75% had an introductory lesson and about 64% were given a concluding lesson (Knippels et al., 2006). In some cases, only the results of the practical were discussed in the final lesson without further reflection on the personal and societal implications of cancer genomics research.

### 3.2.2. Outreach volume and appreciation

From the start of the project in September 2005 until June 2009, the five mobile labs reached 54000 students in 342 different schools, which means that 64% of the Dutch secondary schools were visited and about 35% of all students in upper-secondary biology education experienced one or more DNA labs during their school career. The DNA lab 'read the language of the tumor' reached 188 different schools and 17000 students during the same period.

From the point of view of the research institutes and universities involved, the 'DNA labs on the road' are a powerful outreach activity. After performing the DNA lab, 16% of the students indicate that they consider a study in the natural sciences as a result of having done the DNA lab 'read the language of the tumor' (Knippels et al., 2006).

In general, both teachers and students are very enthusiastic about the module and consider it relevant. They appreciate university students visiting the school and the possibility to work with modern equipment. Students find the practical instructive, interesting and fascinating and they consider the context of cancer research appealing and motivating (score 4.16 on a five-point Likert scale). These findings are confirmed in the evaluation of all five labs performed by Knippels et al. (2006). In this study only 10% of the 1824 students indicated that they did not like the DNA lab.

Teachers experience the DNA lab to be a good addition to the regular curriculum and most of them indicate that they want to continue using the 'DNA lab on the road'.

### 3.2.3. Learning outcomes

Analysis of the materials, classroom observations during the module and interviews with teachers shows that almost all the specific learning goals formulated are reached and that the module does indeed contribute to the students' knowledge of genomics. Results of the questionnaires show that after performing the DNA lab, students consider themselves capable of explaining the importance of DNA-research in hospitals in the context of cancer diagnosis (score 4.25 on a five-point Likert scale). When asked to complete the sentence 'the main message of this DNA lab is…' 80% of the students report '… how DNA and cancer research is performed'.

In interviews students indicate that their biggest learning experience resulted from studying the function of the three genes. The fact that different characteristics of tumors are caused by mutations in different genes and the idea that therapies can be tailored based on analysis of these mutations are a novel insight for secondary school students. After the module, students know that cancer is 'a disease of the genes' but not all students grasped the principle that multiple mutations are needed to turn a healthy cell into a tumor cell. In the questionnaire, about 30% of the students answer that one mutation in the DNA can cause cancer. The exact relation between DNA and cancer is difficult to describe for a lot of students. About 5% of the students state that: 'by analyzing a persons DNA you can see if there is a tumor in the body.' Also the relation 'gene-protein-function' appears to remain unclear to many students. Although this central dogma ought to be prior knowledge even before the introductory lesson, university students that teach the practical report that this concept is one of the most difficult elements in the module. These experiences correspond with studies in molecular biology and genetics education reporting that concepts at the molecular level, such as gene and protein, can be very difficult for students (Gericke & Hagberg, 2007; Lewis & Wood-Robinson, 2000; Marbach-Ad & Stavy, 2000). Relating these concepts to higher-level phenomena, such as cell division (on the cellular level) or cancer (on the organism level) appears also to be very difficult (Dreyfus & Jungwirth, 1989; Flores, 2003; Knippels, 2002; Verhoeff, Waarlo, & Boersma, 2008).

One of the goals of the DNA labs is to increase the notion of societal implications of genomics research. As mentioned before, students consider themselves capable of explaining the importance of DNA research in hospitals in the context of cancer diagnosis (score 4.25). However, an increased notion of societal implications of genomics research also implies better grounded views on ethical dilemmas and enhancement of opinion-forming skills to judge societal implication. In this respect, the study of Knippels et al. (2006) reveals that students report little enhancement of opinion forming skills and better grounded views on ethical dilemmas. Analysis of the instructional design of the module shows that no specific classroom activities are incorporated in the module to enhance opinion forming skills and reflection on ethical dilemmas. Improvements can be made to meet these goals.

### 3.2.4. Effects on attitude

The module stimulates a positive attitude towards DNA-research (see also Klop et al., 2010). All students indicate that they are positive about DNA research. Not surprisingly, 80% of the students report improved diagnosis and treatment of diseases, mainly cancer, as a reason for this opinion.

# 4. Conclusion and discussion

Our results show that most goals of the DNA lab are reached, namely: enhancing up-to-date genomics knowledge; improving the image and attitude towards genomics topics; increasing the notion of societal implications of genomics research and invoking enthusiasm and interest in genomics research.

However, some points for improvement remain. More attention should be paid to students' opinion forming on personal and societal implications of genomics research. This is in line with previous research (Klop et al., 2010). In new versions of the DNA labs, specific lessons on opinion forming have been developed and are now being tested.

Also, the coherence between different biological concepts and biological levels of organization could be made more explicit. This has been attended to in the new versions by including a framework in which students can categorize concepts, relations and questions (Verhoeff et al., 2009). This framework includes the levels of biological organization and has the function of an 'advanced organizer' in the learning process (Ausubel, 1968). The framework is in line with the current pedagogical approach in biology education in the Netherlands, i.e. the use of systems thinking, and learning concepts in the context in which they are used.

Another finding is that the importance of the introductory lesson and final lesson is being underrated by teachers. The study of Knippels et al. (2006) shows that this is the case for all five DNA labs. Teachers indicate that they wish to have more background on current genomics research and support on the content and didactics of the module. To meet this need and to ensure the correct implementation of the introductory and final lessons, a teacher training course was developed in which these aspects are combined. This one-day course is offered twice a year at Utrecht University.

The practical work in the DNA lab 'read the language of the tumor' is guided by third-year bachelor students (age 20-22) of the biomedical sciences program at Utrecht University. Although the results show that teachers and students highly appreciate the way university students teach the lab, some improvements were made in the students' training program. Their training and participation in the DNA lab is now embedded in an optional 10-week (2 days/week) university course on science communication offered by the Cancer Genomics Centre, of which two weeks are used for training and reflection and eight for teaching the lab in schools. In this way the DNA lab offers students a unique training and practical experience in science communication within their university curriculum.

In summary, the context of cancer research is very much appreciated by the students and teachers. Students can perform and understand the techniques and the materials and equipment are interesting and appealing to them. Most of the cognitive and affective goals are reached and improvements have been made to optimize the module. Thus we wish to conclude that the DNA lab 'read the language of the tumor' is a good example of relevant and up-to-date genomics education. Initiatives with similar goals have recently started in Europe. For instance, in France the Strasbourg University PhD School Life and Health Sciences launched an initiative called 'OpenLab' (http://www-ed-sdvs.u-strasbg.fr/openlab/). The European Molecular Biology Laboratory offers in-house training for secondary school teachers called 'the European Learning Laboratory for the Life Sciences' (ELLS) (http://www.embl.de/training/scienceforschools/). We hope that these initiatives can inspire other research institutes, didactics experts and teachers to cooperate in designing relevant and up-to-date genomics education.

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# Paper II:
# Modelling molecular mechanisms: a framework of scientific reasoning to construct molecular-level explanations for cellular behaviour

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## Abstract

Although molecular-level details are part of the upper-secondary biology curriculum in most countries, many studies report that students fail to connect molecular knowledge to phenomena at the level of cells, organs and organisms. Recent studies suggest that students lack a framework to reason about complex systems to make this connection. In this paper, we present a framework that could help students to reason back and forth between cells and molecules. It represents both the general type of explanation in molecular biology and the research strategies scientists use to find these explanations. We base this framework on recent work in the philosophy of science that characterizes explanations in molecular biology as mechanistic explanations. Mechanistic explanations describe a phenomenon in terms of the entities involved, the activities displayed and the way these entities and activities are organized. We conclude that to describe cellular phenomena scientists use entities and activities at multiple levels between cells and molecules. In molecular biological research, scientists use heuristics based on these intermediate levels to construct mechanistic explanations. They subdivide a cellular activity into hypothetical lower-level activities (top-down approaches) and they predict and test the organization of macromolecules into functional modules that play a role in higher-level activities (bottom-up approaches). We suggest including molecular mechanistic reasoning in biology education and we identify criteria for designing such education. Education using molecular mechanistic reasoning can build on common intuitive reasoning about mechanisms. The heuristics that scientists use can help students to apply this intuitive notion to the levels in between molecules and cells.

# 1. Introduction

Many biological disciplines have extended their scope towards the molecular level. This 'molecularization' of biology (Kay, 1996; Morange, 1998, p. 172) adds molecular-level details to phenomena that traditionally have been studied only at higher levels, such as embryology, neurobiology and evolution. Our understanding of many biological phenomena that until recently could be described only at the level of the population, whole organisms or cells is now revolutionized with new insights at the macromolecular level (Moore, 1993).

In upper-secondary biology education, this macromolecular level is part of the curriculum. Students learn the structure and properties of DNA, RNA and proteins and they are taught how DNA codes for RNA and proteins. However, according to (Duncan & Reiser, 2007) they are not explicitly taught how this knowledge can be used when explaining phenomena at the level of the cell or higher levels of biological organization.

For instance, in genetics education, it appears that the molecular details of DNA and proteins add very little to students' understanding of genetic phenomena. Lewis and Kattman (2004) report that the majority of British students in their study (age 14–16) state that genes are important for the determination of characteristics. However, most students did not appear to hold any coherent understanding of the biological mechanisms by which this might be achieved. They report that students treat genes as small particles containing a trait or characteristic and they discuss the implications for education when students take this notion with them in further study (Lewis & Kattman, 2004). Marbach-Ad and Stavy (2000) report similar findings. In their study, many students used concepts and terms from the molecular level such as gene and DNA, but they were unable to explain mechanisms and intermediate stages that link genes to the biological phenomena they are involved in. Furthermore, less than half of the 12th graders in their study were able to explain the function of RNA. These and many other studies (e.g. Duncan & Reiser, 2007; Venville & Treagust, 1998) show that students have difficulty understanding how genes determine traits, even after they have been taught how genes code for proteins via RNA. One of the problems in upper-secondary genetics education is that the message about gene function appears to be twofold: classical genetics education emphasizes that genes determine hereditary traits, whereas in molecular genetics education the message is that genes code for proteins. Although these two accounts of gene function are related, it appears to be very difficult for students to combine the two messages into one overall framework that makes gene function intelligible (see also Lewis & Kattman, 2004). Duncan and Tseng (2011) stress that to understand the relationship between genotype and phenotype it is critical to understand that the genetic code does not directly specify observable effects, but that these effects are driven by interactions at lower organizational levels. Current genetics education, being focused mainly on memorization of terms and processes (AAAS, 2005; Duncan & Reiser, 2007; Verhoeff, Boerwinkel, & Waarlo, 2009), fails to connect the molecular level to higher-level phenomena. Students in undergraduate life sciences curricula encounter similar problems (Duncan, 2007).

To link genes with traits, at least three distinct 'how' questions are needed. To understand 'how genes determine traits', one needs to understand 'how genes code for proteins', 'how proteins are involved in cellular processes' and 'how cellular processes contribute to phenomena at higher levels of biological organization'. Not all of these 'how' questions are unfamiliar to students in upper-secondary education. The first 'how' question is answered by the central dogma in molecular genetics, i.e. that genes code for proteins via the processes of transcription

and translation. Several teaching strategies have been developed to teach this central dogma, and it is a standard part of upper-secondary biology curricula in the Netherlands and many other countries. The third 'how' question concerns the ability to connect different levels of organization in biology. The importance of the notion of organizational levels has been stressed by many authors (e.g. Duncan, 2007; Knippels, 2002; Rappoport & Ashkenazi, 2008; Verhoeff, Waarlo, & Boersma, 2008; Wilensky & Resnick, 1999), and educational strategies such as the 'yo-yo strategy' by Knippels (2002) have been developed to enhance students' ability to connect different levels of organization. The second 'how' question also concerns connecting levels, in this case connecting the cellular level to the molecular level. However, the relationship between molecules and cells is not straightforward. Cellular processes can hardly ever be explained by the action of single genes and proteins; rather, the concerted action of many macromolecules brings about an effect at the cellular level (Boogerd, Bruggeman, Hofmeyr, & Westerhoff, 2007). The studies of Marbach-Ad and Stavy (2000) and Duncan and Reiser (2007) have provided strong indications as to why detailed information about genes and proteins does not automatically help students to explain how genes function: students consider the macromolecular level in the cell as a collection of very complex chemicals (Dreyfus & Jungwirth, 1990), but they might miss what Morange (2008) calls the 'molecular vision'. This vision entails the idea that behaviour of the living cell emerges from the orchestrated functioning of macromolecules (Morange, 2008). As (Dupré, 2009, p. 43) puts it: *'One cannot infer the behaviour of a cell by treating it as a bag of chemicals...'*. Discrete biological function can only rarely be attributed to an individual gene product, in the same sense that the main function of haemoglobin is to transport gas molecules in the bloodstream (Hartwell & Hopfield, 1999). Most biological functions arise from interactions of many components; they are system-level properties instead of properties of individual gene products. (Duncan & Tseng, 2011) indeed identify that students lack a robust understanding of the functioning of proteins as parts in complex systems when reasoning about genetic phenomena. They stress the need for a framework for reasoning about complex systems that can help students to understand how cellular phenomena can emerge from the interactions of molecules in general and proteins in particular.

# 2. Aim and approach of the study

Molecular and cell biologists study the behaviour of macromolecules within the context of a living cell and they try to discover the relationships between these levels of biological organization. Mental models and heuristics that experts use can be informative for designing education (Glaser, 1999). Therefore, we suggest that if we characterize more precisely what these scientists present as explanations and how they construct these explanations, this might help educators to better design education that links molecular interactions to cellular processes. In this study, we thus seek to formulate educational design criteria based on the analysis of the goals and strategies in molecular and cell biology research.

The research questions in this study are:

1. *What characterizes scientific explanations that aim at understanding cellular processes in terms of molecular interactions?*
2. *Which heuristics are used to construct these explanations?*
3. *What educational design criteria can be derived from the analysis of these scientific explanations and heuristics?*

We present a literature review on the philosophical foundations of molecular biology and the closely related fields of molecular cell biology and molecular systems biology. The philosophy of science is concerned with both the nature of scientific explanations and the strategies scientists use to construct these explanations. Based on the philosophy of molecular biology, we propose a framework representing the characteristics of molecular explanations of cellular processes. Heuristics used to construct these explanations can also be represented in this framework. Research on the process of bacterial chemotaxis will serve as an example to show that our findings reflect scientific practice and that historical and contemporary scientific explanations of chemotaxis fit in our framework. The framework aims to provide criteria for the design of educational activities that help students to connect cellular-level phenomena to the molecules that constitute the cell.

# 3. Explanations and heuristics in molecular and cell biology research

## 3.1. Explanations in biology

Characterizing scientific explanations has been focus of philosophical attention and debate throughout history. Woodward (2010) provides an overview of models of scientific explanations and the historical developments starting from the deductive–nomological (D-N) model (Hempel & Oppenheim, 1948) that shows how laws of nature play a central role in scientific explanations. The D-N model has received criticism, and different accounts of scientific explanations have been developed since then. Attempts to unify these different accounts of explanations over different scientific disciplines raised the debate in the philosophy of biology about the status of biology as an autonomous discipline (Mayr, 1996). One of the issues still the subject of a lively debate is the question of whether biological explanations can be reduced to explanations in chemistry and/or physics (see, for instance, Dupré, 2009; Fox Keller, 2009). Both Fox Keller and Dupré claim to be materialists. Fox Keller (2009, p. 21) states that *'as a materialist I am committed to the position that all biological phenomena, including evolution, require nothing more than the working of physics and chemistry'*, and Dupré formulates his standpoint as: *'I do not believe there is any kind of stuff in the world other than the stuff described by physics and chemistry'* (Dupré, 2009, p. 33). We subscribe to this materialist view. However, this view does not imply that all biological explanations can be replaced by the type of explanations used in chemistry and/or physics. Both Fox Keller and Dupré deny that all biological explanations could be derived from the theories or laws of physics or chemistry. This issue of theory reduction has been subject to debate in the philosophy of science for many years, and the dominant view within the philosophy of biology is that no such derivation is possible (Dupré, 2009). To clarify the focus of our study, we will give a brief overview of different types of biological explanation by characterizing the types of questions biologists try to answer. Then we can specify what type of questions molecular and cell biologists try to answer as a starting point to characterize the type of explanations that connect cellular and molecular-level phenomena.

Mayr (1961) first made the crucial distinction between evolutionary (or ultimate) and functional/developmental (or proximate) explanations, as answers to 'Why?' and 'How?' questions. The functional biologist *'is vitally concerned with the operation and interaction of structural elements. His ever-repeated question is "How?" How does something operate, how does it function?'* (Mayr, 1961, p. 134). Mayr uses the question-word 'why?' to refer to evolutionary explanations in biology. 'Why' in this case is a synonym for 'How did the phenomenon come to be, in the light of evolution?' Ariew (2003) reconsiders Mayr's 'ultimate/proximate' distinction and he specifies more precisely the different types of questions that 'evolutionary' and 'proximate' explanations provide answers to. According to Ariew, evolutionary explanations answer questions about the prevalence and maintenance of traits in the population such as 'Why is something prevalent?' and 'Why will something continue to persist?' Proximate explanations answer causal questions about developmental and physiological processes. They are causal as they issue in functional analyses of a system's causal capacities (including developmental analyses) whereby the function of a trait is its causal capacity. Proximate explanations thus answer questions such as 'How does something get built?' and 'How does something operate?' What Ariew calls the functional analyses of a system's causal capacities (how does something operate?) is in line with Cummins' characterization of

functional analysis: *'To ascribe a function to something is to ascribe a capacity to it which is singled out by its role in an analysis of some capacity of a containing system'* (Cummins, 1975). Note that in addition to functional analyses Ariew includes developmental analyses (how does something get built?) in his account of proximate cause. According to Ariew, functional and developmental explanations together form a category of individual-level causal explanations that answer questions about an organism over its lifetime. This contrasts evolutionary explanations that answer population-level questions about the diversity of life over many generations.

## 3.2. Mechanistic explanations in molecular and cell biology

In the introduction, we concluded that explanations of systemic properties at the cellular level in terms of the behaviour of the molecular component within that system are effectively missing in current biology education. These types of explanations are typically proximate explanations; they try to answer the question 'how does the system operate?' These are causal explanations that are constructed from functional analyses of the causal roles of proteins as parts in complex systems (Boogerd, Bruggeman, Richardson, Stephan, & Westerhoff, 2005; Craver, 2001; Cummins, 1975). Boogerd et al. (2005) conclude that explanations of systemic properties are typically mechanistic explanations. In their analysis of the upcoming field of systems biology, Boogerd et al. (2007) conclude that the search for explanations for systemic properties can be characterized as the attempt to construct mechanistic models of the behaviour of the system. Darden (2006) shows that contemporary biologists indeed often seek to discover mechanisms, and Bechtel and Abrahamsen (2005) also state that the term biologists most frequently invoke in explanatory contexts is 'mechanism'. Both Bechtel and Abrahamsen (2005) and Machamer, Darden and Craver, abbreviated as MDC (Machamer, Darden, & Craver, 2000), argue that this concept of 'mechanism' is central for the philosophical understanding of the biological sciences. The importance of mechanisms in biology and molecular biology in particular was stressed by several authors in the early days of molecular biology (Bechtel & Richardson, 1993; Brandon, 1984; Burian, 1996; Crick, 1988; Kauffman, 1970; Wimsatt, 1972), but it has only received serious attention in the philosophy of science since MDC's publication (Machamer et al., 2000). Since then, the amount of literature on what is called 'the new mechanistic perspective' (Darden, 2007) is rapidly growing. Mechanistic explanations play a central role not only in molecular biology (see Darden & Craver, 2002) and molecular systems biology (Boogerd et al., 2007) but also in all other 'molecularized' disciplines such as cell biology (Bechtel, 2006), immunology, neurobiology (Craver, 2002), developmental biology and evolution (Skipper & Millstein, 2005). Darden (2008) emphasizes that the work on mechanisms in philosophy of biology did not originate as a response to past work in philosophy in science but from considerations of the work of biologists themselves, especially in molecular biology, biochemistry and cell biology. She confirms that the philosophical work on mechanisms reflects the research goals and activities that are recognizable in the way biologists work and communicate about their work. Several authors have used historical reconstructions to show that indeed the scientific discourse in molecular biology and related fields can be characterized as a search for mechanisms. Gilbert and Mulkay (1984) reconstructed the quest for the molecular mechanism responsible for ATP synthesis based on interviews with the scientists involved. Their work clearly shows that alternative models of possible mechanisms coexisted and that experimental results contributed

to the refinement of one model while weakening the plausibility of others. Darden and Craver (2002) applied the MDC framework (Machamer et al., 2000) to the case of the discovery of the mechanism of protein synthesis, and Craver (2002) describes the experimental strategies to construct multi-level mechanisms in the neuroscience of memory.

When looking at review articles in molecular and cell biology, the central role for mechanistic models is easily recognizable. In many articles, models of mechanisms are summarized in graphical representation (Bechtel & Abrahamsen, 2005). An example of such a representation can be found in Fig. 1. It is adapted from a review article (Baker, Wolanin, & Stock, 2006) that summarizes the current state of knowledge of the process of bacterial chemotaxis. The figure shows a simplified representation of the mechanistic model, which explains how bacteria can change the direction of their movement depending on the availability of nutrients. Scientists have been working on the mechanistic explanation for this process since the early 1960s and the model is still being refined.

The example of chemotaxis shows the role of the mechanistic model in molecular biology research: the mechanistic model aims to explain the phenomenon by describing how the orchestrated activities of its components bring about the phenomenon (Bechtel & Abrahamsen, 2005; Glennan, 2002; Machamer et al., 2000).

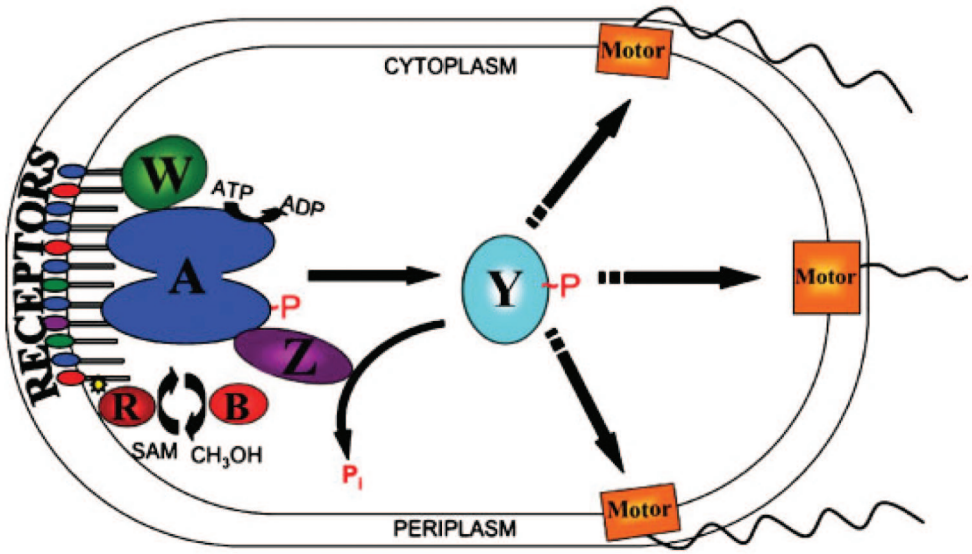


*Fig. 1 Model of the mechanism of bacterial chemotaxis as presented in Baker et al. (2006). After binding attractant or repellent molecules, the receptors activate intracellular proteins that mediate the level of phosphorylated CheY protein (depicted as Y-p). High concentrations of phosphorylated CheY change the rotation of the molecular motor that drives the flagella. This causes the bacterium to start tumbling instead of swimming smoothly, thereby changing its orientation.*

## 3.3. Structure of molecular mechanistic explanations

Having said that mechanistic explanations play a central role in molecular biology, we will first elucidate the definition of mechanism used in this paper. Then we will show how mechanistic explanations connect phenomena at the cellular level with the molecules involved in the phenomenon. The definition of a mechanistic explanation used in this paper is based mainly on the work of Machamer et al. (2000). In their publication, they describe mechanisms as follows: *'Mechanisms are composed of both entities (with their properties) and activities. Activities are the producers of change. Entities are the things that engage in activities. The organization of these entities and activities determines the ways in which they produce the phenomenon.'* Glennan (2002) and Tabery (2004) emphasize that activities only take place due to the interaction of entities. We include the term interaction in our account of mechanism to emphasize that an entity can have the capacity to display a certain activity but that the actual activity only takes place when the entity interacts with another entity with the appropriate properties. At the molecular level, the entities typically engaging in mechanisms are the gene product (proteins, RNAs) interacting with each other, with DNA and with all sorts of small metabolites. Molecular activities are typically ascribed to gene products. For instance, if a protein catalyses the hydrolysis of ATP, ATPase activity is assigned to this protein. An inventory and categorization of activities of gene products can be found in the Gene Ontology database (Ashburner et al., 2000). General activities ascribed to gene products in this database are for example: catalytic activity, binding activity, transporter activity and enzyme regulator activity (www.geneontology.org).

Machamer et al. (2000) stress that *'Entities often must be appropriately located, structured, and oriented, and the activities in which they engage must have a temporal order, rate, and duration.'* Bechtel and Abrahamsen (2005) also emphasize the central role of organization in the mechanisms. Organization of entities and activities specifies when and where the entities and activities are present. Darden (2008) specifies in more detail the organizational features of mechanisms, based on the case of molecular biology. Table 1 shows the features of temporal organization (when) and spatial organization (where) that Darden specifies. Organization can only be assigned to the entities and activities when they are part of the mechanism as a whole. In molecular biology research, hypotheses about the organization of entities and activities are formulated and tested, sometimes even without knowing the exact properties of the entities and activities involved. We therefore explicitly include the term 'temporal and spatial organization' of entities and activities in our description of mechanistic explanations.

*Table 1 Organizational features of entities and activities in mechanistic explanations (adapted from Darden, 2008)*

| Spatial arrangement of entities and/or activities | | Example in cell biology |
|---|---|---|
| Localization | Where are the entity and/or activity located? | Receptor proteins are located at the plasma membrane |
| Structure | Are entities grouped into physical structures? | Histone proteins are grouped into nucleosomes |
| Orientation | How are entities positioned relative to other entities? | α- and ß-Tubulin heterodimers are positioned plus end to minus end in microtubules |
| Connectivity | What entities and/or activities are connected? | G-protein-coupled receptors connect to G-proteins when activated |
| Compartmentalization | Are entities and activities located in compartments? | ATP synthase enzymes are located in mitochondria |
| **Temporal aspects of entities and/or activities** | | **Example in cell biology** |
| Order | In what sequence are activities and entities present? | Ribosomes cannot assemble until the small subunit binds a mRNA molecule |
| Rate | At what rate or speed do activities take place? | Conversion rate of ADP into ATP by ATP synthase enzymes depends on the concentration of protons |
| Duration | How long are activities and/or entities present? | In a signalling cascade downstream signals depend on the duration of receptor stimulation |
| Frequency | Are activities and entities present at a certain frequency? | The frequency in the activity of cyclin-dependent kinases depends on the presence of different cyclin proteins during the cell cycle |

Mechanisms are active and productive (Bogen, 2008), i.e. the interactions of entities lead to a series of activities because they change the properties or the organization of entities, thereby causing new activities. The overall activity of the mechanism can be defined as the overall change between setup and termination conditions. The use of setup and termination conditions when describing biological mechanisms is a simplification, as many biological mechanisms have a cyclic nature and most mechanisms are part of larger dynamic systems that continuously adapt to changes. For instance, in the case of chemotaxis, the mechanism will never 'start' or 'finish'. The chemotaxis mechanism is continuously active, even in the absence of attractant or repellent chemicals. Chemical interactions with the receptors just lead to a different productivity, i.e. the motor starts rotating the other way around.

Craver (2001) emphasizes the multi-level nature of biological mechanisms. He shows that multi-level mechanisms can be presented in terms of hierarchically organized entities and activities. An entity that displays a certain activity at level L consists of lower-level entities that display certain activities at level L−1. At the same time, the entity at level L is part of a higher-level entity at level L+1 that displays its activity because of the organized activities of the entities at

level L. Richardson and Stephan (2007) also stress that mechanistic explanations connect entities at different levels of biological organization, and that the hierarchical ordering of entities is typically in terms of part/whole relationships.

In Fig. 2, we present the general account of mechanistic explanations we will use in this paper. We adopt the MDC approach (Machamer et al., 2000) of hierarchically organized entities and activities to describe multi-level mechanisms, with the following additions. In our model, we explicitly separate organization from the entities and activities. We also separate interactions from activities, although the two are causally linked. Furthermore, we emphasize that activities produce change in the organization and properties of entities, thereby causing the productive continuity that constitutes the higher-level activity.

In biology in particular, most mechanistic explanations span multiple levels. What entities and activities are studied at what level of organization depends on the research goals and varies among biological disciplines. In molecular biology, explanations typically describe the activities and organization of molecules in the cell to explain certain activities in the cell. Activities at the cellular level are often described with the cell as the subject; for example, the cell divides, the cell metabolizes glucose, the cell moves. Activities at the molecular level, for example protein activities, are typically formulated with a molecule as the subject; for example, the receptor binds a hormone or the enzyme hydrolyses ATP. Protein activity starts with the protein binding one or more other molecules. This causes the protein to change conformation, thereby allowing new intra- or intermolecular chemical bonding. The subsequent chain of breaking and forming of chemical bonds in and between the molecules ends in a relatively stable state in which the protein, its binding partner(s) or both have undergone molecular changes. The difference between the condition before interaction and the relatively stable condition after interaction is what is called the molecular activity. In the case of enzyme activity, the substrate is changed while the enzyme typically returns to its original conformation, whereas in other protein activities, such as binding of a ligand to a receptor, the protein remains in a changed conformation, as long as its binding partner stays bound. In either case, the changes caused by one molecular activity are prerequisite for subsequent activities within the mechanism; in other words, the termination conditions of one activity form the starting condition for the next. Alberts' metaphor (Alberts, 1998) of viewing a cell as a factory that contains a network of interlocking assembly lines in which protein machines do the job stems from this mechanistic view on protein function. Molecular biologists try to find molecular-level explanations for cellular activities, but hardly any cellular activity can be explained by the activity of a singular protein or other molecule. Most cellular activities arise from interactions among many components. Hartwell and Hopfield (1999) introduce the term 'functional module' as a critical level of organization in between cells and molecules. These modules have discrete functions in the cell that arise from the interactions among their molecular components (proteins, DNA, RNA and small molecules). By definition, a functional module is a molecular ensemble whose function is separable from those of other modules. It is constituted from a fraction of the cell components that together form a discrete functional entity. As examples of such functional entities, Hartwell and Hopfield (1999) presents ribosomes as well as signal transduction cascades. In the case of ribosomes, the functional module is a structural unit, spatially separable from other cell components, while in the case of a signal transduction cascade, the module is not a fixed structure but a highly dynamic, transient interplay between a set of proteins. Although the cell contains many structural elements, varying from protein complexes of a few proteins to huge organelles composed of thousands of different

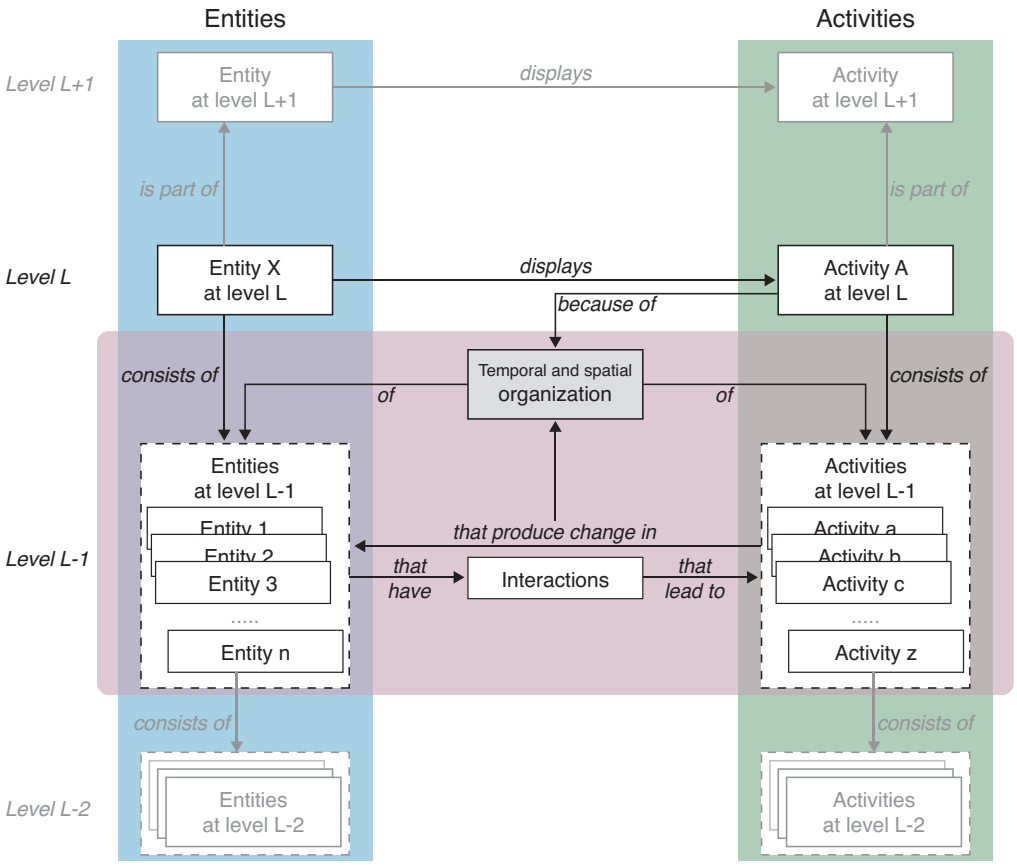


*Fig. 2 General structure of multi-level mechanistic explanations that consist of hierarchically organized entities and activities.*

molecules, Hartwell and Hopfield (1999) stress that what is considered a functional module does not depend on the size, structure or complexity of the molecular ensemble. A group of cell components, either being proteins, protein complexes or (parts of) organelles, can be considered a functional module when the components work together to accomplish a relatively autonomous function in the cell. Assigning modular activities is thus a form of functional analysis (Cummins, 1975) of the cell's components. Some modules, such as ribosomes, have a relatively stable organization during function, while others, like signal transduction pathways, are highly dynamic and transient molecular ensembles, not detectable as a structural unit in the cell. In line with the framework of mechanistic explanations presented in Fig. 2, functional modules can be considered as entities even though many modules are not stable structures. Here the term 'entity' refers to the molecular ensemble as a whole, to which a certain modular activity can be assigned. To characterize modules as 'entities' displaying specific 'modular activities' makes it possible identify organizational levels in between cells and molecules based on the function of groups of cell components. These functional levels offer an alternative to the structural subdivision into organelles traditionally used in upper-secondary cell biology text books.

In the case of bacterial chemotaxis, this functional analysis would lead to the following reasoning: if the bacterial cell moves towards more favourable locations, then there must be a group of molecules in the cell that together constitute a functional module that moves the cell towards more favourable locations. This is what is depicted in Fig. 1 as the chemotaxis system. Note that in this figure the components of chemotaxis are not all depicted in terms of individual molecules. What is assigned the 'motor' is in itself a collection of many molecules that together display motor activity. Here we see that the overall entity (the chemotaxis system or module) is functionally dissected into smaller modules. In the case of chemotaxis, the overall system constitutes a sensor module, a signalling module and a motor module. We use the term submodules (Hofmann, Spahn, Heinrich, & Heinemann, 2006) to stress that modules in the cell can be functionally subdivided into smaller modules down to the level of the macromolecules. Scientists often use these intermediate levels between cells and molecules to describe what kind of activities take place in the cell. For example, the terms 'transcription' and 'DNA replication' refer to modular activities, and 'transcription machinery' (Orphanides & Reinberg, 2002) and 'replication machinery' (Alberts, 2003) refer to the functional modules that carry out these functions.

If we apply this account of cellular, modular and submodular, and molecular levels to our framework of multi-level mechanisms, this results in a schema (Fig. 3) that explains the connections between the entities, activities and organization at the different levels from cells to molecules. In Fig. 4, we present the same figure elaborated for bacterial chemotaxis. Note that we have included only molecular interactions in the figures and have left out modular and submodular interactions. Although modules have interactions in the sense that the output of one module serves as input for another module, these inputs and outputs are themselves molecular interactions that can be described in terms of the molecules of one module that interact with molecules of the other. In the case of chemotaxis for instance, the receptor molecules in the sensor module interact with the CheA protein in the signalling module, and the CheY protein in the signalling module interacts with a protein in the motor module (Baker et al., 2006).

Note that Fig. 1 (cartoon-like) and Fig. 4 (multi-level) both present in a simplified way the current state of knowledge of molecular explanations for bacterial chemotaxis behaviour. For many cellular processes, only partial molecular explanations are available. Some activities can be specified in terms of molecular activities, while others can be described only in functional terms that specify higher-level activities. In other cases, the involvement of molecules in a process is known but the connection to other molecules and the positioning in the intermediate levels is far from understood. Scientists try to fill these gaps by gathering new information that can be placed in one of the boxes represented in Fig. 3. The question remains as to which heuristics and experimental strategies scientists use in their search for new information that can be added to the mechanistic explanation they are working on.

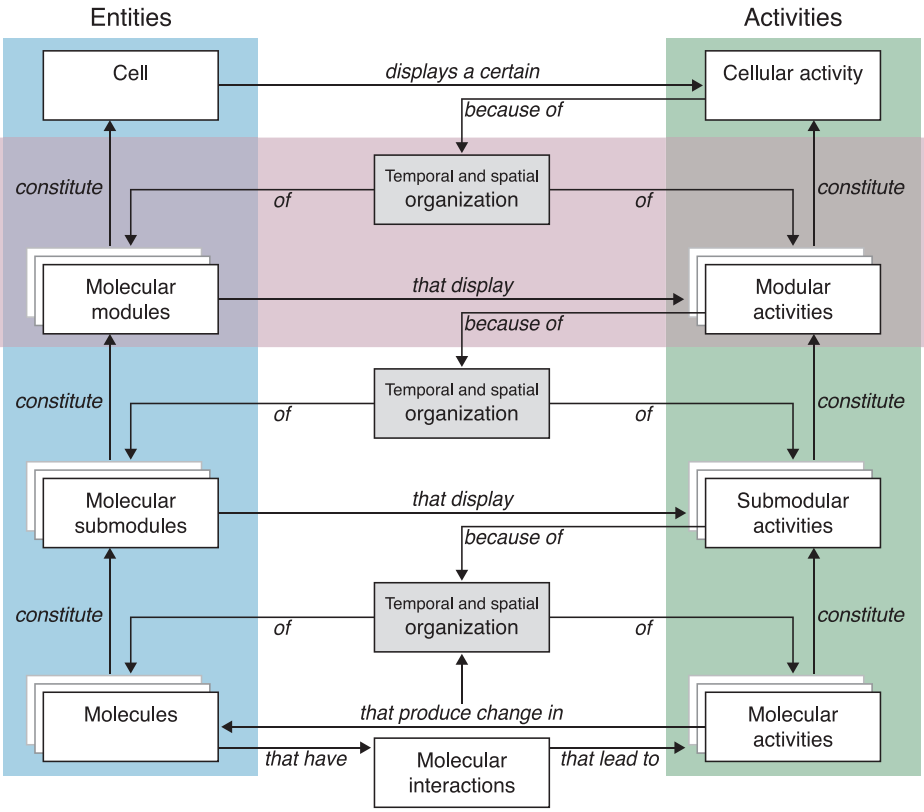


*Fig. 3 A multi-level mechanistic explanation describes a cellular activity in terms of the properties, activities and organization of interacting modular, submodular and/or molecular entities.*

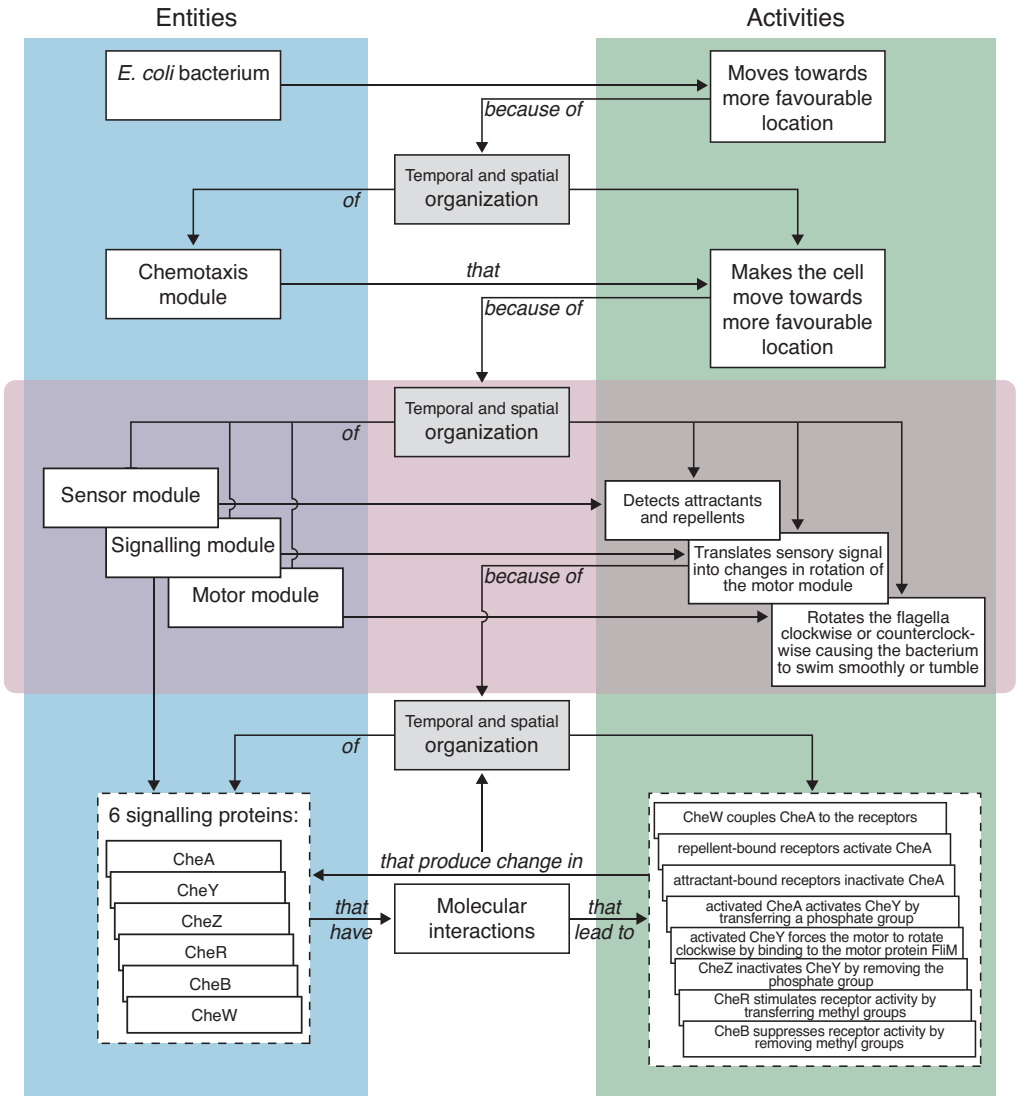


*Fig. 4 Multi-level mechanistic explanation of the chemotaxis behaviour of an E. coli bacterium, elaborated from the signalling module down to and including the molecular level. Data on temporal and spatial organization are not detailed in this figure.*

## 3.4. Heuristics to construct molecular mechanistic explanations

According to Darden (2002) scientists' strategies are aimed at discovering entities and activities and defining the relationships between them and their spatial and temporal organization, thereby filling the gaps between setup and termination conditions of the mechanism. One must find an activity for each entity and an entity for each activity. Also (Bechtel & Abrahamsen, 2005) conclude that strategies to construct mechanistic explanations follow directly from the conception of mechanism as described in Fig. 2: the scientist must identify the parts in the mechanism, determine what activities they perform, and figure out how they are organized so as to generate the phenomenon.

### 3.4.1. Formulating mechanistic research questions

Bechtel (2006) and Darden (2006) show that molecular and cell biologists typically search for mechanistic explanations. This central aim provides a first heuristic used by these scientists, since it specifies the type of research questions they formulate. Their ever-repeated question is 'How does it work?' Early work on chemotaxis offers a nice example of the mechanistic research questions that scientists formulate to better understand the phenomenon they study. Adler (1975) presents six 'how' questions that need to be answered to provide a mechanistic explanation of chemotaxis:

1. *How do individual bacteria move in a gradient of attractant or repellent?*
2. *How do bacteria detect the chemicals?*
3. *How is the sensory information communicated to the flagella?*
4. *How do bacterial flagella produce motion?*
5. *How do flagella respond to the sensory information in order to bring about the appropriate change in direction?*
6. *How is the information integrated, in the case of multiple or conflicting sensory data?*

Note that all six questions ask for explanations of activities. The activities to be explained can be recognized by the verbs 'move', 'detect', communicate' 'produce', 'respond' , 'bring about' and 'integrate'. Question 1 asks for a description of the overall chemotaxis activity in terms of the behaviour of the cells. Philosophers writing about mechanism stress that describing the phenomenon under study in terms of the activity that the overall mechanism displays is a first and essential step in constructing mechanical explanations (Darden, 2002; Richardson & Stephan, 2007). In the case of chemotaxis, the behaviour of motile bacteria responding to environmental cues was described by the end of the 19th century by Pfeffer (1884), Engelmann (1881) and other biologists. These observations were the starting point for Adler's effort in the 1960s to unravel the underlying mechanism (Adler, 1966).

### 3.4.2. Functionally subdividing the overall activity

In questions 2, 3 and 4, Adler divides the total activity into partial activities that together make up the total chemotaxis activity. He hypothesizes that there are three modules with distinct activities: a sensor module that detects chemicals, a signalling module that communicates sensory information and a motor module that produces motion. The strategy of functionally subdividing the overall activity into partial activities that are carried out by lower-level modules is

a research strategy that Darden (2002) calls 'modular subassembly'. Hypothesizing these modules can be inspired by analogous modules or types of modules, and experiments can confirm or refute the existence of these types of modules. In the case of chemotaxis, Alder's hypothesis concerning a signalling module that functions more or less separately from the sensory module was supported by experiments with mutant bacteria. It was observed that many mutants lacked the ability to respond to one specific chemical but responded normally to others, while a few of his mutants did not respond to any chemical. Adler concluded that the former lacked specific types of sensors in their sensor module, while in the latter, signalling of all sensors was blocked because of an error in the signalling module. In more recent review articles, this distinction into three modules is still used in the descriptions of the mechanism of chemotaxis (see, for instance, Baker et al., 2006). Fig. 5 shows the strategy of first describing the overall behaviour as an activity of a modular entity in the cell, followed by dissecting this activity in submodular activities.

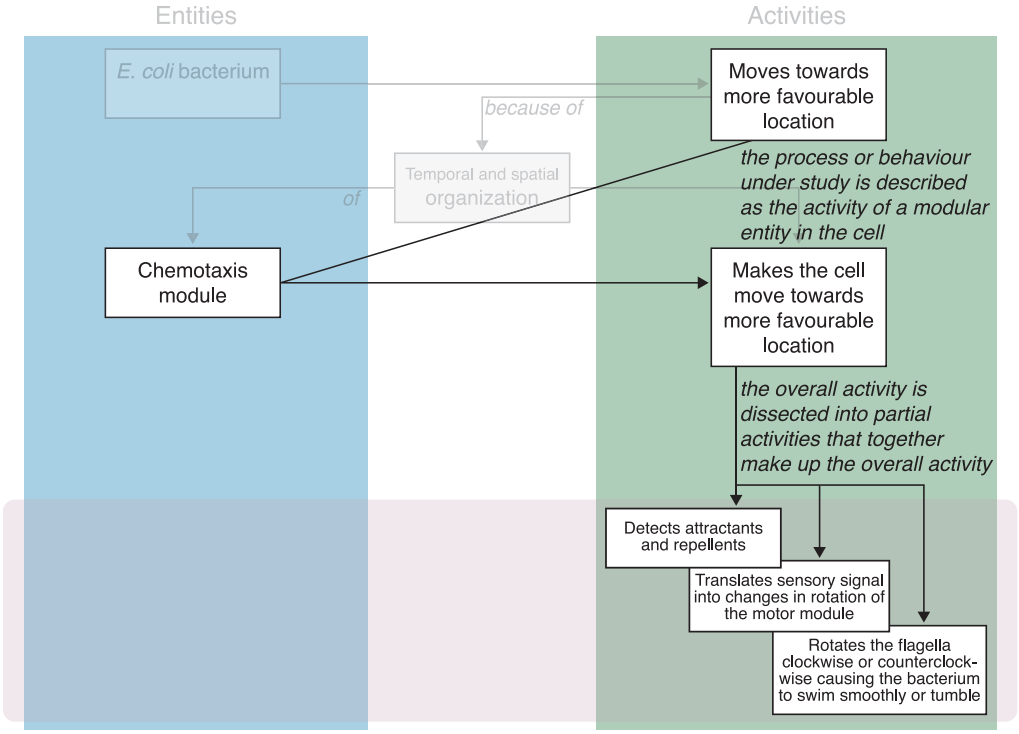


*Fig. 5 The strategy of modular subassembly in chemotaxis research: first, the overall behaviour is described as the activity of a modular entity and then this modular activity is functionally subdivided into partial activities*

## 3.4.3. Hypothesizing mechanistic schemas from activities

When Adler started studying chemotaxis, no mechanisms or components of the mechanisms were known. To answer the question of how chemicals are detected, he postulated two possible mechanisms: either the attractants themselves are detected, or the attractants are first metabolized and then some metabolite of the attractant is detected. By analysing the chemotactic activity of

mutant bacteria, he was able to show that extensive metabolism of the attractants is neither required nor sufficient for chemotaxis. Without knowing any of the molecular components in the mechanism, he could demonstrate that there must be entities located in the cell membrane that directly sense attractants, which he called 'chemoreceptors'. With this conclusion, the first partial sketch of the mechanism was postulated (see Fig. 6).

As shown in this example, predictions can be made about the properties of entities in the mechanism even without knowing its exact composition. This is a reasoning strategy that Darden (2002) calls 'schema instantiation'. First, a rather abstract draft of the mechanistic description of an entity, which she calls a schema, is proposed by considering the behaviour and constraints of the entity as a whole. This schema is then gradually specified and adapted based on experimental results. The construction of these schemas can be inspired by analogies in similar mechanisms in the same or neighbouring fields of research or in the history of science. They can also be inspired by the roles that hypothetical lower-level entities or activities are expected to play. Schema instantiation thus starts by hypothesizing what the entity could look like given the activity it displays. This complements the strategy of subdividing activities because that strategy only provides a subdivision in functional terms, which does not yet contain information about properties of the entities that might display this activity.

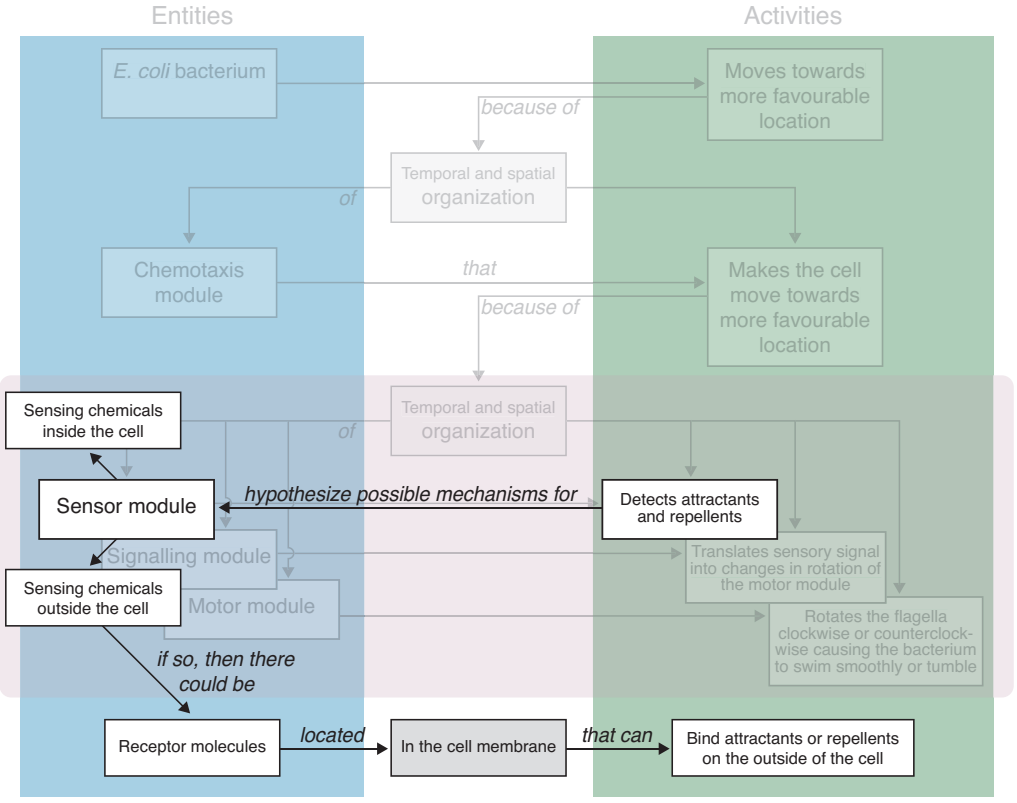


*Fig. 6 The strategy of hypothesizing possible mechanisms to explain the sensing of chemicals in chemotaxis research.*

### 3.4.4. Predicting molecular properties from activities and vice versa

In the period when researchers were trying to identify the molecular component involved in chemotaxis, they had to analyse mutant bacteria that displayed abnormal chemotaxis behaviour. Mapping of the mutated genes in these bacteria led to the identification of several proteins that were essential in chemotaxis. In this way, more and more molecules were added to the 'parts list' of the chemotaxis mechanism. However, it was not immediately clear which activities the proteins displayed and what the role of these proteins was in the mechanism. In many cases, the properties of the proteins predicted a type of activity they could be involved in, while in other cases experiments revealed the presence of certain activities that led to the search for a protein that could display this activity. For instance, Silverman and Simon (1977) describe the finding that some of the proteins become methylated. These proteins later turned out to be receptor proteins. The observation that these proteins become methylated led to the search for and identification of chemotaxis proteins that can transfer and remove methyl groups and to new research to find out what the role of methylation in chemotaxis is. The relationship between the identification of this new molecular activity and the research questions it generates is depicted in Fig. 7.

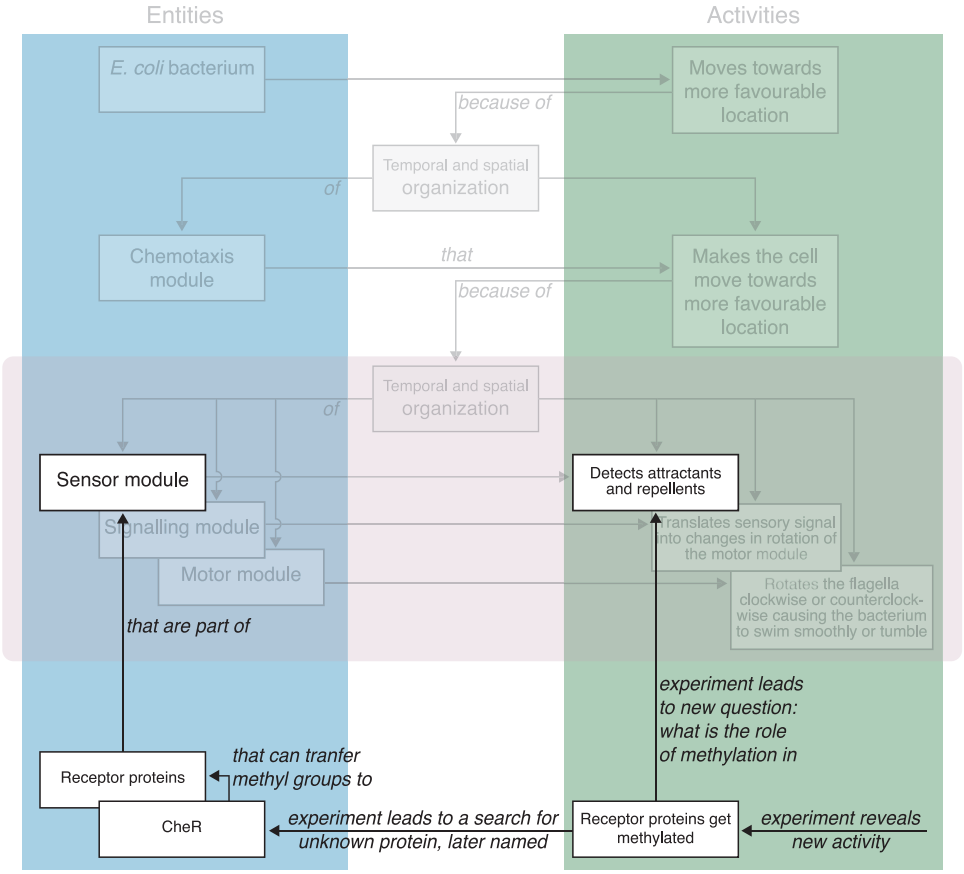


*Fig. 7 The strategy of backward chaining in chemotaxis research: finding a new activity in the chemotaxis mechanism leads to the postulation of new entities and their role in higher-level activities.*

The reasoning strategy in Fig. 7 is an example of what Darden (2002) calls 'backward and forward chaining'. It can help scientists to link molecules to activities and vice versa. Forward and backward chaining is based on the fact that a mechanism has a productive continuity. Interactions of molecules lead to activities and due to these activities the properties and organization of molecules change or new molecules arise. Forward chaining uses the early stages of a mechanism to reason about the types of entities and activities likely to be found in the next stage. Backward chaining reasons from the entities and activities in later stages in the mechanism to predict entities and activities found earlier (Darden, 2002). For instance, the identification of a kinase suggests a role for phosphorylated proteins in a later stage in the mechanism (forward), while the identification of phosphorylated proteins suggests kinase activity earlier in the mechanism (backward). Both forward and backward chaining can be used to predict activities from identified molecules or to predict properties of molecules from identified activities.

### 3.4.5. Hypothesizing and predicting organization in the mechanism

The strategies identified so far focus on the identification of entities and activities and the relationship between them. However, Bechtel (2006) emphasizes that this is only part of the effort. Both entities and activities are organized within the mechanism. Getting a grip on these organizational aspects within molecular mechanisms is probably most challenging for molecular biologists (Harold, 2005). Organizational aspects of entities and activities depend heavily on the mechanism as a whole, and rarely can explanations be given solely in terms of the properties of isolated components (Powell & Dupré, 2009). However, the hypothesized mechanisms as well as information on the properties of entities and activities in the mechanism can be of great heuristic value when discovering organizational aspects. For instance, when Adler proposed chemoreceptors to sense the chemicals on the outside of the cell, this immediately implied that these receptors were located in the cell membrane. As the motor modules are located on the other side of the bacterium, somehow components in the mechanism had to transfer the signal from one side of the cell to the other. Years later it appeared that this role is played by CheY proteins that are not membrane bound but diffuse through the cytoplasm from the receptor side of the cell to the motors.

### 3.4.6. In summary

We identified five heuristics that scientists use to construct mechanistic explanations:
*1. Asking how questions.*
*2. Functionally subdividing activities.*
*3. Hypothesizing mechanistic schemas.*
*4. Predicting molecular properties from activities and vice versa.*
*5. Hypothesizing and predicting organization in the mechanism.*

We can conclude that all these heuristics use existing knowledge about activities, entities or organizational aspects to predict and test for unknown activities, entities or organization. The activities, entities and organizational aspects sought can be at the same level, for instance if the chemical properties of a protein predict a molecular activity or its location. But the knowledge at one level can also be used to predict and test activities, entities and organization at higher levels (bottom up) or lower levels (top down). With the goal to fill the gaps and to solve inconsistencies in the model of a multi-level mechanism in the cell, scientists reason back and forth between entities, activities and their organization, and up and down between different levels. As shown with the example of chemotaxis in Figs 5, 6 and 7, reasoning and research strategies can be displayed in our model of multi-level mechanisms using arrows that connect one box that represents the input information with another one that represents the output information that the strategy aims for.

# 4. Mechanistic reasoning to fill the gap between cellular and molecular-level phenomena in biology education

Our analysis shows that scientists in molecular biology model molecular mechanisms to explain cellular processes. The first two research questions are:

1. *What characterizes scientific explanations that aim at understanding cellular processes in terms of molecular interactions?*
2. *Which heuristics are used to construct these explanations?*

These can thus be answered as follows:

1. Biological explanations of cellular processes are typically mechanistic explanations. Models of the molecular mechanism explain how a cellular process works by showing how the relationships between the consisting molecular entities, their activities and their spatial and temporal organization together bring about the process. Often intermediate levels are used to show how interacting groups of molecules, called molecular modules, have their own level of organization and fulfil specific functions in the overall process.
2. Five heuristics (summarized in section 3.4.6) show how scientists reason mechanistically between cells and molecules. They formulate mechanistic research questions and model molecular mechanisms to answer these 'how' questions. With the goal being to fill the gaps and to solve inconsistencies in the model, they reason back and forth between molecules, molecular activities and their organization, and they reason up and down between different functional levels between cells and molecules.

In the introduction, we described how relating the cellular level to the molecular level in biology is a crucial but very difficult step for students. Our analysis shows that connecting the molecular and cellular levels entails a form of mechanistic reasoning, because it requires relating the behaviour of wholes at multiple levels to the properties, activities and organization of their parts and vice versa (Machamer et al., 2000). Our characterization of molecular mechanistic explanations and the heuristics that scientists use now makes it possible to reinterpret the problem in terms of students' difficulties in reasoning about molecular mechanistic explanations and thus providing criteria to address them. This brings us to research question 3:

*What educational design criteria can be derived from the analysis of these scientific explanations and heuristics?*

We answer this question by first reinterpreting learning difficulties in terms of the knowledge and reasoning skills needed for reasoning about molecular mechanistic explanations. In the last section we will further specify these needs into educational design criteria by adapting the scientific heuristics identified in section 3.4 for educational use.

We will focus on proteins as the active entities at the bottom level and on protein-based modules at multiple levels between proteins and cells, in line with the work of Duncan and colleagues (Duncan, 2007; Duncan & Reiser, 2007; Duncan & Tseng, 2011). This offers the opportunity to refer explicitly to gene function when discussing mechanisms in the cell, thereby bridging the gap between genes and cells that we identified in the Introduction.

## 4.2. Reinterpretation of students' learning problems

Mechanistic reasoning means reasoning about mechanistic explanations (Russ, Scherr, Hammer, & Mikeska, 2008). We will use the term 'molecular mechanistic reasoning' to refer to the reasoning skills needed to construct and to understand molecular mechanistic explanations. Russ et al. (2008) use a case in physics to show that mechanistic reasoning is abundantly present in student reasoning, even in very young students. Reasoning about mechanisms seems to be quite intuitive. It relates to the question 'How does it work?' Even very young children are familiar with the fact that 'wholes' consist of 'parts' and that in many cases interactions of the parts make up the whole. A bike, for instance, can be described mechanistically by describing the parts and their role in the whole (Grotzer, 2003). However, the study of Abrams and Southerland (2001) suggests that mechanistic reasoning in biology education, and especially in molecular biology education, is not as abundantly present as Russ et al. (2008) report. These findings do not necessarily contradict. Mechanistic reasoning is the basis for molecular mechanistic reasoning, but from our analysis we can identify characteristics of molecular mechanistic explanations that complicate students reasoning. These factors may prevent students from using this intuitive notion of mechanism to explain phenomena in the cell.

### 4.1.1. Mechanistic explanations: 'How does it work?' is not an obvious question in cell biology education

For molecular and cell biologists the main question is 'how do cell processes work?' However, Abrams and Southerland (2001) describe how students in the biology classroom tend to neglect physical mechanisms when asked to explain biological phenomena. They tend to focus on the function of a phenomenon, rather than wondering which physical mechanisms explain the phenomenon, and they often rely on teleological and anthropomorphic explanations (Kampourakis & Zogza, 2008; Tamir & Zohar, 1991; Zohar & Ginossar, 1998). In cell biology education, this tendency can be recognized for instance when explaining chromosome transport during mitosis. Most students will be perfectly satisfied with the explanation that chromosomes are sorted and pulled to the centromeres because they need to be distributed equally to form two identical daughter cells. The question how a cell manages to sort and pull chromosomes does not arise in the students' minds. Apparently, the need to explore the causal explanations that answer 'how a cell works' is not self-evident in the biology classroom.

Abrams and Southerland (2001) suggest that one of the reasons for this is the tendency of biology teachers to focus on the benefits of a phenomenon rather than on the cause, and Kampourakis, Pavlidi, Papadopoulou, and Palaiokrassa (2011) emphasizes that teachers should be aware of the difference between proximate and ultimate causes to distinguish effectively between 'how?' and 'why?' questions in the classroom.

### 4.1.2. Molecular interactions: Students do not consider protein interactions as basic causal events in the cell

To be able to reason mechanistically about protein activities in cellular processes, students need to understand that protein interactions can be considered the basic causal events in the cell. As described in section 3.2, protein activities can be understood from the underlying principle that proteins undergo conformational changes when they interact and that these conformational changes allow the next step in the activity of the protein. That means that, in principle, all protein

activities can be described causally in terms of the result of binding, changing conformation and breaking and forming chemical bonds. These interaction-induced conformational changes can be considered the basic causal events at the molecular level, comparable to the physical interactions between parts in daily-life machines that cause the machine to function. This machine-like view of proteins is very different from the view of molecules in chemical reactions as taught in traditional chemistry education. Conformational changes play only a minor role in explanations of the simple chemical reaction used in the chemistry classroom. We suggest that current education about proteins does not provide students with the ideas that the interaction-induced conformational changes of proteins are the basic causal events in living cells. Upper-secondary biology education focuses on the structural and functional aspects of proteins but fails to show the causality in protein activities.



### 4.1.3. Functional levels: Students are unfamiliar with the multiple functional levels in between cells and molecules

In cell biology research, multiple functional levels are distinguished between cells and molecules. Activities at different levels are ascribed to groups of interacting molecules, called functional modules. However, students' images of the inner workings of a cell are based mostly on the structural subdivision of cells consisting of organelles and cytoplasm, since most biology text books present organelles as the organizational level in between cells and molecules. Although organelles are indeed functional units in the cell, only presenting the activities of organelles to explain cellular function has some major disadvantages. First, only part of cellular functions can be assigned to specific organelles. Secondly, many different molecular activities are linked to the same organelle (ATP synthase activity is only one of the many protein activities in mitochondria). Thirdly, the traditional structural subdivision ignores the fact that there are many more functional levels in between cells and macromolecules. Students should be enabled to think up and down between these functional levels to eventually connect activities at the cellular level to molecular activities and vice versa. This bottom-up and top-down reasoning relates to what Knippels calls the 'yo-yo strategy'. She developed this educational strategy to help students to think up and down between levels of biological organization. However, Knippels' strategy was not elaborated for connecting the cellular level to the molecular level. Our framework demonstrates that the traditional levels of cells, organelles and molecules are insufficient to describe how molecules contribute to the functioning of the cell. Knippels' yo-yo strategy can be extended down to the molecular level when cellular activities at the top are viewed as the result of hierarchically ordered mechanisms, with protein activities as the basic units at the bottom and submodular and modular activities as the intermediate levels, necessary to connect these top and bottom activities.

### 4.1.4. Molecular modules: The abstract, dynamic and transient nature of molecular modules complicates students' reasoning.

Scientists identify modules not on structural features but on the discrete functions of groups of molecules. Often these modules are highly dynamic and transient. This complicates reasoning about functional modules, since these cannot always be represented as a structural 'thing'. This abstract and dynamic nature of functional modules adds to the already abstract nature of the macromolecules that constitute these modules. Macromolecules and their activities need to be imagined or represented by using visual modules, such as graphics or animations. Many

publications in science education emphasize difficulties related to visualizing abstract concepts such as molecules (Cook, Wiebe, & Carter, 2008; Ferk, Vrtacnik, Blejec, & Gril, 2003; Gilbert, Reiner, & Nakhleh, 2008; Mathewson, 1999; Schönborn & Anderson, 2006; Yarden & Yarden, 2010).

### 4.1.5. Temporal and spatial organization: Students are unfamiliar with many organizational aspects of proteins and protein activities

Organization is what distinguishes a mechanism from just a collection of parts. This will not be surprising to students, since the same holds true for any mechanism they know. However, organization in the cell is much more complicated to grasp than the composition of a man-made device. Once assembled, the organization of a man-made device is relatively stable, but as mentioned before, organization in the cell is highly dynamic. One of the aspects of this dynamic nature that is relatively unfamiliar to students is the stochastic nature of protein interactions. Students need to understand that protein activities depend on random collisions to get a grip on the organizational aspects mentioned in Table 1. It explains for instance why the speed and frequency of protein activities is influenced by the concentration of the interacting molecules and it makes intelligible that proteins located in the same compartment will interact when they can move freely, while interactions are limited when proteins are bound to membranes or other structures.

## 4.2. From scientific heuristics to design criteria for molecular and cell biology education

In the Introduction, we concluded that students in upper-secondary and even in undergraduate biology education show little awareness that all cellular phenomena emerge from molecular interactions. Although knowledge about the structural and chemical properties of macromolecules is part of most upper-secondary science curricula, students seldom link these properties to the functional roles of molecules in higher-level phenomena (Duncan & Tseng, 2011). Here, we restated this problem in terms of difficulties related to multi-level mechanistic reasoning between the cellular and molecular level. From the previous section, we can conclude that these difficulties ask for educational approaches in which:

- Students are guided towards causal-mechanistic instead of functional explanations.
- Students learn how to explain machine-like protein activities from molecular interactions.
- Students are familiarized with the multiple functional levels in between cells and molecules.
- Students are familiarized with the abstract, dynamic and transient nature of molecular modules.
- Students explore the organization of proteins and protein-based modules.

In this section we will further specify these criteria by adapting the heuristics identified in research question 2. These heuristics represent concrete examples of scientists' molecular mechanistic reasoning, and will inform the design of education aiming at the acquisition of this reasoning.

4.2.1. Raising 'how' questions about cellular activities

The first heuristic identified shows that the ever-repeated question that molecular and cell biologist ask is 'How does it work?' We discussed in the previous section that this is a question that does not automatically occur to students. We therefore suggest that explicitly raising 'how'

questions about cellular activities is an important task for educators in molecular and cell biology. Activities that encourage students to visualize what is actually going on inside a cell might be very useful as a starting point for raising these types of questions. More and more animations and graphics are becoming available that show the complexity of living cells (see (McGill, 2008). These animations are visual models and artistic impressions in which choices have been made about how to represent reality. We suggest that discussing what is realistic in these representations and what is not might be very powerful in raising all sorts of 'how' questions when used in the appropriate educational setting.

### 4.2.2. Explaining protein activities from molecular interactions

The heuristic of predicting activities from molecular properties and vice versa builds on the notion of protein interactions as causal events. For instance, if ATP is involved in an activity, then the protein involved must have a binding site for ATP. The other way around, if the structure of a protein reveals a DNA-binding domain, the protein activity will probably involve the binding of DNA, for instance in transcription activation. These kinds of reasoning exercises to think back and forth between proteins, their interactions and their activities could help students to better understand that protein interactions are the causal events in cellular processes. In the biology classroom, the functioning of proteins can be conceptualized without detailed chemistry, for instance by using the machine metaphor. However, we suggest that at least for a few examples, chemical details must be presented, since forming and breaking chemical bonds in and between molecules is the basis for all conformational changes that are used to explain the machine-like functioning of proteins. Educational activities that show how the same basic chemical principles used in the chemistry classroom can be used to explain how proteins act in a machine-like way might help students to see that basic chemistry can lead to complex machine-like behaviour of proteins and protein-based modules.

### 4.2.3. Exploring levels by functionally subdividing cellular activities

We suggest that intermediate levels of functional modules and submodules can form stepping stones when reasoning from molecular to cellular activities and vice versa. If students are trained to use mechanistic reasoning to explore the functional levels between cells and molecules, this could help to make intelligible for them the fact that the interactions of molecules can lead to such complex cellular behaviour. One of the great educational advantages of using intermediate levels of functional modules is that students can hypothesize these functions from cellular activities without detailed molecular knowledge. Starting from a cellular activity, the question to be raised is 'What activities are needed to accomplish this overall activity?' These type of questions resemble the scientific heuristic that Darden (2002) calls 'modular subassembly'. For instance, in the process of cell division, students can hypothesize that the content of the cell must be duplicated, and that the content must be separated into two portions. These two activities are probably too comprehensive to typify them as modular activities but they can be further subdivided, for instance by asking which cellular components should be duplicated. The different components such as DNA, proteins, mitochondria and membranes all have their own duplication mechanisms, some of which could be interesting to elaborate, for example the DNA replication mechanism and the role of DNA polymerase in it. In this way, education about the DNA replication mechanism connects to the overall process of cell division, thereby extending Knippels yo-yo strategy down to the level of molecular activities.

### 4.2.4. Hypothesizing mechanistic schemas

Students may hypothesize about the characteristics of the mechanism by which certain modules function. The question to be raised is: what type of mechanism could accomplish this activity? These hypotheses can be inspired by analogies from daily life. For instance, if, in mitosis, chromosomes are pulled towards the centrioles, this could involve either rail-like mechanisms, pulley-like mechanisms or maybe both. This reasoning strategy resembles the scientific heuristic that Darden calls 'schema instantiation' (Darden, 2002).

### 4.2.5. Articulating the role of organization in protein-based mechanisms

Identifying how proteins and their activities are organized in protein-based mechanisms is one of the most challenging tasks for scientists. They predict and test organizational aspects from the properties of the proteins (for instance, a DNA-binding domain suggests localization in the nucleus) and they use their hypotheses about the working of the mechanism to predict how parts in the mechanism should be organized. These two strategies can both be used in education to explain that not only is the presence of specific proteins required to establish specific activities in the cell, but also that these proteins have to be organized in a way that makes it possible for the mechanism to function. By exploring the organization of proteins, questions may arise about the origin of the organization of proteins and other cellular components. Some of these questions ask for a developmental explanation. For instance, the question 'What causes this protein to be present in one cell while it is absent in another?' asks for exploring mechanisms of transcription regulation that are central to development. We suggest that educators can use this link between mechanisms and the origin of organization in the mechanisms to show how genes and development are linked.

## 4.3. Using mechanistic reasoning to read and construct models of protein-based mechanisms

Mechanistic models and images are not completely unknown in cell biology education. Most upper-secondary curricula already present mechanisms in the cell, mostly by means of cartoon-like models. What has become clear from our research is that students lack the knowledge base to interpret these models correctly, and that therefore presenting these models does not contribute to understanding how molecular interactions explain cellular processes. For instance, arrows in cartoon-like models indicate the activities in the mechanism. Without knowledge of protein interactions, these arrows remain meaningless. Molecular mechanistic reasoning thus allows more adequate interpretations of the molecular graphics and animations already used in education. Furthermore, students may use molecular mechanistic reasoning to generate ideas and hypotheses about the mechanisms underlying biological phenomena that have not yet been explored down to the molecular level. Molecular mechanistic reasoning thus offers students the cognitive tools to fill the gap between the molecular level and higher levels of biological organization.

Designing and testing activities based on the criteria we identified will be the next step to further develop the concept of molecular mechanistic reasoning in molecular and cell biology education.

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# Paper III:
# Molecular mechanistic reasoning: towards bridging the gap between the molecular and cellular level in life science education

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## Abstract

In most modern science curricula, students learn about DNA, RNA and proteins. However, many studies report that students fail to connect these molecular-level details to phenomena at the level of cells, organs and organisms. It is proposed that students are not sufficiently equipped and encouraged to reason about complex and emergent systems behaviour to bridge the gap between the molecular level and phenomena at higher levels of biological organization. In this study, we explore the potential of a new educational approach that is based on encouraging molecular mechanistic reasoning, which entails hypothesizing, constructing and interpreting mechanistic explanations for (sub)cellular phenomena, while taking into account the physical and chemical principles that drive changes at the bottom level of molecular interactions. We present the theoretical basis for a learning trajectory based on molecular mechanistic reasoning and we show in a small-scale test of the educational approach that it is within reach for pre-university students (aged 17–18) to develop a sound understanding of the multi-level mechanistic nature of cell activities as well as the physical and chemical principles that determine how molecular mechanisms work. We argue that this insight is indispensable to bridge the gap between the molecular and cellular level in life science education.

# 1. Introduction

## 1.1. The gap between the molecular and cellular level in life science education

The molecular life sciences are progressing at a dazzling speed. More and more cellular phenomena are described and understood in terms molecular interactions. Furthermore, biological disciplines that traditionally focused on higher levels of biological organization extend their scope towards the molecular level and in some cases, such as genetics, ecology and evolutionary biology, new molecular insights dramatically remodel traditional scientific ideas. Science educators, researchers and curriculum developers have been thinking about the implications of these scientific advances for science education (e.g. AAAS, 2005; Boerwinkel & Waarlo, 2009; Moore, 2007). One of the educational challenges is that, although in most modern science curricula students learn about DNA, RNA and proteins, these concepts at the molecular level still remain isolated facts and that molecular knowledge hardly contributes to more sophisticated explanations of biological phenomena (Duncan & Reiser, 2007; Marbach-Ad & Stavy, 2000). As a result, memorization and rote learning is often reported as the strategy used by students when presented with molecular-level concepts (Anderson & Schonborn, 2008; Momsen, Long, Wyse & Ebert-May, 2010; Stanger-Hall, 2012).

Relating molecular-level concepts to phenomena at the level of cells, tissues, organs and organisms refers to what Knippels (2002) calls the yo-yo strategy: zooming in and out to identify and relate structures and their functions at different levels of biological organization. However, in Knippels' work, the molecular level was not included. In previous work (Van Mil, Boerwinkel & Waarlo, 2013), we explored the relationship between proteins and cells and we showed that the relationship is not straightforward; the activities of cells can hardly ever be inferred directly from the activity of individual proteins. Groups of interacting proteins form molecular modules with distinct modular activities and multiple modules work together to form multi-module activities. Furthermore, these modules are very dynamic, often transient and, as a consequence, not recognizable as stable structures in the cell. When applying the approach of explaining the activities of cells by identifying the function of underlying structures, the most obvious underlying structures are the cell organelles. This is what happens in traditional introductory cell biology education: all organelles are identified and assigned a function to explain how a cell works (Verhoeff, Waarlo & Boersma, 2008). However, only few cell activities can be explained directly from the activity of organelles (for instance: how to explain cell division from organelle activities?) and the activity of organelles cannot be explained from the activity of individual proteins. Therefore, yo-yo-ing from cells to proteins and back is not just a matter of identifying underlying structures and describing their function, as is the traditional educational approach at higher levels of biological organization. The emergent nature of these higher-level activities does not allow for assigning such a single underlying causal agent. Nonetheless, many students in cell biology education explain cellular function only by referring to organelle function and are satisfied with this explanation (Barak, Sheva, Gorodetsky & Gurion, 1999). We suggest that the focus on the functional questions 'why?' or 'what is it for?' in biology education (Abrams & Southerland, 2001) contributes to this tendency. Here we want to explore the educational potential of studying cellular phenomenon from a different perspective: all cellular phenomena emerge from molecular interactions. Although this might be interpreted as a strong reductionist

approach, we claim the opposite: only with a systems view on molecular events (Nurse, 2003; Powell & Dupré, 2009; von Wulfingen, 2009) can molecular biology education succeed in making intelligible to students that very complex cellular behaviour can emerge from simple molecular interaction.

In traditional molecular biology education, the molecular level, obviously, does play a prominent role. Processes such as DNA replication and protein synthesis are covered in much detail (AAAS, 2005). However, despite these molecular-level details, the central role that proteins play in all cellular processes remains largely unnoticed (Duncan & Tseng, 2011) and as a consequence the molecular level remains isolated from the (sub)cellular level. We suggest that a very important reason for this is that students lack a more general account of how molecular interactions can lead to very complex cellular behaviour, resulting in an explanatory gap between the molecular and (sub)cellular level.

The question addressed in this article is: How can the explanatory gap between the (sub)cellular and molecular level be bridged in life sciences education? Important to clarify is what we mean by 'bridging the gap'. In general, connecting levels of (biological) organization implies using information from one organizational level to better understand or even explain what happens at another level. What we aim for is that students are able to use their knowledge about molecular interactions in their explanations of (sub)cellular behaviour and vice versa that studying (sub) cellular behaviour triggers them to think about the molecules involved in this behaviour.



## 1.2. Expert reasoning about molecules when explaining (sub)cellular phenomena

In search of an educational strategy to bridge the gap, our earlier research analysed the nature of molecular-level explanations for (sub)cellular phenomena and the reasoning strategies that experts in molecular biology. In this research (Van Mil et al., 2013), we characterized the relationship between molecules and (sub)cellular phenomena using the term 'molecular mechanisms', based on the work of Machamer, Darden and Craver. They characterize mechanisms in general as: *'Mechanisms are composed of both entities (with their properties) and activities. Activities are the producers of change. Entities are the things that engage in activities. […] The organization of these entities and activities determines the ways in which they produce the phenomenon.'* (Machamer, Darden & Craver, 2000, p. 3).

In line with general accounts of emergence used by scientists (Wimsatt, 2000), Machamer, Darden and Craver stress that organization is what distinguishes a mechanism from just a collection of parts and they subscribe to the view of others (Bechtel & Abrahamsen, 2005; Glennan, 2002; Tabery, 2004) that interactions between the entities are essential to produce the changes in the mechanism that they call 'activities' (Darden, 2008). In molecular mechanisms, the entities are molecules, and proteins in particular are presented as 'the entities that produce change' via interacting with other molecules. Molecular mechanistic explanations thus include references to the organization of the molecules involved, the interactions between the molecules and the changes these interactions induce. More and more of these detailed molecular mechanisms are becoming available these days. However, when scientists construct mechanistic explanations for sub(cellular) behaviour, they do not, or cannot, always describe all the detailed molecular changes, if only because the phenomena are too complex. As a consequence, they also use entities and activities at intermediate levels between 'activities of cells' and 'biochemical changes'. For instance, with the term 'protein activity' (e.g. catalytic activity, binding activity,

transporter activity and enzyme regulator activity), they characterize the capacity of individual proteins to induce an overall effect that consists of multiple biochemical changes (Ashburner et al., 2000) and the term 'modular activity' is used to characterize the overall effect that is jointly displayed by multiple proteins, such as transcription or signalling (Hartwell & Hopfield, 1999; Koch, 2012). These terms are also used in upper-secondary education; however, the difference is that experts are constantly aware of how changes at these intermediate levels relate to the basic principles of molecular organization and interactions and they use these principles when they hypothesize, construct and interpret mechanistic explanations for (sub)cellular phenomena. We can thus conclude that, in contrast to students, experts can bridge the gap because they can reason mechanistically between cells and molecules, thereby relating entities and activities and their organization at multiple intermediate levels, while being constantly aware of the chemical and physical principles that drive the changes in these mechanisms. This is what we call molecular mechanistic reasoning. It entails hypothesizing, constructing and interpreting mechanistic explanations for (sub)cellular phenomena, while taking into account the physical and chemical principles that drive changes at the bottom level of molecular interactions.
In Van Mil et al. (2013), we concluded that students in upper-secondary and even undergraduate life science education students are neither sufficiently equipped nor encouraged to use molecular-level concepts and principles in their reasoning about (sub)cellular phenomena. We suggest that fostering student reasoning based on expert reasoning in life science research might be a powerful strategy to help students to bridge the gap between the molecular and cellular level. Molecular mechanistic reasoning could make intelligible to students how complex cellular behaviour can emerge from simple molecular interactions.

## 1.3. Triggering molecular mechanistic reasoning in life science education

### 1.3.1. Reasoning about mechanisms is intuitive

Molecular mechanistic reasoning can be considered as a domain-specific case of mechanistic reasoning. First, let us consider what we mean with general mechanistic reasoning strategies. From the general Machamer, Darden and Craver account for mechanisms, we can extract that mechanistic reasoning entails identifying or hypothesizing the interactions and organization of underlying entities and activities in order to construct causal chains or networks with the goal to explain a phenomenon. Darden, Craver and others in the field of philosophy of science (Bechtel & Richardson, 2010; Boogerd, Bruggeman, Hofmeyr & Westerhoff, 2007; Craver, 2002; Craver & Bechtel, 2007; Darden, 2006) have used this account of mechanistic explanations to describe heuristics, reasoning strategies, and research approaches that experts in the life sciences use when they hypothesize, construct and interpret mechanistic explanations. In general, we can distinguish between top-down, bottom-up and causal chaining approaches, based on the direction in which relationship between entities, activities and organizational aspects in multi-level mechanisms are explored (Bruggeman & Westerhoff, 2007; Craver & Bechtel, 2007; Darden, 2002). Upward and downward approaches search for part-whole relationships across levels, while causal chaining (also called forward/backward chaining) searches for causal relationships within one level. Although the studies in which these approaches are characterized focus on the work of experts, mechanistic reasoning appears to be rather intuitive. It builds on the notion of

parts and wholes and causality and it is what people do when they reason about the working of a machine, a car engine or other daily-life mechanisms. Many studies show that even in very young children mechanistic reasoning can be recognized (for a review, see Grotzer, 2003). Russ, Scherr, Hammer & Mikeska (2008) describe mechanistic reasoning in a physics classroom and they recognize all the elements of mechanisms of the Machamer, Darden and Craver framework in classroom discussions with first-grade students.

## 1.3.2. Reasoning about mechanisms in the cell is more complex

As demonstrated in Van Mil et al. (2013), molecular mechanistic reasoning uses the same reasoning strategies as mechanistic reasoning in general; top-down, bottom-up and chaining approaches are distinguishable here as well. The reason that molecular mechanistic reasoning is not as intuitive as mechanistic reasoning in general is that causality and organization in molecular mechanisms is difficult to understand. Causality is a central aspect in mechanisms, and in reasoning about daily-life mechanisms, intuitive causal knowledge about the physical world (Brown, 1993; diSessa, 1993; Klaassen, Westra, Emmett, Eijkelhof & Lijnse, 2008) can be used to predict and interpret how entities and activities in the mechanisms are causally linked. However, causality at the bottom level of molecular interactions is not so self-evident (Powell & Dupré, 2009), since molecules behave differently from daily-life objects. For molecular mechanistic reasoning, the general mechanistic reasoning strategies explained earlier thus need to be supplemented with domain-specific knowledge about the properties of molecules in the cell and the physical and chemical principles that determine behaviour of these molecules in mechanisms in the cell. In the next sections, we specify which aspects of these domain-specific characteristics of mechanisms in the cell can be expected to be new or confusing to students in upper-secondary education and, therefore, can form obstacles for molecular mechanistic reasoning.

### 1.3.2.1. Proteins are the basic entities in molecular mechanisms and protein interactions form the basic activities

Molecular mechanistic reasoning requires an understanding of the protein interactions which are the basis for all complex cellular behaviour. Although students learn about proteins, upper-secondary biology education presents proteins as functional units (e.g. transporters, catalysts or building blocks) that operate in isolation. However, the underlying physical and chemical principles that explain how protein interactions in general can be seen as basic causal events remain hidden, which prevents students to consider protein interactions as the basis of all complex cellular behaviour.

### 1.3.2.2. The hierarchical, multi-level nature of mechanisms in the cell is difficult to recognize and imagine

Molecular modules are groups of proteins to be identified by a collectively displayed activity (Hartwell & Hopfield, 1999). However, molecular modules are often not recognizable as structural units in the cells and the highly dynamic and transient nature of these modules makes it very difficult to imagine or represent how these modules work. The organizational levels traditionally used in upper secondary education, namely cells, organelles and molecules, are insufficient to describe how molecules contribute to the functioning of the cell. Although some modular activities such as DNA replication, transcription and translation are covered, students

are not encouraged to use these intermediate levels to think upward and downward to relate activities at the cellular level to molecular interactions and vice versa.

### 1.3.2.3. Organization in the cell is complex and highly dynamic

Organization is what distinguishes a mechanism from just a collection of parts (Wimsatt, 1972). However, organization at the molecular level is not self-evident. Organization of molecules is based on physical and chemical principles. Although the particulate model of nature is covered in most secondary physics and chemistry curricula, many studies report difficulties when students have to apply principles such as Brownian motion, molecular collisions and attraction and repulsion between particles in their explanations for natural phenomena (Garvin-Doxas & Klymkowsky, 2008; Odom, 1995; Robic, 2010; Williamson & Abraham, 1995). Furthermore, in the biology classroom, little attention is paid to the application of these principles to biological systems. For instance, one of the principles unfamiliar to most students is the stochastic nature of protein interactions (Jenkinson & McGill, 2012) which is an essential element in understanding the 'where' and 'when' of protein activities (Ellis, 2001).

These domain-specific characteristics of mechanism in the cell pose obstacles for students to apply intuitive mechanistic reasoning when explaining cellular activities. In our opinion, it is this lack of knowledge about the molecular entities in the cell and the principles that determine how these molecules behave which keeps students from applying their intuitive notions about mechanisms and mechanistic explanations to the levels between molecules and cells. Moreover, when knowledge of underlying parts and principles is missing, it is comprehendible that students are not inclined to ask 'how questions' to explain cellular activities, and biology education itself does not encourage this either. In current cell biology education, much more focus is put on function of structures and phenomena in the cell than on physical mechanisms (Verhoeff et al., 2008), and therefore students are not used to pose these kind of 'how does it work' questions about cellular processes (Abrams & Southerland, 2001). In Van Mil et al. (2013), we conclude that these domain-specific aspects about mechanisms in the cell lead to five obstacles that show why students are not sufficiently encouraged and equipped to apply their intuitive notion of mechanisms to the levels between molecules and cells:

1. *'How does it work?' is not an obvious question in cell biology education.*
2. *Students do not consider protein interactions as basic causal events in the cell.*
3. *Students are unfamiliar with the multiple functional levels in between cells and molecules.*
4. *The abstract, dynamic and transient nature of molecular modules complicates students' reasoning.*
5. *Students are unfamiliar with many organizational aspects of proteins and protein activities.*

## 1.3.3. Characterization of molecular mechanistic reasoning

We have defined molecular mechanistic reasoning as *hypothesizing, constructing and interpreting mechanistic explanations for (sub)cellular phenomena, while taking into account the physical and chemical principles that drive changes at the bottom level of molecular interactions.*

In the previous section, we clarified what is meant by reasoning about mechanistic explanations in general and we showed how the need to take into account the physical and chemical principles complicates reasoning about mechanisms in the cell. We can now better specify what characterizes molecular mechanistic reasoning if we combine the general mechanistic reasoning approaches with the domain-specific characteristic of mechanisms in the cell. This leads to a set

of domain-specific reasoning strategies that are helpful to explore mechanistic explanations for cellular behaviour.

**Top-down**

- Identify a (sub)cellular phenomenon to be explained and ask relevant how-questions about it.
- Subdivide a (sub)cellular phenomenon functionally to identify underlying activities.
- Hypothesize relevant mechanistic schemas, for instance by using metaphors or comparisons.

**Causal chaining**

- Identify/hypothesize the involvement of proteins or protein-based modules.
- Identify/hypothesize activities of proteins or protein-based modules.
- Link protein or module activities into causal chains or recognize gaps in the causal chain.
- Apply the physical and chemical principles of molecular interactions as a basis for causality in the mechanisms.
- Apply the physical and chemical principles of molecules as a basis for organization in the mechanisms.

**Bottom-up**

- Combine entities, activities, organization and causality into a mechanistic model that accounts for a (sub)cellular phenomena.



## 1.4. The intended use of molecular mechanistic reasoning

As mentioned before, we consider molecular mechanistic reasoning essential to bridge the gap between the cellular and molecular level. In different aspects of the work of experts, molecular mechanistic reasoning can be recognized (Van Mil et al., 2013), but if we take a closer look at upper-secondary life science education, students are in fact already confronted with many assignments in which molecular mechanistic reasoning plays a role. Below, we discuss three examples that are generally regarded as relevant and suitable for upper-secondary life science students. These three assignments are used in our study and a detailed description can be found in the appendix. We argue here that if students are not properly trained to recognize and apply the physical and chemical principles that drive changes at the bottom level of molecular interactions, the mechanistic reasoning that these assignments aim for remains superficial or students may tend to stick to functional or even teleological and anthropomorphic reasoning.
These assignments are based on different tasks in which scientists use mechanistic reasoning. Assignment 1 asks for *questioning* and *hypothesizing*, assignment 2 asks for *interpreting scientific models* and assignment 3 asks for *(re)constructing a model*. Many of the molecular mechanistic reasoning elements we identified in the previous paragraph can be recognized in these assignments, although called upon in different ways. For instance, assignment 2 explicitly shows proteins while in assignment 1 students have to hypothesize the possible role of proteins or protein-based modules. We do not aim for expert level reasoning in upper-secondary students, but we suggest that meaningful mechanistic reasoning about cellular phenomena can only be established if students are provided with an intelligible account for the domain-specific aspects of molecular mechanisms.

### 1.4.1 Assignment 1: 'Pose questions and ideas to explain the crawling of a neutrophil'

Students look at a microscopic time-lapse movie that shows a neutrophil chasing a bacterium[1]. They are asked to formulate all questions they can come up with and provide possible answers to the questions[2].

### 1.4.2. Assignment 2: 'Interpret textbook graphics of molecular mechanisms'

Students interpret two graphical representations of molecular modules (Fig. 1 and Fig. 2) taken from a standard upper-secondary science reference book that students are allowed to use during the regular biology exams.

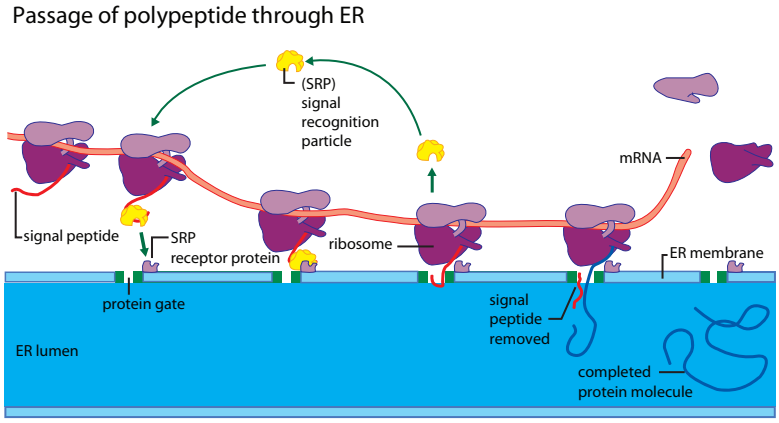


*Fig. 1: Graphical model of the mechanism of protein translocation as depicted in a students' standard reference book. (adapted from BINAS havo/vwo, 5th edition, Groningen: Wolters-Noordhoff)*

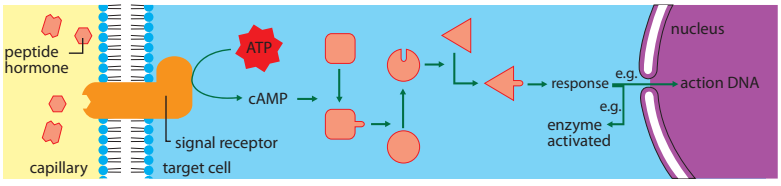


*Fig. 2: Graphical model of the mechanism of action of peptide hormones as depicted in a students' standard reference book (adapted from BINAS havo/vwo, 5th edition, Groningen: Wolters-Noordhoff)*

1. http://www.youtube.com/watch?v=OWUmXx5V_wE, narration muted.

2. A comparable assignment is used by Duncan & Tseng (2011) and they report that, prior to their intervention, the explanatory mechanisms that students provided did not include proteins and were based on human-like sense of smell or touch, and often unexplained or nerve/brain-like ability to signal and command. After their intervention however, students showed substantial gains in their understanding of the central role of proteins, providing explanatory schemas of how proteins contribute to the phenomenon appeared to be difficult.

# 2. Aim of the study

## 2.1. Focus of the study: proof of principle

We aim at stimulating molecular mechanistic reasoning to bridge the explanatory gap between the molecular and cellular level. From the above, we conclude that mechanistic reasoning in general will not be the main problem since it is rather intuitive, but that students lack an intelligible account for proteins interactions, molecular modules and molecular organization. Our hypothesis is that if they are trained to recognize and use these domain-specific aspects in their reasoning, they are capable of constructing meaningful mechanistic explanations for cellular phenomena through applying general mechanistic reasoning strategies namely top-down reasoning, causal chaining and bottom-up reasoning. This hypothesis is tested through the development and subsequent testing of a series of lessons based on molecular mechanistic reasoning.

## 2.2. Research question

*Can students in upper-secondary education learn to use molecular mechanistic reasoning to bridge the explanatory gap between (sub)cellular activities and molecular interactions?*

We approach this question from three perspectives successively:

1. *Can we design and effectuate a learning trajectory that guides students meaningfully through the multi-level mechanistic relationship between cell activities and molecular interactions?*
2. *Does the learning trajectory stimulate students to use molecular mechanistic reasoning when they interpret and construct explanations for (sub)cellular activities?*
3. *Do students experience molecular mechanistic reasoning as helpful to connect the molecular and cellular level concepts?*

For the first perspective, we describe and evaluate the design and the outcomes of the lesson series. We want to know whether the lessons in which general mechanistic reasoning strategies are applied to the domain-specific characteristics of molecular mechanisms do indeed guide students meaningfully through the multi-level mechanistic relationship between cell activities and molecular interactions. For the second perspective, we use the assignments that were presented in the previous section. Each assignment asks for different aspects of molecular mechanistic reasoning and together they provide an overall picture of students' tendency and ability to apply molecular mechanistic reasoning. As a comparison, assignment 1 is used before and after the lessons. For the third perspective, we focus on students' metacognition on molecular mechanistic reasoning, using responses in interview and reflection sessions during and after the lessons. In these sessions, students are explicitly asked to express their thoughts about the meaning, value and usefulness of the lessons.

# 3. Towards a learning trajectory to bridge the gap

To see whether molecular mechanistic reasoning is within reach for upper-secondary science students, we developed a series of lessons that guide students through the construction of molecular-level explanations for cell activities. This paragraph describes the guidelines which led to the designed learning trajectory. The guidelines concern:

- using mechanistic reasoning strategies to guide students
- the role of the teacher
- the role of visual literacy in the lesson series
- the examples used in the lesson series.

## 3.1. Using mechanistic reasoning strategies to guide students

Mechanistic reasoning strategies can be recognized in the work of experts (Bruggeman & Westerhoff, 2007; Darden, 2006; Van Mil et al., 2013), but the main argument to use the reasoning strategies as a meaningful guide in teaching is that these strategies are based on the intuitive notions of parts and wholes and causality (Grotzer, 2003), and therefore can be called upon also in novices to explore unfamiliar mechanisms. The strategies include:

*1. Top-down*: Downward reasoning starts with an entity or activity and searches for relationships with underlying activities or entities. Typical questions in downward reasoning are: What does it consist of? What parts are involved? How could it be established? What is needed to accomplish this? Two downward reasoning strategies that can help to specify the search for underlying parts and activities are: (a) subdividing activities into the lower-level partial activities and (b) hypothesizing mechanisms based on analogies.

2. *Bottom-up*: Upward reasoning starts with an entity or activity and searches for relationships with higher-level entities and activities. Typical questions in upward reasoning are: What is it part of? What role does it play? How does it contribute?

3. *Causal chaining*: Causal chaining entails reasoning within one level in the mechanisms. It starts with an entity that displays a certain activity and searches for causal relationships with preceding and subsequent activities. Typical questions are: What happens next? What happened before? What things were involved in causing this? What things are affected by this?

The design should do more, however, than just follow these general mechanistic reasoning strategies. Previously we argued that difficulties to apply molecular mechanistic reasoning will not be caused by mechanistic reasoning as such, but by a lack of domain-specific knowledge 'at the bottom', related to protein interactions, molecular modules and molecular organization. The design will have to provide this knowledge. The design therefore contains the three phases (top-down, exploring the bottom and bottom-up) that together form a learning path in which students explore the construction of molecular mechanistic explanations for cell activities. In

this path, the domain-specific characteristics of mechanisms in the cell are introduced only at moments that students encounter that their existing knowledge is insufficient to construct meaningful mechanistic explanations (Klaassen, 1995).

## 3.1.1. Phase 1: Top-down

### 3.1.1.1. Start at the level of the organism

The lesson series can be characterized by a sequence of logical steps that need to be taken by students to fill the gap between molecular interactions and cellular activities. In phase 1, we start with a top-down approach to familiarize students with asking how-questions in search for underlying explanations. The first step in this phase is that students descend towards the cellular level, taking phenomena at the organism level as a starting point. For students, we define the cellular level by using the term 'cell activity': an activity that can be assigned to cells using the statement: the cell (verb). For instance: the cell divides, the cell dies, the cell produces insulin, etc. This first step intends to raise students' awareness that they can use prior knowledge and logical reasoning to identify that cell activities are underlying activities in phenomena at the organism level. This is in line with the work of Knippels (2002) and Duncan and Tseng (2011), who started at the organism level with the aim to descend to lower levels.

### 3.1.1.2. Confront the knowledge gap

The next step in the top-down phase invites students to descend further, using two top-down strategies: subdividing and hypothesizing. The expected result of this step is that students realize that many cell activities identified in the first step cannot be explained using their prior knowledge about the parts in the cell. Students know that cells consist of organelles and cytoplasm, but these entities will not help them to explain cell activities such as 'cell division' or 'cell death'. In this way, the downward reasoning strategy of assigning activities to underlying structures that was helpful to descend from the organism level will now show its limitations. An example of this is that students know that 'mitosis' is part of 'cell division', but they cannot name a specific part of the cell that can be held responsible for mitosis. It is thus an explicit goal in this phase to confront students with the fact that the knowledge and explanations about (sub)cellular activities they have relied on so far are not sufficient to provide an intelligible explanation for most of the cell activities they came up with. This resembles the problem-posing approach developed by Klaassen (1995), which is based on the idea that students should be brought in a position in which they want to extend their knowledge in a certain direction, because they need it to solve a question or problem that they experience as relevant. In this case, students realize that to *explain* the cell activities they identified, they need to extend their knowledge to unknown 'smaller' entities and their activities in the cell.

## 3.1.2. Phase 2: Exploring the bottom level

### 3.1.2.1. Provide knowledge on proteins and molecular dynamics

Since the students experience the limitations of the downward reasoning strategy at the end of phase 1, the beginning of the second phase is to offer them an alternative approach: determine

a bottom level that can be used as a starting point for bottom-up reasoning to fill the gap. However, it will not be self-evident how the level of molecular interactions can be used as a basis for this bottom-up reasoning. Although students know that cells consist of molecules, their perspective on molecular interactions is limited. They know from chemistry classes that molecular interactions are the basis for chemical reactions that lead to new or altered molecules, but how changes in molecules can be the basis for activities at intermediate levels between the micro and the macro is an aspect that is hardly addressed in upper-secondary chemistry education (Meijer, Bulte & Pilot, 2009)

Therefore, in this phase students are familiarized with the molecular dynamics principles and we introduce 'colliding, binding and changing shape' as an account for cause and effect of protein interactions. This means: proteins and other molecules move and collide frantically (if not attached to other structures). If the molecules fit (which is determined by the shape and the spatial distribution of chemical groups) they bind, and when they bind a reshuffling of chemical bonds takes place, which changes the shape and thus the binding properties of the molecules involved. This change in shape allows for new interactions that were not possible before. To understand this account, students need to be aware of a number of physical and chemical principles that we call 'molecular dynamics principles'. The principles that we consider to be essential are:

- Brownian motion
- random walk
- molecular collisions
- molecular recognition
- conformational change
- self-assembly.

### 3.1.2.2. Chain molecular interactions into activities of proteins and protein-based modules

As the next step, we use causal chaining approaches to chain molecular interactions into activities of proteins. In this way, students experience how subsequent causal changes in molecules form the basis of activities that are commonly described as protein activity, such as an ion channel transporting ions, a receptor detecting a hormone or an enzyme catalysing the conversion of glucose. Next, the same causal chaining approaches are used to explore the interdependency between proteins. The change that is the effect of one protein activity can be the cause for the next. In this way the concept of protein-based modules is established and students see that with the same causal chaining approach the interdependency between modules can be explained.

An essential element in mechanisms is the organization of parts and their activities. In the case of molecular mechanisms, the previously described molecular dynamics principles are fundamental for understanding the organization of molecular and modular entities and activities in the cell. We suggest that the 'colliding, binding and changing shape' account can make intelligible to students how temporally and spatially ordered activities can emerge from collisions of molecules, which provides the fundament for reasoning about the organization in molecular mechanisms[3]. As a result of phase 2, students understand how complex activities can emerge from the exact same basis of the 'colliding, binding and changing shape' of proteins and other molecules.

## 3.1.3. Phase 3: Bottom up

### 3.1.3.1. Gradually increase in complexity

In phase 3, bottom-up reasoning will be used to explain cell activities, thereby closing the gap between molecular interactions and cell activities. However, cell activities differ widely in terms of complexity. Some cell activities that students are familiar with can be explained from the activity of a singular protein. For instance, cells excrete ions because protein pumps in the cell membrane pump ions out of the cell. Here, explaining the protein activity suffices to explain this cell activity in terms of molecular interactions. However, this holds for only a fraction of the cell activities that students are familiar with. Therefore, we choose three levels of increasing complexity to show that, in all cases, the same bottom-level principles apply regardless of increasing complexity. In addition to an example in which the activity of one protein can explain the cell activity, we show how in some cases the cell activity can explained from the activity of a multi-protein module and how in the third case the cell activity can be explained by combining the activities of multiple protein-based modules. By using these three complexity levels, we expect to make intelligible to students that interactions between proteins are the basis for cell activities and that entities and activities at intermediate levels, such as protein-based modules, are used to handle complexity.

In Fig. 3 we present an overview of the phases that we identified to allow students to experience step-by-step how to use molecular mechanistic reasoning to bridge the gap between cell activities and molecular interactions. It displays for every phase the connection to be sought for and the reasoning strategies used to explore these connections.



3. Note that organization of proteins and protein activities is essential information in molecular mechanistic explanations, but that these organizational aspects need not be explained *per se* in order to use them in the explanations. For instance, it is not indispensable to know how a receptor ended up in the cell membrane, in order to use its localization to explain how a cell responds to a hormone. Interestingly, many of these organizational aspects give rise to new mechanistic questions (e.g. about protein sorting), often closely related to questions about development and cell specialization.

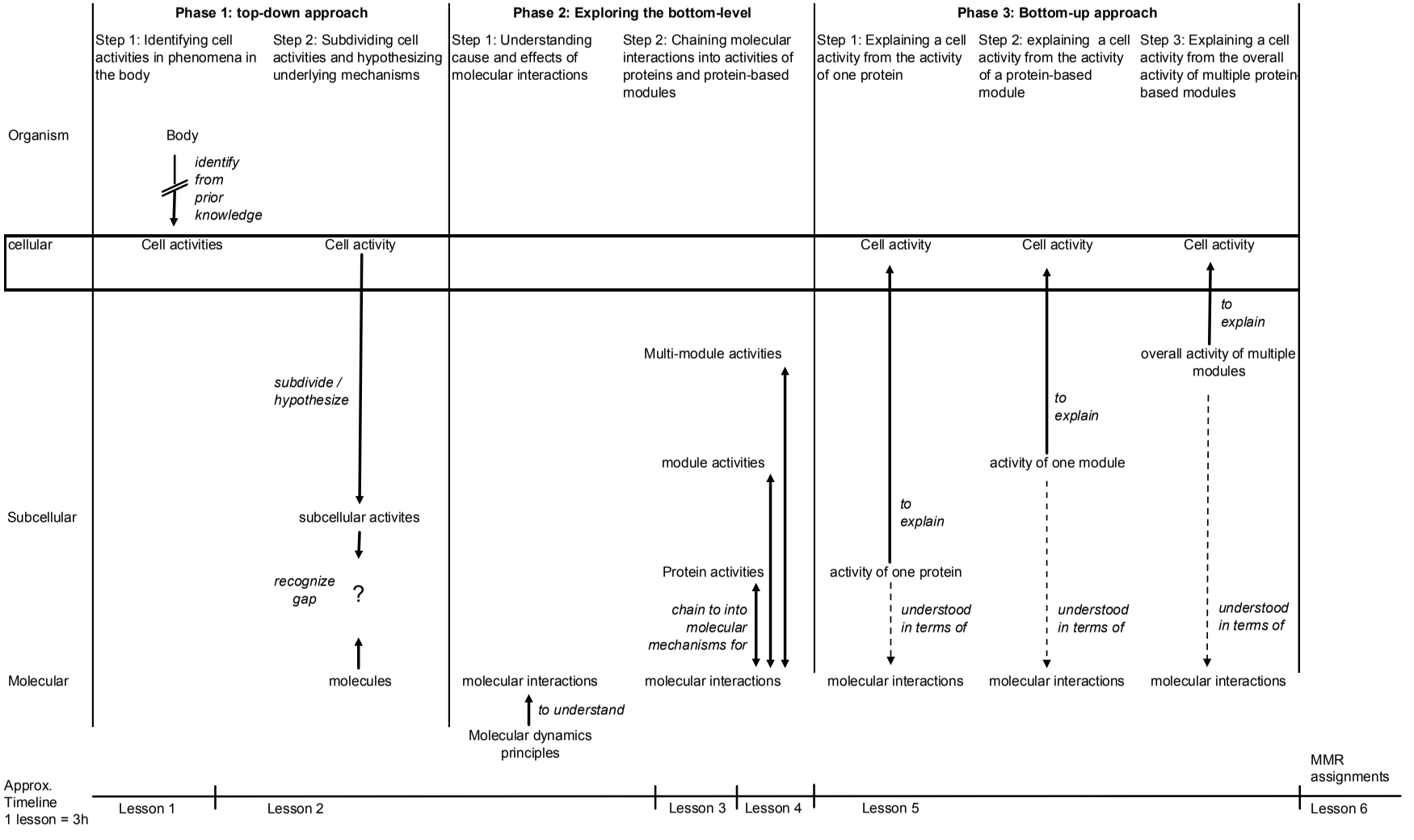


*Fig. 3: Schematic overview of the reasoning strategies and connections between levels sought for in each phase in the design*

## 3.2. The role of the teacher

It will not be self-evident to students that the goal of the lessons, i.e. understanding the multi-level mechanistic relationship between (sub)cellular activities and molecular interactions, is within their reach. We expect that they are used to accept (sub)cellular activities as given facts, without questioning the underlying molecular mechanisms (Abrams & Southerland, 2001). Therefore, the first important role of the teacher in these lessons is to constantly challenge the students to pose how-questions over and over again. It is to be expected that students experience some discomfort when the role of the teacher is not 'providing the correct answers' but 'raising new questions'. Secondly, the teacher must demonstrate and explain the reasoning steps that she expects the students to be able to take when reasoning about these how-questions. Students probably will not feel equipped to contribute to answering these questions, so the role of the teacher is to guide this process by modelling and scaffolding the reasoning steps that are within reach for the students at that stage in the lesson series. This role resembles the cognitive apprenticeship approach of Collins, Brown, and Newman (1989).
Each of the phases in the design consists of four subsequent roles of the teacher and associated teaching activities, based on the cognitive apprenticeship approach (Collins *et al.*, 1989):

- *Orientation*: the teacher offers a perspective on the progression that students will make in this phase. She emphasizes the endpoint of the previous step and helps students to formulate the question that they will work on to make the next step.

- *Modelling*: the teacher demonstrates the reasoning strategies needed to make this step and offers the content knowledge needed to handle these strategies. In doing so, she explains her thinking and encourages the students to join him in his reasoning.

- *Scaffolding*: the teacher guides the students in practising the reasoning strategies and applying the content knowledge needed, either by verbal instructions and questions or by hints and guiding questions in assignments

- *Articulation*, *reflection and exploration towards the next step:* the students express in their own words the reasoning strategies they used to make the step. The teacher helps the students to look back on the starting point of this step and to reflect on how the reasoning strategies contributed to the progression they made. Then the teacher helps students to identify the questions that remain to be explored in the next step.

The teacher roles are here presented in a sequential order. However, at some moments in the trajectory, the modelling and scaffolding role of the teacher will alternate a few times within one phase, for instance in phase 1, when two downward reasoning strategies are used subsequently.

## 3.3. The role of visual literacy in the lesson series

Mechanistic models provide an explanation for an activity by showing the organization, interaction and causality in underlying entities and activities (Darden, 2007). In molecular and cell biology, visual models are often used as a simplified way to display mechanisms in the cell. The scope of these visual models can range from displaying simplified sketches of very complex

cellular activities to visualizing the most detailed bottom-level molecular changes. If we want our students to reason mechanistically about (sub)cellular activities, this entails being able to read and use the visual language that is used to communicate about these mechanisms, at least to a certain extent. We suggest that, to encourage mechanistic reasoning about (sub)cellular activities, modelling and scaffolding the interpretation of molecular mechanistic models is an indispensible element in the lessons. This scaffolding should be combined with encouraging students to reflect on the way that the general elements of mechanisms (entities, activities, interactions, causality, organization) as well as the molecular principles (movement, crowding, conformational changes) are or are not depicted in the models.

The visual models used in the lessons are either static cartoon-like diagrams or animations. Both have their strengths and their weaknesses (Gilbert, Reiner & Nakhleh, 2008; McClean et al., 2005; McGill, 2008). Here we focus on strengths and weaknesses related to encouraging molecular mechanistic reasoning. Obviously, in an animation it is easier for the learner to recognize changes in time and space. In static mechanistic models, arrows or other visual conventions need to be used, which can be confusing for the learner, for instance because arrows can either mean changes in time or space or both (Heiser & Tversky, 2006). On the other hand, the seemingly very realistic representations of (macro)molecular entities in many modern molecular movies can result in students accepting the model as a realistic display, without questioning the simplified or even non-depicted mechanisms and principles underlying the displayed events. Furthermore, in static representations, the learners can determine their own pace and go back and forth between start-up and termination conditions since all the aspects in the model stay accessible constantly, whereas in animations it is more difficult to interpret all the information as a coherent whole. In the lessons we use both types of models when encouraging molecular mechanistic reasoning. To allow students to interpret both type of models and to recognize that the same type of events are displayed, students first need to be familiarized with the multiple ways that proteins are represented. In the 3D molecular animations we use, proteins are mostly depicted using molecular surface representations. This way of representing proteins emphasizes the complex compositing and structure of proteins, and it allows for showing very realistically the influence of interactions on the structure of proteins. The cartoon-like graphical schemes (such as Fig. 1 and Fig. 2) represent proteins with simplified geometrical shapes. This allows for depicting the chain of molecular events in one scheme without too much detail. However, in these static cartoon-like models, almost all of the molecular dynamics principles need to be inferred by the students. Because the 'colliding, binding and changing shape' account is grounded in the molecular dynamics principles, we start with animations in which these molecular dynamics principles are displayed clearly. Once students master this account as a basis for protein interactions, we introduce animations in which these principles are depicted less obviously or even in a misleading way and students are encouraged to reflect critically on the these animations. The most demanding step from the perspective of molecular mechanistic reasoning is interpreting the cartoon-like schematic representations of mechanisms. Not only do almost all of the molecular dynamics principles need to be inferred by the students, but also very often entities and activities that are needed for a intelligible mechanistic explanation are simply not depicted. By encouraging molecular mechanistic reasoning, we hope that students start to recognize these gaps in visual models of molecular mechanistic explanations. Thus, the use of visual models is not only inevitable in our strategy because these models provide the mechanistic explanations central in the lessons; working with the models also shows students

that molecular mechanistic reasoning is a crucial competence to give meaning to these types of molecular -level representations. In other words, molecular mechanistic reasoning contributes to a domain-specific visual literacy by providing a framework that applies to all models of protein-based mechanisms, regardless if the representation is static and schematic or dynamic, three-dimensional and highly stylized.

## 3.4. The examples used in the lesson series

When we presented the phases in the lesson series, we concluded that we can show students the relationship between cell activities and molecular interactions at three levels of complexity. The cell activity can be explained from the activity of:

- one type of protein
- one molecular module
- multiple interdependent molecular modules

This means that the most coherent way to design the lesson series is to choose three examples representing these three different degrees of complexity that can guide students through all the steps of the lesson series: starting with a phenomenon at the level of the organisms, identifying cell activities involved in this phenomenon and then trying to explain these cell activities in terms of molecular interactions using molecular mechanistic reasoning. By choosing three different examples and applying the same steps to every example, we can show that the examples differ in the degree of complexity, but that the same principles apply and therefore the same downward and upward reasoning strategies can be used. We used three examples in which students identified cellular activities with the goal to find out how these cellular activities can be explained based on protein interactions. The three examples can be found in Table 1.
In phase 1, all three phenomena are introduced. Step 1 starts with the teacher modelling top-down reasoning the symptoms in the case of cystic fibrosis (CF) and familial hypercholesterolaemia (FH) to identify the malfunctioning cell activity 'excreting chloride ions' and 'taking up LDL-cholesterol'. Wound healing is introduced after students have practised top-down reasoning with

*Table 1: The exemplary cell activities that students identify from cystic fibrosis, familial hypercholesterolaemia and wound healing represent three different levels of complexity in mechanistic explanations*

| *Phenomenon* | *Cell activity in healthy individuals* | *Complexity level* |
|---|---|---|
| Cystic fibrosis | Mucous cells excrete chloride ions | One protein explains the cell activity |
| Familial hypercholesterolaemia | Liver cells take up LDL-cholesterol | The cell activity can be explained from the activity of a multi-protein module |
| Wound healing | Fibroblasts secrete collagen when stimulated with the hormone TGF-β | The cell activity can be explained from the combined activities of multiple protein-based modules. |

LDL = low-density lipoprotein; TGF = transforming growth factor.

some other disease-related phenomena, namely cancer, HIV/AIDS and diabetes. The motive for students to use top-down approach in these examples is that they try to reconstruct the focus that scientists choose when studying diseases: e.g. in studying cancer they focus on cell division, in studying diabetes they focus on the production of insulin, etc. The goal is that students recognize that phenomena such as wound healing comprise many cell activities that all could be focus of scientific research. In step 2, the teacher continues on 'cell division' that was identified as one of the activities in wound healing and models top-down reasoning to identify subcellular activities by subdividing 'the cell divides' and by hypothesizing mechanisms that could explain the transport of mitochondria. Students apply this hypothesizing strategy on CF. They formulate mechanisms that could explain the secreting of chloride by using the metaphors of a real-life pump. The motive for students to apply top-down reasoning to descend further into the cell is that they feel that just identifying cell activities is not a satisfactory explanation for the disease. At the end of phase 1, students conclude that they cannot explain 'secreting chloride', 'taking up LDL-cholesterol' and 'dividing' satisfactorily with their prior knowledge about cell organelles. Additional knowledge is needed about entities in the cell that can serve to explain the cell activity. This forms the motive to 'jump' to the bottom to learn about the bottom-level entities and activities. Students realize that this knowledge may help them to approach the problem from the other side by building the explanation bottom-up.

In phase 2, the three examples do not play a prominent role. Activities of protein and protein-based modules are presented and explained in terms of molecular interactions rather isolated from the three examples. The bottom level of molecular interactions is presented as a basis for constructing explanations for cell activities, but constructing these explanations will only be done in the next phase. Nonetheless, most protein and module activities that are chosen to explain in detail play a role in (sub)cellular activities which are familiar to students, and the animation entitled 'Inner life of the cell' is used to show proteins and protein activities in their cellular context. The motive for students to continue to the next phase is the fact that after this phase they still do not know how to explain CF, FH or wound healing although they are offered a new perspective: bottom-up reasoning.

In phase 3, the gap between the identified (sub)cellular activities in the three examples and basic principles of molecular interactions is closed, starting with the simplest example, CF. In CF, one protein activity can explain the cell activity. However, students realize that that cannot be the case in all cell activities. The case of FH seems to be more complex. Although scientists have identified one protein malfunctioning in most patients, the activity of this protein cannot explain the cell activity. At this point, students see that they need to combine the activity of multiple proteins into one modular activity to explain how LDL-cholesterol is taken up by liver cells. The example of wound healing is the most complex. Not only many cell activities are involved, but even if students focus on explaining one of these activities, they see that multiple modular activities have to be combined to explain how fibroblasts start excreting collagen when stimulated with the hormone TGF-β.

Fig. 4 shows the general scheme that is used in phase 3 to identify the gap to be filled in the explanation of the three phenomena. In the lessons, the three examples serve as a context to explore the more general question 'how do cells work?' and we expect that phase 2 in particular makes clear that the principles and concepts are more widely applicable than just these three disease-related phenomena. As part of research question three, the student interviews will be used to evaluate if indeed students feel that what they learned has wider applicability.

## 3.5. Outline of the activities in the lesson series

In the results section, we describe for each phase the rationale in the intended trajectory and we indicate crucial learning activities in the design. A detailed description of all activities, including an overview of how the activities are sequenced in the modelling, scaffolding and reflection phases of each step, is available on request.

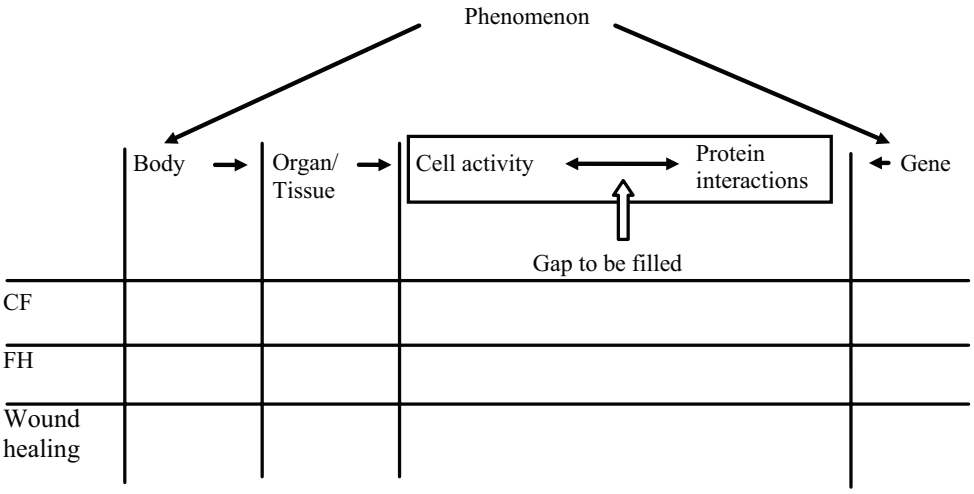


*Fig. 4: The general scheme used in the lessons and the gap that is filled in the three examples*

# 4. A small-scale test of the trajectory

In the empirical part of this study, we focus on a small-scale test round in which we search for a proof of principle to answer the question: *Can students in upper-secondary education learn to use molecular mechanistic reasoning to bridge the gap between (sub)cellular activities and molecular interactions?* . With this test round, we want to find out whether and how the theoretically established trajectory can be enacted in practice, whether the goals are within reach for students and whether the students experience the trajectory as building a meaningful bridge between the molecular and the cellular level. With this test round, we want to find out whether and how the theoretically established trajectory can be enacted in practice, whether the goals are within reach for students and whether the students experience the trajectory as building a meaningful bridge between the molecular and the cellular level.

Our theoretical analysis shows that domain-specific knowledge and insights related to protein interactions, molecular modules and molecular organization are key elements in bridging the gap and we claim that these aspects are currently not sufficiently explained in most biology curricula. Therefore, we first want to know if these new goals are meaningful and within reach for students in upper-secondary education. It is to be expected that not only students but also many teachers are unfamiliar with using these elements in their teaching about molecular concepts. Because in our design, the teacher has a specific role in modelling explicitly the questions and reasoning central in each step, we decided to first test the design in a somewhat artificial educational setting in which the teaching was done by the principal researcher (first author), who is the designer of the lesson series and an expert in the field of molecular cell biology. By doing so, we diminish distortions due to transfer to a teacher who is yet unfamiliar with the aims and focus of the trajectory. However, we are aware that this limits the study, because it leaves open questions about the transferability and usability by teachers who have not been trained to recognize, explain and reflect on molecular mechanistic reasoning with their students. This can be considered a possible area for further research if indeed molecular mechanistic reasoning seems to be feasible and helpful for upper-secondary students in life science education.

## 4.1. Participants and set up of the lessons

The lessons were part of a project in which schools from the region of Utrecht, the Netherlands, were invited to allow some of their pre-university science students to participate in innovative science modules offered by Utrecht University. Teachers in the participating schools invited students from their interdisciplinary science course 'Nature, Life and Technology', an optional course in most Dutch upper-secondary science curricula, to participate. Students who volunteered could choose between three courses offered, of which our lesson series called 'The Molecules of Life' was the only life science module (the others were physics modules). Twelve students (nine girls and three boys) from five different schools chose to participate. The science curriculum of all students included the regular biology and chemistry classes. At the beginning of the lessons, the students did not know most of the other students, they did not know the teacher and the university was an unfamiliar environment to them.

For 6 weeks, the students came to Utrecht once a week for a 3-hour lesson. When working on assignments, most students worked in pairs from the same school. Five lessons were used for the teaching activities in the trajectory and in the sixth lesson students worked individually on the

assignments. Next to serving a research goal, the assignments were used to grade the students if this was requested by the school.

## 4.2. Data collection and analysis

Multiple data sources were used.

- Observations during all the lessons, guided by the detailed lesson plans that include the scenario for the intended trajectory. Observations were done by a second researcher (second author), who was not involved in the design and teaching of the lessons.

- Video and audio recordings of the lessons, including audio recordings of all the conversations between two selected pairs of students.

- Students' completed worksheets. During the lessons, students used provided worksheets to write down their answers individually or in couples. Each student collected the worksheets in an individual workbook that was kept by the teacher until the next lesson. The molecular mechanistic reasoning assignments before and after the lesson series were filled out individually on provided worksheets.

- Transcripts of semi-structured interviews with students performed during the third and sixth lessons. The 30-minute interviews were video- and audiotaped and transcribed verbatim. The interview protocols can found in the appendix. Eight students were interviewed in pairs from the same school by the second researcher, about the following aspects:
  - their prior knowledge
  - their perception and appreciation of the different learning activities
  - their understanding of central terms introduced the lessons, such as cell activity, modular activity and protein activity
  - their ideas about the terms for reasoning strategies used in the lessons such as downward, upward, backward-forward, subdivide.

- Video and audio recordings of a 30-minute evaluative group discussion with all students guided by the teacher after the lessons. The following aspects were discussed.
  - what students learned in the lesson series
  - whether students found the lessons valuable
  - how the students' image of the cell changed.

As mentioned in the 'aim of the study', we approach the research question from three perspectives and the different data sources play different roles for each perspective. In Table 2, the data sources for each perspective are shown.

*Table 2: Data sources used for each perspective*

| *Perspective* | *Observations* | *Recordings of the lessons* | *Completed worksheets* | *Interview transcripts* | *Recordings of evaluative group discussion* |
|---|---|---|---|---|---|
| 1: Trajectory | X | X | X | X | |
| 2: Use of molecular mechanistic reasoning | | | X | | |
| 3: Metacognition on molecular mechanistic reasoning | | X | | X | X |

For the first perspective, the designed and effectuated trajectories are compared. To see where theory and practice diverge, we need to provide detailed and coherent descriptions and reflections on the intended and observed progression in the trajectory. For the second perspective, students' use of molecular mechanistic reasoning is analysed. For this, we focus on the assignments at the beginning and at the end of the lesson series. For the third perspective, we interpret students' statements about their perception of the learning trajectory. In the following section, we describe for each perspective how the different data sources were used.

### 4.2.1. Perspective 1: The learning trajectory

The perspective in this part is: Can we design and effectuate a learning trajectory that guides students meaningfully through the multi-level mechanistic relationship between cell activities and molecular interactions? Therefore, in the first part of the results section, we report about the intended path and outcomes in each step in the lesson series. For each step we answer two questions:

*A: How was the intended path in this phase designed and executed?*

*B: How did students progress through this phase?*

To answer question A, the intended trajectory that was elaborated in the lesson plan was compared to the actual execution. The teacher and observer discussed the observed differences after each lesson. If necessary, adaptations for the next lessons were made to execute the scenario for the lessons with the highest possible fidelity.

To answer question B, both the actual observable outcomes as well as students' expressed perceptions and understanding of the activities and strategies in the lessons are used. Observable outcomes were captured in students' writings and drawings in the completed worksheets as well as students' questions, remarks and answers in the lessons. Students' perceptions and understanding were captured in the interviews as well as in their video- and audiotaped responses during the lessons.

In the analysis, the intended outcomes as formulated in the designed trajectory (question A) were guiding. Depending on the intended outcome, the most obvious data source was chosen to start the analysis. For instance, for the outcome in phase 1 'students can mention cell activities in general terms', we chose students' worksheets as the most direct indication because students worked on assignments that explicitly asked to formulate cell activities. However, the outcome

in phase 3 'students realize that in principle all cell activities can be explained in terms of molecular interactions' appeared to be best evaluated by starting with students' responses and questions in classroom discussion. In all cases, we moved back and forth between the different data sources to get the most accurate evaluation for each outcome. For instance, classroom observations and video data were used to interpret whether responses during the interviews were specific for one student or that it applied to more students. In other cases, answers in students' worksheets, guided us towards analysing specific moments in the video data in search for better understanding or more general patterns in students 'reasoning. In general, we aimed to capture for each expected and unexpected outcome students' quotes, questions and answers that were informative to better understand the reasoning path that (one or more) students took, thereby revealing strengths, difficulties, and unforeseen effects in our design. Given our goal to gain better understanding of the opportunities and pitfalls in the trajectory, both general tendencies shown in most students, as well as ideas and confusions of individual students can be very informative. In the results section, we alternate between these types of results in an attempt to provide the most insightful aspects of the trajectory in that phase.

Here we show with an example how the analysis processes worked. In the results section of phase 2, we conclude that after, explaining protein activities in terms of 'colliding, binding and changing shape', students already expected that multiple proteins can cooperate to perform higher-level activities. How did we come to this conclusion? The analysis starts with the intended outcome as formulated in the intended trajectory: 'Students understand how molecular interactions are the basis for protein activities, module activities and multi-module activities'. In the design, we first identified cell activities and then 'jumped' to explaining protein activities. The term 'molecular modules' would only be introduced later and we did not expect that students would infer higher-level activities already from explaining individual protein activities. However, we noticed in the data from interview I, collected directly after working on individual protein activities (lesson 3), that, in all four interviews, students indicated that they expected that after understanding proteins they would learn how proteins can cooperate. For instance:

*Interviewer: And working your way upward, what do you mean with that?*

*Monica: Well, now we look at … eh … Well, for instance you have a protein and then each time you look at a larger level. So, one protein, and then proteins that cooperate and then larger each time.*

and

*Interviewer: What do you mean with looking at it from the other side?*

*Mike: If you have cell activities (left hand moves to left) at the one hand and protein activities at the other hand (right hand repeatedly to right). With cell activities you start thinking smaller and smaller. With protein activities you start thinking larger and larger. What does the protein do that relates to the cell activity?*

From the interview fragments, we conclude that students expect that upward reasoning entails understanding how proteins at the bottom cooperate to form 'larger activities'. However, these expressed expectations can be based merely on the explicit use by the teacher of terms such as 'bottom level' and 'upward reasoning'. It does not necessarily mean that this expectation is based on students' understanding of how proteins work. To get better insight if this expectation

was also (partly) inferred from the 'colliding, binding and changing shape' account, we looked back in the video data to check students' responses when explaining singular protein activities. We specifically looked for chaining approaches with which students question or discuss the events before or after individual protein activities. We identified several of these moments, most of which occurred when watching the animation 'Inner life of the cell'. For instance, when the teacher showed that the disintegration of protein fibres in the cytoskeleton can be explained from the successive change in shape of the fibre-proteins, Mike suggested that this cascade might be triggered for instance by a protein that cuts the fibre. These moments in which students indicate that they expect interdependency of protein activities complement our first findings from the interview, which led to our statement: 'after explaining protein activities in terms of "colliding, binding and changing shape", students already expect that multiple proteins can cooperate to perform higher-level activities.'

### 4.2.2. Perspective 2: Students' use of molecular mechanistic reasoning

In the second part of the results section, we analyse the assignments that students worked on at the start and at the end of the lesson. We use these assignments for our second perspective: *Do students use molecular mechanistic reasoning when they interpret and construct explanations for (sub) cellular activities?* Assignment 1 (the neutrophil movie) is used twice in the lesson series. At the start of lessons students work on the assignment, and at the end the exact same assignment is repeated. During the lessons, the assignment is not discussed, nor do students know that the same assignment will be repeated at the end of the lesson series. Since assignment 1 is also used as a starting assignment at the beginning of the lessons, we can compare students' tendency and ability to use molecular mechanistic reasoning at the start and at the end of the lesson series.
In students' completed worksheets, we look for the molecular mechanistic reasoning and, in the case of assignment 1, we also look at the differences between the first and the second time that students worked on the assignment. In the introduction, we identified the elements of molecular mechanistic reasoning as a domain-specific translation of the general Machamer, Darden and Craver account for mechanistic explanations. Russ et al. (2008) used this account to recognize elements of mechanistic reasoning in a physics classroom discussion. Our approach of recognizing molecular mechanistic reasoning resembles the approach of Russ et al. However, instead of searching for general mechanistic reasoning elements as Russ does, we use our domain-specific translation of these elements because our claim is not that mechanistic reasoning as such is the problem, but that only when students are familiarized with the domain-specific aspect of mechanisms in the cell, meaningful mechanistic reasoning can occur. Therefore, our analysis questions are:

- *How-questions*: Does the student identify a (sub)cellular phenomenon to be explained and ask relevant how-questions about it?
- *Subdividing*: Does the student subdivide a (sub)cellular phenomenon functionally to identify underlying activities?
- *Hypothesizing*: Does the student hypothesize mechanistic schemas, for instance by using metaphors or comparisons?
- *Entities*: Does the student identify/hypothesize the involvement of proteins or protein-based modules?

- *Activities:* Does the student identify/hypothesize activities of proteins or protein-based modules?
- *Chaining*: Does the student link protein or module activities into causal chains or recognize gaps in the causal chain?
- *Causality*: Does the student apply 'colliding, binding and changing shape' as a basis for causality in the mechanisms?
- *Organization*: Does the student apply the molecular dynamics principles of molecular interactions as a basis for organization in the mechanisms?
- *Model*: Does the student combine entities, activities, organization and causality into a mechanistic model that accounts for a (sub)cellular phenomena?

As mentioned in the introduction, these elements are not called upon in the same way in each assignment. In the results section, we will first indicate for each molecular mechanistic reasoning element how it plays a role in the three assignments. Then we discuss for every the element if and how it appears in students' completed worksheets. Finally, we analyse if students use the element in coherence. Molecular mechanistic reasoning can be considered a domain-specific reasoning framework and it can be recognized in expert reasoning (Van Mil *et al* ., 2013). By looking at whether students make coherent use of the different aspects of molecular mechanistic reasoning, we can judge whether students (partly) adopt molecular mechanistic reasoning as a framework that helps them in their reasoning about cells.

### 4.2.3. Perspective 3: Students' metacognition on molecular mechanistic reasoning

In the third part of the results, we discuss perspective 3: *Do students experience molecular mechanistic reasoning to be helpful to connect the molecular- and cellular-level concepts?* ? Data for this part were mainly collected during the interviews at the end of the lesson series. In the interviews, students were encouraged to reflect on the three examples and indicate the differences in complexity of the cell activities under study. Furthermore, after the interviews a group discussion with all the students was arranged by the teacher. The discussion focused on students' ideas about cells and how this view changed during the lessons. In addition, students' ideas, comments and questions about cells or the role of molecules in the cells that came forward during the lessons were used.

# 5. Results

In three sections, we describe our empirical finding about the series of lessons. First we describe for each phase the intended path and the observed outcomes (perspective 1). Then, we report about the assignments that students worked on before and after the lessons to see how they use the different aspects of molecular mechanistic reasoning (perspective 2). In the final section, we report how students reflected in the interviews and classroom discussions on the use of molecular mechanistic reasoning to bridge the gap between cell activities and molecular interactions (perspective 3).

## 5.1. Perspective 1: The learning trajectory

### 5.1.1. Results of phase 1: Top-down approach

In phase 1, we apply top-down reasoning starting at the organism level. We aim to let students realize that with their current knowledge the top-down approach stops at the level of cells and cell organelles even though they know that cells consist of molecules. Identifying cell activities in phenomena in the body is the first step. The second step is subdividing these cell activities and hypothesizing underlying mechanisms

#### 5.1.1.1. How was the intended path in phase 1 designed and executed?

**Step 1**

In the modelling phase, the teacher shows students how to descend from symptoms in CF and FH via affected organs and tissues to the malfunctioning cell activities.

In the scaffolding phase, students apply the same strategy for other diseases, guided by the question 'What cell activities would you expect scientists to study when working on cancer, HIV/AIDS etc.?' All cell activities mentioned by the students are collected, and students are asked to group and reformulate these cell activities into a general list that covers all cell activities. Finally, the example of wound healing is explored to show that in many phenomena in the body several cell activities are involved at the same time, Students identify cell activities in wound healing in a wound healing animation[4].

The activities in this step take about 2 hours in lesson 1 and 1 hour in lesson 2.

**Step 2**

Starting from the list of cell activities, students experience two top-down strategies to descend further down from the cellular level to subcellular activities, guided by the question 'how do cells do this?' Fig. 5 shows how the teacher guides students through the subdividing of the activity 'the cell divides'. This example builds on students' prior knowledge such as about mitosis and DNA replication but also introduces new activities such as transport of organelles.

The teacher uses 'transport of organelles' to show how scientists use the second top-down reasoning approach: they use metaphors and comparisons to hypothesize about mechanisms

4. See http://www.youtube.com/watch?v=FraKUUetOpc

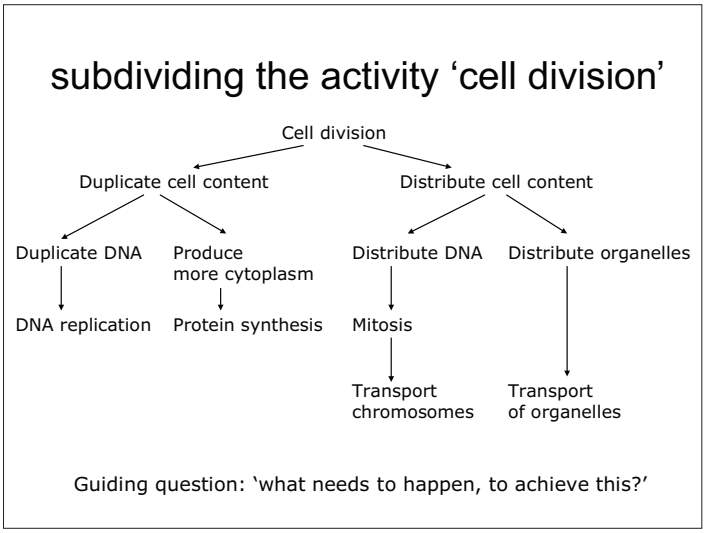


*Fig. 5: Scheme used in the lessons when applying the subdividing strategy to the activity 'cell division'*



that could explain the activity. These hypotheses can be based on comparisons or metaphors from familiar mechanisms, such as man-made devices. The teacher discusses that if something needs to be moved to a specific location, the mechanism could look like 'random move and catch', or 'guided by a track', and if it is guided it could be 'pushed on a rails' or 'pulled by a rope'.

Students practise the subdividing strategy by identifying activities that are part of mitosis in a standard graphical representation of the mitotic phases, guided by the question 'what needs to be done from one phase to the next?' The hypothesizing strategy is, for instance, applied to the example of CF. Students are asked: 'what could a mechanism look like that enables cells to pump chloride out of the cell?' Students are encouraged to make a comparison with a real-life pump and formulate 'design characteristics' for such a pumping mechanism.

In the reflection, students are asked if these strategies helped them to better understand 'how cells do things' and if they regard the identified cell activities as to be explained by the strategies in this phase. We expect that students recognize that still many how-questions remain and that they feel that the identified cell activities are not explained yet. Probably they know that molecules must be at the basis of the hypothesized activities, but they will not be unable to bridge the gap between molecular interactions and subcellular activities because of a knowledge gap on molecular interactions. The identification of this gap is the motive to 'jump to the bottom' in the next phase.

Step 2 takes a relatively small part of the whole design, covering 30 minutes in lesson 1, about 30 minutes for a homework assignment and 30 minutes in lesson 2. Hypothesizing is applied to the case of CF and FH and subdividing is mainly applied to aspects of cell division, being a cell activity in wound healing.

### 5.1.1.2. How do students progress through phase 1?

**Students' progression in summary**

The activities in step 1 enable students to apply downward reasoning from phenomena in the body to identify cell activities. They can describe in general terms the activities that can be attributed to individual cells. In some case, students mention activities that cannot be attributed to individual cells; these are in fact activities at a higher organizational level.

Students recognize that cell activities can be subdivided in partial activities, and they can explain why the terms 'descending' and 'downward reasoning' are used for this strategy. In graphical representations of cellular activities, students can identify partial activities that are directly visible, but they have trouble with inferring unrepresented partial activities by subdividing the cellular activity. Students use metaphors to hypothesize about an underlying mechanism. They come up with metaphors and comparisons themselves easily, but they indicate that they cannot judge if these comparisons make sense.

**Step 1 in detail**

*Students can apply downward reasoning from phenomena in the body to cell activities*

The activities in phase 1 are designed to promote downward reasoning from phenomena in the body to identify cell activities. When discussing CF, the step from mucus-producing tissue to cells that secrete chloride ions is scaffolded by asking students about the difference in composition between healthy and thick mucus. Students are able to conclude that composition differs mainly in water content. With some scaffolding questions from the teacher, students make a link to osmosis, from which they can conclude that a high salt concentration outside the cell is needed to create water transport out of the cell. The last step is provided by the teacher: in the case of CF, the cells lack the ability to secrete chloride ions to sufficiently promote water transport. From this, students conclude: in healthy individuals, mucus cells secrete chloride ions and in CF patients this cell activity is affected.

In the case of FH, the teacher starts with observable symptoms and complaints that patients might encounter (e.g. heart failure) before descending to the underlying causes: high levels of LDL-cholesterol in the blood caused by liver cells failing in the uptake of LDL-cholesterol particles. In this case, the switch in focus at the organ level from an 'error in the heart' to an 'error in the liver' does not surprise students. Although they cannot predict it themselves, they are perfectly aware that the activity of one organ can influence the activity of another. This relates to students' intuitive notion in mechanistic reasoning that we will also exploit when reasoning about molecular mechanisms.

*Students can mention cell activities in general terms*

Table 3 presents the cell activities formulated by the students in the examples of cancer, diabetes and HIV/AIDS. Taking into account that some activities are mentioned in more than one example (e.g. 'cells divide' is mentioned in all three examples), the 12 students came up with 21 different cell activities in this assignment.

When asked to come up with their own examples of diseases and disorders and then identify underlying cell activities, students are capable mentioning several cell activities (median 8 per student) as part of various disorders (median 3 per student). They have little trouble to distinguish between phenomena in the body and cell activities.

*Table 3: Cell activities mentioned in students' completed worksheets (N = 12)*

| *I expect that cell biologists working on … , study how cells …)* | | |
|---|---|---|
| Cancer | Diabetes | HIV/AIDS |
| Divide (12) | Produce insulin (11) | Neutralize or destroy (9) |
| Attach (9) | Build up and break down substances (9) | Recognize foreign agents (8) |
| Die (9) | Respond to hormones (8) | Take up (6) |
| Produce ATP (9) | Produce hormones (7) | Get infected by viruses (2) |
| Communicate (8) | Secrete substances (e.g. insulin) (3) | Defend and fight viruses (2) |
| Move (8) | Take up substances (3) | Protect against disease and pathogens (2) |
| Grow (2) | Produce and/or use energy (ATP) (3) | Break down (1) |
| Copy DNA (1) | Store substances (e.g. sugar/energy) (2) | Attach to HIV (1) |
| Respond (1) | Communicate (1) | Divide (1) |
| Take up and use nutrients (1) | Divide (1) | Grow (1) |
| Take up growth hormone (1) | | Move (1) |
| ~~Become malignant (1)~~ | | Pass on traits (1) |
| ~~Change (1)~~ | | ~~Produce blood (1)~~ |
| ~~Function as a cancer cell (1)~~ | | ~~Weaken immune system (1)~~ |
| | | ~~Loose viability (1)~~ |

Strikethrough = 'not a cell activity', based on: 'not assignable to a single cell' or 'not contributing to a healthy body'.

In both the provided examples as well as in their own examples, students formulate some cell activities that were marked in the analysis as 'not a cell activity' (strikethrough in the table). These concerned two types of activities: (1) the activity cannot be attributed to individual cells, e.g. 'producing blood' and 'digesting food', and is in fact an activity at a higher organizational level; and (2) the answer does not describe an activity that is functional in a healthy body, e.g. 'become malignant' and 'weaken the immune system'. Although these answers provide a correct completion to the provided sentence, they do not fit in a list that describes cell activities that contribute to the healthy functioning of the body.

To help students to distinguish between cell activities and higher-level activities, teacher and students together compose a list of cell activities in two steps. First, the teacher collects all the cell activities that students come up with in their own examples and if necessary reformulate them in terms of scientifically accepted cell activities: e.g. 'cells clump together' (in wound healing) is reformulated as cells 'migrate' and 'attach'. Then, students are asked to extract the

general terms that cover all the activities they have. This resulting list of 15 general cell activities can be found in Table 4.

Students are encouraged to add more activities to the list, but their previous assignment already covered all the terms they could think of. When analysing the animation of wound healing, students manage to recognize all the cell activities from the list in one or more stages of wound healing (Table 4).

Students experience the approach as a way to connect knowledge at different levels: *teacher just states that cells divide, it is less easy to understand why a cell divides. It all relates to each other.'* (Monica, classroom discussion). Moreover, students indicate that a first step in the process of constructing an explanation for phenomena visible at the organism level, requires the subdivision of the phenomenon into smaller (underlying) parts by formulating and answering how-questions. . *'From choosing diseases, we continued with asking how-questions: how is the disease manifested [at the organisms level], what activities play a role in the cell. Thus we went working our way towards smaller levels. The cellular level, eventually'* (Monica, interview I). Students indicate that they understand how to apply the approach. However, they state that they are not used to approach biological topics in this way and they experience it as being encouraged to think for themselves. *'Here, you want to know "how" it happens and in biology class you just accept that it happens.'* (Jody, interview I).

*Table 4: General cell activities with examples from wound healing as mentioned in the classroom discussion*

| *General terms for cell activities* | *Cell activities recognized as part of wound healing* |
|---|---|
| Cells recognize … | Immune cells recognize bacteria |
| Cells take up … | Immune cells take up bacteria |
| Cells store … | Red blood cells store oxygen |
| Cells break down … (= 'burn' in case of sugar, etc.) | Cells break down collagen |
| Cells build up … | Fibroblasts build up collagen |
| Cells produce … | Cells produce growth factors |
| Cells secrete … | Cells secrete growth factors |
| Cells regulate … | Cells regulate growth of blood vessels |
| Cells divide … | Epithelial cells divide to fill the gap |
| Cells die … | Platelet cells die to form the scab |
| Cells contract … | Muscle cells in blood vessels contract |
| Cells move/migrate … | White blood cells move/migrate through vessel wall |
| Cells attach … | Epithelial cells attach to fill the gap |
| Cells communicate … | Cells communicate to 'know' when to divide |
| Cells specialize/differentiate … | Cells specialize/differentiate into skin cells |

Step 1 appears to be a good preparation for step 2, 'descending towards the subcellular level'. It is obvious to students that if you want to explain a cell activity, you will have to descend further down into the cell to identify underlying parts and activities. The term 'cell activity' enables to distinguish between 'what cells do' (the cell activity) and 'what happens inside the cell' (subcellular activities). This seems a trivial distinction, but in the strategy it is crucial: we first define 'what cells do' as a starting point to meaningfully explore 'what happens inside the cell'. By doing this we build on students' intuitive notions that explaining 'how it works' entails exploring underlying parts and activities – one of the basic building blocks in mechanistic reasoning.

**Step 2 in detail**

Students feel that identifying the affected cell activity in step 1 still does not provide a satisfactory explanation for the disease: *'When having identified the cellular activity that malfunctions in the disease, you still don't know what actually goes wrong. Or how the cellular activity can be explained. So we have to go even further to study that.'* (Alice, interview I).

*Students can use hypothesizing and subdividing, but they feel they do not learn much from it*

When the teacher shows how the cell activity 'dividing' can be subdivided, students recognize underlying activities that they are familiar with, such as mitosis and DNA replication. However, in the scaffolding phase, students have to apply the strategy themselves to the activity 'mitosis'. Using a standard scheme of the mitotic phases, students are asked to mention all the events between prophase and telophase. Students' worksheets demonstrate that they mention underlying activities based on the text accompanying the figures, but they hardly apply the subdividing strategy to infer underlying partial activities that are not depicted in the figures, e.g. 'if membranes are formed, there will probably be production of phospholipids'. Only Jane noted that 'DNA replication takes place' entails 'unwinding and opening of the double helix' and 'coupling and pairing of new nucleotides'. When discussing the assignment, the teacher encourages students to identify more underlying activities. With a little help, most students succeeded in mentioning more underlying activities. However, in the interviews we can see that some students still stick to the assumption that these partial activities in the process of mitosis can presumably be explained by the activity of cell organelles.

*Linda:* *Mitosis. And then you had to write down everything that happened in between, from the one figure to the next.*

*Interviewer:* *So we could call mitosis a cell activity. What then are these steps in between?*

*Alice:* *The activity of the organelles …? [looks doubtfully]*

*Linda:* *[looks doubtfully as well]*

Transport of organelles, vesicles and chromosomes is used to model the hypothesizing strategy. The teacher questions the movements of vesicles or mitochondria to specific sides of the cell and students conclude that this cannot be explained from molecular motion or diffusion. The teacher hypothesizes through using an analogy that things in the cell could be actively pulled or pushed to specific locations using 'ropes' or 'rails' to guide the direction. Students recognize the pulling mechanism from prior lessons about mitosis, although they have no idea how 'pulling' could be established in the cell.

In the scaffolding phase, the case of CF is used to let students hypothesize about a mechanism that could transport chloride ions against the concentration gradient. One student came up with a mechanism comparable with a double door in a submarine. When asked to explain why the double door was needed, he said that otherwise everything could just diffuse in and out of the cell. Then the teacher reminded him that just opening the doors subsequently would also lead to other substances leaving and entering the cell, and that his suggestion could not explain transport of chloride ions against the concentration gradient. The following classroom discussion led to three 'design criteria' for the mechanism: the transport mechanisms must be

selective (only chloride ions), it will require energy (ATP was mentioned from prior knowledge) and will probably have an inlet at the intracellular side and an outlet at the extracellular side.
We can see that students are capable of formulating hypotheses about 'how activities could be established', although they need external encouragement and feedback on their ideas, because they lack more specific content knowledge to critically reflect on the value of their ideas. In the case of FH, students mention that LDL transport might also be achieved by a pump mechanism and the teacher encourages them to think of more mechanisms to take up substances. Students answer with the term 'endocytosis' based on prior knowledge, but do not use a hypothesizing strategy to reason about it.
From the interviews it became clear that activities in step 2 of phase 1 are hardly mentioned by students when asked to recall what they have done. When reminded by the interviewer, they do not value these activities as very important in the overall lesson series and most students report that they did not learn much in this step.
Some students indicate that they experienced the use of comparisons and metaphors to reason about the question 'how could it happen?' as 'just making up something'. During the classroom activities they repeatedly respond with: *'how can I know that? I am just guessing …'* In the interviews, students cannot remember applying the strategy, so they do not experience it as a very explicit approach in the lessons.
At the end of phase 1, many students are still prone to assign subcellular activities to the cell as a whole or one specific part (organelle) in the cell, but on the other hand they understand the message of the subdividing strategy:

*Interviewer: What do you conclude from that [applying the subdividing strategy]?*

*Monica: That one activity consists of multiple activities. So that an activity is not an isolated activity, but that each activity can de divided in multiple activities.*

In conclusion, although students cannot memorize the exact reasoning skills (namely subdividing and hypothesizing) to identify subcellular activities, they do report that it is necessary to dig deeper into cellular activities to provide an explanation for cell activities. Students have little prior knowledge of protein activities and, in addition, at this point lack the conceptual knowledge about how molecules are involved in subcellular activities, so it is not surprising that they hypothesize that known cell organelles will close the gap between molecules and (sub)cellular activities. When students are confronted with the fact that most cell activities cannot be explained from the activity of organelles, this creates an important content-based motive for the next phase: exploring which bottom-level entities can account for activities that cannot yet be explained. We can conclude that the two strategies encourage students to continue questioning and reasoning at a point where they usually accept presented (sub)cellular activities as given facts. However, students feel that they did not have the content knowledge to apply the strategies meaningfully and therefore do not value it as relevant. Despite the students' discomfort, the effects of step 2 are valuable, because the activities confront students with the need to know more about the cell's constituents if they want to make scientifically more appropriate hypotheses and subdivisions. This 'need to explore the bottom level' is the most important outcome of phase 1. Although students cannot judge how knowledge about yet-unfamiliar underlying entities will help them exactly, they implicitly use their notion of mechanisms to conclude that 'we need to descend

further, to really know how it works'. Since they know in general terms that cells consist of molecules, 'jumping' to molecules as the bottom-level entities to explore how molecules can provide a fundament for explaining activities in the cell, makes sense to them.

## 5.1.2. Results of phase 2: Exploring the bottom level

The identification of the gap at the end of phase 1 is the starting point for phase 2. Students realize that somehow cells are built up from molecules, but they lack the conceptual knowledge and additional reasoning strategies to explain how molecules are involved in cell activities. Here we introduce proteins as the 'working parts' in the cell and interactions between proteins (and other molecules) as the 'causal events' in the cell. The overall learning goal in phase 2 can be summarized as 'students can explain how molecular interactions can lead to protein activities and activities of protein-based modules'. This goal contains two elements that are subsequently addressed:

*Step 1: Understanding cause and effects of molecular interactions*

*Step 2: Using these insights to explain how molecular interactions can lead to activities of proteins and protein-based modules.*

Because of the interrelation of these goals, we describe the design and outcomes of step 1 and 2 in conjunction.

### 5.1.2.1. How was the intended path in phase 2 designed and executed?

**Step 1: Understanding cause and effects of molecular interactions**

Students are introduced into the basic conceptual knowledge about the composition and the resulting chemical properties and structure of proteins. This introduction is not much different from standard introductory biochemistry courses. Many visual models are used to emphasize the three dimensional structure and the diversity in shape and size of proteins, and students are familiarized with the different ways of representing proteins. Next, students are introduced to the basic molecular dynamics principles using animations and videos of experimental set ups that simulate the principles[5]. The molecular dynamics principles we consider crucial as a basis for molecular mechanistic reasoning are:

- Brownian motion
- random walk
- molecular collisions
- molecular recognition
- conformational change
- (self-)assembly.

5. Brownian motion, random walk and molecular collisions, see:
http://www.youtube.com/watch?v=FAdxd2Iv-UA and http://www.youtube.com/watch?v=PtYP8uoN0lk
Molecular recognition and Self-assembly, see:
http://www.youtube.com/watch?v=X-8MP7g8XOE and http://www.youtube.com/watch?v=YbpTusoDEgA
Conformational changes, see:
http://www.molecularstation.com/molecular-biology-images/505-protein-pictures/50-hemoglobin-animation.html

These principles allow students to understand the cause and effects of interactions between proteins. We do not intend to use detailed chemical knowledge. Therefore, we offer students a simplified 'summary' of the cause and effects of molecular interactions after introducing them the underlying molecular dynamics principles. We present 'colliding, binding and changing shape' as the basic causal chain of events that underlie all higher-level activities and we think this suffices as a basis for molecular mechanistic reasoning in these introductory lessons.

**Step 2: Chaining molecular interactions into activities of proteins and protein-based modules**
In this step, students learn to explain the activity of receptors, enzymes, membrane transporters, transcription factors and, in principle, all other proteins by describing the protein activity as causal chain of forming and breaking chemical bonds and interactions in and between the molecules involved. First, the teacher models the use of these molecular dynamics aspects to evaluate an animation of 'receptor activity'[6]and highlights the aspects that are not or incorrectly displayed in the animation. Next, the teacher constructs a scientific explanation for this event from molecular dynamics principles by chaining the cause and effects of these molecular interactions into an activity that scientists call 'receptor activity'. Students then apply the 'colliding, binding, changing shape' account to explain the activity 'kinesin protein walks along a microtubule' using an animation[7]. After students have explained this protein activity in detail, the teacher discusses the definition of a protein activity: *a protein activity is an event based on changes in the shape of a protein, that are caused because specific interactions with other molecules lead to rearrangement of chemical bonds in and between molecules.* Student use this definition to identify protein activities in the animation 'Inner life of the cell'[8]. To practise with explaining other protein activities, students explain binding, activating/inactivating, coupling/splitting and pumping using the 'colliding, binding and changing shape' account. In the reflection in this phase, the teacher emphasizes that all protein activities can be explained in terms of breaking and forming chemical bonds in and between the molecules involved in the activity. Linking these molecular events into a causal chain provides a scientific explanation for 'what proteins do'. The teacher generalizes this by providing a 'protein activity dictionary', which contains a description of all protein activities in general terms accompanied with an explanation that describes the causal chain of molecular interactions. The effect of a protein activity is a new molecular configuration and this new configuration might allow for a next protein activity. This causal link between protein activities is the basis for molecular modules. To practise this causal chaining approach, students are asked to organize a given set of proteins (including information about their activity, interacting partners and localization) into a working signalling module. To do this, they need to reason backwards and forwards between the different protein activities to build the causal chain. When doing this, they need to be aware of the molecular dynamics principles that determine when, where and how the given proteins interact.
In the reflection, the teacher shows that students have been confronted before with modular activities in the cell, by showing the schemes from the reference book used in regular biology classes. Transcription, translation, glycolysis, citric acid cycle – familiar to students – are now presented as modular activities in the cell, and students are guided towards the fact that the

6. See http://www.youtube.com/watch?v=Ms_ehUVvKKk
7. See http://www.youtube.com/watch?v=4AnPVuzF7CA
8. See http://multimedia.mcb.harvard.edu/

'molecular output' of one module (e.g. transcription) can be the trigger to start the next (e.g. translation). They see that modules can be chained into multi-module activities, with molecular interactions being the causal link between the two modules.
The activities in phase 2 take most of the time in the design. Phase two covers about three lessons of 3 hours each plus two homework assignments of about 30 minutes each.

### 5.1.2.2. How did students progress through phase 2?

**Students' progression in summary**
After step 2, students grasp the idea that all protein activities can be explained mechanistically in terms of colliding, binding and changing conformation. However, students are not very precise when verbalizing these explanations themselves. Apart from that, it seems within reach of these students to explain how molecular interactions can be the basis of protein activities. This is an insight that students report to be new and meaningful. Furthermore, students infer themselves that more complex activities can arise when multiple proteins cooperate and they consider it to be possible to use protein activities to explain cell activities. So the outcome of this step is that students can explain how molecular interactions can be the basis for more complex activities by using mechanistic reasoning in which they specify activities in terms of causal chains and parts-wholes.
The term 'molecular modules' is explained by the teacher only after students use the 'colliding, binding and changing shape' account to explain how protein activities can influence each other. Students express that they find it a logical term for a group of cooperating proteins. Since principles that causally link one protein activity to the next are the same principles that link one modular activity to the next, students recognize 'colliding, binding and changing shape' as a general 'reasoning tool' to explain causality at all levels between cells and molecules

**Step 1 in detail**
*Students regard the molecular level as 'the bottom' and they can apply molecular dynamics principles when reasoning about molecules*
During phase 1, students expect the organelles to be the parts in the cell that can explain cell activities, but when they are confronted with cell activities that cannot be explained in terms of organelles, they actually find it a very logical step to jump to 'molecules as the bottom level':
*'that [the jump] was because eventually we got stuck … with the 'how-question'. Then it is useful to start at the smallest level and working our way up so you eventually end up with a good … [explanation].'* (Monica, interview I) and they realize that the level of the organelles was skipped: *'yes, in fact you skip a few things, namely the mitochondria, organelles. You skip the organelles. You go directly to the smallest things present in a cell.'* (Kate, interview I)
Students do not show any trouble understanding the presented molecular principles. When discussing the animations and simulations, the teacher encourages the students to reflect critically on the representations of the molecular dynamics principles. Students recognize that in many representations in which molecular recognition is depicted, random walk and collisions are not depicted but replaced with a seemingly directed movement. For instance, when looking at the 'receptor activity' animation, several students mentioned that the hormone should not be depicted as if *'the hormone binds directly at the right spot at the receptor … it should first bump and turn a few times until it fits in the right way'* (Monica), *'The atoms in the proteins don't vibrate'* (Tanja), *'The hormone flies directly to the receptor'* (Alice), *'It should go back and forth until it bumps into the receptor'*

(Jake) (all in classroom discussion). However, some incorrect use of the principles occurs. For instance, while electromagnetic forces do play a significant role in molecular recognition, some students tend to use these forces as well to explain directed movement of molecules over longer distances, for instance a positively charged protein pump that 'sucks in' all the negatively charged chloride ions like a magnet.

For a basic understanding of the 'colliding, binding, changing shape' account, it is very important that students understand how the three-dimensional shape of a protein can change dramatically when only a few of its atoms interact with another molecules. This principle is discussed using a visualization of the two conformations of haemoglobin (with and without oxygen bound). Mike explained it by using the expression of a 'chain reaction' between the bonds in the protein:

*Teacher:* *how can the whole protein change shape if oxygen only changes a few bonds?*

*Mike:* *If these bonds change, this causes a chain reaction [in the protein] because all the other bonds should adapt because they cannot react [bind] in the same way they used to do.*

We can conclude that, from step 1, most students understand the principles that underlie the 'colliding, binding and changing shape' account, which is an essential step for explaining protein activities in step 2 of this phase.

**Step 2 in detail**

*Students can recognize and explain protein activities from molecular interactions*

At the beginning of phase 2, students mention in their worksheets that they know some proteins (mainly receptors, enzymes and transport proteins such as haemoglobin). However, they also report that they did not know how proteins work. In the interviews after the lessons about protein activities, students frequently report 'colliding, binding and changing shape' as a newly acquired insight and they consider it an intelligible and satisfactory explanation for protein activities. *'In biology class we learn that a receptor binds a protein, but not exactly how the protein can bind, how the interaction occurs' … 'For example you read 'a receptor binds with this' and now I think "O yes, I know how that works, I learned that". And normally you just read it; you think 'oke', and you move on'*
(Alice, interview II).

After having defined a protein activity and using this definition to identify protein activities in the animation 'Inner life of the cell', students report they were surprised that proteins actually perform so many activities in the cell: *'Proteins basically do everything that happens in the cell'* (Jake, interview I). Most surprising is the 'walking' of the kinesin protein. After analysing the 'kinesin walking' animation, they regard a mechanistic explanation in the term 'colliding, binding and changing shape' a plausible and meaningful explanation for this activity.

After the scaffolding phase, students hold the opinion that in principle all protein activities can be explained mechanistically in terms of colliding, binding and changing conformation: *'To explain protein activities you need three basis principles: changing shape, binding and colliding'* (Kay, interview II). They report this insight to be new and useful in their reasoning about proteins.

When the teacher shows that binding of a hormone causes a conformational change in a receptor, which enables the intracellular part of the receptor to interact with molecules that did not fit before hormone binding, it is made clear to the students that molecular interactions can

be viewed as causal events, in the sense that one interaction allows for the next. This causality is not difficult to grasp for the students. However, one misconception can arise: students do not see that the conformational change of a protein only remains as long as the molecular interaction persists. They think that the collision with a molecule (a hormone or another protein) is enough to change proteins from an inactive into an active conformation. This appears to be problematic later in this step when students interpret graphical representations of a signalling cascade as an example of a protein-based module.

Explaining the 'walking' of a kinesin protein in terms of colliding, binding and changing shape appears to be relatively easy after the modelling phase. This is mainly due to the fact that this animation displays most of the molecular dynamics aspects very accurately, in contrast to most molecular animations available. The only obstacle for students is their idea that ATP would transfer its energy to the protein, thereby facilitating the conformational change of the protein. This idea conflicted with the animation in which not the hydrolysis but the binding of ATP causes the conformational change and then the conformational change causes the ATP to be hydrolysed into ADP and P.

From the assignments in which students build explanations for other protein activities we can see that the activity 'catalysing' remains vague to students. Applied to the specific case of 'splitting a molecule', they can see that if a molecule binds to a protein that subsequently changes shape, this could stretch the bound substrate, causing an chemical bond in the molecule to break. However, in the case of 'connecting two molecules' it is difficult for students to see how binding to a protein could cause two molecules to form a connection, which does not occur when the two molecules bump into each other without the presence of the protein. In the case where students explain how a protein could pump molecules out of the cell, many students forget to include the binding and hydrolysis of ATP. They describe that the binding of a molecules caused the protein to flip, thereby 'spitting out' the molecule on the other side of the membrane. They apply the 'colliding, binding and changing shape' account as they were taught, and they do not see a necessity to incorporation of ATP in the mechanism. In fact, many proteins display major conformational changes without the involvement of ATP. After discussing the general definition of a protein activity, recognizing these activities in the animation 'Inner life of the cell' is relatively straightforward.

*Students regard the concept of 'molecular modules' a logical next step*

Interestingly, even before having been introduced to molecular modules, students report that 'colliding, binding and changing shape' could also form the basis for explaining how proteins cooperate and some of them actually hypothesize that cooperation of proteins can probably explain more complex higher-level activities in the cell. For instance, before the start of step 2, Kay used the term 'protein complexes' for the cooperation of proteins and he considered whether it makes sense to label these 'protein complexes' as units that are responsible for a specific activities. He stated: *'In the cell there are a lot of those things [proteins] that cooperate, for example to produce insulin. I don't know … maybe you can call it "the insulin cell organelle"'* (Kay, interview I). From the use of the word 'organelle' we can see that Kay values these 'cooperation proteins' as an intermediate level between (sub)cellular activities and molecules, in the same way that he considers the organelles to be responsible for certain (sub)cellular activities.

From this, it is not surprising that students adopt the term 'molecular module' as a logical organizational level between cells and molecules at the end of phase 2. They find it apparent

that the module as a whole is responsible for an activity and that a module is not just a collection of proteins. They can reflect on the importance of a specific distribution of the proteins and protein activities in space and time to make the module work, and the term 'domino-effect' (Linda, interview II) is used to explain what happens when a protein is missing in a module. Furthermore, the students are not surprised that these modules are not necessarily identifiable as structural units in the cell. The molecular dynamics principles from phase 2 clarifies to the students that proteins do not need to be clustered structurally to work together as module.

The introduction of the term 'molecular modules' helps students to get a grip how multiple proteins can work together thereby displaying a joint activity. In addition, they report that the molecules that form the output of one module can serve as an input for another module thereby chaining molecular modules into even larger activities. As a result of phase 2, the majority of the students nuance the idea of cell organelles as the only intermediate level between cellular activities and molecules and they refer to the 'colliding, binding and changing shape' of proteins when reasoning about causal events in the cell.

It is noteworthy that after explaining protein activities in terms of 'colliding, binding and changing shape' students already expect that multiple proteins can cooperate to perform higher-level activities. As a consequence, students adopt the term 'molecular module' as being a logical follow-up on the principles they learned in step 1. The most powerful example of a molecular module is a 'signalling cascade'. It is clear to the students that the overall modular activity is 'transmitting a signal from the membrane to the nucleus', and it is also clear that multiple protein activities are necessary in a specific order to complete this activity. Students experience organizing the different proteins of the signalling module into a causal chain as working on a puzzle that needs to be solved. When reflecting on the term 'molecular module', students are surprised to see that some of the (sub)cellular activities that they knew from prior knowledge, such as the cell cycle, mitosis and glucose metabolism, can be characterized as the result of the activities of molecular modules, although they immediately recognize that the term applies to these activities. When students are familiarized with the term molecular module and they have seen some examples, it is no surprise to them that modules influence and depend on each other via molecular interactions. Since modular activities consist of protein activities and protein activities emerge from molecular interactions, students understand that the causal links between modules can be explained in terms of molecular interactions.

### 5.1.3. Results of phase 3: Bottom-up approach to explain cell activities

At the end of phase 2, students know that activities of proteins and protein-based modules arise from molecular interactions. The next step is using these activities as building blocks in the attempt to close the gap between molecular and cellular activities. How activities of proteins can be used to explain 'what cells do' is the central question in phase 3.

The intended outcomes in this phase can be summarized as *Students find it intelligible that all cell activities constitute from molecular interactions.* By applying bottom-up reasoning, students realize that in principle all cell activities can be explained in terms of molecular interactions, if spatial and temporal organization is included in the explanation. Furthermore, students realize that complexity increases dramatically, with increasing number of interactions and therefore intermediate organizational levels are used in these explanations to handle complexity.

### 5.1.3.1. How was the intended path in phase 3 designed and executed?

In phase 3, students use the molecular dynamics explanations of protein activities and modular activities from phase 2 to build explanations for the cell activities in the three examples identified in phase 1. The advantage of these three examples is that students are already familiarized (in our lessons or from prior knowledge) with most protein activities and modular activities that serve as building blocks to construct the explanations. These building blocks are: an ion pump (CF), a receptor (FH), vesicle formation (FH), signalling, transcription, translation, protein sorting and exocytosis (wound healing). The main task for students is to explain the start and end situation of the activity (Table 5) and then chain these building blocks into a mechanism that accounts for the change between the start and the end.

The three examples represent three levels of increasing complexity. As complexity increases the level of detail in which the examples are worked out decreases. To explain how 'mucous cells secrete chloride ions', students explain the protein activity 'pumping chloride ions' in terms of the multiple molecular interactions, To explain how 'liver cells take up LDL-cholesterol', the multi-protein modular activity scientifically referred to as 'receptor-mediated endocytosis' is explained in terms of the multiple protein activities, and to explain how 'fibroblasts excrete collagen when stimulated with TGF-β', students explain this multi-modular activity in terms of the activities of the multiple molecular modules involved.

**Step 1: The cell activity can be explained from the activity of one protein**

In the case of CF, students explain the cell activity 'pumping chloride ions' by designing a chloride pump using the design criteria for a pumping mechanism they hypothesized in phase 1. To do this they have to use the principles for protein activities learned in phase 2.]

**Step 2: The cell activity can be explained from the activity of a multi-protein module**

In the case of FH, students study multiple representations of the molecular mechanism of clatherin-mediated endocytosis (graphical schemes and animations[9]) ) to explain the activity 'taking up LDL-cholesterol'. They are asked to combine information from these different sources to build a causal chain of molecular events that can explain how 'taking up LDL-cholesterol' works.

**Step 3: The cell activity can be explained from the combined activities of multiple protein-based modules.**

To explain the secretion of collagen, in the case of wound healing, the students are offered the general representations of five modular activities taken from the reference book they use in all regular science classes. These schemes represent signalling, transcription, translation, translocation to the ER and exocytosis. With these schemes they have to construct the causal chain of events from the start (binding of TGB-β to the receptor) to the end (collagen being outside the cell).

In phase, 3 bottom-up reasoning shows how explanations of cell activities at the three levels of complexity all use basic principles of molecular interactions. In doing so we show how scientists use intermediate functional levels between molecules and cells in their explanatory models in order to deal with complexity and we make intelligible to students that very complex activities can emerge from basic molecular interactions.

*Table 5: Start and end situation of the causal chain to be build so as to explain the cell activity in the three examples differing in complexity*

| *Phenomenon* | *Cell activity in healthy individuals* | *Start situation* | *End situation* | *Complexity level* |
|---|---|---|---|---|
| Cystic fibrosis | Mucous cells excrete chloride ions | Chloride ion in the cell | Chloride ion outside the cell | One protein explains the cell activity |
| Familial hypercholesterolaemia | Liver cells take up LDL-cholesterol | LDL-cholesterol outside the cell | LDL-cholesterol inside a vesicle in cell | The cell activity can be explained from the activity of a multi-protein module |
| Wound healing | Fibroblasts secrete collagen when stimulated with the hormone TGF-β | No TGF-β, no collagen outside the cell | TGF-β present, collagen outside the cell | The cell activity can be explained from the combined activities of multiple protein-based modules. |

LDL = low-density lipoprotein; TGF = transforming growth factor.

The activities in phase 3 take about one lesson of 3 hours.

### 5.1.3.2. How did students progress through phase 3?

**Students' progression in summary**

In this step, the students realize that the gap between the molecular and cellular level can actually be bridged. The use of multiple complexity levels helps to show that sometimes molecular-level explanations for cellular activities can be very simple, but that in most cases many molecules are involved.

By starting with a simple example (CF) and showing that the same principle applies to more complex activities (in FH and wound healing), students see that in principle all cell activities can be explained mechanistically in terms of molecular interaction. The concept of molecular modules, being groups of interacting proteins that together display an overall activity, contributes to the dynamic image of activities in the cell and to the concept that molecular interactions can lead to activities ranging from relatively simple to very complex.

Students experience the use of intermediate organizational levels to handle complexity as helpful. They can distinguish between molecular interactions, protein activities, modular activities, multi-module activities and cell activities. However, they have difficulties to place organelle activities in this hierarchy.

**The three complexity levels in details**

*Students recognize the increasing complexity in the three examples*

The tasks that students work on in this phase were designed to show that in all three examples the cell activity identified in phase 1 can indeed be explained in terms of 'colliding, binding and changing shape', although it was not our intention to teach all the molecular details of the mechanisms involved. Levels of sophistication and details vary amongst student couples, but all couples manage to recognize start and end of the activity and to provide a chain of events that

9. e.g. http://www.youtube.com/watch?v=PifagmJRLZ0&NR=1

happened between start and end situation. In some cases, students just mention that one event follows on a previous event as without providing a causal explanation for the link between these events. For instance, after watching the animation that explains LDL-cholesterol[10], Jody and Mike formulated the steps in their worksheets as:

*Step 1: LDL-cholesterol collides with the receptor and binds*
*Step 2: The receptor changes shape*
*Step 3: Adaptin collides and binds to the receptor*
*Step 4: Adaptin and the receptor change shape*
*Step 5: Clathrin collides and binds to adaptin*
*Step 6: HSC70 and auxilin collide and bind to clathrin*
*Step 7: This causes the clatherins to reshape into a circle with the above-mentioned proteins in the middle.*

Whereas Linda and Jane wrote:

*Step 1: Cholesterol is bound to LDL-cholesterol particles outside the cell*
*Step 2: This complex binds to the LDL-receptor*
*Step 3: Because of the binding the receptor changes shape, an adaptor protein binds, the protein changes shape again and clathrin can bind.*
*Step 4: Clathrins can bind to each other via the leg domain. This causes the membrane to reshape. A 'closed cage' is formed, a kind of ball.*
*Step 5: HSC70 and auxilin bind to the closed cage, which causes the closed cage to disintegrate, resulting in only the ball remaining (without the skeleton)*

From interview II, it becomes clear that the students consider the three examples as increasingly complex phenomena. When asked in what way the three examples differ. Alice and Linda reported:

*Alice:* *This one [CF] is pretty easy*

*Linda:* *The cell activity is the protein activity. This is just easy. Then, with FH it goes one step further with a module activity involved.*

*Alice:* *Yes, and multiple protein activities*

*Linda:* *Yes, and then wound healing is really a whole list with all …*

*Alice:* *… cell activities. And with those a lot of module activities are involved. And with those a lot of protein activities are involved.*

*Linda:* *It gets, let's say, increasingly complex.*

Alice and Linda summarized the increasing molecular complexity of CF, FH and wound healing in terms of proteins and protein-based modules involved. From their report, it becomes clear that they understand that some phenomena can be explained by the activity of a single

10. See http://www.youtube.com/watch?v=eRslV6lrVxY,
http://www.youtube.com/watch?v=-ZFnO5RY1cU and
http://www.youtube.com/watch?v=PifagmJRLZ0&NR=1

protein whereas in other phenomena multiple cellular activities are involved. Similar awareness of increasing complexity can be found in other interviews. Most students can express the hierarchical nature of cell activity, modular activities and protein activities accurately:
*: 'And here [wound healing] a lot of cell activities are involved in order to realize the process, and therefore a lot of modules too, and therefore even more protein interactions since it goes down [into the cell] and thus an increasing number of proteins [are involved].'* (Monica, interview II).
However, in the lesson series, we do not pay explicit attention to organelles and organelle activities. Some students recognize that organelles can be considered very complex molecular modules. For instance Monica explained: *'well, a mitochondrion consists of proteins and also some other substances. So that is actually cooperation between several molecules, including proteins.'* For others, the terms 'molecular module' and 'organelle' could not yet be connected meaningfully:

*Interviewer: Would you call an organelle such as a mitochondrion a module activity?*

*Linda: Ehm … well, I don't know. I don't think so.*

*Alice: No, I also don't think so*

*Interviewer: No? Why not?*

*Alice: Well, are there also many proteins involved? (She asks Linda)*

*Linda: Eh, not that I can see from the biology lesson! Maybe there are proteins involved, but not that I know now …*

(Interview II)

Interesting to see here is that Alice and Linda questioned the role of proteins in a mitochondrion. If proteins are involved, they would call it a molecular module. Apparently, when answering this question Anna and Linda did not make the link that for all activities in the cell, including organelle activities, protein activities are the basis. Although they seem to be very close to realizing that organelle activities also emerge from protein activities, they show in their response that that idea is not consolidated yet.

## 5.2. Perspective 2: Students' use of molecular mechanistic reasoning

To present our findings about students' use of molecular mechanistic reasoning (our second perspective), we first show how the molecular mechanistic reasoning elements play a role in the assignments. Then we report for each of the elements if and how students use this element in their assignments. To conclude, we consider if and how student use the elements in conjunction in each assignment so as to be able to judge students' use of molecular mechanistic reasoning as a coherent domain-specific reasoning framework.

### 5.2.1. Recognizing molecular mechanistic reasoning in the assignments

In Table 6, we show for each element how it plays a role in the three assignments. We can conclude that assignment 1 (neutrophil movie) hardly provides any direct clues that guide students towards using molecular mechanistic reasoning or even mechanistic reasoning in general. Therefore, assignment 1 provides interesting clues whether students show a tendency to reason mechanistically before the lessons and if this reasoning is enriched by the molecular-level principles from the lessons. Assignment 2 contains many direct references to elements needed to reason mechanistically (entities, activities and organizational aspects) and the assignment specifically asks for mechanistic reasoning within one level. Most interesting question related to assignment 2 is whether students succeed in applying the molecular-level principles consequently to build scientifically sound interpretations. Also, in the third assignment, many mechanistic elements are presented in the text. However, the entities and activities presented in this assignment are at many different levels of organization and the relationships with molecular-level principles need to be inferred by the students. A very interesting question related to assignment 3 is whether students manage to relate these terms at different organizational levels into coherent mechanisms and if they use the molecular-level principles to explain these mechanisms.

### 5.2.2. Students' use of molecular mechanistic reasoning elements in the assignments

#### 5.2.2.1. How-questions: Do students identify a (sub)cellular phenomenon to be explained and ask relevant how-questions about it?

The different type of questions that students ask and ideas that they bring forward when confronted with (sub)cellular phenomena is most prominent in assignment 1. The first time students (n = 12) work on assignment 1, on average they pose three questions. Out of a total of 37 questions from all students, 20 (54%) are clearly questions about underlying causes or mechanisms, mostly formulated as how-questions. Other questions are mainly functional questions. All students except one wrote down: 'Why does the neutrophil chase the bacterium?' Suggested answers to this question varied from 'to destroy the bacterium' and 'the bacterium is dangerous for the body' to 'the bacterium is food for the neutrophil'. Without such suggestions it is impossible to judge whether they might still aim at explaining underlying mechanisms when they write 'why?'

In general, we can conclude that already in the pre-test a tendency to question the underlying mechanisms that can explain the behaviour of the neutrophil can be recognized because all students formulate relevant 'how-questions' about the behaviour. However, not much mechanistic reasoning occurs when after posing the question. Most students do not add any idea or hypothesis to their how-questions.

An obvious difference between the first and second time that students work on assignment 1 is that the second time we see an enormous increase in the total number of questions as well as the proportion of how-questions. Students (n = 12) on average asked nine questions and of out of a total of 107 questions, 81 (76%) referred to underlying causes or mechanisms.

In assignment 2 and 3, students are explicitly asked to write down the how-question that the

graphic and the article tried to answer. Remarkable here are the differences in how students formulate these questions. About the newspaper article, most students (seven out of twelve) formulate rather broadly that the article explains 'how the sperm cell finds its way to the egg'. However, Kate was more specific: 'How does calcium trigger extra activity of the flagellum of

*Table 6: The way the molecular mechanistic reasoning elements are called upon in the three assignments*

| ***Molecular mechanistic reasoning element*** | *1 Neutrophil movie* | *2 Textbook graphics* | *3 Newspaper article* |
|---|---|---|---|
| *How-questions* | | | |
| Identifying phenomenon to be explained and ask how-questions | Type of questions open to students | Explicitly asked for in assignment | Explicitly asked for in assignment |
| *Subdividing* | | | |
| Functionally subdividing of phenomenon | Implicitly stimulated by asking for 'as many questions as possible' | Not needed to interpret the graphics | Useful but not explicitly asked for |
| *Hypothesizing* | | | |
| Hypothesizing mechanisms e.g. using metaphors/analogies | Implicitly stimulated by asking 'what are your ideas?' | Useful if students recognize gaps or unexplained activities | Useful if students recognize gaps or unexplained activities |
| *Entities* | | | |
| Identifying/hypothesizing<br>• Proteins<br>• Protein-based modules | All underlying entities need to be hypothesized | Proteins are schematically depicted. The scheme in total represents module | Some proteins and hormones mentioned in the text.<br>Many gaps to be filled |
| *Activities* | | | |
| Identifying/hypothesizing<br>• Protein activities<br>• Modular activities | All underlying activities need to be inferred or hypothesized | Some protein activities depicted by arrows.<br>The scheme in total represents modular activity | Some protein activities and higher-level (modular) activities mentioned in the text<br>Many gaps to be filled |
| *Chaining* | | | |
| • Linking activities into causal chains<br>• Recognizing gaps in the chain | Every sequence of events needs to be inferred | Arrows indicate the chain of events<br>Some gaps remain | Some sequence of events mentioned in the text<br>Many gaps remain |
| *Causality* | | | |
| Appling 'colliding, binding, changing shape' as a basis for causality | Unlikely to be used, because all proteins need to be hypothesized | Most binding events indicated with arrows. Some conformational changes depicted | Mostly higher-level causal terms used in the text, such as 'activating' and 'opening' |
| *Organization* | | | |
| Applying molecular dynamics principles as a basis for organization | If and how molecular dynamics principles apply needs to be inferred | If and how molecular dynamics principles apply needs to be inferred | If and how molecular dynamics principles apply needs to be inferred |
| *Mechanistic models* | | | |
| Combining entities, activities, organization and causality into mechanisms to explain (sub)cellular activities | Not explicitly asked for, but a mechanistic model can be part of students' ideas | Model is provided, no modelling by the students | Drawing and description of the model explicitly asked for |

the sperm cell' and Ellen formulated the 'how-question as: 'How progesterone around the egg causes the sperm cell to move faster/speeds up its flagellum, to get sperm and egg together.' Both Kate and Ellen showed with their formulation that they are aware that the mechanism discussed in the article only explains a specific aspects of the overall activity 'sperm cells finding the egg'. Similar variation is formulating the exact how-question can be found when students analyse the graphical explanations in assignment 2 and we can conclude from both assignments that students differ in how accurately they formulate the activity that is actually discussed in a mechanistic model.

### 5.2.2.2. Subdividing: Do students subdivide a (sub)cellular phenomenon functionally to identify underlying activities?

The subdividing strategy is most prominent in assignment 1. The second time that students work on the assignment, almost all students subdivide the phenomenon in more detail into underlying activities.
For instance, in the first test Monica wrote:
*Why does the neutrophil chase the bacterium?*
*How does the neutrophil know that it must chase the bacterium?*

While in the second test she wrote:
*How does the neutrophil move?*
*How does the neutrophil know where to go?*
*How does the neutrophil know that it must chase the bacterium?*
*How does the neutrophil enclose the bacterium?*
*How does the neutrophil change its velocity?*

The first time, Monica identifies the activity 'chasing the bacterium'. The second time, she adds questions about different aspects of the movement of the neutrophil that she does not mention in the pre-test. Not only does she question the mechanism that makes the neutrophil move, she also subdivides 'chasing' into 'moving', 'knowing where to go' and 'changing velocity'. This suggests that she regards these aspects as different underlying activities that each can be explained mechanistically.

### 5.2.2.3. Hypothesizing: Do students hypothesize mechanistic schemas, for instance by using metaphors or comparisons?

The strategy of using metaphors or comparisons to hypothesize mechanistic schemas can be recognized most prominently in assignment 1. Two aspects of the phenomenon in assignment 1 seem to be powerful in triggering thinking about underlying mechanisms. One is the nature of the physical link between the bacterium and the neutrophil. Already, in the first time, many students realize that 'there must be something' like a substance or vibrations that allows the neutrophil to sense the bacterium. Some students refer to some form of sense organ that the neutrophil must have. *'The neutrophil can smell the bacterium. It has a certain odor. Or it hears the bacterium, because of vibrations, a certain frequency, or the amount of echo (like a bat). Or some kind of sixth sense.''* (Kate, first test). One other student also used vibrations in the liquid as an explanation.

The other powerful aspect that triggers mechanistic reasoning is the movement and reshaping of the cell. One student compared the movement of the neutrophil with snail and two students refer to the use of cilia as a motility mechanism they known from other organisms.
The first time that students worked on assignment 1, the ideas about underlying mechanisms remain relatively superficial. Students use very general terms such as 'substances' or comparisons and metaphors that are not further specified. Given students' prior knowledge, this is not surprising. Their knowledge about organelles is not useful to explain how cells sense bacteria, move or reshape. Without more specific information about underlying entities and activities, students use metaphors and comparisons in their reasoning, but as can be expected these are formulated in general terms and students can hardly reflect on the explanatory value of these hypotheses.

### 5.2.2.4. Entities: Do students identify/hypothesize the involvement of proteins or protein-based modules?

Assignment 1 does not include any clues about the involvement of proteins in this phenomenon, so students need to hypothesize or infer the involvement of proteins from prior knowledge. The first time that students worked on the assignment, none of them mentioned the involvement of proteins, although after the assignment students indicate that they already had some prior knowledge about proteins. They knew about receptors recognizing substances, and enzymes catalysing chemical reactions, but apparently this assignment does not trigger students to use this knowledge. The second time, all the students refer to proteins a few times, mainly as the receptors signalling a bacterial substance and motor-protein being involved in the movement of the cells. This is not surprising since the lessons focus totally on the role of proteins in cellular phenomena and these examples are explicitly covered in the lessons.
In assignments 1 and 3, some students hypothesize a module to bridge a gap in the explanation. For instance in assignment 1, Mike wrote: *'It finds the bacterium by sensing this substance with a receptor and then activating a module that causes the neutrophil to change direction.'*

### 5.2.2.5. Activities: Do students identify/hypothesize activities of proteins or protein-based modules?

In assignment 2, modules are the objects to be explained and students are directly confronted with proteins in the graphical representation. One of the questions to students is: 'Formulate precisely the how-question central in this scheme'. Here we see that some students are very imprecise in formulating what the module actually does. For instance, Kay wrote that the signalling cascade schema explains *'how the DNA is turned into action'*
We see that students tend to refer to module-like activities when they judge that activity to be explained cannot be done by singular proteins or other molecules. However, we feel that many students overestimate the 'explanatory power' of singular proteins. For instance, Kate wrote: *'The neutrophil binds to certain substances with receptors … this makes a certain protein change shape in such a way that the neutrophil moves.'*

### 5.2.2.6. Chaining: Do students link protein or module activities into causal chains or recognize gaps in the causal chain?

In assignment 2, students are explicitly asked to use the word 'because of' , thereby' and 'if … then' to build a causal chain and they are encouraged to write down a question if they notice an explanatory gap in the chain. All of the students discover numerous gaps that are not explained in the scheme. For instance, in the protein translocation scheme Mike wrote: *'I don't know how the signalling peptide gets loose from the protein'*. We can see that students are aware that activities are causally linked via molecular interactions and they propose how this can be established. For instance Jane suggested *'when the receptor is activated a substance is released in the cell that binds to "peddle proteins" that start moving'* (Jane, second test).

### 5.2.2.7. Causality: Do students apply 'colliding, binding, changing shape' as a basis for causality in the mechanisms?

In assignment 2, students apply 'colliding, binding and changing shape' pretty accurately. If we take a close look at how students interpret the scheme of the signalling cascade, we notice an unforeseen limitation of this 'colliding, binding and changing shape' account. Students are told to avoid the term 'activate' thereby encouraging them to formulate more precisely what happens with the depicted proteins in the signalling module. They use colliding, binding and changing shape consequently but most students think that the depicted shift from an inactive into an active state of a protein can be explained from the 'collision' between two proteins. They do not see that most proteins return to their original conformation after the interaction and that the conformational change only remains as long as an interacting molecule stays bound to the protein (for instance cAMP or a phosphate group that is added).

In assignments 1 and 3, the terms 'colliding, binding and changing shape' are not used very consequently by the students. They tend to describe activities with higher-level terms such as 'activate' and 'sense'. However, because often students accompany these terms with references to molecular interactions, such as 'the receptor senses the bacterial substance', or progesterone activates the calcium channel, we have the impression that they are aware that molecular interactions are the basis for all activities.

### 5.2.2.8. Organization: Do students apply the molecular dynamics principles of molecular interactions as a basis for organization in the mechanisms?

Most students apply jittering motion and collisions consequently to explain localization, movement and recognition of molecules in their explanations. The use of directed movement to explain spatial changes is totally absent in the assignments after the lessons, whereas during the lessons students often use statements such as 'it just goes there' or even 'it wants to go there' if they notice a spatial change for instance in a molecular animation. In a few cases, students indicate in the assignments that they cannot explain movement and recognition, where in fact the molecular dynamics principles would suffice. For instance, Alice wrote in assignment 2: *'I don't know how it can be that the recognition protein (SHM in the drawing) finds the signalling peptide and how the protein complex ends up at the protein channel.'*

### 5.2.2.9. Model: Do students combine entities, activities, organization and causality into a mechanistic model that accounts for a (sub)cellular phenomenon?

Students' ability to (re)construct a mechanistic model that can account for the phenomenon described can be recognized in assignment 3. All the information in the newspaper article needs to be combined to describe and draw a mechanistic model. Great differences can be seen between students, mainly in their tendency and effort to be very precise, i.e., not leaving gaps in the model. Students' drawings mainly show entities and their relative position in the mechanism. Most students use arrows to indicate spatial changes, for instance a flow of calcium ions, and they also use arrows to indicate interactions, for instance progesterone binding to the ion channel. Obviously, activities are much more difficult to indicate in a drawing. Most students refer in their written text to activities and causality but leave out these aspects in their drawings. In general, we can say that constructing detailed mechanistic models from presented data requires an integration of all the aspects of molecular mechanistic reasoning. The newspaper assignment shows that this is a very demanding task. All students try to combine the information in the text into a coherent mechanism and all of them succeed in constructing a chain of events from the egg producing progesterone to the sperm cell swimming faster. However, how detailed they work out the different steps varies, for instance, *'progesterone appears to activate the calcium channel in the membrane, thereby opening it'*. Eight out of twelve students adopt this formulation as one step, without further clarification, whereas four students use the 'colliding, binding, changing shape' account to clarify in more detail what is meant by 'activating the calcium channel'.
Furthermore, six students recognize explicitly that the article provides no information on how calcium causes the flagellum to rotate faster, while the others just quote the article that 'if calcium enters the sperm cell, the flagellum starts rotating faster'.

### 5.2.2.10 Students' use of molecular mechanistic reasoning as a coherent domain-specific reasoning framework

Looking at assignment 1, we can conclude that, even before the lessons start, students show a tendency to reason mechanistically about cell activities, although their reasoning remains superficial. Furthermore, we see that students provide different types of explanations. We can recognize many 'upward' questions about the role or function of the phenomenon. In answering these 'upward' questions, students are imprecise in their formulations and they use anthropomorphic and teleological speech to express their ideas about the role of the phenomenon, such as *as 'the neutrophil wants to catch the bacterium because it is dangerous to the body'*. It is tempting to interpret these results as non-mechanistic responses. However, from the perspective of multi-level mechanistic reasoning, 'upward' questions are expressions mechanistic reasoning as well. 'Upward' questions ask for the role that the activity (chasing the bacterium) plays in the larger system it is part of (e.g. the body). Obviously, it provides a different type of explanation than the 'downward' question 'how does it work?', but in fact also these upward questions strengthen our assumption that mechanistic reasoning as such is not the problem. However, at the start of our lessons, students appear unaware of the distinction between different types of explanations, and our focus on mechanisms in the cell trigger them to focus more on these 'downward' questions the second time they work on the assignment.
It is difficult to judge whether students are constantly aware of the general account for causality

in terms of 'colliding, binding, changing shape' when they use terms for higher-level activities in their reasoning. In assignment 2 we see that, although explicitly asked for, many students take causal shortcuts by using words such as 'transport, produce, activate' without explaining these terms by using 'colliding, binding changing shape' or otherwise referring to molecular dynamics.

## 5.3. Perspective 3: Students' metacognition on molecular mechanistic reasoning

To address perspective three, we report in this section about the ideas that students express about the use of molecular mechanistic reasoning to bridge the gap between cell activities and molecular interactions. These findings are mainly based on the interviews at the end of the lesson series and the group discussions during the lessons. We focus on students' thoughts about the meaning, value and usefulness of the lessons.

The lack of conceptual knowledge and the lack of encouragement to reason about mechanistic explanations for cellular behaviour in traditional biology education are reflected in many student quotes, for instance, *'I just never questioned how cells can do things.' and 'Normally, in biology lessons they just tell you that it happens. Now you have to think how it happens.'* In the designed learning trajectory, we attempt to intertwine both aspects to enable students to bridge the gap between the molecular and cellular level. Question remains whether students experience their learning trajectory as successfully connecting the previously separated cellular and molecular domains, and whether they have the feeling that they learned a new way of reasoning to make this connection.

In response to the question 'How would you explain your classmates what you have learned in these lessons', Ellen responds: *'I would say that we looked at diseases and that from the disease we reasoned towards organs and cell activities, and from the bottom, from the gene that is mutated or damaged, back to cell activities. Say, establish that connection. How it works, ultimately.'* (evaluative group discussion). In Ellen's response we can see that she recognizes that connecting the cellular level to higher and lower levels was central and that cellular phenomena were approached both top-down and bottom-up. Alice responds: *I would say that I learned a different way of looking at things and not just accepting everything, but also critically looking at "how about that?" And "how does it work?" … Normally if you read something in the biology book, that a protein binds, you think "ok, fine", but if you have learned this, you think "ah, ok, that is how it is" and "this is how it works". Because normally you know not only that it happened, and not how it happens'* (evaluative group discussion). When being asked whether they have learned new things, the most prominent response is: *'we didn't learn much new, we just got deeper into the things that we already knew'*. Jane formulates this point as: *: 'I would say that I didn't learn much, in terms of new content, but, let's say, acquiring a new way of thinking. But that is not learning, it is more … I don't know. It is not that I know new things now. I still know the same'* (evaluative group discussion). From the point of view of metacognition, it is interesting to see that Jane considers learning as acquiring new knowledge. Since the lessons did not depend on much new knowledge, she says *'I didn't learn much'*. However, this does not mean that students did not experience the lessons as valuable. Most students said that usually their learning approach for these subjects at secondary school was based on memorization and rote learning. They value the approach of *'learning to think'* (Kate, interview II), *'searching for the underlying reasons'* (Monica, interview II), for instance because *'If it is logical, it is also easier to remember.'* (Monica, interview II), and *'by thinking in a different way you automatically discover new things to learn'* (Jane, evaluative group discussion).

Students saying *'now I really know how it works'* regard an explanation in terms of molecules

that 'collide, bind and change shape' as more fundamental than the higher-level activities that they had learned before. This feeling of a more fundamental insight comes from the fact that 'binding, colliding and changing shape' applies to all higher-level activities. It is generalizable and it can be used as a basis for reasoning about any activity in the cell. It is the understanding of this general basis for all mechanisms in the cell that makes students saying that *'proteins actually perform everything in the cell'* (Kay, interview I). These and other students' reports during the lessons show that they experience that cellular activities can be explained in terms of protein activities, which subsequently can be explained in terms of 'colliding, binding and changing shape'. That they consider interactions between molecules as a plausible and intelligible bottom-level that underlies all (sub)cellular activities and that proteins actually *'do all the work in the cell'* shows that students value molecular mechanistic reasoning as connecting molecular interactions at the 'bottom' to higher-level (sub)cellular activities at the 'top'.
It appears that students, after having explained how they value the molecular complexity of the three examples, are convinced that also the basis of very complex activities lies in the interaction of molecules. For example:

*Interviewer: and [what do you do] when you apply downward reasoning?*

*Monica: Then you start with a phenomenon and you end up at the molecular interactions. In other words, you just end up at a point at which you cannot go deeper. Then it just ends.* (Interview II)

In Monica's response we can see that she is convinced that at the level of molecular interactions you cannot go deeper. Although from an expert's point of view this is actually not the case, it shows that she feels that an explanation in terms of molecular interactions is somehow more fundamental than explanations in higher-level terms.
The focus on molecular interactions as a basis for living systems also provokes fundamental questions in some students:

*Kay: When is something alive?*

*Teacher: Cells are the smallest living units. Viruses are not alive.*

*Kay: Now I just find it very strange to call things 'alive'.*

*Teacher: Why?*

*Kay: because actually, it is all just a chemical reaction (other students laugh)*

*Alice: So we are all chemical reactions[…]*

*Teacher: The message is not: you are just a big bag of molecules. We have also used a very central word, and that was organization. You cannot just throw a bunch of molecules together and than say 'now, this is life'. If it is not organized in a way that is exactly right… And by the way, how come cells are organized in that way?*

*Jane: Because of evolution*

*Teacher: Yes, evolution is actually inside the cells.*

Here, we see that the focus on explaining cell activities in terms of underlying molecular mechanisms can provoke very central questions in biology.

# 6. Conclusions

In this study, we present the contours of an educational approach that encourages molecular mechanistic reasoning. The motive to explore the educational potential and feasibility of such an approach is based on the theoretically established starting point that bridging the gap between cellular and molecular level activities asks for reasoning about multi-level mechanisms that are built up from molecular interactions. Therefore, the research question in this study is:
*Can students in upper-secondary education learn to use molecular mechanistic reasoning to bridge the explanatory gap between (sub)cellular activities and molecular interactions?*

We approached this question from three perspectives.

1. *Can we design and effectuate a learning trajectory that guides students meaningfully through the multi-level mechanistic relationship between cell activities and molecular interactions?*
2. *Does the learning trajectory stimulate students to use molecular mechanistic reasoning when they interpret and construct explanations for (sub)cellular activities?*
3. *Do students experience molecular mechanistic reasoning as helpful to connect the molecular and cellular level concepts?*

The three perspectives show that the approach guides students meaningfully through the multi-level mechanistic relationship between molecular interactions and cell activities, although some questions and bottlenecks remain. In Table 7, the findings from the result section are summarized in an overview of the achieved effects as well as the remaining questions and bottlenecks that we have identified.

*Table 7: Overview of the achieved effects, strong points, remaining questions and bottlenecks in the design*

| *Aims in each phase in the design* | *Achieved effects* | *Remaining questions and bottlenecks in the design* |
|---|---|---|
| Phase 1: top-down approach | | |
| Step 1: Identifying cell activities in phenomena in the body | The term 'cell activity' makes sense to students and helps to define 'the cellular level' | Sometimes, confusion about what activity can or cannot be assigned to individual cells (e.g. producing blood) |
| | Students produce a list of general cell activities easily | |
| | Descending from the organism level helps to relate cell activities to phenomena in the body (e.g. diseases) | |
| Step 2: Subdividing cell activities and hypothesizing underlying mechanisms | Subdividing helps to elicit and integrate knowledge about cell processes | Learning activities not experienced as informative and essential in the design by students |
| | Subdividing helps to realize that the 'How?' question can be asked each time again at an underlying level of causation | |
| | Subdividing and hypothesizing evoke the need for a 'bottom level' | |

| | | |
|---|---|---|
| Phase 2: Exploring the bottom level | | |
| Understanding cause and effects of molecular interactions | 'Colliding, binding, changing shape' is experienced as logical and fundamental | Makes great demands on students' visual processing; more practice required |
| Chaining molecular interactions into activities of proteins and protein-based modules | The terms protein activity and modular activity make sense to students and help to recognize 'intermediate levels' based on activities instead of structures | Makes great demands on students' abstract reasoning: more practice required<br>Some organelles are difficult to place in a hierarchy of activities |
| Phase 3: Bottom-up | | |
| Explaining cell activities of increasing complexity | Students can explain examples of cell activities at different complexity levels, by applying the terms protein activity and modular activity. | With increasingly complex activities students stick to less detailed explanations, hardly using 'colliding, binding changing'. It remains unclear whether they are nonetheless aware of the physical and chemical basis when reasoning about these activities |
| Overall | Students experience molecular mechanistic reasoning as 'learning how it really works' and 'going deeper into the things we already knew'<br>Students report that the lessons focused on 'learning to think' and 'logical reasoning' and that this also helps to remember the content | Students report that they did not learn much because they do not experience acquiring molecular mechanistic reasoning as 'learning new things' |
| Molecular mechanistic reasoning assignments | Students demonstrate all the elements of molecular mechanistic reasoning<br>Students pose more how<br>questions after the lessons<br>All students provide mechanistic accounts for the phenomena under study, but the level of detail differs widely<br>Students hardly refer to directed movement and intentional behaviour of the cell or its constituents | Difference between students in the level of detail in their explanations; how to challenge students not to be too easy-going<br>Difficult to judge whether students are indeed aware of underlying physical and chemical principles when they use higher-level activities such as 'activate', 'respond', 'sense', 'produce' |

Perspective 1 sheds light on the achieved effects during the learning trajectory and the questions and bottlenecks that remain. In general, we see that after identifying cell activities as partial activities in the body, students find it self-evident that explaining these activities entails 'descending deeper into the cell' and they regard 'descending' a strategy towards better understanding. This is the core intuition for mechanistic reasoning: changes, in this case 'cell activities', have a cause, and in many cases this cause can be better understood by descending to underlying mechanisms and explore the more fundamental causal relationships that drive these mechanisms (Cummins, 1975). In the learning trajectory, we make this intuition productive, by first confronting the students with the limitations of their ideas about how changes in the cell are caused. They can subdivide activities and use analogies to reason about underlying mechanisms, but they indicate themselves that this does not lead to better understanding and as a consequence they do not

experience these activities as informative. At the same time, students are aware that somehow molecules must be involved in all these activities, but they indicate that they have no idea how molecules can cause these activities. Here, the explanatory gap reveals itself and students express a need to better understand how molecules are involved. In fact, these students regard molecules as the logical candidates to form the bottom-level entities when explaining cell activities, but they lack an intelligible account for causality and organization at this bottom level to be able to use molecules to understand the mechanisms that constitute 'higher-level' activities in the cell. In the lessons, we provide such a basic account by using 'binding, colliding and changing shape' to describe cause and effect of protein interactions. This account is easily grasped by the students and it appeares to be useful as a basis to understand increasingly complex mechanisms, from the activity of individual proteins to the joint activity of multiple protein-based modules. Some limitations of the 'colliding, binding and changing shape' account appear. For instance, some students interpreted 'changing shape' as mere deformation due to the collision, without including binding as the cause for a rearrangement of chemical bonds. Despite the limitations of the simplification, the 'colliding, binding, changing shape' account made intelligible to these students how protein interactions can be the basis of complex molecular mechanisms. In the final step of the trajectory, students used these molecular mechanisms at different complexity levels to explain the cell activities that were identified at the start and as a result they indicated that they found it logical that the same bottom-level principles of molecular interactions apply to all cell activities. However, when explaining more complex activities, students tend to rely on higher-level causal terms such as 'produce, respond, activate' and it remains difficult to judge whether they are aware of underlying physical and chemical principles when using these terms. Students obviously reason mechanistically when they use these terms. However, our account for molecular mechanistic reasoning includes that students are aware of the physical and chemical principles that drive changes at the bottom level of molecular interactions. It is questionable if these six 3-hour lessons provided students with enough examples and practice to transform their tendency to accept and subscribe all kind of (sub)cellular activities without questioning the physical and chemical principles that underlie these activities.

Perspective 2 addresses the question was whether indeed students are stimulated to use molecular mechanistic reasoning when they interpret and construct explanations for (sub)cellular activities. The analysis of molecular mechanistic reasoning assignments shows that, before the lessons, students pose some mechanistic how-questions, but their reasoning in answering these questions is very superficial. After the lessons, much more mechanistic questions were posed and from these questions we can see that students better subdivide cellular phenomena into (hypothetical) underlying activities. Furthermore, when interpreting graphical representations of molecular mechanisms students search for causality, they recognize gaps and use molecular dynamics principles in their reasoning about causality and organization. They hardly refer to directed movement and intentional behaviour in their explanations. However, many students are not very precise and consequent in applying these principles, and we suggest that much more molecular mechanistic reasoning practice is needed in interpreting, constructing and hypothesizing explanations for (sub)cellular activities.

Perspective 3 shows that students experience this way of reasoning about cells as a new perspective. One aspect they mention as being new is the focus on 'explaining' in contrast to 'just being told how it is'. Another remarkable observation is that, before the lessons started, students have not experienced an explanatory gap between 'what cells do' and what 'molecules

do'. Although they know that cells consist of molecules, they report never having thought about how cells do things. This relates to students responses about the traditional biology lessons. Students experience the traditional cell biology and molecular biology lessons as being told 'what happens', without questioning 'how it happens'. However, it seems that most of them do not see this as a problem, although they report memorization and rote learning as strategies they use when learning about (sub)cellular and molecular activities.

In general, we can conclude that an intelligible account for the cause and effect of molecular interactions is indispensable for bridging the gap between the molecular and cellular level. We show that this account can be introduced in a meaningful way, which means that it is used to construct mechanistic explanations for cell activities that in the perception of students cannot be explained satisfactorily without this account. Students experience this as a new, useful and generally applicable perspective on how cells work. For students to use molecular mechanistic reasoning consequently and precisely when reasoning about (sub)cellular activities, much more practice is needed, but this study shows that applying molecular mechanistic reasoning strategies meaningfully in the domain of cell biology is within reach for students in upper-secondary life science education.

# 7. Discussion

In this section, we reflect on the potential and limitations of the presented approach. Furthermore, we discuss the limitations of the study and suggestions for further research.

The approach is shaped by the consequent use of term 'activity' for all productive changes above the level of molecular interactions, whether it be protein activities, modular activities, organelle activities, cell activities, organ activities or activities of the body. The inspiration for using this term comes from the philosophy of science (Bogen, 2008; Machamer, 2004) and we focus here on the educational potential and pitfalls of using this term in molecular and cell biology education. We have showed that students experienced the term 'activity' as a logical label for 'the things cells, organelles, proteins and protein-based modules do'. We have also showed how the term 'cell activity' helps students to define the cellular level and to distinguish between activities that are higher and lower than the cellular level. At the molecular level, the use of the term 'protein activity' is very common in biochemistry and molecular biology research as well as in education. However, we think that without a sound understanding of the fundamental physical and chemical changes, proteins only remain functional units 'that do the work' and there is a risk that the term 'protein activity' might be interpreted by students as intentional. In our opinion. this is not a meaningful connection between the molecular and cellular level because it does not connect to students' chemical and physical knowledge about molecules. Although we agree with Duncan and Tseng (2011), Roseman, Caldwell, Gogos & Kurth (2006) and others that for mechanistic understanding in genetics (and all other biological disciplines that include the molecular level) students should be aware that 'proteins do the work of cells' (Roseman et al., 2006), we stress the importance of an intelligible account for the physical and chemical basis for 'carrying out work in the cell'.

We have tested if the simplified 'colliding, binding and changing shape' account could provide this insight and we were interested if students experience it as more fundamental than explanations in terms of 'activities'. This appears to be the case and we suggest two aspects contribute to this: first, activities can appear in infinitely diverse forms, while 'colliding, binding, changing shape' applies to all activities regardless the complexity of their appearance. The account thus has wider and more general applicability (Grotzer & Mittlefehldt, 2012) than terms for specific protein activities. Second, the causal events 'colliding' and 'binding' and 'changing shape' are intuitive and can easily be mapped onto events in the world around us. This means that it is to be expected that students do not feel the need for further explanation to accept these events as plausible and intelligible (Grotzer & Mittlefehldt, 2012). However, in the lessons we see that our simplified 'colliding, binding and changing shape' account for causality is somewhat misleading when interpreting molecular events. 'Changing shape' can be interpreted by students as the result of the collision causing plastic deformation, analogues to the deformation of a car in an accident, instead of a new energy state that depends on attractions and repulsions between atoms in the molecules. An educational strategy to highlight this difference could be to compare the effect of protein interactions to bending a spring with a magnet or other analogues for elastic deformation.

In our approach, visual literacy plays an important role because of the spatiotemporal nature of mechanistic models. Many visual models, especially animation, suggest goal-directed and intentional behaviour of molecules in the cell and we suggest that it is an important task for

educator to empower their students to interpret these events as non-intentional and based on randomness. Scholl and Tremoulet (2000) give an overview of a long tradition of experiments that demonstrates that people infer causality but also intentionality if abstract objects move in certain patterns. These precepts appear to be fairly fast, automatic and irresistible and it is suggested that this notion precedes higher-level cognitive processing. This suggest that it take quite some effort to actively counterbalance the impression of intentional behaviour that arises from such animations (see also Jenkinson & McGill, 2012). Therefore, explicitly and consequently practising molecular mechansitic reasoning is also indispensable for interpreting moleuclar movies (McGill, 2008). Actively 'reading' different representations of molecular mechanistic models can be stimulated if students are familiarized with all the conventions that are used to depict, describe or even tacitly ignore the elements of molecular mechanistic explanations in schematic models as well as animations.

One could argue that there is a pitfall of students drawing deterministic conclusions due to the focus on explaining the behaviour of living systems in terms of physical and chemical principles. However, the solution for this should not be found in treating the molecular level as a separate domain that hardly contributes to our understanding of living systems. From our findings, we argue that carefully designed cell biology education based on molecular mechanistic reasoning can help students to understand how complex cellular behaviour can emerge without the need to rely on vitalism, although this was not an explicit goal in our approach.

With this study we do not claim to present a ready-to-go educational approach that overcomes all the obstacles identified in Van Mil et al. (2013). Concepts at the molecular level remain abstract and getting a grip on the dynamic and transient nature of molecular mechanisms is undoubtedly a very demanding and time-consuming effort for students in the life sciences. However, with these series of six lessons we show that a sound understanding of the multi-level mechanistic nature of cell activities as well as the principles that determine how these underlying molecular mechanisms work can form the basis for meaningful learning about these abstract and dynamic concepts.

We organized a somewhat artificial environment to test our design. Students volunteered to attend the lessons and the teaching in this study was done by the principal researcher who was the designer of the trajectory and an expert in molecular and cell biology. However, these aspects hardly interfere with our goal to provide a proof of principle. The setting we chose enabled us to work for a considerable amount of time with a relevant group of students from different schools. Since molecular mechanistic reasoning as learning goal is not yet part of current biology or chemistry curriculum, it would be very difficult to claim such a long period of teaching in a regular biology or chemistry classroom.

In this study, we have worked with a group of 12 motivated science students and we see differences in the level of sophistication students' reasoning within that group. It is beyond the scope of this study to provide explanations for the differences between individual students, for instance by comparing their grades in the regular biology and chemistry classes, although it would be interesting to see which characteristics contribute to the student's ability to adopt molecular mechanistic reasoning easily. Results with a group of 12 motivated students do not provide a proof that all students in various educational settings will adopt and appreciate this approach. However, the aim was to see whether this theoretically underpinned approach could indeed do what is was supposed to do and to learn about the opportunities, limitations and

pitfalls when making the translation from theory to educational practice. Now that we know that an approach based on explaining molecular mechanistic reasoning can help students to bridge the gap between the molecular and cellular level, the next question can be addressed: How can molecular mechanistic reasoning be incorporated as an explicit learning goal throughout the different stages in life science curricula? Furthermore, it would be interesting to explore if the explicit use of the term 'activity' that we adopted from the Machamer, Darden and Craver account for mechanisms could also help students in others domains of biology or science education in general to 'think in levels'.

# 8. Implications for teaching

Molecular level details are part of most life science curricula for upper-secondary students. In this study we show that it is within reach for students to connect molecular level concepts meaningfully to their knowledge about cellular behaviour. However, this does not happen automatically. We consider the connection between molecular and cellular level concepts 'meaningful' if students see how their knowledge about molecules helps them to better understand or even explain how (sub)cellular activities emerge. This means that ideally every time a (sub)cellular activity is discussed, the mechanistic relationship with underlying molecular changes is explained. In many different topics in upper-secondary education, cells and their activities play a role. As Cohen & Yarden (2009) already suggest, the cellular level needs to be integrated in biological knowledge by explaining the role of cells at many different moments in the curriculum and in connection to many different biological phenomena. The same holds true for the molecular level, only at a later stage in the curriculum. We suggest that a basic understanding of the bottom-level principles that drive molecular mechanisms is indispensable for meaningful learning about molecules in the cell and we show that this is within reach for students. However, after an introduction of the molecular level, which can be done as shown in this study, ideally the mechanistic relationship between cells and molecules is explained every time that cellular behaviour is discussed in different topics; for instance, in the immune system, digestion and the nerve system. Here, we see an important role for the teacher to include also the downward 'how' instead of focusing mainly on the upward 'why' of (sub)cellular activities in these topics (Abrams & Southerland, 2001).

We thus propose that when proteins in the cell are introduced, at the same time the molecular dynamics principles should be included to provide an account for molecular causality and organization. The simplified 'colliding, binding, changing shape' account appears to be a good starting point for this. Furthermore, the intermediate levels of molecular modules should be introduced and be distinguished from cell organelles by well-chosen examples. We show that together this allows students to build a general understanding of the physical basis for (sub) cellular activities and makes intelligible the central role that proteins play in all cellular activities. Based on our findings, we support the idea to introduce the central role of proteins even before the introduction of the relationship between genes and proteins in molecular genetics (Roseman et al., 2006). This is a very unusual approach for most biology teachers. However, from the point of view of conceptual coherence and meaningfully connecting prior knowledge, it makes sense to argue that the role that genes play in the cell can only be understood if the central role of proteins in cell activities is understood. We think that by inverting the introduction of genes and proteins, the much-reported superficial speech about genes 'telling the cell what to do' may be counterbalanced with a mechanistic alternative of the genes producing[11] the bottom-level entities that play a central role in mechanisms in the cell.

11. Note that, for understanding how genes can produce proteins (via transcription and translation), a sound understanding of molecular mechanisms is indispensible as well. This strengthens our suggestion that it makes sense to first teach about the central role protein interactions before introducing the mechanisms of transcription and translation in molecular genetics.

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# Appendix 1:

## Assignments used to recognize molecular mechanistic reasoning (perspective 2) and supportive molecular mechanistic reasoning guidelines available for students

### Assignment 1: 'Pose questions and ideas to explain the crawling of a neutrophil'

Students watch a microscopic time-lapse movie that shows a neutrophil chasing a bacterium (http://www.youtube.com/watch?v=OWUmXx5V_wE, narration muted) and they write down their questions and ideas to explain this cell activity.

#### Translated text of Assignment 1

In the movie you see a neutrophil (a type of white blood cell) chasing a bacterium. Imagine you are a scientist and you are being asked to explain this phenomenon. Write a research plan in which you work out as detailed as possible the following questions:

- Which questions do you want to answer with your research?
- What are your current ideas/hypotheses, which can help you to answer these questions?

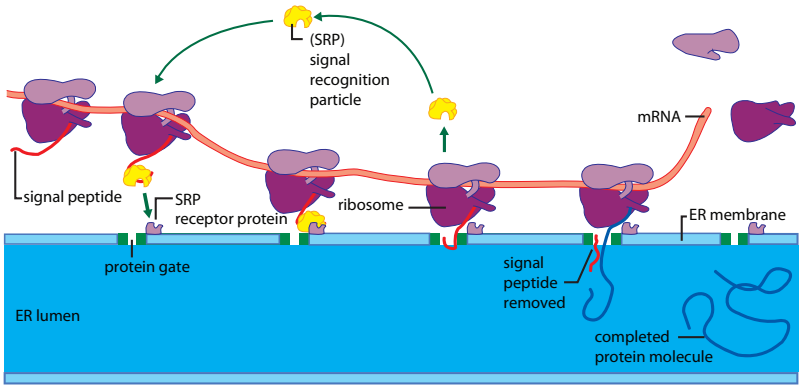
### Assignment 2: 'Interpret textbook graphics of molecular mechanisms'

Students interpret two graphical representations of molecular modules taken from a standard upper-secondary science reference book that students are allowed to use during the regular biology exams.

#### Translated text of Assignment 2a

Look at the figure below: passage of a newly formed amino acid chain through the endoplasmic reticulum (adapted from your reference book Fig. 70I).

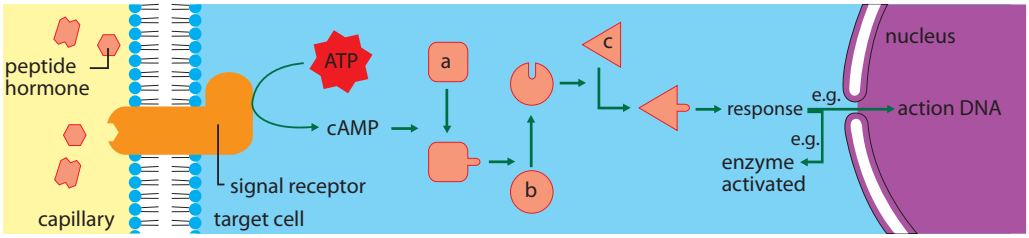
Passage of polypeptide through ER



- What is the how-question that this scheme attempts to answer?
- What are the start and the end situations in this scheme?
- Describe all the steps in between by using the words 'because of …', 'if … then' and 'thereby'.
- Also note all the steps that you lack information about or of which you don't know how it works, by writing down: 'I don't know whereby/how/what happens if …' . Try to add a hypothesis, formulated as: 'It could be that …'

## Translated text of Assignment 2b

Look at the figure below: a signalling cascade (adapted from your reference Fig. 89B).




Describe, using the questions below, the molecular mechanistic explanation that is depicted in this scheme, *without* using the words 'activate' and 'signal'.

- What is the how-question that this scheme attempts to answer?
- What are the start and the end situations in this scheme?
- Describe all the steps in between by using the words 'because of …', 'if … then' and 'thereby'. Do this without using the words 'activate' or '(in)active' or 'signal'. For the steps in which protein a, b and c, you choose yourself what happens exactly, but here as well don't use the words 'activate' or '(in)active' or 'signal'.
- Also note all the steps that you lack information about or of which you don't know how it works, by writing down: 'I don't know whereby/how/what happens if …' . Try to add a hypothesis, formulated as: 'It could be that …'.

## Assignment 3: 'Reconstruct (in text and drawing) the mechanistic model discussed in a newspaper article'

Students read an article from the science section of a national newspaper and write down and draw a model for the mechanism that is discussed in the article. The article explains the directed swimming of sperm cells by describing how progesterone released from the ovaries activates an ion channel in sperm cells which causes rapid movement of the sperm tail. (The translation of the newspaper article is available on request. The article did not contain a graphical representation).

## Translated text of Assignment 3

Read the newspaper article 'Calcium channel bring together sperm and egg' published 20 March 2011

Describe and draw the molecular mechanism that is described in the article, using the hints below.

- Describe as specifically as possible the how-question that these scientists tried to find an answer to.
- List all the components that are involved in the mechanisms and make a schematic drawing of the mechanism.
- Describe step by step what happens by using the words 'because of …', 'if … then' and 'thereby'.
- Also note all the steps that you lack information about or of which you don't know how it works, by writing down: 'I don't know whereby/how/what happens if …' . Try to add a hypothesis, formulated as: 'It could be that …',

## Supportive guidelines available for students while working on the molecular mechanistic reasoning assignments

**In molecular mechanistic reasoning, you make flexible use of:**

- Downward reasoning: which smaller activities does this activity consist of?
- Upward reasoning: which larger activity is this activity part of?
- Forward/backward reasoning: what is the preceding activity (backward) and what is the subsequent activity (forward)?

**Useful questions for mechanistic reasoning:**

- What is the phenomenon under study? What is the central how-question?
- What (type of) entities play a role?
- Which activities play a role?

**For downward reasoning**

Which smaller activities does this activity consist of?

Which suggestions/hypotheses do you have about the underlying mechanism?

**For upward reasoning:**

Which larger activity is this activity part of?

In which larger activity does this entity play a role?

**For forward/backward reasoning:**

What are the start and end situations?
What (type of) entities play a role?
How are the entities organized (place and time)?
Do I get in logical steps (based on molecular interactions) from start to end?

Which molecules interact? How is that depicted or described?
What happens because of the interaction? How is that depicted or described?
What entities and/or activities play a role, but are not depicted or described?
Which how-questions remain unanswered? What gaps remain in the causal chain?
Which suggestions/hypotheses do I have about the unanswered how-questions?

# Appendix 2:
## interview protocols

### Protocol for the semi-structured interview with four student couples at the end of lesson 3

**Subdividing activities of the body into cell activities**

- *What did you do in lesson 1? (Practised a specific way of thinking? How would you call that?)*
- *How did you reason about those cells? What would you call 'activities of the body?' And what would you call 'cell activities'?*
- *To which contexts did you apply this reasoning? (HIV/cancer/diabetes/own ideas about disease/phenomenon)*
- *Do you see a pattern in what you did? How would you call that yourself?*
- *You started with thinking 'smaller'. In what way did you think 'smaller'?*
- *What is this 'subdividing' strategy useful for according to the teacher? (What do they report about the work of cell biologists/molecular biologists? Why do scientists use this subdividing strategy?)*

**From cell activities to partial (subcellular) activities**

- *What thinking steps did you make after subdividing activities in the body into cell activities?*
- *The teacher looked at your examples of cell activities that you identified in the body, and subdivided some of these activities in even smaller partial activities. Did you also practise this yourself? In what way?*
- *What was the question that remained?*
- *Did you also come up with that question yourself? Why is that question important, do you think (for scientists)?*
- *Is there something you realized then, which you didn't see before?*
- *What particles do you expect scientists to examine? (Possibly they mention proteins, because of the focus in the last lesson)*
- *Why proteins?*
- *The teacher mentioned a few times 'reasoning from the top to the bottom' and 'from the bottom to the top'. What do you think the teacher means with that?*

**Hypothesizing mechanisms**

- *In the lessons the teacher mentioned the example of cystic fibrosis. Could you briefly repeat what goes wrong in CF?*
- *If needed: The defect is in a pump that transports chloride ions. You hypothesized with the teacher what such a pump could look like.*
  *Why is that important?*
  *What can you do with it?*
  *Did you practise this yourself? (Cell division: how can chromosomes be transported?)*
  *What is the difference between the situation in CF and the examples you practised with? (Monogenetic disease, wound healing: cell division = much more proteins involved than in the case of CF)*
- *What do you think, is the benefit of hypothesizing for scientists who are interested in the molecules in the cell?*

**Taking a closer look at the functional players in the cell: the working parts**

- *What was the next step in the lesson series after descending from the body to cell activities and partial activities in the cell?*
- *The teacher emphasized that there was a 'jump' to proteins, after subdividing cell activities into partial activities. What do you think the teacher meant with that 'jump'? Why is the jump needed?*
- *What do you think is in between?*
- *Why do you think the teacher skips that part?*

**Proteins: new conceptual knowledge**

- *What do you know about transcription and translation?*
- *What did you learn about proteins in regular biology classes?* (Composition, type of functions in the cell? How proteins perform these functions?)
- *If you know that DNA codes for proteins, what content did the teacher cover at the end of lesson 2 and what did the teacher aim to teach you?* (Proteins, biochemical properties, molecular interactions as a basis for protein activities)

**Reflection: find out to what extent students can formulate the reasoning steps and see if they can hypothesize what is to come in the next lessons**

- *What would be your general answer to the question: which activities did you do in lesson 2 and what did you practise with those activities?*
- *Which reasoning steps did you make?*
- *Where did you stop?*
- *What is still missing?*
- *Why would the teacher postpone that step (and make a jump instead?)*

## Protocol for the semi-structured interview with four student couples at the end of lesson 6

**What strategy was central in the lessons?**

- *At the moment of the first interview, 'protein activities' had just been introduced. Then you got new knowledge about protein activities in the cell. What new knowledge followed on that?*
- *What did you learn next? What was the central approach? What lesson activity did you learn the most from?*
- *I asked in the first interview what you think the teacher would mean with 'the jump'. In your opinion, is that question answered or the gap filled? If so, what does the teacher mean with that 'jump'?*
- *You are used to learn from a textbook. In these lesson series that is not the case. The teacher taught you a way of reasoning. What was the line of reasoning that was central? How would you call that? Which terms do you use in that reasoning?*

Interviewer explains to students: *'These lessons are designed to learn a way of reasoning that allows you to explain cell activities using the molecular level. I want you to reconstruct how you experienced this way of reasoning.'*

**Reconstructing the lesson series: how do students report on the molecular mechanistic reasoning skills that they have learned?**

We use a scheme to reconstruct the phases in the design. Students use cards as pieces in a puzzle to put together the scheme. Students are told that the scheme represents the line of reasoning that was central in the lesson series. Central activity in the interview: students put the pieces of the scheme together and while doing so, they are stimulated to express their considerations. The interviewer will also ask what students remember about that step in the lesson series.

**Reconstructing the thinking steps**

Material: worksheet A (containing a table with empty cells)

First students fill the upper row of the table, with cards that represent the thinking steps that were central in the lessons. Students are provided with cards showing the terms to be used, and they place the cards above the proper column. While doing this, the interviewer asks:

- *Why do you choose this order? Students are stimulated to think aloud.*
- *What did you learn about molecular interactions?*
- *What did you learn about protein activities?*
- *What exactly is a functional module?*
- Questions about organization:
  - *What did the teacher tell about organization? What do you think 'organization' means?*
  - *How did you practise this?*
  - *What did you learn from that?*
  - *How could you integrate 'organization' in this scheme?*
  - *Where in the scheme is 'organization' important?*
  - *How does a functional module distinguish from an ordinary group of proteins?*
  - *Why is a whole series of protein activities not yet necessarily a functional module?*
- General: *What knowledge (represented in this scheme) was completely new to you?*

**Reconstructing the complexity levels in the explanations for the three examples (CF, FH and wound healing)**

After completing the top row in the scheme, students are asked to fill in the scheme as completely as possible for the examples CF, FH and wound healing. For CF this is straightforward, but in FH and wound healing we expect hesitation and confusion because many proteins/modules are involved. The interviewer highlights that in each phenomenon eventually one cell activity was central in the lessons:

CF: mucous-producing cells secrete chloride ions

FH: liver cells take up LDL-cholesterol

Wound healing: fibroblasts secrete collagen when stimulated with TGF-β.

- *What is the difference between the three phenomena? Why did the teacher choose these examples?*

The interviewer encourages the students to highlight the difference in the scheme. Possibly by using arrows: CF can be explained using one protein activity, FH can be explained using one module activity and in wound healing many cell activities and underlying module activities are involved.

Next, students are asked to place the three phenomena in the scheme. To see the order they choose. The interviewer asks: *'why do you choose this order?'* 'why do you choose this order?' If students do not provide a reason, the interviewer asks them explicitly to order the examples in increasing complexity. Again: *'why do you choose this order?'* Note that step 2B in the interview continues with the scheme that contains the thinking steps that students place in the top row in step 2A.

**Metacognition: How do students report about learning the reasoning strategies in molecular mechanistic reasoning: downward/upward/forward/backward reasoning.**

- *Where do you start when you reason upwards? (Genes/proteins?)*
- *Which questions do you pose/which thinking steps do you use?*
- *What is your starting point in downward reasoning (about a disease)?*
- *Which questions do you pose?*
- *What is forward/backward reasoning? (The aspect of time plays an important role. What happened before this activity and what happens next in the chain?)*
- *In which reasoning step does the aspect of time play an important role?*
- *If you transfer these reasoning strategies to your biology classes at school; do you think you can and will use it? If so, what other 'things' could you explore down to the molecular level? what are those 'things'?*

**Concluding questions:**

- *What was new in these lessons? How would you summarize that?*
- *How can you use this knowledge in regular biology classes?*
- *Have you done this before? If so, in which situations?*
- *Did these lessons have an effect on you? Do you think that you can apply it elsewhere? If so, in what situations?*

# Samenvatting

In dit proefschrift beschrijf ik mijn onderzoek naar het leren en onderwijzen van de moleculaire basis van levensprocessen. Ik heb gekeken wat leerlingen in de bovenbouw vwo leren over cellen, over de processen die plaatsvinden in cellen en over de moleculen betrokken bij die celprocessen. De hoeveelheid details is overweldigend, maar het is de vraag of deze details bijdragen aan het begrip over de werking van cellen, weefsels en organen. In veel vakdidactisch onderzoek is al beschreven dat leerlingen moeite hebben om moleculaire begrippen als DNA, RNA en eiwitten te gebruiken in hun denken over cellen. In **hoofdstuk 1** beschrijf ik dat dit knelpunt in het biologieonderwijs ook naar voren kwam tijdens de evaluatie van het Reizende DNA-lab 'Lees de taal van de tumor'. Utrechtse studenten Biomedische Wetenschappen verzorgen dit mobiele practicum, waarin leerlingen uit 4-6 havo/vwo DNA-mutaties in tumorcellen opsporen, om vervolgens conclusies te trekken over de effecten van deze mutaties op het gedrag van de cellen en de gevolgen die dit heeft voor de diagnose en behandeling van een kankerpatiënt. Dit blijkt een uitdagende, maar pittige opgave. Vooral het verband tussen een gen, het eiwit waarvoor dit gen codeert en de rol die dit eiwit speelt in een specifiek celproces, is voor veel leerlingen moeilijk te leggen. Hoewel kennis van cellen (cellulair niveau) en de begrippen DNA, RNA en eiwitten (moleculair niveau) tot de standaard examenstof behoren op havo en vwo, lijkt het erop dat het huidige biologie- en scheikundeonderwijs er onvoldoende in slaagt deze twee organisatieniveaus betekenisvol met elkaar te verbinden. Ik heb me daarom in dit onderzoek afgevraagd waarom kennis over moleculen en kennis over cellen zo moeilijk met elkaar in verband te brengen zijn en ik heb gezocht naar kansen voor het onderwijs om de relatie tussen moleculair en cellulair niveau te verhelderen.

In **hoofdstuk 2** specificeer ik het geschetste probleem. Ik bespreek bevindingen uit de vakdidactische literatuur en ik besteed specifiek aandacht aan twee biologiedidactische proefschriften die eerder verschenen bij het Freudenthal Instituut voor didactiek van Wiskunde en Natuurwetenschappen. Ik stel vast dat het denken in biologische organisatieniveaus van veelal neerkomt op het toekennen van functies van duidelijk te identificeren onderdelen in het geheel. Zeker wanneer er vanaf het niveau van het menselijk lichaam afgedaald wordt naar lagere organisatieniveaus, gebeurt dat veelal door het bespreken van de individuele functies van organen, weefsels, cellen en tot slot de organellen in de cel. De moeilijkheid zit hem echter juist in het feit dat met kennis over de losse onderdelen, het nog steeds heel moeilijk is om een verklaring te geven voor wat het geheel doet. Inzicht in de organisatie van de onderdelen ten opzichte van elkaar en de interacties die ze daardoor met elkaar kunnen aangaan, is essentieel om te begrijpen hoe het geheel werkt. De *structuur* van het lichaam kun je dus wel beschrijven door aan te geven welke organen erin zitten, maar om de *werking* van het lichaam te begrijpen, zul je naast de functie van die individuele organen ook moeten aangeven hoe organen met elkaar in verbinding staan en elkaar beïnvloeden binnen het lichaam. Dit gegeven, dat veel eigenschappen van een systeem (bijvoorbeeld een lichaam, een orgaan of een cel) niet begrepen kunnen worden door alleen losse onderdelen te bestuderen, wordt in de filosofie *emergentie* genoemd. De termen *organisatie* (hoe verhouden de onderdelen zich ten opzichte van elkaar in plaats en tijd) en *interactie* (welke onderdelen beïnvloeden elkaar op welke manier) blijken cruciaal te zijn om het ontstaan van emergente eigenschappen beter te begrijpen.

In **hoofdstuk 3** beschrijf ik mijn focus en onderzoeksvragen. Hieruit blijkt hoe het emergentie-perspectief uit hoofdstuk 2 een belangrijke basis is voor het onderzoek in dit proefschrift.

Wetenschappers in de life sciences bestuderen cellen namelijk vanuit het idee dat het complexe gedrag van cellen (cellen kunnen delen, hormonen produceren, signalen doorgeven etc.) voortkomt uit simpele interacties van moleculen. Ze proberen het gedrag van cellen te verklaren door zo precies mogelijk te beschrijven wat er op welk moment en op welke plek gebeurt als de betrokken moleculen interacties aangaan. Het doel van deze studie is om te verkennen hoe leerlingen in de bovenbouw van het vwo ook gestimuleerd kunnen worden om moleculaire interacties als basis te gebruiken voor hun denken over celprocessen. Dat is de reden van de titel van dit proefschrift: *Learning and teaching the molecular basis of life*.

De onderzoeksvragen zijn:

1. *Hoe gebruiken wetenschappers hun kennis over moleculen in de cel om het gedrag van cellen te verklaren en hoe zien de verklaringen die ze presenteren er uit?*
2. *Hoe kan deze typering van het werk van wetenschappers helpen bij het ontwerpen van onderwijs over de moleculaire basis van levensprocessen?*
3. *Is het mogelijk om een leertraject te ontwerpen waarin leerlingen het gedrag van cellen en de interacties van moleculen in die cel betekenisvol met elkaar verbinden?*

In **hoofdstuk 4** beantwoord ik onderzoeksvraag 1 door middel van een filosofische en historische analyse van het werk van wetenschappers in de life sciences. Centraal in dit hoofdstuk staat de term 'moleculair mechanistische verklaring'. In een mechanistische verklaring beschrijven wetenschappers hoe moleculaire onderdelen in de cel onderling interacties aangaan, en hoe deze interacties de moleculen veranderen, waardoor ze nieuwe interacties aan kunnen gaan die daarvoor niet mogelijk waren. Ze beschrijven dus ketens van moleculaire gebeurtenissen die, als je ze in totaliteit beschouwt, een beeld geven van het gehele proces. Opvallend is dat dit type verklaring vaak wordt weergegeven in schematische plaatjes en recentelijk ook steeds vaker in animaties. Deze plaatjes en animaties zijn dus eigenlijk mechanistische *modellen* die antwoord proberen te geven op de vraag *hoe* een celproces tot stand komt. Wetenschappers hebben de inhoudelijke kennis en vaardigheid om deze modellen te lezen en te interpreteren als een weergave van mechanismen die gebaseerd zijn op moleculaire interacties. Deze plaatjes en animaties worden in versimpelde vorm ook veel in het life science onderwijs gebruikt. Echter, als we deze plaatjes en animaties kritisch bekijken wordt duidelijk dat leerlingen op het vwo veel van de benodigde kennis en vaardigheden missen om deze modellen te interpreteren als een mechanistische verklaring gebaseerd op moleculaire interacties. Ik concludeer in hoofdstuk 4 dat dit moleculair mechanistische perspectief bij leerlingen ontwikkeld moet worden om een betekenisvolle brug te kunnen slaan tussen het moleculaire en cellulaire niveau en ik geef aan welke punten extra aandacht nodig hebben in het onderwijs om dit perspectief te ontwikkelen.

**Hoofdstuk 5** beschrijft het belang, maar ook de kansen die ik zie om dit moleculair mechanistisch denken bij leerlingen te stimuleren. Op de eerste plaats moet het voor leerlingen duidelijk zijn dat ze naar onderdelen in de cel gaan kijken om zo een verklaring te vinden voor iets dat tot op dat moment voor hen onverklaard was. Met andere woorden: je kunt wel weten dat een cel van alles doet, maar als je wilt weten hoe de cel dat bewerkstelligt, dan zul je moeten kijken naar wat er met onderdelen in die cel gebeurt. Dit wordt aangeduid als 'het gebruik van een verklaringscontext' als motief om af te dalen naar lagere organisatieniveaus. Op de tweede plaats moet het voor leerlingen ook duidelijk worden dat de veranderingen die onderdelen in de cel ondergaan, gebaseerd zijn op voor hen begrijpelijke fysisch-chemische veranderingen. De basis hiervoor moet gelegd zijn in de scheikundeles, maar in de biologieles moet duidelijk worden dat de regels van de scheikunde ook gelden voor complexere veranderingen zoals die in een cel plaatsvinden.

Leerlingen kunnen natuurlijk niet alle kennis verwerven die een expert gebruikt om complexe veranderingen in de cel te begrijpen. Daarom doe ik in dit hoofdstuk de suggestie om leerlingen een vereenvoudiging aan te bieden, waarmee ze toch voldoende inzicht hebben in de oorzaak van interacties tussen moleculen in de cel en wat er gebeurt bij zo'n interactie. Deze vereenvoudiging richt zich op het type moleculen dat een belangrijke rol speelt in verklaringen van celbiologen: *de eiwitten* en kan als volgt worden samengevat. Door warmtebeweging verplaatsen eiwitten zich willekeurig door een cel heen. Door deze beweging botsen ze constant tegen elkaar en tegen andere moleculen. Eiwitten hebben specifieke vormen en als eiwitten met de juiste vorm op de juiste manier tegen elkaar botsen, kunnen ze door hun chemische eigenschappen aan elkaar binden. Dat binden heeft echter tot gevolg dat de beide eiwitten van vorm veranderen, omdat de atomen waaruit de eiwitten bestaan zich herrangschikken. Dat leidt ertoe dat het van vorm veranderde eiwit nieuwe interacties kan aangaan die voor de interactie niet mogelijk waren. Er is dus sprake van kettingreacties, waarbij de interacties moleculaire veranderingen veroorzaken, zoals moleculen die gesplitst worden, van vorm veranderen of gekoppeld worden, en waarbij de ene interactie dus de volgende mogelijk maakt. Uit dit basisidee van kettingreacties volgt ook dat meerdere eiwitten samen kunnen werken in een moleculaire modules met een specifieke functies in de cel, zoals het activeren van een bepaald gen. Vaak zijn deze modules niet als vaste structuren in de cel te herkennen, maar komt het effect tot stand doordat de betrokken moleculen kris-kras door de cel of het celcompartiment bewegen.
Deze manier van redeneren, die ik *moleculair mechanistisch redeneren* noem, is nodig om plaatjes en animaties van moleculaire processen in de cel betekenis te kunnen geven. Daarom zal in een lessenserie waarin het moleculaire niveau gebruikt wordt om het cellulaire niveau beter te begrijpen, zowel het aanleren van deze manier van redeneren als het toepassen hiervan bij het lezen van plaatjes en animaties veel aandacht moeten krijgen.
In **hoofdstuk 6** beantwoord ik onderzoeksvraag 3 door het ontwerpen en uittesten van een lessenserie waarin moleculair mechanistisch redeneren centraal staat. De lessenserie is erop gericht leerlingen te laten nadenken over moleculen in de cel, en dan met name de eiwitten, op een manier die hen in staat stelt om plaatjes en animaties van celprocessen te kunnen interpreteren als mechanismen van interacterende eiwitten en eiwitcomplexen. De vraag in dit hoofdstuk is of dit doel haalbaar is voor leerlingen in het vwo en of ze deze manier van denken over moleculen in de cel ervaren als hulpmiddel om beter grip te krijgen op de vraag hoe het kan dat een cel allerlei levensprocessen vertoont.
De lessenserie is getest met een groep van 12 5 vwo-leerlingen die zes keer een 3-uur durende les hebben gevolgd, waarbij ik zelf de docent ben geweeest. Tijdens de lessen zijn video- en audio-opnames gemaakt en de werkbladen van de leerlingen verzameld. Daarnaast heeft een tweede onderzoeker alle lessen geobserveerd en op twee momenten interviews met de leerlingen afgenomen.
Met het ontwerpen en testen van de lessenserie wil ik in essentie het volgende te weten komen: gebeurt er in de lessen wat ik verwacht dat er zal gaan gebeuren op basis van de theoretische inspiratie waarop het lesontwerp gebaseerd is? In het analyseren van de resultaten richt ik me dan ook op de volgende vragen: gebruiken leerlingen het moleculair mechanistisch redeneren zoals bedoeld en ervaren ze het als een bruikbare manier van denken waarmee ze de verbinding kunnen leggen tussen 'wat cellen doen' en 'wat moleculen doen'?
Op basis van deze eerste test kan ik een aantal algemene uitspraken doen die betrekking hebben op hoe de lessen gewerkt hebben. Ten eerste worden de leerlingen zich bewust van het feit dat

ze wel weten wat cellen in het lichaam doen (in de lessen noemen we dat de 'celactiviteiten'), maar dat ze niet kunnen verklaren *waardoor* cellen dat doen. Vervolgens geven de leerlingen aan dat ze het vanzelfsprekend vinden dat je naar onderdelen in de cel gaat kijken, als je wilt weten hoe een celactiviteit tot stand komt. Dit is wat ik noem 'de mechanistische intuïtie' die ik in mijn theoretische analyse beschreven heb en op dit moment in de lessenserie bewust inzet. Leerlingen beseffen dat ze, zoals ze het zelf formuleren, 'omlaag', 'kleiner' of 'dieper' moeten zoeken naar de onderdelen om tot een verklaring te kunnen komen. Tegelijkertijd beseffen ze dat ze al behoorlijk wat onderdelen van de cel kennen (voornamelijk organellen), maar dat die kennis bij de meeste celactiviteiten ontoereikend is om met een plausibele verklaring te komen.
Vervolgens worden in de lessen eiwitten geïntroduceerd als deeltjes (macromoleculen) in de cel waarvan je iets moet weten om wél tot acceptabele verklaringen voor celactiviteiten te komen. Leerlingen accepteren het als een gegeven dat losse moleculen in de cel continu door elkaar bewegen en tegen elkaar aan botsen, en ze begrijpen dat moleculaire veranderingen (bijvoorbeeld een chemische reactie) pas plaatsvinden als de betrokken moleculen precies 'in elkaar' passen, dankzij hun atomaire samenstelling. Het blijkt voor leerlingen nieuw te zijn dat eiwitten van vorm veranderen als ze interactie aangaan met andere eiwitten, maar ze kunnen dit gegeven gebruiken om vormveranderingen te gaan zien als schakels in moleculaire mechanismen die bepalend zijn voor waar en wanneer er iets gebeurt in de cel. Het blijkt in de lessen dat ook zonder gedetailleerde (bio)chemische kennis leerlingen dit mechanistische principe van eiwitten kunnen toepassen. Als de basis van eiwitinteracties gelegd is, gebruiken leerlingen dit inzicht om plaatjes en animaties waarin celprocessen zijn weergegeven te interpreteren. Ze leren kijken naar de plaatjes en animaties als *een weergave van schakelingen die op eiwitinteracties gebaseerd zijn*. Deze manier van kijken en interpreteren stelt ze in staat om voor eenvoudige celactiviteiten die met plaatjes of animaties verbeeld worden een begrijpelijke en plausibele verklaring op te stellen, namelijk: de celactiviteit kun je zien als een schakeling van eiwitinteracties, oftewel een *moleculair mechanisme*. Leerlingen geven aan dat deze manier van redeneren over cellen nieuw voor ze is en dat ze zich niet eerder afgevraagd hebben hoe cellen werken. Ze hebben de lessen ervaren als 'je afvragen hoe het zit' in plaats van 'gewoon te horen krijgen dat het zo is'. Ik interpreteer dit als een effect van het feit dat het stellen van mechanistische vragen en construeren van mechanistische verklaringen (de zogeheten verklaringscontext) centraal staat in de lessen.
De lessen onderstrepen dus de kracht van het basisidee, namelijk dat de mechanistische verklaringscontext (oftewel: hoe werkt het?) een krachtige, en misschien wel de enige, manier is om leerlingen op een betekenisvolle manier te laten verkennen hoe cellen op moleculair niveau werken. Toch roepen de lessen ook nieuwe vragen op en is het ontwerp nog niet perfect.
In **hoofdstuk 7** kijk ik terug op het ontwerp, de lessenserie en de manier waarop ik die onderzocht heb. Ik bespreek de inzichten die ik centraal heb gesteld in de lessenserie en de didactische keuzes die ik gemaakt heb. Zo heb ik ervoor gekozen om eiwitinteracties te versimpelen aan de hand van de termen 'botsen, binden en van vorm veranderen'. Hoewel de leerlingen deze termen gingen gebruiken bij het interpreteren van plaatjes en animaties, blijft het de vraag in hoeverre de term 'van vorm veranderen' ook begrepen is door de leerlingen. Hebben ze het ter kennisgeving aangenomen en geleerd wanneer ze het moeten toepassen of heeft het ook betekenis gekregen doordat ze nu weten waardoor eiwitten van vorm veranderen als ze binden? En wat is eigenlijk precies het effect van dat 'van vorm veranderen'? Dat blijkt nogal te verschillen per eiwit. Het ene eiwit knipt door de vormverandering een gebonden molecuul in kleinere moleculen, terwijl een ander eiwit door vormverandering juist twee gebonden moleculen aan elkaar koppelt. De

leerlingen kunnen meestal uit het plaatje of de animatie wel aflezen welk effect plaatsvindt, maar ze krijgen geen verklaring waarom juist dat effect optreedt. Hier raakt de lessenserie de biochemie en ik ben van mening dat dit het punt is waarop de kennis te gedetailleerd wordt voor de biologielessen op middelbare school. Echter, een betere aansluiting tussen de vwo-scheikunde lessen over chemische reacties en de biologielessen over het moleculaire niveau zou het begrip zeker ten goede komen.
Verder concludeer ik dat er in de didactische opzet van de lessen nog stappen te maken zijn. In deze eerste verkenning is er omwille van de tijd voor gekozen om op een zeker moment in de lessen simpelweg mee te delen dat leerlingen zich gaan verdiepen in eiwitten, omdat dat de deeltjes zijn waarvan ze iets moeten weten als ze verder willen werken aan de centrale vraag 'hoe werken cellen?'. Het zou echter nog krachtigere lessen opleveren als leerlingen zelf de benodigde kennis ontdekken en inpassen in de antwoorden waarnaar ze op zoek zijn. In toekomstig onderzoek zou dit aspect uitgewerkt kunnen worden.
Mijn onderzoek roept de vraag op hoe docenten op de middelbare school toegerust kunnen worden om het moleculair mechanistisch redeneren in hun lessen in te passen. In **hoofdstuk 8** doe ik hiervoor een aantal suggesties en bespreek ik wanneer het moleculaire niveau in de biologie betekenisvol geïntroduceerd zou kunnen worden. Ik suggereer dat in het huidige biologieprogramma al meer dan genoeg celprocessen aan bod komen waarmee de verbinding met het moleculaire niveau gemaakt kan worden. De vele plaatjes en animaties die daar momenteel al voor gebruikt worden, vormen een uitstekend uitgangspunt om de vraag op te roepen: wat gebeurt hier eigenlijk? Het oproepen en behandelen van deze vragen in de les vraagt echter nogal wat van de docent en hoewel het geen onderdeel van dit onderzoek was, heb ik de indruk dat veel docenten niet voldoende vertrouwd zijn met de mechanistische kijk op eiwitten en eiwitinteracties om op deze manier met visuele modellen van celprocessen aan de slag te gaan. Verder zullen docenten zich ook bewust moeten worden van de beperkingen die deze modellen hebben. Het gevaar van verfeitelijking van wat er te zien is in de plaatjes en animaties ligt continu op de loer en de docent zal in staat moeten zijn de leerlingen hier keer op keer op te wijzen en ze op die manier te trainen kritisch te kijken en redeneren.
Op basis van mijn bevindingen suggereer ik dat het moleculaire niveau in de biologie betekenis kan krijgen vanaf 4 vwo, mits geïntroduceerd in een verklaringscontext, waarin de vraag 'hoe doet een cel dat?' centraal staat. Ik stel zelfs dat de moleculaire werking van eiwitten eerder geïntroduceerd zou kunnen worden dan de organellen in de cel, om zo in een vroeg stadium leerlingen al een alternatief te bieden voor het toeschrijven van menselijke eigenschappen of doelgerichtheid (de cel wil, moet, besluit etc.) aan cellen of celorganellen.
Tot slot suggereer ik dat expliciet gebruik maken van mechanistisch redeneren niet alleen kansen biedt voor celbiologieonderwijs, maar ook voor het natuurwetenschappelijk onderwijs in het algemeen. De natuurwetenschappen richten zich op het beter begrijpen van objecten en gebeurtenissen in de wereld om ons heen. Wetenschappers stellen vragen en construeren verklaringen als antwoord op die vragen. Daarom stel ik dat ook in het natuurwetenschappelijk *onderwijs* de verklaringscontext centraal zou moeten staan. Dat je voor het opstellen van deze verklaringen vaak op zoek moet naar onderliggende onderdelen en processen, oftewel *'the mechanisms at work'* zal geen leerling verbazen. De uitdaging ligt in het ontwerpen van onderwijs dat productief gebruik maakt van deze intuïtie, zodat nieuwe deeltjes en processen geen losstaande feitjes blijven, maar worden geïntroduceerd om iets dat tot dan toe voor lief werd genomen, beter te begrijpen.

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# Curriculum vitae

Marc van Mil was born on May 19th 1978 in Heerlen, the Netherlands. After finishing his pre-university secondary education (vwo) *cum laude* at Eijkhagen College in Landgraaf he studied Biotechnology at Wageningen University. In 2005 he graduated *cum laude* for his master's degree in which he combined his interest in molecular and cellular biotechnology with a specialization in science communication and education.

From 2005 to 2008 Marc worked for the Cancer Genomics Centre (CGC) at the University Medical Centre Utrecht on several educational and outreach projects. In collaboration with the Freudenthal Institute for science and mathematics education (FIsme) at Utrecht University he designed and set-up one of the 'DNA labs on the road' called 'Read the language of the tumor'. In this project, students in the Biomedical Science program of Utrecht University take laboratory equipment into secondary schools to teach about cancer research. Furthermore, he designed the course 'Molecules in Life' for Junior College Utrecht and he has been teaching it on a yearly basis since 2006. In 2008 this course was approved for national use in the Dutch Nature, Life & Technology (NLT) curriculum.

In 2008 Marc applied and was awarded a grant from CSG Centre for Society and the Life Sciences to start a PhD study at the Freudenthal Institute in collaboration with the Cancer Genomics Centre. The results of this genomics education research project called 'Education for visual literacy in a genomics world' are presented in this thesis.

Since 2013 Marc works on educational innovations in the Biomedical Sciences bachelors' program in the University Medical Centre Utrecht.

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## Selected conference papers, posters and symposium contributions

**Van Mil, M.H.W. , Postma P.A., Boerwinkel, D.J. & Waarlo, A.J.** (2012). *Molecular mechanistic reasoning: towards bridging the gap between the molecular and cellular level.* Paper presented at the ERIDOB conference in Berlin, 17-21 September.

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Universiteit Utrecht

Although learning about DNA, RNA and proteins is part of the upper-secondary biology curriculum in most countries, many studies report that students fail to connect molecular knowledge to phenomena at the higher level of cells, organs and organisms. It is proposed that students are not sufficiently equipped and encouraged to reason about complex and emergent systems behaviour to bridge the gap between the molecular level and phenomena at higher levels of biological organization. This study explores the potential of a new educational approach that is based on encouraging molecular mechanistic reasoning, which entails *interpreting cellular phenomena as the overall result of the interactions between underlying physical entities.* It builds on recent work in the philosophy of science that characterizes explanations in molecular cell biology as molecular mechanistic explanations. In this study we focus specifically on the interactions of proteins as a basis for higher level cellular phenomena. The study presents the theoretical basis for a learning trajectory based on molecular mechanistic reasoning and it shows in a small-scale test of the educational approach that it is within reach for pre-university students (aged 17–18) to explore meaningfully the multi-level mechanistic nature of cell activities as well as the physical and chemical principles that are at the basis of molecular mechanisms in the cell. In the presented approach students are challenged to interpret cell biology animations and graphics as mechanistic explanations for cell activities, which make these visual models a powerful educational tool for developing the multi-level mechanistic perspective on cellular behaviour. It is argued that this perspective helps students to get a grip on cellular complexity in life science education.