# The Immune System as a Complex System: Description and Simulation of the Interactions of its Constituents

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*Wo Gefahr ist, wächst*
*das Rettende auch.*

Friedrich Hölderlin

**Abstract**

Progress in biotechnology has enhanced our knowledge about finer details of structures and interactions in cell biology. Particularly in immunology, the development of theories and models has, however, not kept pace with experimental achievements. One of the reasons for this may be that – because of the unsufficient power of computers – it has long been impossible to investigate complicated (since realistic) models of cellular cooperation within the immune system by means of computer simulations.
The work presented here describes the software package SIMMUNE, which allows to model and simulate multicellular biological systems on the scale of membrane receptor mediated cellular interactions. It is capable of including the molecular components of these systems (as, e.g., membrane receptors or freely diffusing signal molecules) with realistic numbers of molecules, reaction rates, and diffusion coefficients.
As theoretical biology in general, the development of SIMMUNE has profited by the transfer of concepts and techniques from theoretical physics.
In contrast to, e.g., simpler differential equation models which simulate the dynamics of populations of agents using global rate equations, which means, without taking into account any spatial structure, SIMMUNE strictly observes the principle of locality of information by simulating local, receptor mediated interactions. Since this approach does not a priori impose any non-local structure on the systems being simulated, SIMMUNE simulations are apt to serve as starting points for multiscale analyses of simulated immune responses. Such analyses will be useful for the development of effective theories of immunological processes and will hence contribute to making the complexity of the immune system more comprehensible.

**Zusammenfassung**

Der Fortschritt in der Biotechnologie hat unser Wissen um die Kenntnis feiner Details von Strukturen und Wechselwirkungen in der Zellbiologie bereichert. Vor allem in der Immunologie hat die Entwicklung von Theorien und Modellen jedoch nicht Schritt gehalten mit den experimentellen Errungenschaften. Einer der Gründe hierfür mag sein, daß es – aufgrund der unzureichenden Rechenleistung der Computer – lange Zeit nicht möglich war, komplizierte (weil realistische) Modelle der zellulären Kooperation innerhalb des Immunsystems durch Computersimulationen zu untersuchen.

Die vorliegende Arbeit beschreibt das Softwarepaket SIMMUNE, das es gestattet, vielzellige biologische Systeme auf der Skala der durch Membranrezeptoren vermittelten zellulären Wechselwirkungen zu modellieren und simulieren. Es ist in der Lage, die molekularen Komponenten dieser Systeme (wie z.B. Membranrezeptoren oder frei diffundierende Signalmoleküle) unter Verwendung realistischer Molekülzahlen, Reaktionsraten und Diffusionskoeffizienten zu berücksichtigen.
Wie in der theoretischen Biologie im Allgemeinen, hat sich auch bei der Entwicklung von SIMMUNE die Übertragung von Konzepten und Techniken aus der theoretischen Physik als nützlich erwiesen.
Im Gegensatz zu, beispielsweise, den einfacheren Differentialgleichungsmodellen, die die Dynamik von Populationen von Agenzien durch globale Ratengleichungen, d.h. ohne die Berücksichtigung räumlicher Struktur modellieren, beachtet SIMMUNE durch Simulation lokaler, durch Zellmembranrezeptoren vermittelter Wechselwirkungen strikt das Prinzip der Lokalität von Information. Da dieser Ansatz den simulierten Systemen keine nichtlokale Struktur a priori aufprägt, eignen sich Simulationen mit SIMMUNE als Ausgangspunkt für Mehrskalenanalysen simulierter Immunantworten. Solche Analysen werden der Entwicklung effektiver Theorien immunologischer Vorgänge dienen und somit dazu beitragen, die Komplexität des Immunsystems überschaubarer zu machen.

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# Chapter 1

# Introduction and Survey

## Reductionism and effective theories in physics and biology

New technologies have begun to turn the formerly descriptive then empirical science of the living into an experimental discipline. Faced with the rapidly growing amounts of data about components and interactions in biological systems biologists tend to look with envy to physics with its simple laws and its comprehensible objects of investigation.
But simplicity and comprehensibility in modern physics are not generous presents of nature; rather, they were achieved by the technique of reductionism and the formulation of effective theories.
Classical mechanics of macroscopic bodies is an example of an effective theory. We can use it to calculate the dynamics of a game of billiards neglecting all processes on the molecular level and below. Using a reductionistic approach we would also neglect the influence of the moon's movement on our game while still obtaining results of almost arbitrarily high precision.
Reductionism is the foundation of most of the achievements of modern biology too. The investigation of isolated tissues, cells, and molecules has brought forth quantifiable knowledge from reproducable experiments.
Reductionism has, however, been less successful when used in efforts to predict the *behavior* of biological *systems*. Non-trivial interaction with its environment may in fact be regarded as one of the defining properties of a living organism.
Much more than in physics, effective theories (or effective models) were applied in biology usually as a consequence of missing knowledge about finer details and not as a chosen tool for simplification. It is a widespread convic-

tion that we would be able to understand (and predict) the behavior of many complicated biological systems if we only had more detailled data about the inner structures of their constituents.
The adaptive immune system – existing in every vertebrate's body – is such a complicated system. Its complexity arises from the wealth of different components (cell types, specialized molecules) and the great variety of interactions between them. Understanding the cooperative behavior of the immune system's components to the extent to be able to predict (and manipulate!) immune responses is still on the wishlist for the future[1]. Reductionism cannot help us immediately here. We have to look for effective theories and have to exploit the power of computers in our efforts to handle the huge amount of experimental data.
From the earliest days of theoretical immunology, concepts from theoretical physics have proven to be useful in tackling the complexity of the immune system. Spin system formulations have been used to model mathematically the mutual activation/deactivation of immune system cells; methods from statistical physics were employed in search for macroscopic state variables describing immune responses on the cell population or systemic level. The principle of the spatio-temporal locality of information which lies at the heart of modern physics had however not yet been applied rigorously – and based on realistic, experimentally determined parameters – to immune system modeling.
Even biologists who know about the local nature of receptor mediated interactions often tend to describe immunological interactions on the population level rather than as spatially and temporally localized events.
The software package SIMMUNE which is introduced in this work was designed to allow for simulations of the immune system (and, more generally, of systems consisting of large amounts of interacting cells) based on data that describe cellular interactions on the scale of membrane receptor mediated signaling. As a consequence of recently initiated projects like the *Alliance for Cellular Signaling*[2] the traditionally raised objection against detailled

[1] Immunology and vaccine research still are practically two rather separate branches of biology and medicine.

[2] Excerpt from the program summary of the Alliance for Cellular Signaling [5]:
*The overall goal of the Alliance for Cellular Signaling is to understand as completely as possible the relationships between sets of inputs and outputs in signaling cells that vary both temporally and spatially. The same goal, stated from a slightly different perspective, is to understand fully how cells interpret signals in a context-dependent manner. This will involve identification of all the proteins that comprise the various signaling systems, the assessment of time-dependent information flow through the systems in both normal and pathological states, and finally the reduction of the mass of detailed data into a set of*

microscopic simulations, namely, that the necessary microscopic data are still missing, may soon be losing its persuasive power.
SIMMUNE fills the gap between projects like the *Virtual Cell Project* [77] that simulates in detail the intracellular chemistry of single cells and, on the other hand, the (generalized) cellular automaton *IMMSIM* [17] the emphasis of which is on allowing for simulations of quite large cell population rather than on cellular individuality.

## Survey of this work

**Chapter 2** will introduce the basic concepts of immunology and then touch some of the areas of immunological research and discussion that presumably will profit much from SIMMUNE's approach to computer aided modeling of cellular behavior. Readers interested in more complete information on the subject might want to consult [1], [44], [65], or [22].

**Chapter 3** offers a brief survey of some modeling and simulation techniques in theoretical immunology.

**Chapter 4** describes the concept of mechanisms as basic building blocks of local dynamics in simulations of complex systems. It explaines how SIMMUNE is embedded in Mack's theory of complex systems [54].

**Chapter 5** elucidates some of the connections between ideas from Artificial Intelligence, research on abstract cognitive systems and immunology.

**Chapter 6** introduces the center of my thesis, the modeling and simulation software SIMMUNE.

**Chapter 7** describes how to use the software package SIMMUNE.

**Chapter 8** describes the application of SIMMUNE to simulations of leukocyte chemotaxis.

The **appendix** will discuss some of the mathematical and technical issues related to SIMMUNE and list simulation parameters from the simulation examples.

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*interacting theoretical models that describe cellular signaling.*

# Chapter 2

# Immunology

*In this chapter, an introduction to some basic concepts in immunology is provided. Its objective is to make this thesis as self-contained as possible – also for readers with little biological background knowledge. Besides briefly treating the fundamentals of the adaptive immune system, some of those open questions in immunology are touched that might be answered one day (at least partially) by computer based experiments like the ones that can be done with SIMMUNE.*

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## 2.1 The Task of the immune system

The task of the immune system (IS, for short) typically is described as that of a defense system directed against infectious material that has entered the organism. Until recently, the most common operational description of this task was that the IS has to discriminate **non-self** (foreign material, not belonging the organism) from **self** and to eliminate non-self and all sources (like infected cells) of non-self. Now, immunologists have begun to redefine the task of the IS as experimental evidence is growing that the IS may ignore foreign material (like silicon) and on the other hand may attack uninfected, but aberring body cells (like tumor cells).
Modern concepts describe the IS as a signal processing system being capable of recognizing sources of harm or even potential harm: danger [58]. While

those concepts themselves are gaining ground rapidly, the organizational structures that could provide those capabilities remain the subject of very controverse discussions [22], [23], [41], [95].

## 2.2 The components of the immune system

The material components of the immune system consist of a class of cells, the white blood cells – or **leukocytes** – and several classes of molecules. Perhaps the most prominent leukocytes are **T-cells**, **B-cells**, **natural killer cells (NK-cells)**, **macrophages**, and **dendritic cells**.[1] Like all blood cell types, they are produced in the bone marrow. T-cells mature in a lymphoid[2] organ called **thymus**, B-cells start circulating in the blood directly after their production in the bone marrow.
The cells of the immune system interact with each other via signal molecules called **interleukins**. Interleukins are a special class of **cytokines**, small soluble proteins that can induce intracellular processes in a cell after having bound to their corresponding surface membrane receptors on the cell (see, e.g., [66]).
T- and B-cells have specialised receptors with variable binding regions which are used for binding to processed molecular material being presented by other cells, or soluble, free floating molecules, respectively. Besides signal molecules and receptors the immune system possesses a family of cell-independent effector molecules, the **complement**.
Any kind of material the IS rises a response against is called an **antigen**. B-cells, macrophages and dendritic cells have special capabilities to present (potential) antigen to T-cells.
The number of cells of the immune system in a human body is estimated to be of the order of $2 \times 10^{12}$ which makes the immune system comparable in mass to major organs like the liver.

## 2.3 Cellular biochemistry, intercellular communication

All intercellular communication in multicellular biological systems is based on the binding of typically highly specific ligands to receptors in the cellular surface membrane. The ligands can be free floating molecules or receptors

[1] Immunologists may forgive me that I do not mention any granulocytes. Also, my description of the innate IS is a very abbreviated one.

[2] which means: belonging to the adaptive part of the immune system

situated in the membranes of neighboring cells. The binding kinetics and the strength of the binding between two such molecules are governed by the complementarity with regard to spatial configuration and electrical charge distribution of their binding sites.
Receptor-ligand interactions usually do not involve covalent binding but depend on the formation of hydrogen bonds or the proximity of oppositely polarized parts of the two molecules.
Cell surface receptors are proteins. Their structures are determined by their sequence of amino acids[3] which in turn is encoded in the DNA of the organism. The variety of sidechains encountered in the 20 existing amino acids is the reason for the plethora of possible different three-dimensional protein shapes.
The affinities (reaction equilibrium constants) in cellular receptor biochemistry typically lie in the range of $10^6$ to $10^9$ $\frac{\text{liter}}{\text{mol}}$. For most receptor-ligand bindings the reaction kinetics are strongly asymmetric, with fast complex formation and slow decay[4].
While one side of cell surface receptors protrudes from the membrane into the extracellular milieu, the other side reaches into the cytoplasm (the intracellular milieu). Conformational changes caused by the binding of a ligand induce cascades of intracellular events usually leading to the production of certain proteins, for example further membrane receptors or signalling molecules to be secreted by the cell.
This stimulus-response principle lies at the heart of the simulation software SIMMUNE to be introduced in chapter 6.

## 2.4 Innate and adaptive immunity

Even rather primitive animals (and also plants) have means to defend their organisms agains intruding parasites. Usually, primitive organisms inhabit milieus with rather static characteristics: Adaptivity requires organisms complex enough to provide internal mechanisms of regulation – for the metabolism in case of changing food supply, for the maintainance of body temperature in case of changing climate, etc.. One of the characteristics of an animal's milieu is, of course, the variety of its parasites. Slowly changing parasitic environments allow to use a defense system specialised on recog-

[3]This is at least true for biologically important proteins in their natural physiologic milieu since structural uniqueness and stability are clearly prerequisites of metabolic functionality.

[4]in the case of antibody-antigen binding with half life times in the order of weeks.

nising and attacking the most common parasites.
Molecular defense arsenals similar to what is called the complement in vertebrates can be found in the IS of nonvertebrate animals. Molecules of the complement can aggregate on the surface of bacteria to destroy their outer membranes by establishing perforating channels in the membranes. Aggregates of complement on the surface of microbes also attract macrophages that are capable of swallowing and digesting microbes. Cells of the organisms itself are protected against the formation of complement aggregates on their surfaces by special membrane proteines.

The relationships between pathogens and their potential hosts are mostly well equilibrated by nature [95], [69]. Primitive organisms have reproduction cycles short enough to insure the survival of their species in spite of the loss of many individuals as a result of infection. Their pathogens, on the other hand, replicate fast – because their hosts are short-lived – and without intricate strategies of host deception – because their hosts do not possess intricate strategies of pathogen elimination.
Complement and the cell types that get activated by it are the elements of early immune responses (starting immediately after infection) in higher, vertebrate organisms, too. Many pathogens aiming at infecting complex organisms like vertebrates are, however, able to deceive complement or manage to enter and replicate in the organism's own cells.
As a result[5] vertebrates evolved with immune systems that produce effector cells bearing receptors with varying binding possibilities. These cells are capable of reacting to different molecular aspects of pathogens instead of depending on evolutionary established sets of pathogen determinants. Furthermore, they may memorize the inflammatory context related to the infection that activated them and, if they are long-lived enough, reinduce the successful immune response quickly once the organism encounters the pathogen again (see 2.8).

### 2.4.1 The humoral immune response

Targeted against pathogens floating in the blood or other bodily fluids[6] are soluble receptors called **antibodies**. Antibodies (or **immunoglobulines**) are released by **plasma cells** stemming from B-cells. They are Y-shaped

[5] Strictly speaking it makes no sense to formulate a causality here: which ones were first – the cunning pathogens or the clever means of defense?

[6] the term **humoral** immune response stems from the ancient word for bodily fluids: *humors*

macromolecules with two functional ends: One end (the fork of the Y) is used to bind to noxious chemicals or to the surface of larger pathogens. The molecular shape of this end is the product of (almost) random mutations of certain genetic sequences. Immunologists assume that the range of possible binding characteristics of antibodies is large enough to guarantee that for virtually any molecular shape the right (fitting) antibodies may be produced by a vertebrate organism.
The other end of the antibodies fits to certain receptors on the surface of cell types like macrophages, dendritic cells, natural killer cells etc. . Which cell type offers the right receptor for a given antibody depends on the **isotype** of the antibody. The different antibody isotypes have different functions and are used in different types of tissues.

Upon leaving the bone marrow B-cells carry receptors with variable binding sites on their surface. All B-cell receptors that belong to the same cell have the same binding characteristics. Two different naive[7] B-cells have different B-cell receptors. As soon as a B-cell senses binding events on its B-cell receptors and receives some additional affirmative stimuli (see 2.7) it differentiates into either a memory cell or a proliferating and antibody producing cell. The antibodies that are produced by such a cell have the same binding characteristics as the B-cell receptors of its ancestor had. Cells deriving from a single ancestor are said to establish a **clone**
Antibody producing cells may produce up to 20.000 antibodies per second thus charging their environment with enough antibodies to quickly coat the molecules or viruses they were produced to fight. Once a particle is coated with antibodies it is marked for destruction by macrophages which have receptors for the non-variable end of antibodies.

### 2.4.2 Cell mediated immunity

The other class of receptors that have variable binding sites can be found on T-cells. T-cells use them to scrutinize organism cells for signs of ongoing viral replication[8] or to read out molecular information about the extracellular milieu collected by so called **antigen presenting cells**, (**APC**s) (see 2.4.3 below).

[7] A cell of the IS is termed **naive** if it has not yet reacted to stimuli caused by the influence of pathogen or other material it can react to, given the specific properties of its receptors.

[8] which can only happen inside of cells since viruses have no machinery for protein production and consequently have to abuse the infrastructure of a host cell.

T-cells exist in two flavors: as so called **T-killer** cells and as **T-helper** cells. T-killers focus on suspicious material being produced intracellularly while T-helpers mainly interact with antigen presenting cells. **T-cell receptors** (**TCR**s) do not bind directly to suspicious macromolecules. Instead, they bind to **MHC** receptors[9] on the surfaces of their target cells. There are two classes of MHC receptors: **MHC1** and **MHC2**. Both take small protein (or **peptide**) fragments from intracellular compartments with them on their way to the cell membrane where they present them to T-cells (more precisely, to T-cell receptors). MHC1 gather material that has its origin inside the cell. Every cell of the organism[10] uses this mechanism to indicate what kind of proteins it produces.
The protein fragments being presented on MHC2 entered the cells from the extracellular milieu, captured by receptors like the above mentioned B-cell receptors. MHC2 receptors do hence present a selected summary of the proteins floating around. They are only found on APCs.
Activated T-killers that sense the binding of their TCR to MHC1 (+peptide fragment) assume viral replication or other abnormal processes in the cell carrying the MHC1:peptide complex. They may react by releasing membrane perforating molecules (**perforin**) into the gap the between them and their victim. Once the attacked cell's membrane is perforated, osmosis drives extracellular water into the cell until it bursts. The other method used by T-killers to kill infected or aberrant cells employs the possibility to activate a cellular suicide mechanism – called **apoptosis** – existing in almost every cell type.
An activated T-helper reacts to a binding of its TCR to MHC2 (+peptide fragment) by releasing signal molecules that activate other components of the immune system. B-cells, for example, will get activated by pathogen contact directly only in special cases where the pathogen has the right size and the right repetitive molecular components to cross-link two or more of their B-cell receptors. Activation of B-cells against other pathogens depends on the confirmation of the material's dangerousness by T-helpers.

While all the properties – including receptor specificities – of the innate IS (complement, macrophages etc.) are encoded in the germ line information, the receptor specificities of T- and B-cell populations are shaped[11] by the

[9] MHC stands for 'major histocompatibilty complex'. MHC molecules were discovered during research on donor tissue acceptance/rejection, hence the term histo*compatibility*.

[10] which is active enough to synthesize proteins

[11] For quite a while immunologists believed that antibodies were actually shaped along the templates provided by the shape of pathogens.

pathogen contacts the organisms has in the course of its life, since only those T- and B-cell clones bearing the right receptors to respond to the pathogens receive the necessary signals for their survival (see 2.5). Therefore, the T- and B-cells establish what is called the **adaptive** immune system.

### 2.4.3 Antigen presenting cells

T-cells interrogate all kinds of cells about their protein production; all cells do present (potential) antigen to them[12]. Macrophages, dendritic cells and B-cells, however, present (potential) antigen on their MHC2 as a major part of their functionality. Furthermore, they are able to deliver certain costimulatory signals to the T-cells thereby inducing processes that lead to the activation of the T-cells. This is the reason immunologists termed these cell types *professional antigen presenting cells*. In the literature, *antigen presenting cell* or *APC* mostly means *professional antigen presenting cell*.

## 2.5 T-cell selection

Obviously, T-cells reacting (in a directly destructive manner or by releasing signal molecules) to MHC receptors that present peptide fragments of healthy tissues are dangerous. On the other hand, the organism needs to be able to produce T-cell receptors for any kind of pathogen. The mechanisms the IS uses to escape from this dilemma have long been unknown. The question was whether an *instructive* or *selective* strategy is used [13]. An instructive mechanism would direct the production of T-cell receptors to avoid T-cell receptors with shapes fitting to material of the own body. A selective strategy would allow for the production of T-cells with T-cell receptors that have random binding possibilities but would then discard all potentially dangerous cells.
Burnet [14] developed the theory of **clonal selection** of T-cells. According to this theory which is (at least concerning the existence of the mechanism) generally accepted knowledge in immunology, T-cells have to pass a test for reactivity to *self* in the thymus. Those T-cells that react too strongly to the self peptides being presented by APCs in the thymus are induced to commit suicide. How complete the elimination of (strongly) self-reactive T-cells in the thymus is, of course crucially depends on the completeness of the repertoire of self peptides that is presented in the thymus. Theories have been

[12] Those cells that violate this protocol of behavior by ceasing to display MHC1 receptors on their their surface are suspicious enough to get killed by natural killer cells.

developed postulating mechanisms of self peptides being transported into the thymus from all over the organism.
Interestingly, the thymus not just exerts this negative selection; T-cells that fail to react to MHC:peptide at all also won't leave the thymus alive. The rationale behind this is that such cells would be uselessly circulating the body, and as the immune system can only occupy a rather fixed, small portion of the available space in the organism, these cells would constrain the variety of circulating T-cell receptors.
Since T-cells qualify themselves for being used by the immune system by successful interaction (albeit a weak one) with MHC:self-peptide, TCR binding possibilities of naive T-cells cannot be utterly specific. A question arising here is whether the immune system removes some of this degeneracy during the development of responses against pathogens or whether it can ensure specific responses from degenerate receptors during second and later contact with the pathogen [9].
The selection of T-cells in the thymus must be a delicately balanced process. The mechanisms providing this balance are still largely unknown. Grossmann [40] developed a theory according to which T-cells on their way through the thymus step by step tune their responsiveness to the MHC:peptide they encounter. This model describes T-cell selection as a process which may continue after the cell has left the thymus rather than as a single event.

## 2.6 Antigen recognition

One of the key questions in immunology is: what makes a molecule (be it part of a virus, a bacterium, or perhaps part of the organism itself) an antigen? Taking clonal selection as the sole mechanism of discrimination between potential antigens the process of antigen recognition is boiled down to molecular shape matching. In most of the immunological literature recognition of an antigen by the IS simply means – unless further explanations are given – that T-cells with receptors having high enough affinity for the molecule in question are available in the organism.
Failed efforts to develop vaccines by trying to induce immune responses to purified pathogen fragments were the first indicators that the IS does not judge upon the molecular shape of a potential antigen alone.
So called **adjuvants** must be mixed with the fragments in order to create an efficient vaccine. Adjuvants typically consist of selected material that excites strong reactions of the innate immune system – such as fragments

of classical pathogens, i.e. pathogens the organism's ancestors have been in contact with for a long time. Such material is taken up for example by dendritic cells (which are very effective APCs) and then presented to T-cells together with a combination of interleukins that signal ongoing, damage causing processes of infection.

## 2.7 The spatial organization of the immune system

Biological organisms need to maintain various special molecular (and cellular) milieus for the various needs of their metabolism. Even single cell organisms set up spatial compartments separated from the rest of the organism to achieve this. Selective transports allow for exchange of material between different compartments. Some of the compartments found in vertebrates have been identified to play important roles in the organization of the immune system.
The immune system uses the **central lymphoid organs**, bone marrow and thymus, to produce and mature its cellular components. **Peripheral lymphoid organs** like the **spleen** and the **lymph nodes** provide special milieus for the encounter of lymphocytes and APCs. They are interconnected by a system of **lymphatic vessels**. The lymphatic vessels, similar to the blood vessels, span most of the body. Transport within them is, however, not driven by active pumping; it is a passive tranport, depending to some degree on the movements of the organism's limbs.

Dendritic cells that sampled potential antigen in the peripheral body tissues get stimulated by infection to migrate to the closest lymph node to present processed fragments of the pathogen on MHC1 and MHC2. Naive T-cells after having passed their examination in the thymus continuously circulate from the blood into the peripheral lymphoid tissues and back. In the lymph nodes naive T-cells may encounter APCs that present the right peptide to induce MHC(+peptide):TCR binding. (Professional) antigen presenting cells – like dendritic cells – not only carry MHC1/2 on their surface but also additional receptors that bind to their counterparts on naive T-cells, thereby delivering co-stimulatory signals to the T-cells. Only the combination of MHC:TCR binding and co-stimulatory signals will activate a naive T-cell to become an **armed effector** cell.
Some antigens are presented on APCs that do not by default deliver the necessary co-stimulatory signals for naive T-killers but only for naive T-

helpers. A T-killer response against these kinds of antigen then requires a 3-cell interaction: APC stimulates the activation of a T-helper, which in turn stimulates the APC to deliver the co-stimulatory signals to activate a naive T-killers.
Naive B-cells may get activated through a cross-linking of their B-cell receptors by antigens with highly repetitive structures, like polysaccharides that can be found on the membranes of bacteria. B-cell activation by protein antigens, however, requires the interaction with activated T-helpers which are specific for the same antigen. This interaction also happens in the lymph nodes. A simplified sketch of the scenario is as follows: A naive B-cell presents (potential) antigen on MHC2 molecules. A T-helper cell with the right TCR for the antigen reacts to the MHC2:TCR binding by expressing on its surface a ligand for a certain receptor (called CD40) which in turn is located on the surface of the B-cell. Additionally, the T-helper secretes cytokines into the gap between the two cells. The cytokine pattern that is produced by the T-cell determines the kind (isotype) of immunoglobulines that will be produced by the B-cell once it has undergone the transition to a plasma cell. The mechanisms of this T-helper - B-cell dialogue will be formulated in the language of SIMMUNE in chapter 6.3.4.

The analogy has been drawn between a lymph node and a discussion forum [22]: Different cell types of the IS meet there to exchange information about different aspects of potential antigens by intercellular communication between multiple cells, mediated by specific combinations of receptor signals. Gradually, we are beginning to understand the rules of these discussions and the dependence of the achieved decisions on the nature and order of the signals contributed by the participating cells [32].

## 2.8 Immunological memory

One of the reasons for the importance of the innate IS is that a first-contact response to a pathogen from the adaptive components like B- and T-cells takes several days to develop. During this time the innate IS has to provide enough protection to prevent too much harm being done to the organism.

Immunological memory is the property of the immune system to react much faster and with larger numbers of effectors to a pathogen upon second or later contact with it. The explanation for this property is that after a first-contact response a certain number of lymphocytes with high affinity

receptors for the pathogen remains in the organism in a kind of stand-by state, waiting for reactivation. It has been shown that B-cells may exist in a long lived memory mode that can clearly be distinguished from the state of recently activated non-memory cells. These memory B-cells upon restimulation induce the production of plasma cells that secrete antibodies with high affinity for the original pathogen.
While it is still an open question whether long lived memory T-cells exist, there seems to be a consensus that even as memory cells they would need some repeated stimulation – probably from APCs. If the stimulation is provided via MHC:TCR interactions then the question arises what kind of antigen is presented to the T-cells. It could be the original antigen taken from the original pathogen. But then this antigen would have to be conserved somewhere in the organism. A possible locus of conservation may be dendritic cells in the peripheral lymphatic organs. They might be able to display antigen to T-cells even years after the first infection with it.
Another theory considers the possibility that memory T-cells could be stimulated by **self-antigen** – material from the host organism itself. Of course, this stimulation would have to be well below the threshold of a stimulation that would trigger a full immune response.

*Weil, so schließt er messerscharf,*
*nicht sein kann, was nicht sein darf.*

*For, he reasons wittily*
*that, which may not can not be.*

(Christian Morgenstern)

## 2.9 Autoreactivity and autoimmune diseases

The possibility of memory T-cell stimulation by self-antigen touches one of the most controversely discussed issues in immunology: the question whether T-cells (and B-cells) may interact productively with self-antigen.
If the reaction between T-cells and (potential) antigen were an on/off interaction, widely independent from other signals, and if clonal selection were the sole mechanism controlling T-cell reactivity, then the existence of autoreactive T-cells would be a dangerous failure of the system.
In 1974 Niels Jerne [45] presented his theory of **idiotypic** networks. He argued that, since the variable parts of antibodies are created by random

genetic mutations, antibodies should be treated by the immune system like foreign substances. The randomly generated parts of antibodies are called **idiotopes**; the set of idiotopes characterizing an antibody is called its **idiotype**. The assumed ability of the IS to produce antibodies for any possible molecular shape should lead to antibody reactions of the system to its own antibodies. Once a humoral immune response has been raised against some antigen, the corresponding antibodies should be recognized by other antibodies which in turn would be the target of a third antibody response etc..
Theories have been developed (see 3.4) how networks of interacting antibodies (or, more precisely: B-cell clones) could be employed by the IS to fulfill regulatory tasks like enhancing responses against pathogens, suppressing *self*-targeted clones, or maintaining the memory of pathogens after they had been removed from the system.
These kinds of physiological autoreactivity or even autoimmunity would violate the postulate of non *self*-responsiveness. One would, however, not associate physiological autoimmunity with what Paul Ehrlich termed **horror autotoxicus**.
When Burnet developed his *clonal selection* theory he was convinced that immunological autoreactivity could only be a random mistake of the IS, leading to damage in randomly selected tissues.
The diseases known today to be caused by the IS turning against its own host – **autoimmune diseases** – display typical pathological patterns and target tissues – in contradiction to Burnet's hypothesis. Insulin dependent diabetes, rheumatoid arthritis, and multiple sclerosis and about a dozen other autoimmune diseases represent the IS aberrations diagnosed in the large majority of autoimmune disease patients.

## 2.10 Cognitive aspects in immunology

Inspired by the regularity of disease patterns in autoimmune diseases, I. Cohen developed a theory of cognitive abilities of the immune system [23]. According to his theory, the recognition of antigen and contexts of infection is linked to an internal image the immune system has about its host organism. Cohen termed this image the **immunological homonculus** [24] following the example from neurology where a mapping between certain cortical regions of the brain and the sensorical and motorical control of corresponding regions of the human body has been discovered. The fact that the number of autoimmune diseases is rather small and that hence the reactivity of the IS to certain self-antigens is much higher than to most others, indicates that

in analogy to the neurological homonculus, not all components of *self* are treated with equal importance – or, from the point of view of clonal selection: are ignored with equal consistency.
If this structure is not just a frozen accident of evolution it may well be a frame of reference used by the IS to organize its responses to foreign material (and its tolerance of self). According to one of the major directions in Artificial Intelligence research, the existence of such a framework is the prerequisite for recognition (see chapter 5.3.2).

# Chapter 3

# Models and simulations in immunology

*L'âme des hommes de science est nécessairement candide*
*et elle donne asile à de monstrueuses illusions.*

(Christophe [19])

*This chapter provides a brief survey of some modeling and simulation techniques in theoretical immunology. It treats only those approaches that have – like cellular automata – either some concepts in common with SIMMUNE, or share some technical aspects with SIMMUNE though being rather different from the overal idea.*

---

## 3.1 Reductionistic and integrative models

During the last decades a number of mathematical models and more recently some computer based tools for immune system simulation have been developed. They may be classified according to whether they are reductionistic – limited to the investigation of properties of isolated components of

the immune system, as e.g., the affinities of immune cell receptor molecules – or integrative, trying to model or simulate aspects of immune responses emerging from the interactions of a larger number of different agents (like cells and molecules).

## 3.2 Top-down and bottom-up approaches

The integrative approaches may be divided into two classes: top-down and bottom-up. While bottom-up approaches start on the microscopic level of cellular or even molecular interactions and try to derive mesoscopic or macroscopic properties of the immune system by simulation, the aim of top-down modeling is to be able to identify classes of macroscopic behavior and to speculate on microscopic interaction pathways that are compatible with the large scale dynamics[1].

## 3.3 Reaction-kinetics models

Abstracting from the specific details of interaction between the IS's agents one can formulate systems of coupled differential equations describing how the time development of an agent's concentration in the modeled organism depends on the concentrations of other types of agents. These models may be called *reaction-kinetics* models as they bear resemblance to models of reaction kinetics in chemistry. A very simple example for such a system is the following, which consists of three types of cells: healthy cells $C$, infected cells $I$ that can infect healthy cells and IS cells $K$ that kill infected cells.

$$\begin{aligned} \frac{dI}{dt} &= p_{\text{infect}} IC - p_{\text{kill}} IK - d_I I \\ \frac{dK}{dt} &= p_{\text{resp}} IK - d_K K \\ \frac{dC}{dt} &= s - p_{\text{infect}} IC - d_C C \end{aligned}$$

Using as a shortcut notation $I$, $K$ and $C$ for agent names as well as for their concentrations, the equations above describe a situation that may be interpreted as follows: Infectious agents of type $I$ transform agents of type

[1] Even those endeavors that technically limit themselves to macroscopic modeling must be able to relate their dynamics to known cellular processes and may hence be regarded top-down.

$C$ into new agents of type $I$ upon contact with a rate $p_{\mathrm{infect}}$. Agents of type $I$ get removed (killed) upon contact with agents of type $K$ with a rate $p_{\mathrm{kill}}$. Agents $K$ proliferate upon contact with $I$ with a rate $p_{\mathrm{resp}}$. Agents of all three types die naturally at their specific rates $d_I$, $d_K$, $d_C$. $C$ type agents are produced at a constant rate $s$.

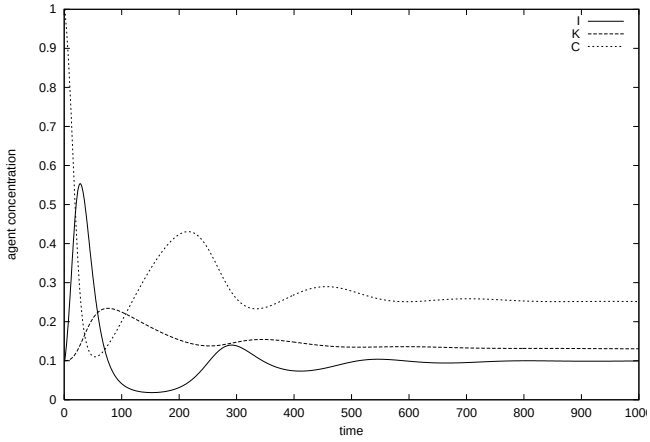


Figure 3.1: time development of agent concentration in the simple IS model

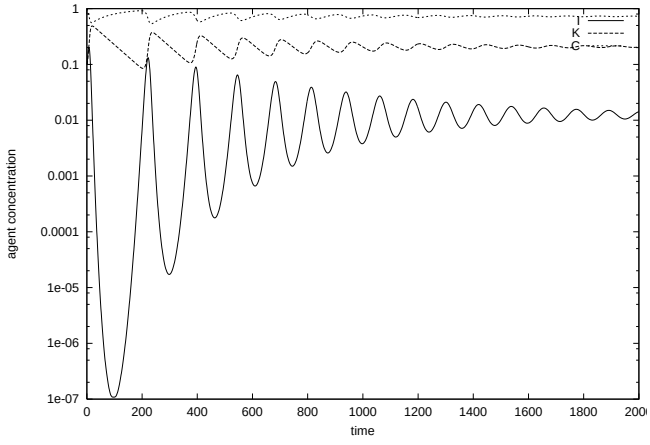


Figure 3.2: The model IS settles down into 'cronic infection' after oscillations with very low concentrations of $I$.

$K$ could be considered to be an immune system cell type being produced as

a response to the appearance of the infectious agent $I$. $C$ may be presenting any possible type of target cell for the pathogen $I$.
Integrating this system of equations yields different kinds of time development for $I$, $K$ and $C$ depending on the parameters $p_{\text{infect}}$, $p_{\text{kill}}$, $p_{\text{resp}}$, $s$, the death rates $d_x$ and, of course, the initial values of $I$, $K$ and $C$.
Fig. 3.1 shows the time development of the agents for one set of parameters (see appendix). At first, the number of infected agents $I$ grows while the number of healthy agents $C$ decreases. Then, as the response from the IS's agents $K$ grows, the number of infected agents declines while $C$ recovers. Finally, the system ends up in a steady state that may be interpreted as a chronic infection: Infectious agents persist even though the IS constantly prepares agents of type $K$ to suppress the infection.
Other sets of parameters may lead to stronger oszillations of the agents concentrations. Fig. 3.2 shows in a half-logarithmic plot a system that settles down into a steady 'chronic infection' state after having gone through states where the concentration of $I$ is very low.

## 3.4 Network models

Niels Jerne's idea of idiotypic networks in the IS triggered a series of models describing the (antibody mediated) interactions between B-cell clones [64], [8], [68] (and references therein).
The common ingredient of these models is the formulation of the stimulatory (or inhibitory) influence of the B-cell clones $B_j$ (or simplified: the different antibodies) in the system on the proliferation of a given clone $B_i$.

$$\frac{dB_i}{dt} = \theta\left[h_i(t)\right], \tag{3.1}$$

with the field $h_i$ experienced by the clone $B_i$:

$$h_i = \sum_j J_{ij} B_j. \tag{3.2}$$

$\theta$ is called the *activation function*. The *interaction matrix* $J_{ij}$ determines the kind of interactions (stimulatory or inhibitory), its structure determines the topology of the idiotypic network. Depending on the topology such models may display behavior reminiscent of immunization, tolerance etc..
However, due to the difficulties that obstruct the experimental confirmation of the importance of idiotypic networks in real immune systems, network models have fallen out of favor during the last years.

## 3.5 Automata models

While in the early days of cybernetics automata were information processing (electro-)mechanical devices, nowadays automata are more abstractly defined as units with data input, data output and a function defining the generation of the output depending on the input and the unit's current state. As such, automata are well apt to serve as building blocks for models of the IS that identify sets of characteristic states (like 'in rest' or 'activated') of components of the IS together with transition rules but which neglect any microscopic details of IS behavior.

### 3.5.1 Automata networks

Networks of automata can be created by connecting the outputs and inputs of different automata, thereby making the state transitions of each automaton depend on the data it receives from those other automata the outputs of which are connected to its input.
Especially prominent a class of automata networks has become that allows only two states for its automata components: boolean automata. They represent the prototype for minimal systems of interacting entities.[2]
Cohen & Atlan [25] used a network of five boolean automata in a model of autoimmunity regulation.

### 3.5.2 Cellular automata

Cellular automata (CA) were invented to investigate how simple building blocks could locally cooperate to produce aggregates with interesting behavior. The building blocks are automata living on a grid. Their rules define how the change in state of a single automaton at the next timestep depends on its own current state and the states of its direct neighbor automata.
Clearly, the most fascinating aspect of CA modeling is the fact that even rather simple transition rules together with local interactions can lead to very complex behavior of automata aggregates. An overview and classification of different types of CAs can be found in [87].

Celada & Seiden [17] developed a CA model of the IS, called IMMSIM where the cells are simulated by automata that may (as a modification of

[2] In physics, similar systems enjoy some popularity, too. There, they are called Ising-spin systems.

the usual CA concept of static correspondence between information and position) carry their state information with them while they are moving on a 2-dimensional grid. Furthermore, their program uses receptor mediated interactions between the objects that represent IS cells. For these reasons, and the fact that a single gridnode may be shared by many cells, Celada and Seiden call IMMSIM a *generalized* cellular automaton.
Depending on the agents (cells, molecules) they encounter IMMSIM's cells may change their state from, e.g.,*naive* to *activated*.
With their simulation program Celada and Seiden investigated a number of IS phenomena, for example *Affinity maturation and hypermutation [...] of the humoral immune response* [18].
The modifications that set IMMSIM apart from classical cellular automata represent an important gain in flexibility. However, in spite of the introduction of receptors, the rules for the cellular interactions are analogous to those of classical cellular automata: cells switch between predefined states and their transitions are determined by the properties of their neighbors.

# Chapter 4

# Dynamics described by mechanisms

*This chapter introduces the concept of mechanisms as basic building blocks of local dynamics in simulations of complex systems. The connection between SIMMUNE and Mack's theory of complex systems is explained.*

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## 4.1 Mechanisms

Meaningful modeling of natural dynamic phenomena must be based on descriptions of experimentally determinable state transitions of the system being investigated. In those cases where it is possible to determine all variables of the system that govern its behavior the transition from one set of values of the variables to another may be formulated as a Markov process: the state of the system at one moment of time depends on the state of the system at the preceding moment of time only. If the description of the system's dynamics is a probabilistic one it's the dynamics of the probability distribution of the states (or of the probability density, for systems being described by continuous variables) which has this Markov property.
Natural dynamical phenomena may be described using formal mathematical quantities (like pressure, temperature and volume of a gas, to pick an example from thermodynamics) or with the help of representations of natural

bodily entities and their material properties. In the latter case the material properties are properties that are located spatially and temporally since the entities which they belong to have a position in space and time.
The famous *Nahewirkungs Prinzip* which states that physical entities cannot influence each other immediately unless they are located at adjacent points in space is manifest in interactions which are mediated by material objects.

Material interactions and the material state changes they induce in the interacting entities are commonly termed *mechanisms*.
Abstracting for the moment from the concept of a mechanical device that induces or experiences state changes I want to define a mechanism of a dynamical object as a markovian state transition together with the explicit description of the dependence of the transition on the values of a particular subset of the variables that describe the object's state. Alternatively, a mechanism may be defined without reference to variables as a set of conditional actions performed on the components of an object where the conditions specify which properties the object needs to possess in order to be subject to the corresponding actions.

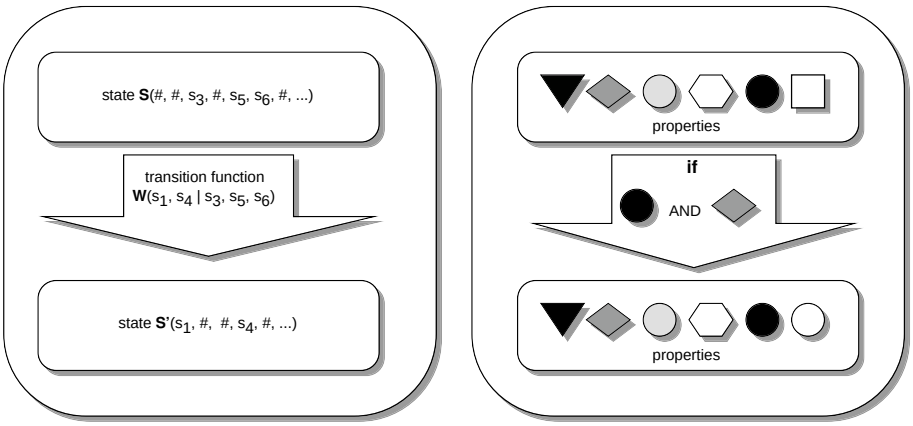


Figure 4.1: two representations of the concept of a mechanism

The goal of microscopic simulations of complex systems is to investigate modes of behavior that emerge as the results of interactions between the microscopic constituents of the systems. Usually, the dynamics in such simulations are defined by describing how the state transitions of an object (that belongs to the microscopic constituents of the system that is to be simulated) depend on the states of its neighbor objects.

Mechanisms, in contrast, describe how an object may transform depending on its own properties. Using mechanisms to formulate the possible interactions and dynamics within a multi-component system may be helpful to avoid conceptual errors in the model one develops. Consider for example the following immunological statement: 'Activated T-killer cells kill infected cells.' It may be used as a basis for simulations à la chapter 3.3 or for simple cellular automata. Using the statement in such a way without further reflection would however disregard some important points:

- (*locality*) The two cells (T-killer and target) have to meet: the killer cell has to be led to the site of infection. (→ chapters 2.7, 8.1)
- (*availability of information*) How does the T-killer cell know that its neighbor is an infected cell – as opposed to some normal, healthy cell? (→ chapters 2.5, 2.6)
- (*specificity*) The killer cell has to possess the right T-cell receptor to bind to MHC:virus-peptide. (→ chapter 2.6)
- (*imperfection*) The killer cell might erroneously kill its neighbor cell during an autoimmune disease. (→ chapter 2.9)

Trying to formulate the T-killer's mechanism that determines the interaction with its potential targets we ask: what are the specific properties of the T-cell's state that make it release perforin or induce apoptosis in its neighbor (see 2.4.2)? Even the (probably) simplified answer that an activated T-killer cell merely needs the signal coming from a binding event on its TCR includes

- *locality* of the interaction: Obviously the cells have to be neighboring each other in order to interact via membrane bound receptors.
- the *availability of information*: The binding event on its TCR *is* the only information the activated T-cell needs in order to 'know' that there is an infected cell that needs to be killed.[1]
- the *specificity* of receptor binding: How strong must the binding be, how many receptors must be occupied?
- the *imperfection*[2] of such a mechanism: Once a cell possesses this mechanism it will perform the mechanism's actions when the conditions are fulfilled regardless of whether its neighbor *really* is an infected cell.

[1] Questioning this brings us back to chapter 2.10 and to 5.2.

[2] from the point of view of an IS that is supposed to act flawlessly – without commiting errors like killing healthy cells.

We might proceed to ask what changes in the state of the T-cell are induced by the mechanism's actions. The T-cell will, for example, start secreting perforin. Since we know that the infected neighbor cell should be the target of the perforin being released, we are led to ask which receptor mediated interactions determine the spatial polarity in the T-cell's perforin secretion. Simply by trying to formulate the ingredients of the mechanisms for the interaction between T-killers and their potential targets, we are brought to some key questions in immunology.

Comparing the cellular-automaton approach with a mechanism-based multi-agent simulation two important differences become obvious:

1. The state transitions of a cell in a cellular automaton depend on the states of the neighbors of the cell. In contrast, the actions of an entity that acts according to a set of mechanisms depend on the entity's own state.

2. While in a cellular automaton rules are given for transitions between a priori defined states, the conditions and actions of a mechanism typically involve only a subset of the state variables.

## 4.2 Mechanism based modeling of complex systems

Mack [55], [56] chose an analogy from biochemistry by linking the conditional actions to entities called *enzymes* that recognize whether a mechanism's conditions are fulfilled and that – if the conditions are fulfilled – perform the mechanism's actions. Although Mack's enzymes may – in models of biological systems – be representations of enzymes in the classical sense, namely of molecules acting as biochemical catalyzers, they are generally used in his *theory of complex systems* [54] to stress the fact that any kind of dynamics in physical, chemical, biological or even sociological systems must be reducable to local transformations mediated by locally acting agents or forces.
Another important ingredient in Mack's theory of complex systems is the assertion that all types of local actions should be decomposable into a set of fundamental actions of which there are only four types:

- actions that *copy* objects,

- actions that *compose* complex objects from more fundamental ones,

- actions that *compose* relations between objects from existing, more fundamental relations[3] and
- actions that *derive* relations between objects from existing relations of other types.[4]

This assertion amounts to postulating that it should be possible to construct any complex system's structure (and its dynamics) with the help of these *atoms* of action.
Modeling the behavior of complex systems it is important to provide such a framework of primitives of local state transitions, since any predictions or conclusions derived from the model can only describe phenomena on scales which are coarser than the scale on which the fundamental mechanisms act: *what is assumed is not explained* [54] [57].

The application of these concepts to IS modeling shows that we need to make sure we have a well-defined description of the fundamental immunological interactions in our model – and that we should not mix different scales of description. A cellular automaton model for single-cell-level simulations of (parts of) the IS that formulates the cell transition rules based on macroscopic, cell population scale dynamics would make little sense.
Note that this does not mean that we are not allowed to let macroscopic parameters like temparature or pH influence the behavior of the objects on the microscopic scale. It only means we should not use as an input on the microscopic scale those (macroscopic or mesoscopic) dynamical properties of the system we want to be able to reproduce or even explain with our simulations.

[3] Think of a friend of a friend becoming your friend.
[4] In chemical reactions, for example, a relation of spatial proximity between two molecules may lead to a relation of chemical binding.

# Chapter 5

# Cognition and the immune system

## 5.1 Expert systems vs. adaptive autonomous agents

Expert systems are systems that contain human scientific[1] expertise in a symbolic representation that allows automated manipulation in such a way that the system would be able to answer a question that had not been encoded explicitely in the system's data content but that could be answered based on the information available in the system.
The key challenges in developing an expert system are the choice of the right symbolic representation, the identification of the fundamental pieces of information, and the formulation of the rules according to which complex assertions may be synthesized from these pieces. The programmer of the expert system thus has to build an image of a certain part of the world.
There are approaches that provide expert systems with user feedback rating the usability of the systems' data output. This feedback is then processed to ameliorate the content or presentation structure of the answers the systems give to their users' questions.

[1] A robot that is capable of producing drawings that look like those of Picasso would not be called an expert system.

The knowledge in expert systems, however, is static in the sense that expert systems do not learn from experience about the specific part of the world they were programmed to 'know'. They do not change their internal image of (their part of) the world.
Adaptive autonomous agents (AAAs) represent the other main approach for systems with rule based knowledge about their world in Artificial Intelligence (AI) research. AAAs are systems that try to fulfill a given set of tasks in a given environment. They decide autonomously how to relate signals from the environment to own activities (reactions). The mechanisms according to which decisions are made can be modified by the automata in response to succes or failure to fulfill their tasks. We may interpret the set of these mechanisms as an AAA's internal image of (its part of) the world, thereby ascribing to AAAs the ability to learn about their environment.

## 5.2 Learning processes in artificial intelligence and immunology

A common sense definition describes learning as a (self-) modification of a system that enables it to perform better upon repetition of a certain task or similar task [36]. In AI research, learning is typically defined as a process by which a system builds a recordable representation of past interactions with its environment. The two definitions are linked by a test situation (repetition of the task) and a cognitive process (recognition of the situation). According to the common sense definition, the adaptive immune system's generation of memory lymphocytes (and the environment necessary to stimulate them) certainly is a learning process, since it enables the IS to respond faster and more efficiently to a pathogen it has previously encountered. We may ask what the recordable representation of a pathogen encounter is in the immune system. Is it just the antigen?
There are important differences between learning concepts for expert systems and automata from AI and the processes we identify as belonging to learning in the immune system. They make it rather improbable that only the initial external stimulus (the antigen) is used to memorize the course of an immune response:

1. In an immune system nearly all signals that are to be processed have their origin inside the system itself, while in AI the signal comes from the interface that mediates between the cognitive device and its environment.

2. All actions of the immune system may be considered to be part of the signal as well as belonging to the signal processing, while in AI there is typically an input level which is separated from the signal processing part of the system.

Furthermore, due to the unavoidable degeneracy of T-cell receptors (see chapter 2.5) a purely antigen-based memory would frequently trigger false alarm: memory cell activation by *self* peptides.

## 5.3 Cognitive processes

### 5.3.1 What does it take to recognize your grandma?

Human cognitive processes are linked to the manipulation of mental images [50]. The question is, whether we recognize for example our grandma by activating a single, isolated representation of her, a fixed image – whether there is something like a 'grandma cell' among the neurons in our brains.
Experience with patients that had undergone neurosurgery has shown that the brain stores information in a distributed, plastic manner. Nuclear magnetic resonance experiments could establish that neuronal activity during the recognition of even simple geometrical shapes involves rather large areas of our brains.
Widely accepted today is the idea that when we recognize a certain object we recall (aware or unaware of it) its position in the network of symbols for known objects that we have inside our mind.
Manipulation of symbol structures instead of fixed images provides our cognition with robustness in two aspects:

1. Robustness towards small changes in the (often visually) conceived signal and

2. robustness towards small changes in the network of symbols which is our internal representation of the world.

We would recognize our grandma even without her favourite hat.

### 5.3.2 Connectionism vs. symbol system approaches

The concepts from AI (expert systems, automata) presented above belong to the class of approaches that define cognition as the manipulation of symbol structures. *Recognition* in this sense is the successful mapping of a stimulus onto an already existent symbol structure.

A second main direction in AI explains (natural) cognition as the massively parallel interaction of signal processing units. According to this concept, which is called *connectionism*, the manipulation of symbol structures is a coarse-scale, derived, abstract – or to stick to the notion introduced earlier: *effective* – theory of cognition. Connectionism defines knowledge as stable or reproducably excitable patterns of activity in multi-component systems. The most prominent realisations of such systems are *neural networks* which try to model cognitive processes with networks of model neurons that mimic the dynamics of natural neural systems like the brain.
Gradually, the two opposing approaches have found their specific applications: while symbol systems are mainly used for automated problem solving, connectionistic systems are used for pattern recognition tasks.

### 5.3.3 What does it take to recognize influenza?

Above, we asked whether there is a 'grandma cell' in our brain. One of the most ardent questions in immunology is whether there is such a thing like an 'influenza cell' in our immune system that is responsible for triggering an immune response once it notices an influenza infection.
According to our image of the IS it uses both of the two *rule based* approaches from AI mentioned above: The innate IS resembles an expert system. It has a 'hard-wired' knowledge of some classical pathogens that may infect us and it has a set of fixed rules how to react to questions about the adequate type of immune response the organism poses by confronting the cells of the innate IS (like macrophages and dendritic cells) with signs of ongoing desctruction caused by e.g. a virus.
Following Grossman's ideas of leukocytes being capable of tuning their responsiveness to the signals they receive [40, 41], we identify the cells of the adaptive IS as adaptive autonomous agents that obtain their education and, later, their strategic signals from the immunological expert system, the innate IS.
It is still unclear whether we will be able to recognize that the IS uses its components to set up a network representation of *self* and antigenic experience to achieve cognitive robustness in a similar way as – presumably – the human mind.

## 5.4 Cognition is in the eye of the beholder

Obviously, a system being capable of performing cognitive processes need not be in possession of conscience.[2] Slightly more involved is answering the question which kinds of observations allow us to conclude that a system has cognitive powers.
Consider ant colonies, for example. Putting a dead beetle in front of an ant hill will typically invoke the following events: First, one or two ants will discover the beetle and will attempt to carry it into the ant hill. If the beetle is too large they will return into the hill to come back with some of their colleagues to give the beetle a second try. If they fail again to move it some of them will again return to the hill and come back with even more ants.
Finally, they will be able to carry their prey with them.
Having witnessed this behavior we might say that the ants have solved their problem and have achieved a cognitive task. However, none of the ants had any idea how many of them it would take to move the beetle. And probably none of the ants even noticed there was some cognitive task to fulfill. The task and the cognitive achievement was existent only in the eye of the human observer.
Similarly, the IS becomes a system not till we describe it as such. We recognize the IS as a system since we see cooperative action of its components. It is known that the cells of the IS are involved in morphogenesis, in 'housekeeping' (like removing all kinds of debris from the body), in destroying aberrant cells etc.. Would any of these tasks attract our attention more than fighting infections, this thesis would perhaps be about, e.g., the 'Housekeeping System'.
A description of the IS in terms like 'immune response' or 'immune memory' is an effective theory. On a slightly finer scale we can formulate an effective theory of the processes during an infection on the cell population level in terms like 'B-cell response', 'T-cell response' etc..
There are, however, no effective theories yet for the scales in between the population level and the microscopic cell level. This thesis is about a tool that might help us to identify such theories.

[2] The subconscious connection we typically draw between the two notions very probably is one of the reasons people tend to be reluctant to ascribe cognitive capabilities to other systems than the human brain.

# Chapter 6

# SIMMUNE

*The modeling and simulation software SIMMUNE is introduced.*

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## 6.1 The concept of SIMMUNE

Among immunologists a consensus exists that the wide range of immune responses emerges not from receptor specificity alone, but from receptor specificity combined with the capability of immune system cells to process and distinguish between different patterns of combined signals and, finally, that while some decisions in the immune system are made on the cellular level many others emerge from intercellular communication.
The software package SIMMUNE to be introduced here was designed to facilitate the definition of models of multicellular biological systems based on single-cell stimulus-response mechanisms as introduced in chapter 4. The data for such stimulus-response mechanisms can be determined experimentally and do hence not depend on hypotheses about higher level structures within the IS. Besides putting simulations from the start on shakier ground, using mesoscopic or macroscopic data for the definition of single-cell behavior would invalidate most predictions derived from simulations of the models – see section 4.2.

The SIMMUNE *modeler* offers a graphical user interface to define molecule properties, cell mechanisms, compartments and the modeled organism as

a set of interconnected compartments. Furthermore, the simulations to be run can be defined: initial concentrations of the agent types involved, which agents to trace during the simulations etc. .
The second component of SIMMUNE is the *simulator*. It takes as input the definition of the models and simulations creates with the *modeler*. During a simulation various types of data – including dynamic data ('movies') – can be saved. Detailled descriptions of the features of the software will be given in chapter 7.

## 6.2 SIMMUNE through the eyes of the physicist

SIMMUNE is a tool to simulate interacting particles that have a continuous spectrum of states. Transitions between the states are probabilistic events triggered by the exchange of messenger particles with limited ranges. Simulations are done in discretized space-time: The particles are living on a grid and their states are (asynchronuously) updated at discrete timesteps. Transitions between the particle states depend on the messenger field intensities – typically involving a threshold field intensity.
The messenger field intensities are calculated by integration of reaction-diffusion equations.

## 6.3 Agents and structures in SIMMUNE

### 6.3.1 Compartments

As mentioned in the first chapter, the immune system uses special compartments for some special tasks like T-cell selection (in the thymus) or lymphocyte-APC interactions (in the lymph nodes).
Simulated organisms in SIMMUNE may consist of several two- or three dimensional compartments. Technically, compartments in SIMMUNE are two- or three dimensional cubic grids with grid spacing of 10 microns.[1] Each volume element (or grid node) may be occupied by at most one simulated cell. No limit is imposed on the number of molecules per volume element since molecules are simulated not as individual entities but by manipulations of local molecule concentrations (see below).
Like their biological counterparts, compartments in SIMMUNE can be connected by selective transports. For each kind of agent (celltype, moleculetype) a permeability of a given intercompartment connection can be intro-

[1] 10 microns is the typical size of cells of the immune system.

duced. Furthermore, a duration of transport can be defined.
Compartments may in principle have any size – in practice, limitations are imposed by the amount of available computer memory.

### 6.3.2 Molecules

The molecule concentrations encountered in immunology range from several molecules up to several tenthousands molecules per cell or cell-equivalent volume element. Simulating single molecules in systems consisting of many thousands of such volume elements is out of question with today's computers. Molecules in SIMMUNE are hence presented by the local concentrations of their molecule type. *Local* means within a given volume element of a simulated biological compartment or an intracellular compartment.
The parameters characterizing a molecule type in SIMMUNE are

- the number of distinct binding sites (called *epitopes*),
- the binding possibilities of the epitopes, including reaction characteristics (affinities, reaction rates),
- the molecule's lifespan,
- the molecule's diffusion coefficient.

### 6.3.3 Cells

Cells interact with their environment via stimulus-response mechanisms: A cell receives a certain molecular signal through its surface receptors and reacts by modifying protein contents (amount and/or distribution) – which may then lead to effects on the scale of the whole cell, like the cell's death or entering the (division-) cellcycle.
The kind of response of a cell to a stimulus depends on the strength of the stimulus: the number of surface receptors being occupied by specific ligands and sometimes the mechanical strength (avidity) of the binding between receptors and their ligands. Furthermore, it may depend on the direction of origin of the stimulus relative to some intracellular direction of reference.
The most elementary description of a cellular response to a given stimulus is the description of the alterations of the cellular protein contents (amount and distribution). Often, however, such a description may be replaced by higher level description – for example of the cell's state of movement. The parameters entering the description of a cellular response are varying, depending on the kind of response. For a response consisting of the secretion

of certain molecules the parameters are: the kind of molecule, the amount being secreted, and the direction (which side of the cell are the molecules secreted to?). Further parameters are the *duration* parameter which determines amount of time between the onset of the response and the changes – as they are defined as the final outcome of the response – effectively being visible in the system, and perhaps a *delay* parameter which defines the (mean) amount of time between the reception of the signals and the onset of the response. Both time parameters are probabilistic parameters: The user of SIMMUNE defines mean values of the duration and (optionally) the delay. The actually used values have a gaussian distribution around the mean with a variance of 0.25 times the mean[2]. The difference between the duration parameter and the delay parameter is that actions which start with no delay take the momentary (at the time the conditions are checked) state of the cell for the parameters they possibly have while a delayed action evaluates the cell's state not before the delay has passed.

### 6.3.4 SIMMUNE's mechanisms of cellular behavior

SIMMUNE allows all of the above mentioned stimulus-response parameters to be specified. Fig. 6.1 shows the prototype of a cellular mechanism. In the language of SIMMUNE the stimuli that are necessary to provoque a certain response from the cell are called *conditions*, the responses are called the *actions* of the mechanism. Arbitrarily many conditions may be combined with the logical AND.
Consider the example of the simplified scheme of the interactions between naive B-cells and T-helper cells as given in chapter 2.7. It is illustrated in figure 6.2. The mechanism of the T-cell is:

- **if** there is a molecule complex TCR:MHC2(+peptide) on any of the sides of the T-helper **then** the T-helper will react by expressing CD40L (the ligand for the receptor CD40 on the membrane of the B-cell) and by secreting a certain pattern of cytokines (one of which may be IL4, as in figure (6.2)) in the direction of the TCR:MHC2(+peptide) stimulus.

The mechanism of the naive B-cell is:

[2]The ad hoc determined variance will be a defineable parameter in future versions of SIMMUNE. The gaussian character of the distribution is however very probably a reasonable choice, since in real cells both time parameters are influenced by a large number of stochastic processes – which according to the central limit theorem leads to a gaussian distribution of their values.

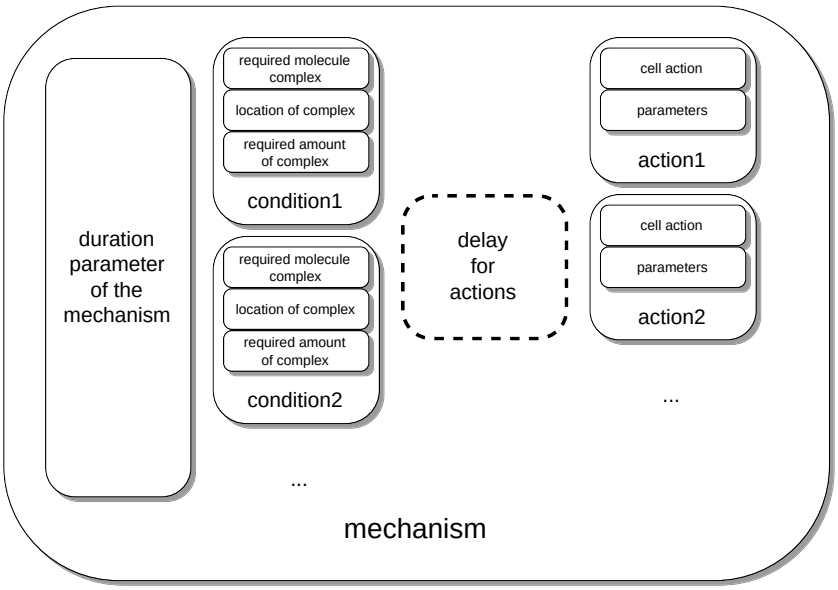


Figure 6.1: Prototype of a cellular mechanism

- **if** there is an CD40:CD40L complex on any of the sides of the B-cell **and if** there is an IL4Receptor:IL4 complex **and if** there is **no** IFN$\gamma$Receptor:IFN$\gamma$ complex[3] **then** it will react by proliferation and becoming an antibody (isotype IgG1) secreting plasma cell.

### Combining conditions by OR, concurrency

Sometimes, different sets of stimuli may lead to the same cellular actions. To model those cases one would like to combine conditions of a mechanism by the logical OR. This is not possible in SIMMUNE; one has to define alternative mechanisms that have different sets of conditions but equal actions – reflecting the fact that real cells may employ alternative *pathways* of cellular activity to attain a certain state. Recalling the definition of mechanisms as given in chapter 4, modeling this behavior requires multiple mechanisms.

Situations may occur where one mechanism depends on properties of those components of a cell which are the target of another mechanism's actions; or where two mechanisms have similar conditions but mutually exclusive

[3] which would suppress the switch to isotype IgG1

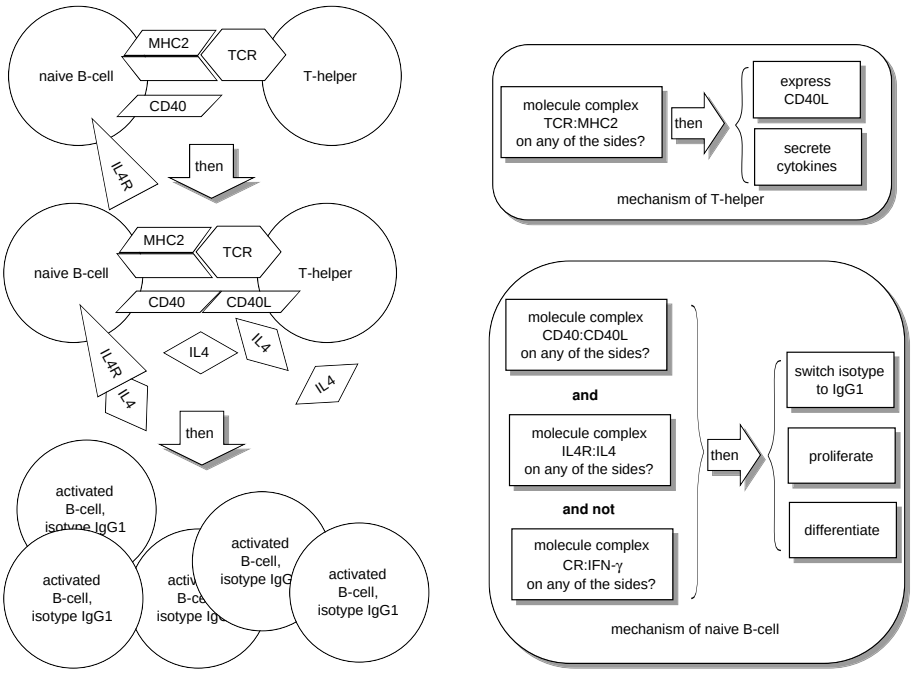


Figure 6.2: Mechanisms of B-cell activation by T-helper cells

actions. The *concurrency* of actions would present a problem in simulation approaches where state transitions are formulated in terms of a priori defined states and where actions may create intermediate states that are not part of the set of those states that are defined as starting points of state transitions. In SIMMUNE, there are no predefined states, actions act on components of cells (like membrane receptors) in such a way that modifications of cellular properties lead to cellular states that simply either fulfill the conditions for the cell's mechanisms or not.
Typically, cells have more than one mechanism. The order in which the single mechanisms check whether their conditions are fulfilled (and initiate their actions), is randomly determined for each timestep.

### 6.3.5 Intracellular structure

Early versions of SIMMUNE tried to model cells as black boxes, or automata with a set of input variables (occupancy of the different types of receptors) and a set of output variables (updated numbers of membrane

receptors, numbers and directions of molecules being secreted). Many immunologically relevant processes involve asymmetrical cellular responses and memory effects. At least partially, the knowledge about the underlying intracellular structures and the processes they are participating in is becoming available (see chapter 8.1 for an example). Thus, instead of employing abstract variables describing cellular polarity and delay differential equations to take into account memory effects, SIMMUNE offers to define intracellular compartments with selective transports between them. Furthermore, the duration of such intracellular transports and whether they lose material or involve conversions of given types of molecule complexes into other types can be determined.
Note that in many cases intracellular compartments and transports will be defined which do not have known biological counterparts but which represent models of the current state of knowledge about intracellular asymmetries.

# Chapter 7

# How to use SIMMUNE

*This chapter describes how to use the components of SIMMUNE, the modeler and the simulator. It is an excerpt from the online documentation that comes with the SIMMUNE package.*

*Soon, a database for mechanisms and cell types as well as updated versions of the software and the documentation will be available for non-commercial use via*

*www.simmune.org*

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## 7.1 Introduction

As mentioned before, SIMMUNE consists of two components: the SIMMUNE modeler (short: 'simmod') and the simulator (short: 'simmune'). Simmod is used to define models of multi-cellular systems by defining the properties of cell types, molecule types, molecule complexes, compartments and the properties of the simulations (which agent types are to be used etc.). During the simulation of a model, information like the amount of cells, molecule concentrations, gradients of molecule concentrations or the spatial distribution of cells can be viewed and saved in various data formats.

## 7.2 The SIMMUNE modeler

When starting, simmod asks whether you want to open an existing model or to create a new one. The simmod main window offers four subwindows:

- *molecules*, where you define molecule types and molecule complexes.
- *cell types*, where you define the cell types in your model.
- *organism*, where you define the compartments your simulation will use and how they are connected to form the simulated organism.
- *simulations*, where you define simulations and review simulations you have already ran.

### 7.2.1 Molecules and molecule complexes

#### Creating new molecules

Click on the 'new molecule' button, define the number of binding sites for the new molecule and whether it should be a membrane bound molecule or not. Double-click the molecule name in the list of defined molecules to edit properties like its lifespan, its diffusion coefficient or its description. After having typed a numerical parameter, you have to press RETURN (or ENTER) to submit the changes. Don't forget to save the changes.

#### Defining a molecule's binding possibilities

To define or modify a molecule's binding possibilities drag it into the left field of the 'define binding possibilities' rectangle. Drag the desired binding partner into the right field. Draw a line between the bindingsites you want to allow binding. Click on the small square of the interconnecting line to activate the binding for modification of its association and dissociation rates. After having typed a numerical parameter, you have to press RETURN (or ENTER) to submit the changes. Save changes! In order to remove a binding possibility, set its association rate to 0.0.

#### Creating molecule complexes

Drag molecule types that you to use for building a molecule complex into the fields of the 'define molecule complexes' area. Drag into the left upper square first. Double-click the small squares of the connecting lines between molecules to set the binding. Give every complex a unique name. If you

want to use molecules in cell mechanisms, you have to turn them into single-molecule complexes. All defined (and saved) complexes will be visible in the cell definition window. Important: You do not have to define all possible complexes. Only those molecule complexes that will appear in the definition of cellular behavior need to be defined here. (In fact, you should only define those which you actually need for the cellular mechanisms as defining complexes adds to the simulation overhead.) During a simulation, molecules that share a common neighborhood will bind to each other according to the binding possibilities and association and dissociation rates you defined for them. To keep the simulations computationally feasible, the size of molecule complexes is limited. A molecule complex will not grow beyond containing five 'fundamental' molecules.

Properties of molecule complexes: The lifespan of a molecule complex that consists of several molecules is set to be the mean lifespan of its constituents. The calculation of diffusion coefficients is slightly more difficult: Assuming spherical shape a molecule of radius $R$ has a diffusion coefficient proportional to $1/R$. When two molecules bind the resulting complex typically does not have a radius that is a simple sum of the radii of its constituents. Usually, it is better to calculate the new radius by first assuming that the mass of the single molecules is proportional to $R^3$, then adding the two molecule masses and calculating the radius (and the diffusion coefficient) of the complex from its mass.

#### Deleting molecule complexes

All defined (and saved) complexes are listed in the *cell types* definition window, too. Use the 'delete molecule complex' button in that window to delete molecule complexes.

### 7.2.2 Cells

#### Creating new cell types

Click on 'new celltype'; give it a unique name. To modify the properties of a cell type double-click it in the list of cell types. It will then appear in the 'cell type definition' field. Don't forget to save the changes you made.

#### Cell parameters

Basic cell type properties are defined by the following parameters:

- **lifespan**: this is the mean life time of a cell of this type.

- **matrix**: a cell that is defined to be a matrix cell will not exert 'brownian' motion. It will, however be able to perform directed movement (see below).

- **migration speed**: the migration speed for directed movement (chemotaxis) in microns per minute of cells of this type.

### Defining a cell's behavior

In SIMMUNE, the behavior of a cell depends on the mechanisms according to which cells of its type act. A mechanism in SIMMUNE consists of a set of conditions and a set of actions and a time scale parameter. During a simulation cells check whether the conditions of a mechanism are fulfilled and if so, performs the mechanism's actions. The time scale parameter specifies how long it will take the cell to complete the action (i.e., how long it will take until the changes introduced by the action become visible in the system). The actual time intervals fluctuate stochastically around the given parameter following a gaussian distribution with a standard deviation of half of the given value.
Mechanisms can have any number of conditions and actions. Cells can have any number of mechanisms. In order to edit a celltype's mechanisms, the celltype must be selected in the 'celltype definitions' field. To edit a mechanism double-click it. It will then be displayed in the 'mechanism definition' field. To replace the old mechanism by the altered one delete the old mechanism and save the new one. Details about cellular mechanisms are given below. Don't forget to save the altered cell properties.

### Cellular mechanisms

There are three types of conditions:

- conditions that check whether a certain molecular complex is (or is not) present at a certain location with a certain amount.

- a 'dummy' condition stating that the mechanism's action should be performed as default behavior.

- a 'dummy' condition stating that the mechanism's action should be performed once, initially when the cell is created.

To change a condition's or an actions parameters, you first have to select it. To use a molecule complex or a celltype as parameter, drag it from the list of

molecule complexes or cell types. Click the headers (for example 'location' or 'amount' to set those parameters. All amounts are numbers. So, 10000 means: 10000 molecule complexes, not moles. Don't forget to press return after having entered a numerical parameter. The locations that have to be defined for some conditions and actions are always relative to the side of the cell that has been set to be the side of reference by previous conditions or actions. In a condition, *SOME_SIDE* will always set (a randomly chosen) side of reference; *ANY_SIDE* will only set a side of reference, if the requested amount of the requested complex can be found at that randomly chosen side of the cell. The other possible locations in a condition do not set a side of reference. To select a cell action from the list of available actions click the header button 'action'. Give the mechanism a unique name and add it to the cell that is currently edited (in the 'cell type definition' field).

### Available cellular actions

Currently, the following cellular action can be used in the definition of mechanisms:

- **produce and secrete molecule**: makes the cell produce the specified amount of the selected molecule type and secrete it to the selected side of the cell. If the cell has a neighbor cell at the chosen side the secreted molecules will stay confined to the gap between the cells until the cells separate.

- **display membrane molecule**: makes the cell display the specified amount of the selected molecule type (which must be a membrane molecule type) on the selected side of the cell's surface.

- **transform into celltype**: makes the cell transform itself into a cell of the specified type. The molecule complexes on the cell's surface remain unchanged.

- **move along grad of**: The differences of the amounts of the specified molecule complex on opposing sides of the membrane are used to define a *move vector*. The move vector is then scaled to have a length that corresponds to the distance a chemotacting cell migrates within the chosen timestep length. During the simulated chemotaxis a cell adds its move vectors from several timesteps to a buffer vector until the absolute value of one of the components of this buffer attains the grid spacing (10 microns). Then the cell moves on one gridpoint in the

(positive or negative) direction of this component of the buffer and the buffer component is reset to 0.

- **move along internal grad of**: like the action above, except that the amounts of molecule complexes from intracellular compartments are used.
- **divide**: makes the cell produce one new cell of its own type and place it in its neighborhood (if there is space available).
- **set delay**: This action is used to make the cell perform certain actions after a delay. It takes as parameter the delay in seconds. To use it in a mechanism define it to be the *first* action in the mechanism's list of actions. Then add the actions that should be performed after the delay.
- **stay attached**: In order to interact with other cells for some longer period of time real cells use adhesion molecules that can make two cells mechanically stick together. In SIMMUNE, no forces between cells are simulated. If you want to make two cells stick together make them perform this action. To re-enable them to separate and move away from each other they have to perform the action 'lose attachment', below.
- **lose attachment**:(see the documentation of the action 'stay attached', above.)
- **die**: makes the cell die. All intracellular molecules will be removed from the simulation.
- **burst**: makes the cell die. All intracellular molecules will be put into the extracellular milieu were the cell was before it died.

Besides exerting chemotaxis, cells may display brownian like (random) motion: if a celltype is not defined to be a matrix type it will move stochastically with a frequency given by the 'cellular motility' parameter (which belongs to the definition of compartments, see below).

### Intracellular compartments and inner transports

In order to make use of intracellular inhomogeneity, one can define internal compartments. When a type of internal compartment is defined, the cell has one of them under the surface of each of its sides. Every cell automatically

has a 'central' compartment.
Inner transports are used to take molecule complexes from one type of internal compartment to the other (or from the surface or the central compartment to some internal compartment). Example: Let a cell have an internal compartment of type 'actin-reservoir'. Then, under the surface of each of the cell's sides one actin-reservoir will be located. An inner transport may now connect the surface of the cell to the actin-reservoir. This inner transport would take (unless 'production' is selected, see below) molecule complexes from the right side of the cell's surface to the actin-reservoir that is located under the surface of the right side of the cell (and analogously for the left, upper, lower, front and back side of the cell). The source compartment and the target compartment of an inner transport may be identical. A simple way to simulate amplifying cascades is to define transports with identical target and source compartments and complexes and setting a 'ratio' (see below) larger than 1.0.

Click 'show extended parameters' to define or modify inner compartments and transports. Drag molecule complexes to define startcomplex and endcomplex.

- **loss** is the relative amount of complexes lost during transport. It has to be between 0.0 and 1.0 . If, for example it is 0.98 then 98 percent of the complexes that start the transport are lost during the duration of the transport.

- **ratio** is the amount (relative to the number of found startcomplexes) of the startcomplexes that is taken away (or, if production is selected) for the transport. It has to be between 0.0 and 1.0 . If, for example it is 0.98 then (if production is not selected) the transport takes away 98 percent of the startcomplexes that are found.

- **rate** is the frequency relative to the cell step time (which is a parameter that is defined once for every simulation) at which new transports are started. If, for example it is set to 0.2, then after every fifth cell step time a new transport starts.

- **delay** is the time it takes for the transport. If, for example it is 30.0 then it takes 30 seconds before the complexes arrive in the target compartment.

- **production** selected means: the startcomplex is not really taken away; rather, the endcomplex is produced in the target compartment.

- **target removal** selected means: the tranported amount is not added to the target compartment; rather, it is substracted from the endcomplex in the target compartment.

### 7.2.3 Compartments

#### Creating new compartments

Click 'create new compartment' and give the new compartment a unique name.

#### Defining compartment properties

A compartment in SIMMUNE is defined by the following properties:

- the **viscosity of its medium** (relative to the viscosity of water). The value of this parameter may be adjusted to alter the 'velocity' of diffusing molecules.
- the **cellular motility** in the compartment. This parameter describes the 'velocity' of the cells that move in a kind of 'brownian' motion. Its value defines how many cell diameters per minute the cell will move randomly.
- the **nature of the edges** of the compartment: If the compartment is 'open' the edges are permeable to molecules (then, molecules will diffuse through the compartment edges as if the compartment edges were imaginary boundaries in an infinite compartment).
- **width**, **height**, and **length** of the compartment. One unit is a (typical) cell diameter: 10 microns.

### 7.2.4 Simulations

#### Opening/creating simulations

When clicking on the 'simulations' tab **for the first time during a simmod session**, simmod asks whether you want to examine or edit an existing simulation or create a new one.

#### Defining which agents should be investigated or manipulated during the simulation

During most simulations, there exist cell types and molecule types the concentrations of which do not need to be monitored or manipulated. In order to be able to monitor or manipulate an agent's concentrations during a simulation run you have to check 'trace' in the list of available cells or molecules by double-clicking after the agent's name.

#### Defining initial concentrations

If you want SIMMUNE to automatically inject certain concentrations of agents when the simulation starts, select the agent's name in the list of available cells or molecules and click the corresponding green arrow button to define the initial concentration.

#### Playing movies of recorded simulations

During a simulation with SIMMUNE you can record the dynamics of the cells as a 'movie' (see the documentation about the simulator). In case you have already done so for the simulation you have selected, the available movies will be listed in pop-up list in the 'movies' window. To view the selected movie click 'open the movie'. Then click 'start' to play it. Should you have saved molecule concentration gradients during the simulation too, you may click on 'select molecule gradients' to obtain a list of available gradients which you can display in the movie window. If you wish to examine cellular movements with some external software, you can transform a SIMMUNE movie into a plain text file containing the x, y, and z coordinates (or only y and z, if the compartment is two-dimensional) of the cells in the movie. The data for the single images of the movies are separated by a line containing '-1' entries. The name of the file is the same as for the movie, except for the ending which is '.gpm'.

## 7.3 The SIMMUNE simulator

Starting simmune, you have to select an existing SIMMUNE model and from that model the simulation you want to run. After you have selected the simulation, it may (depending on the parameters of the simulation and the speed of your computer) take quite a while (e.g., 30 seconds) until the simmune main window appears.
The simmune main window offers three kinds of views:

- the **history view** which shows the history of the concentrations of those molecules and cells in your simulations you chose to trace.
- the **compartment view** which shows a projection of a selected compartment with the cells it contains.
- the **compartment slice view** which shows a single slice of a selected compartment.

### 7.3.1 Basic functionality

The user interface in the simmune main window consists of:

the start button,
the quit button,
the button to switch to the (agent concentration) history display,
the button to switch to the compartment view,
the button to switch to the compartment slice view,
the compartment selector,
the refresh frequency selector,
the button to inject agents,
the button to save simulation data.

Finally, there is a checkbox for switching on and off molecular diffusion. If you switch off diffusion in the middle of a simulation moving cells might have created a spatial distribution of the molecules that represents a snapshot which would not endure if diffusion could act upon it and which – used as a permanent molecule distribution – is highly artificial.

### 7.3.2 History view

The history view of the SIMMUNE simulator shows the history of the amounts of cells and the concentrations of molecules. The amounts of cells are the total amounts of cells of a given type all over the compartment. The molecule concentrations are given as mean concentration in the compartment.

### 7.3.3 Compartment view

The compartment view of simmune shows a projection of the selected compartment. Front-left is the front edge of the compartment, back-right is the back edge.

### 7.3.4 Injecting agents

Clicking the 'inject' button will produce a small window with selectors for the agent type to inject, the injection amount and the location where to inject the agents.
When the agent type to inject is a cell type, the injection amount is given relative to the total (not just the unoccupied) positions for cells at the selected location. If, e.g., the compartment has the dimensions 1x81x81 (width x height x length) and the selected location is the right edge of the compartment, an amount of 10 per cent will result in injecting (up to) 0.1 x 1.0 x 81 = 8 cells. As the positions are selected randomly from the positions of the chosen location, the actually injected amount will be less because the cells at occupied positions will not be replaced by the cells that are to be injected.
When the agent type to inject is a molecule type, the injection amount is given in mols.
If the selected amount is 'from file' clicking 'inject now' will let you choose a file containing cell location data or molecule concentration data. (To learn how to create such files, see Saving simulation data, below.) Be careful to use only data files that were created for compartments with dimensions indentical to the compartment which is currently selected (future versions of SIMMUNE may check this ...).
If the selected location is 'spread' the whole compartment is the target of the injection.
If the selected location is 'center' the target of the injection is just the center position of the compartment. When the agent type to inject is a cell type the injection amount will automatically be one cell.
If the selected location is 'by mouse' the target of the injection will be a single cell position (to be selected by mouse click after you pressed 'inject now'). When the agent type to inject is a cell type the injection amount will automatically be one cell.

### 7.3.5 Saving simulation data

Clicking the save button (the one with the camera symbol) will open a small window that offers to choose the data format (and what kind of data are to be saved), the name for the data and (except for view image copies) for which agent types these data should be saved. For each simulation there is a subdirectory in the directory containing all the model data. This subdirectory has the name of the simulation (plus '.sim' extension). Within

the subdirectory there is (if simulation data have been saved) a subdirectory called 'Data'. Within this 'Data' directory there is a subdirectory for each compartment for which data have been saved ('gnuplot data', 'simmune data', 'molecule concs' and 'cell locations' – see below). Images (postscript or bitmap, see below) will be saved in the simulation directory in a subdirectory called 'Images'. Movies have their own subdirectories in the simulation directory.

The following data formats are available:

- **postscript** (Unix version of SIMMUNE only): makes of postscript image copy of the view that is currently active. The name for the data will be the filename for the data (plus '.ps' extension).
- **bitmap**: makes of bitmap ('*.bmp') image copy of the view that is currently active. The name for the data will be the filename for the data (plus '.bmp' extension).
- **gnuplot data**: writes the history of the concentrations (amounts) of the selected agent types to text files. (file name ending: '.sgp') .
- **simmune data**: writes the history of the concentrations (amounts) of the selected agent types to binary files. (file name ending: '.sdt') .
- **simmune movie**: will record a movie showing the movements of the cells of the selected types. In order to provide a (memory-) economic way of recording slowly advancing processes, the time interval between single pictures of the movie can be specified. It has to be at least as long as the cell step time.
- **molecule concs.**: writes the information about the local concentrations of the selected molecule types to files which may be used to inject prepared molecule distributions into simulations. (see also Injecting Agents.) (file name ending: '.scn')
- **molecule gradient**: writes the information about the lines of equal molecule concentrations to files which may be used to display those lines of equal molecule concentrations in movies.
- **cell locations**: writes the information about the locations of cells to files which may be used to inject prepared cell distributions. (file name ending: '.scl') .

# Chapter 8

# Application of SIMMUNE

*In this chapter, the application of SIMMUNE to modeling leukocyte chemotaxis is described. The model that is used employs such features of SIMMUNE as intracellular compartments and transports.*

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## 8.1 Chemotaxis

### 8.1.1 Chemotaxis in the immune system

*Chemotaxis* is the directed movement of cells in concentration gradients of certain types of molecules, so-called *chemoattractants*. Different types of cells may react to different types of molecules as chemoattractants. Chemoattractant cytokines are called *chemokines*.
Chemotaxis is an important ingredient of many fundamental biological processes. The movement of cells during embryological tissue formation is controlled by it as is the recruitment of specialized IS cells to sites of infection or immunological information exchange (see chapter 2.7).
There are basic differences between bacterial chemotaxis and leukocyte chemotaxis (see, for example, [44]). Here, only leukocyte chemotaxis will be treated.
The cellular events underlying chemotaxis may be divided into membrane based and intracellular processes. The primary event is the binding of chemoattractants from the extracellular milieu to membrane receptors. Many

signaling pathways that link binding events on membrane located receptors to intracellular processes make use of so-called G-proteins that are attached to the intracellular ends of the membrane receptors. A binding event on the extracellular receptor end causes the G-protein to get detached from the receptor and initiate an intracellular cascade of messaging molecules. In the case of chemotaxis some of these messaging molecules interact with certain types (see [16]) of the *actin* molecules in the cell.
Actin is the substance that is responsible for most of a cell's structural stability. In cells it exists as monomers, called *G-actin*, and as polymeric, filamentous *F-actin*. F-actin is the main component of the cellular sceletton, the cytosceletton. But actin is not only used for tasks of static nature: leukocytes move by extending actin stabilized pseudopods and contracting them again. Using fluorescence microscopy it was possible to show that the leading edge of chemotacting cells is an area of high actin polymerization and depolymerization.
It is becoming evident that some of the products of the intracellular messaging cascade control these processes by competing with ubiquous molecules that cap actin monomers, thereby preventing them from polymerization.

During the last 25 years extensive observational data have been accumulated describing leukocyte chemotaxis and chemokinesis (the movement of cells in homogeneous concentrations of chemoattractants) under many different conditions. The first assays [11] established the existence of the phenomenon. Later assays with constant chemotactic gradients were aiming at establishing the basic knowledge about the precision of leukocyte chemotaxis [91],[92], the connection between cell morphological changes and direction and speed of migration [93], [94].
Chemotacting leukocytes can sense and respond to relative chemoattractant concentration differences of down to 1% across the cell diameter. In chemoattractant concentrations of more than 10 times the dissociation constant of the binding between the chemoattractant and the membrane receptors leukocytes lose their orientation.
Recently, experiments have been made to investigate complex cellular properties like chemotactic memory in temporally [4], [21] and spatially [29],[30] changing chemotactic fields.
Chemotacting cells are not only capable of simple upgradient movement. They may move downward of local strong gradients in favor of shallow gradients emanating from distant sources of chemoattractants. The intracellular processes that can account for these abilities are only beginning to be explored.

### 8.1.2 Simulating leukocyte chemotaxis with SIMMUNE

Ever since the classic paper of Keller and Segel [48], various mathematical models of chemotaxis and chemokinesis have been derived, aiming at reproducing experimental data and predicting yet to be discovered intracellular mechanisms. Tranquillo et al. [85], [86] included modeling of stochastic receptor-ligand binding into their models.
Common to these models is, however, the need to omit either collective aspects (like, e.g. the mutual influence of migrating cells – mediated by the perturbation of the chemotactic field by the migrating cells) or intracellular mechanisms during chemotaxis. Furthermore, the necessity to keep the models numerically tractable led to the assumption of very simple (and static) chemotactic fields.
SIMMUNE is capable of calculating the temporal evolution of chemoattractant concentrations during the passage of chemotacting cells. It takes into account details like diffusion limitation of receptor-ligand binding or the influences of cell number densities and amounts of surface receptors on the local concentrations of the chemoattractant.
Besides being interesting in themselves, the chemotaxis experiments with SIMMUNE serve to establish chemotaxis as a standard building block for SIMMUNE simulations of immunological processes that include the spatial organization of agents by means of chemotaxis.

At present, there is no hope for a mathematical/computational model describing in detail the intracellular processes involved in leukocyte locomotion. Simulations have to employ 'effective' processes, which do not neccessarily have their real biological counterparts. While the use of such effective models is unavoidable at the current state of knowledge (and computer power) care must be taken to take into account the time delays introduced by omitted (because unknown) processes.
It is known that the regulation of the cellular response to external chemoattractant happens by processes like removal of ligand-bound chemoattractant receptors, their phosphorylation (which lowers the affinities for those intracellular molecule complexes that initiate the signalling cascades) and the redistribution of free receptors to the surface from intracellular 'pools'.
It was shown [93] that the rate of receptor recovery is a first-order function of the receptors missing (i.e. the difference between the initial and the current number of receptors) and that receptor number increase after chemoattractant induced membrane receptor loss does not involve protein synthesis, that is, does not require the building of new receptors. As this

behavior was observed for different initial numbers of receptors, receptor recovery can not be regulated by a hard-coded standard number of receptors but rather by time consuming processing of internalized ligand-bound receptors before they are brought back to the cell surface. Taking this into account, mechanisms describing regulation of surface receptor numbers must include a time delay parameter.

As described in chapters 6 and 7, SIMMUNE offers to define intracellular compartments with selective transports between them. Furthermore, a transport duration may be defined.
The cell model we[1] used to simulate the processes involved in leukocyte chemotaxis is illustrated in figure 8.1. The cell in our model contains a central compartment (marked (4) in the figure) working as a buffer pool for G-actin. It is connected by diffusion to peripheral G-actin reservoirs (2) which in turn exchange G-actin with the actin-polymerization compartments (3) through active intracellular transport. G-actin in the actin-polymerization compartments is induced to polymerize by messenger molecules which are produced as a result of binding events on the chemoattractant receptors. Occupied receptors are taken away from the surface; they are internalized and brought back to the surface – stripped of their ligands – after some delay.

The relative amounts of F-actin in the four actin-polymerization compartments are used to calculate the vector that determines the direction of cell movement: The difference of F-actin in the right side actin-polymerization compartment and the left side actin-polymerization compartment is the first component of this 'move vector'. The second component is given analogously by the top/bottom difference. Finally, the move vector is scaled to have a length that corresponds to the distance a chemotacting cell migrates within the chosen timestep.[2]
This rescaling is done to overcome one of the limitations stemming from the fact that the cells in our simulations are moving on a grid. During the simulated chemotaxis a cell adds its move vectors from several timesteps to a buffer vector until the absolute value of one of the components of this buffer attains the grid spacing (10 microns). Then the cell moves on one gridpoint in the (positive or negative) direction of this component of the buffer and the buffer component is reset to 0. This scheme allows the simulated cells

[1] Eric Kunkel (Stanford Medical School) and the author

[2] We make the assumption that the migration speed (not its direction) during chemotaxis is independent of the concentration of chemoattractant.

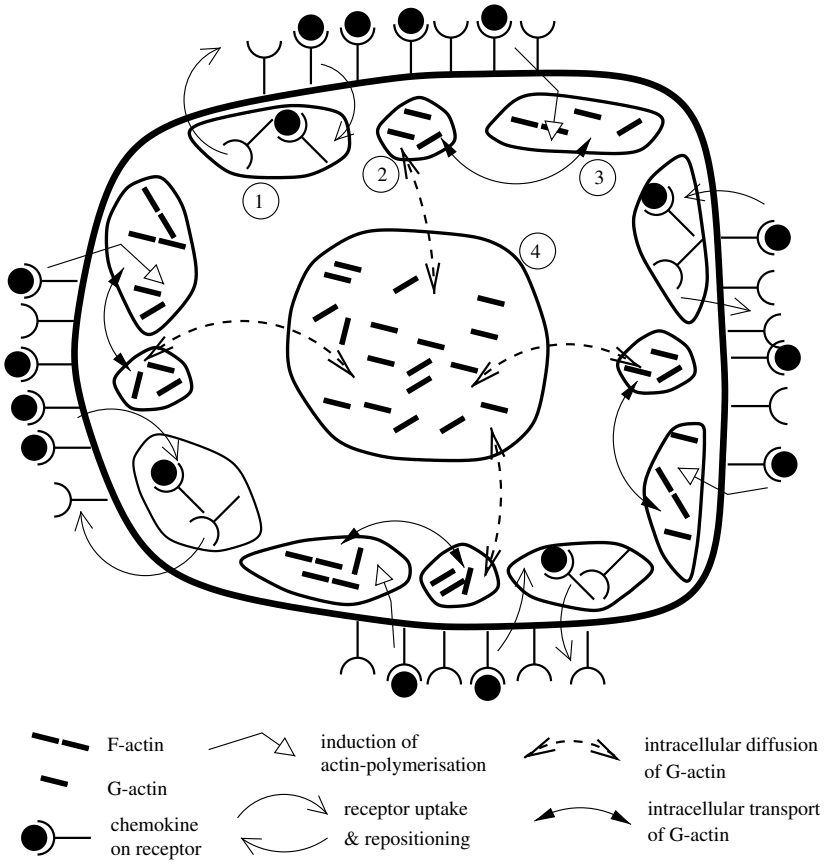


Figure 8.1: Model of a chemotacting cell

to actually move along the gradient of the chemoattractant they perceive instead of being confined to movement along just that axis of the grid that corresponds to the direction of the strongest intracellular chemotactic signal.

### The triangle assay

The intracellular processing of the primary chemoattractant signals they receive via their membrane receptors enables chemotacting cells to reach their destination through complicated patterns of overlapping concentration gradients of different chemoattractants.
In order to investigate how leukocytes react to conflicting chemotactic signals Foxman et al.[29] used two different chemoattractants, LTB4 and IL8, in a

set up in which two sources of chemoattractant and the initial position of the leukocytes formed an equilateral triangle.
Experiments were done with the two sources of chemoattractant filled with

1. on the left: LTB4, on the right: none
2. on the left: none, on the right: IL8
3. on the left: LTB4, on the right: LTB4
4. on the left: IL8, on the right: IL8
5. on the left: LTB4, on the right: IL8.

The results are shown in figure 8.2. In the two assays with identical chemoattractant in the two sources the majority of cells migrated furthest either to the left or the right source. In the assay with two competing chemoattractants, in contrast, the leukocytes migrated mainly in the direction intermediate between the two sources.

Figures 8.3 and 8.4 show the results obtained with SIMMUNE simulations of the assays. Though still quite simple, the model used for the intracellular processes is capable of reproducing the observed characteristics of leukocyte chemotaxis.
The figures also show, how the chemoattractant gradients are perturbed by the migrating cells. For the assay with two different types of chemoattractants the two sources released different amounts of chemoattractant. Nevertheless, the cells managed to sense the geometrical information presented by the two sources.
To model the fact that during the 'real' experiments chemoattractant was released continuously from the two sources, the simulations used two types of cells, secreting CK1 and CK2.

The size of the compartment used for the simulations was 243 x 243 x 3 volume elements – corresponding to a thin, square slice of 2.43 millimeters sidelength. The compartment was chosen to be not just two-dimensional, since the medium on the petri-dishes used in the 'real' experiments also has a certain depth. Simulating chemotaxis in a truly two-dimensional compartment will cause the cells to produce very strong perturbations of the chemoattractant gradients, since molecules have one spatial dimension less to 'swim' around cells.

With the parameters chosen for the intracellular transports, the simulated cells get disoriented around 10 times the dissociation constant ($10^{-9}$ $\frac{\text{mol}}{\text{liter}}$) of the receptor-chemoattractant binding.

In the appendix A.2.2 the configuration file for the chemotacting cells is shown as it is created by the SIMMUNE modeler and parsed by the simulator. The parameters for the cells' mechanisms and internal transports can be read off.

For the intracellular transports:

- *production of the messenger molecule* (transport names: 'Messenger_From_CK1', 'Messenger_From_CK2'): An intracellular transport is used to produce the messenger molecule, called 'ActinB', as a response to the cell sensing occupied chemoattractant receptors. Once per second for each occupied receptor an ActinB molecule is produced and transported to the 'FActinCompartment' (marked as (3) in figure 8.1). The transport takes three seconds.

- *active transport of actin* (transport names: 'Actin' and 'Actin2'): between the 'FActinCompartments' and the peripheral G-actin reservoirs (marked (2) in the figure) G-actin is transported in both directions. Every second 30% of the G-actin is taken up and brought to the target compartment. The transport takes one second.

- *receptor recycling* (transport names: 'CK1R_Recycling', 'CK2R_Recycling'): The process by which chemotacting cells internalize occupied receptors, strip them from their ligands and bring them back to the membrane is modeled by a transport with a delay of 600 seconds. Once per second 5% of the occupied receptors are removed from the surface.

For the cellular mechanisms:

- *initialization of the cell's chemoattractant receptors*: On every side of the cell 10000 receptors for the chemoattractants 'CK1' and 'CK2' are put at the moment the cell is created.

- *establishment of the cell's G-actin content*: At the moment the cell is created 10000 G-actin molecules are produced in the central compartment (marked (4) in the figure).

- *chemotactic cell movement*: The messenger 'ActinB' can bind to the G-actin (simply called 'Actin' in the model) to form the molecule complex 'ActinB_Actin' in the 'FActinCompartment'. The amounts of this complex in the 'FActinCompartments' are used for the calculation of the movevector as described above.

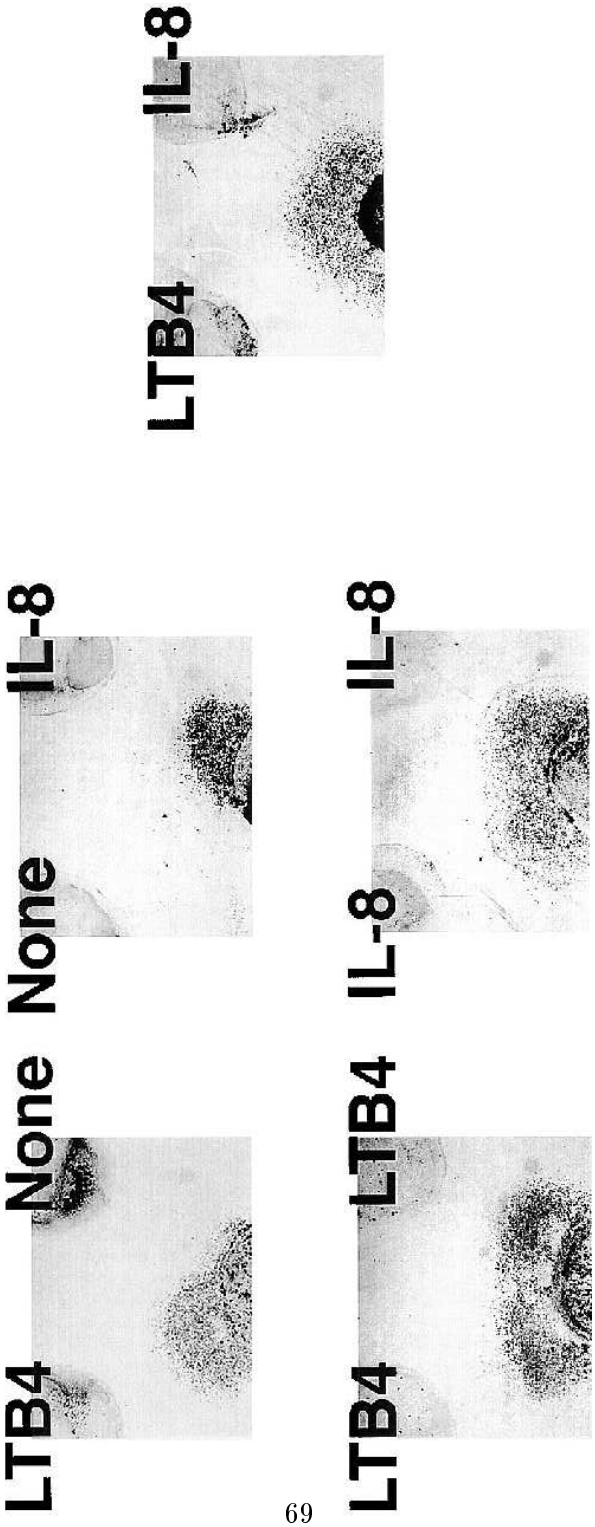




Figure 8.2: Results of the 'triangle' assay from [29]

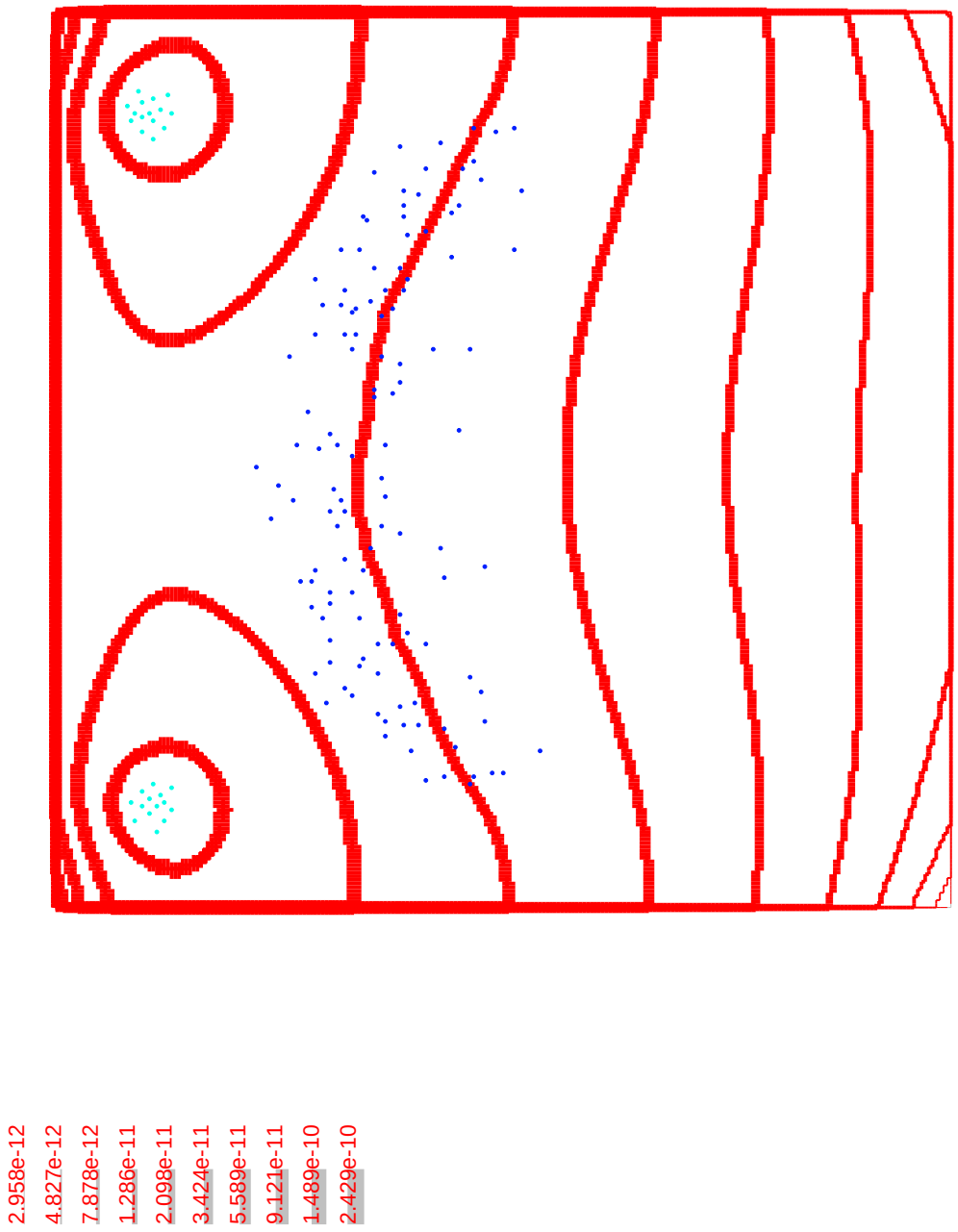


Figure 8.3: Simulation result corresponding to experiment 3 or 4 from the triangle setup (identical chemoattractants IL-8 or LTB4 in the two sources). Each blue dot represent a single cell. In red the lines of equal concentration of the chemoattractant are shown. The concentrations are given in mol/liter.

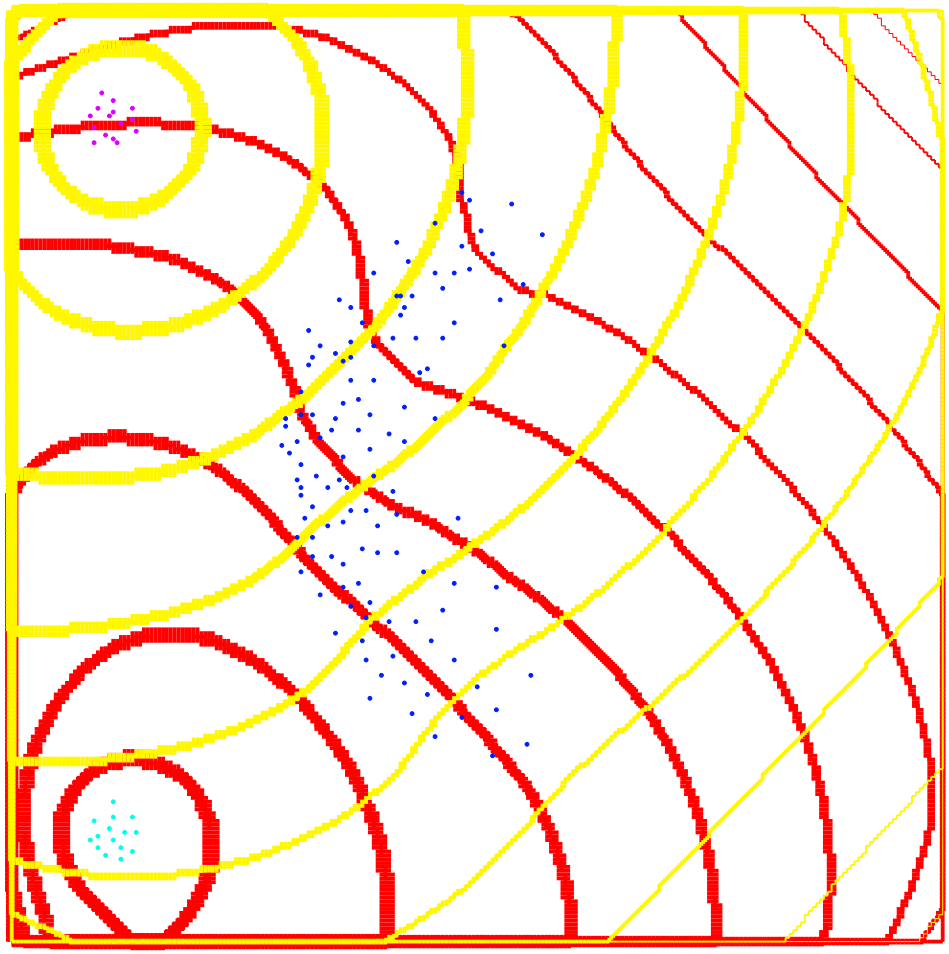


Figure 8.4: Simulation result corresponding to experiment 5 from the triangle setup (two different chemokines). Each blue dot represent a single cell. In red and yellow the lines of equal concentration of the chemoattractant are shown. The concentrations are given in mol/liter.

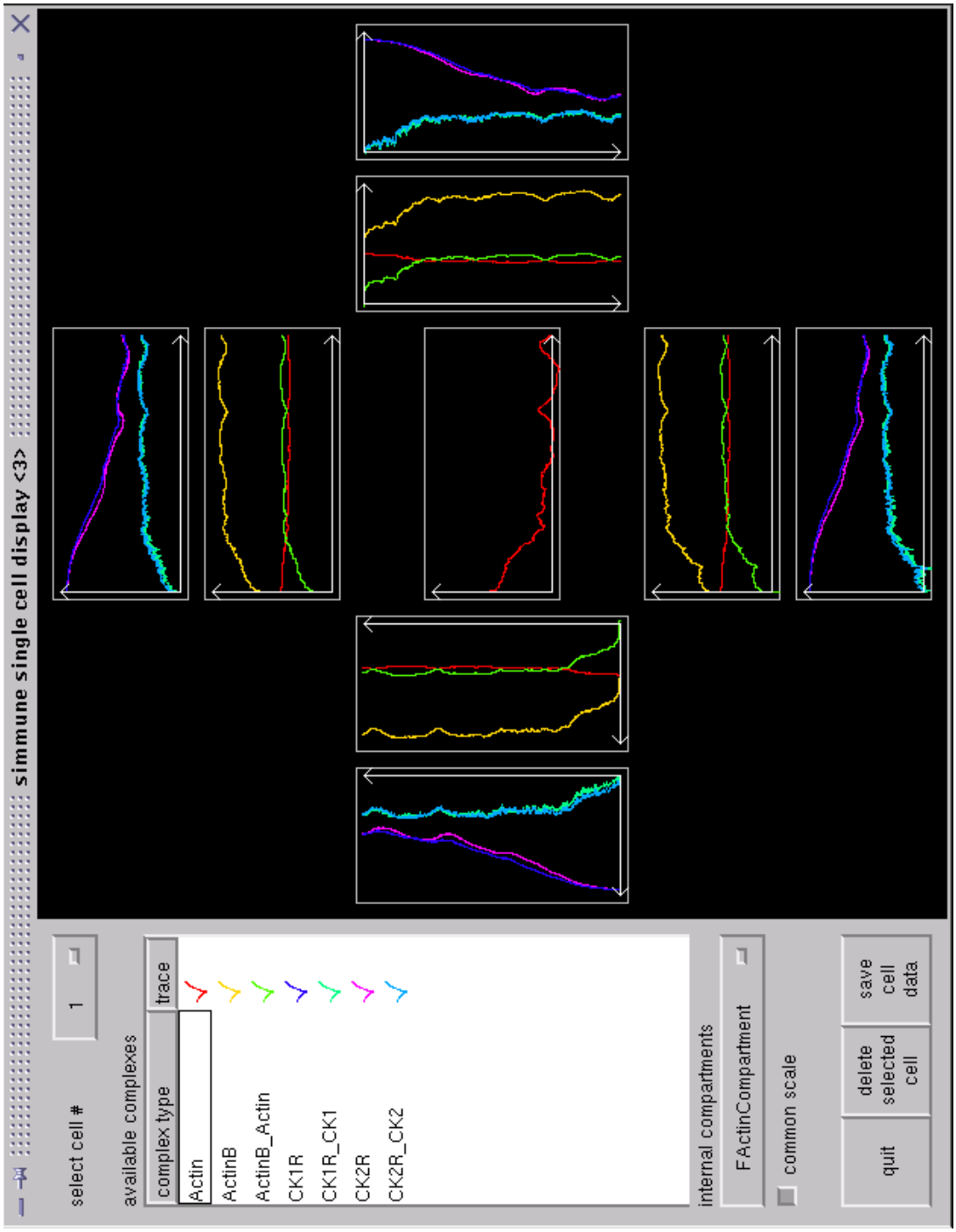


Figure 8.5: Evolution of the amounts of the molecules on the cell membrane and the intracellular compartments during chemotaxis. The peripheral boxes represent four membrane sides. Underneath them the FActinCompartments are shown. The central box represents the central compartment of the cell.

Figure 8.6: A screenshot of the cell type definition window of the SIMMUNE modeler.

# Appendix A

# Technicalities

## A.1 Reaction-Diffusion in SIMMUNE

As mentioned in chapter 6, the spatial compartments in SIMMUNE are simulated as 2- or 3-dim. cubic grids with a grid constant of 10 microns. Each volume element of such a grid may be occupied by a cell or a (technically) unlimited amount of molecules. The molecules within a single volume element of the grid are assumed to be homogenously distributed.
During a simulation of a 2-dim. compartment of, for example, 2 mm side-length molecule concentrations have to calculated in 40.000 volume elements for each single time step. Obviously, it is important to use algorithms that perform those calculations as fast as possible. Instead of using a single standard algorithm, SIMMUNE tries to dynamically determine the most efficient method that still yields results of acceptable accuracy.

The macroscopic, deterministic equation governing the dynamics of the (local) concentration $c_A$ of a molecule $A$ is the reaction-diffusion equation

$$\frac{\partial c_A}{\partial t} = D_A \nabla^2 c_A + f_R\left(c_A, c_B, c_C, ...\right), \tag{A.1}$$

where $c_B, c_C, ...$ denote the (local) concentrations of the bindingpartners of A, $D_A$ is the diffusion coefficient of A, and $f_R$ is the function describing

the change per time unit of $c_A$ due to chemical reactions. To numerically[1] integrate this equation it has to be discretized in space and time. A spatial discretization is already imposed by the grid structure of the spatial compartments in SIMMUNE. The timestep length used for the discretization of time cannot be chosen a priori. It depends (once the size of the volume elements is fixed) on $D_A$ and the reaction rates of the chemical reactions of A and will typically be a dynamical parameter of the solution algorithm. Situations may occur during simulations that would make a straightforward numerical integration of (A.1) inappropriate or unnecessarily expensive:

1. Sometimes, the molecular reactions happen on timescales much smaller than those of cellular actions and those governing diffusion. The molecular concentrations may then be assumed to be locally in chemical equilibrium all the time. Therefore, it may be sufficient to calculate equilibrium concentrations instead of integrating reaction rate equations. This, of course, will save a lot of computation time.

2. There may be certain areas of the compartment where little or no molecular reactions occur. The diffusion part may then be solved with algorithms that treat the size of the volume elements as dynamical parameters.

3. The local molecule concentrations can sometimes be rather low. This means that only few molecules will be located within a single volume element. A deterministic treatment of such a situation would ignore important statistical fluctuations in the reaction and diffusion processes. We need to switch to algorithms that are capable of dealing with the stochastic nature of molecular interactions.

The appropriate strategies for the three situations above will now be discussed:

### A.1.1 Fast molecular reactions: Equilibrium assumption

For systems with constant temperature $T$ and pressure $P$ chemical equilibrium is characterized by the minimum of the Gibb's potential $G$. In what follows I will derive an expression of the derivative of $G$ along the directions of the chemical reactions in the system that can be used in an algorithm to search for the minimum of $G$.

[1] An analytic solution of the reaction-diffusion equation can be given only for some special cases.

Together with the convexity of $G$ in single phase systems the necesary and sufficient condition for chemical equilibrium is

$$dG|_{T,P} = 0. \tag{A.2}$$

Consider a system of N substances $(X_1, X_2, ..., X_N)$ connected by R chemical reactions. Let $\mathbf{n} = (n_1, n_2, ..., n_N)$ of denote the N-tupel of particle numbers of the substances. Every chemical reaction may then be defined by a N-dimensional so-called *stoichiometric vector*. The entries of a stoichiometric vector are the multiplicities of the substances produced during the reaction. If, for example, the first reaction in the system is

$$X_1 + X_2 \mapsto X_4, \tag{A.3}$$

the corresponding stoichiometric vector would be $\mathbf{v}_1 = (-1, -1, 0, 1, 0, ..., 0)$.

Starting from a given set $\mathbf{n}^0$ of particle numbers, any other stoichiometrically allowed state $\mathbf{n}$ may be attained by adding a linear combination of stoichiometric vectors:

$$\mathbf{n} = \mathbf{n}^0 + \sum_{j=1}^{R} \mathbf{v}_j \xi_j. \quad \xi_j \in \mathbb{R} \tag{A.4}$$

(A.2) then translates into

$$\left(\frac{\partial G}{\partial \xi}\right)_{T,P} = \mathbf{0}. \tag{A.5}$$

Per definition, the chemical potential $\mu_i$ of $X_i$ is

$$\mu_i = \left(\frac{\partial G}{\partial n_i}\right)_{T,P,n_{k\neq i}}. \tag{A.6}$$

Hence, with

$$\left(\frac{\partial G}{\partial \xi_j}\right)_{T,P,\xi_{k\neq j}} = \sum_{i=1}^{N} \left(\frac{\partial G}{\partial n_i}\right)_{T,P,n_{k\neq i}} \left(\frac{\partial n_i}{\partial \xi_j}\right)_{\xi_{k\neq j}}, \tag{A.7}$$

one may write

$$\left(\frac{\partial G}{\partial \xi_j}\right)_{T,P,\xi_{k\neq j}} = \sum_{i=1}^{N} \mu_i \nu_{ij}, \tag{A.8}$$

where

$$\nu_{ij} = \left(\frac{\partial n_i}{\partial \xi_j}\right)_{\xi_{k\neq j}} \tag{A.9}$$

simply is the $i$-th entry in $\mathbf{v}_j$.

It can be shown (see, e.g. [12]) that in dilute solutions

$$\mu_i = \phi_i(P,T) + kT \ln(c_i), \tag{A.10}$$

where $c_i = n_i/n_{total}$ is the concentration of $X_i$ and $\phi_i$ is some function of P and T and $k$ is Boltzmann's constant.
The condition for equilibrium, (A.5), may thus be rewritten as

$$\sum_{i=1}^{N} \nu_{ij}\phi_i(P,T) + kT \sum_{i=1}^{N} \nu_{ij} \ln(c_i) = 0, \tag{A.11}$$

or

$$\begin{aligned} \sum_{i=1}^{N} \nu_{ij}\phi_i(P,T) &= -kT \sum_{i=1}^{N} \nu_{ij} \ln(c_i) && \text{(A.12)} \\ &= -kT \ln\left(\prod_{i=1}^{N} {c_i}^{\nu_{ij}}\right) && \text{(A.13)} \\ &= -kT \ln K_j, && \text{(A.14)} \end{aligned}$$

where the last equation is the definition of the equilibrium constant $K_j$ of the $j$-th reaction. It is also known as the law of mass action.
For $T, P = const.$ $K_j$ and $\phi_i(P,T)$ are constants, so we obtain an expression for the derivative of $G$ along the direction of the $j$-th reaction which is valid also outside the equilibrium – and which depends on parameters only that are usually known: the equilibrium constants $K_j$ of the chemical reactions involved.

$$\left(\frac{\partial G}{\partial \xi_j}\right)_{T,P,\xi_{k\neq j}} = kT \left(\sum_{i=1}^{N} \nu_{ij} \ln(c_i) - \ln K_j\right). \tag{A.15}$$

Starting from a given initial set of concentrations $\mathbf{c} = (c_1, c_2, ..., c_N)$ one may now move along the negative gradient of $G$ (as a function of $\xi$) to minimize $G$.
A straightforward implementation of this steepest descend method would be the iteration of the following scheme:

1. At the current position, calculate $\vec{d} = -\nabla G$.
2. Calculate the position of minimal $G$ along the direction of $\vec{d}$ and take it as the next position.

In many cases, this approach will need unnecessarily many small steps if the line minimum of $G$ isn't determined with very high precision – which also would be very time consuming. Consider, for example a $G$ that has the shape of a narrow valley (Fig. A.1).

The solution to this problem is to construct the direction of each line

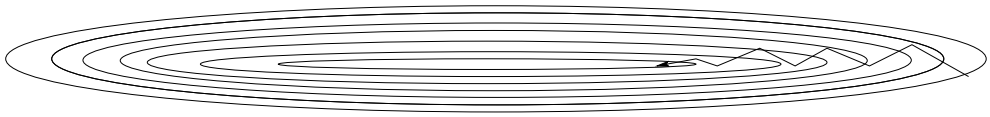

Figure A.1: steepest descend method in a narrow valley

minimization such that movement along that direction will not spoil the improvements of the approximate solution made by previous line minimizations. Obviously, one has to search for directions of descend perpendicular to the previously used ones – or at least to the last previous one. This approach is called *conjugate gradient* method. Implementations can be found in [70].

SIMMUNE uses a condensed version of the conjugate gradient approach that according to our experience works nicely. Instead of calculating line minima of $G$ and explicitely constructing conjugate directions, once a direction of descend is determined, a line minimization is made for the canonical scalar product of the currently used gradient (determining the current direction of descend) and the local gradient along the line. The position, where a local gradient is found that (approximately) has the minimal scalar product with the currently used gradient is chosen as the starting-point for the next gradient descend (and scalar product line minimization) – see Fig. A.2.

### A.1.2 Multigrid treatment of the diffusion equation

In areas of the compartment where no molecular reactions occur, the solution of the diffusion equation can considerably be accelerated by algorithms that iteratively increase the volume elements of the spatial discretization.

A straightforward discretization of the 1-dim. diffusion equation

$$\frac{\partial c}{\partial t} = D\nabla^2 c, \tag{A.16}$$

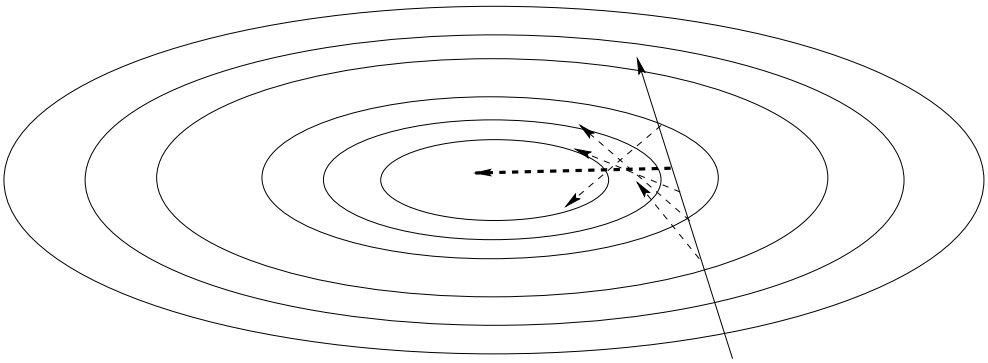

Figure A.2: SIMMUNE's condensed conjugate gradient method

for the diffusion of a molecule with local concentration $c$ and diffusion coefficient $D$ is:

$$\frac{{c^{n+1}}_{(j)} - {c^n}_{(j)}}{\Delta t} = D\left(\frac{{c^n}_{(j+1)} - 2{c^n}_{(j)} + {c^n}_{(j-1)}}{(\Delta x)^2}\right). \tag{A.17}$$

Here, ${c^n}_{(j)}$ is the molecule concentration in the $j$-th volume element at timestep n. $\Delta t$ is the timestep size, $\Delta x$ is the distance between the centers of two neighboring volume elements.
(A.17) can now be used to iteratively integrate (A.16) over some finite time interval. Suppose, we have homogenous Dirichlet boundary conditions, that is: let $c$ vanish on the boundaries of the (here 1-dim.) area where we wish to solve (A.16). The eigenmodes of the difference equation (A.17) are then

$${\nu^{(k)}}_{(j)} \sim \sin(kj\Delta x), \quad k \in \mathbb{N} \tag{A.18}$$

and the corresponding eigenvalues are

$$\xi_k = 1 - \frac{4D\Delta t}{(\Delta x)^2}\sin^2\left(\frac{k\Delta x}{2}\right). \tag{A.19}$$

The stability requirement $|\xi_k| \leq 1, \forall k$, leads to the condition

$$\Delta t \leq \frac{(\Delta x)^2}{2D}. \tag{A.20}$$

It can easily be shown that the mean square displacement $\overline{(\Delta x)}^2$ of a molecule by diffusion during a time interval $\tau$ is $2D\tau$. So the physical interpretation

of (A.20) is that the maximum allowed timestep is about the diffusion time across the size of the volume element.

Now, (A.19) shows that the fast oscillating modes within an approximative solution of (A.16) disappear rather quickly under iteration of (A.17). These fast oscillating modes represent local fluctuations of the molecule concentrations. The practical consequence is that after some iterations we may switch to a coarser grid: a grid with larger volume elements. And, if we increase the diameter of the elementary volume elements by a factor 2, the allowed timestep for the iteration grows by a factor 4. The step from grid to grid itself may also be iterated, leading to fast progress along the time axis.
Such an approach is called a *multigrid algorithm*. Multigrid algorithms are widely used tools for the solution of discretized differential equations. While typically much emphasis lies on finding the right *restriction-* and *interpolation* operators – which transport the approximate solution from the fine grids to the coarse grids and back – SIMMUNE uses the most simple operators that respect mass conservation: The restriction operator simply sets the molecule concentration in a coarse grid volume element to the mean concentration of the smaller volume elements within it. The coarse grid volume elements in SIMMUNE are not overlapping, that is, they contain disjunct sets of fine grid volume elements. After the coarse grid iterations are done, the concentrations in the fine grid volume elements are set to the concentration of the coarse grid volume element that contains them. The errors that are introduced on the fine grid by this interpolation scheme are then corrected by several iterations of the updating procedure (A.17) on the fine grid. The actual number of iterations needed depends on the diffusion coefficient of the molecule type. It is chosen such that the time advances several times the diffusion time across the fine grid volume element.

Below, the diffusion coefficients and the resulting max. timesteps of two chemokines (interleukins that induce directed cellular motion) (see also chapter 8.1) are given.

| molecule type | diffusion coefficient $[m^2/s]$ | max. timestep $[s]$ |
|---|---|---|
| fNLLP | $1.0 \times 10^{-9}$ | 0.05 |
| IL-8 | $1.0 \times 10^{-10}$ | 0.5 |

In three dimensional space, each volume element (except for those on the boundaries) has six instead of two (1-dim. case) neighbor elements, so the max. timestep gets reduced by a factor three. An updating step of the system that increases the simulated biological time by e.g. 10 seconds would thus

become computationally quite expensive if calculated on the original grid, without using coarser grids.

### A.1.3 Stochastic approaches

#### The Master Equation

The microscopic, molecular events leading to the macroscopic (and deterministic) equation (A.1) are of stochastic nature. The assumption of very large molecule numbers and the negligeability of stochastic fluctuations underlying the formulation and usage of (A.1) are often not justified in biological systems. In order to take into account the deviations of the behavior of real molecular ensembles from the idealistic dynamics goverened by the macroscopic reaction-diffusion equation one has to go back to the formalisms describing microscopic (as opposed to population based) stochastic state transitions.
The most general formulation of such a transition is given by its *Master Equation* (*ME*). Consider again the system of chemical reactants ($X_1$, $X_2$,..., $X_N$) with particle numbers $\mathbf{n}$=($n_1$, $n_2$,...,$n_N$) from (A.1.1), above[2]. The Master Equation[3]

$$\partial_t P(\mathbf{n}, t|\mathbf{n}', t') = \sum_{\mathbf{m}} \left[ W(\mathbf{n}|\mathbf{m}, t) P(\mathbf{m}, t|\mathbf{n}', t') - W(\mathbf{m}|\mathbf{n}, t) P(\mathbf{n}, t|\mathbf{n}', t') \right] \tag{A.21}$$

describes the time derivative of the probability of finding the system in the state characterised by the set of molecular particle numbers $\mathbf{n}$ at time $t$, given the system is in state $\mathbf{n}'$ at time $t'$. Here, $W(\mathbf{n}|\mathbf{m}, t)$ is the momentary (at time $t$) transition rate from state $\mathbf{m}$ to state $\mathbf{n}$.
The probability of finding the system in the state with particle numbers $\mathbf{n}$ at time $t$ is completely determined by the probabilities of the system's states at time $t'$. Processes that display this kind of behavior are called *Markov* processes[4].

[2] I will first consider a subsystem without diffusion: the elementary volume element of SIMMUNE's spatial grid. Within such a volume element the molecule concentrations are assumed to be homogenous. I will hence not take into account the spatial aspect of reaction-diffusion systems

[3] The sum $\sum_{\mathbf{m}}$ is to be understood as a summation over all stoichiometrically allowed states of the system.

[4] It should be noted that the assumption of well-stirredness of the reactants has to be made for a ME-formulation, too. Would the occurring chemical reactions introduce major inhomogeneity in the system the reactions would no longer be markovian processes. Kuzovkov & Kotomin [52] investigated reaction induced inhomogeneity and derived ex-

While, in general it may be difficult to find expressions for the transition rates $W$, these can usually be determined for chemical reactions: they are simply the reaction rates. Writing down (A.21) for a given system of chemical reactions hence is straightforward. Solving it analytically isn't, typically.

Gillespie [33] has developed a Monte Carlo method to simulate the stochastic time evolution of chemical systems. Stundzia & Lumsden [83] extended his method to incorporate molecular diffusion. The computational expense of the Gillespie algorithm is, however, proportional to the number of molecules (not molecule types!) in the system. And, since it requires the calculation of two random numbers per reaction step, it can practically only be used for very small molecule numbers.

**Expansion of the Master Equation – the Fokker-Planck equation**

The Master Equation was introduced here to take into account the stochasticity of molecular reactions. Faced with the technical difficulties that obstruct the solution of the Master Equation, one might ask whether it is possible to find a mathematical description of multistate systems with given transition rates that preserves some of the benefits of it while being more accessible to fast numerical equation solvers.
Kramers [51] derived an expansion (improved by Moyal [60] and put on rigorous grounds by van Kampen [46]) of the ME leading to a *Fokker-Planck* equation (*FPE*) that in turn can be translated to a stochastic differential equation.

By defining the *jump rate* $t(\mathbf{m}|\mathbf{n}) = W(\mathbf{n}+\mathbf{m}|\mathbf{n})$ one can rewrite the ME (A.21) as[5]

$$\partial_t P(\mathbf{n}, t|\mathbf{n}', t') = \sum_{\mathbf{m}} \left[ t(\mathbf{m}|\mathbf{n}-\mathbf{m}) P(\mathbf{n}-\mathbf{m}, t|\mathbf{n}', t') - t(\mathbf{m}|\mathbf{n}) P(\mathbf{n}, t|\mathbf{n}', t') \right] . \tag{A.22}$$

Expanding $[t(\mathbf{m}|\mathbf{n}-\mathbf{m})P(\mathbf{n}-\mathbf{m}, t|\mathbf{n}', t')]$ around $\mathbf{m} = 0$ yields[6] the *Kramers-*

pressions for effective reaction rates. Their approach can however not easily be adopted for the simulation of reactions between free-floating and membrane located molecules.

[5] $t(\mathbf{m}|\mathbf{n})$ will be nonzero only for jumps $\mathbf{m}$ that obey the stoichiometric constraints.

[6] $\left(\frac{\partial}{\partial \mathbf{n}}\right)^k$ is to be understood as a shortcut notation of the mixed partial derivatives appearing in the multidimensional Taylor expansion.

*Moyal* expansion:

$$\partial_t P(\mathbf{n}, t|\mathbf{n}', t') = \sum_{k=1} \frac{(-1)^k}{k!} \left( \frac{\partial}{\partial \mathbf{n}} \right)^k [\alpha_k(\mathbf{n}) P(\mathbf{n})] , \tag{A.23}$$

where

$$\alpha_k(\mathbf{n}) = \sum_{\mathbf{m}} \mathbf{m}^k t(\mathbf{m}|\mathbf{n}) \tag{A.24}$$

are the *jump moments* of the system. Terminating the expansion after the second term yields a Fokker-Planck equation

$$\partial_t P(\mathbf{n}) = -\sum_{i=1}^{N} \frac{\partial}{\partial n_i} \alpha_1(\mathbf{n}) P(\mathbf{n}) + \sum_{i=1}^{N} \sum_{j=1}^{N} \frac{1}{2} \frac{\partial^2}{\partial n_i \partial n_j} \alpha_2(\mathbf{n}) P(\mathbf{n}). \tag{A.25}$$

There is, however, no obvious reason why this truncation should produce meaningful results, i.e. why it should be possible to neglect the higher orders in the expansion[7].

**System size expansion of the Fokker-Planck equation**

In this section a system size expansion of the Fokker-Planck equation will be presented that puts the truncation of the expansion of the ME on firmer ground. It was first given by van Kampen [46] (and in the manner presented here in [47]). Gillespie [34] showed that the system size expansion and similar approaches are valid only for systems with chemical reactions that change the number of molecules by not more than one per reaction, that is, for systems where the elements of the stoichiometric vectors $\mathbf{v}$ have absolute values of $\leq 1$. For the purposes of SIMMUNE this is however no harmful constraint since receptor-ligand interactions only involve (per binding or dissociation step) one molecule of each type.

In a Fokker-Planck equation the first part – containing the first order derivatives – is sometimes called the *drift* part while the second part is called the *diffusion* part. The first part corresponds to the deterministic part of the dynamics to be modeled while the second part describes the stochastic fluctuations.
Now, let $\phi(t)$ be the solution of the differential equation describing the deterministic part of the dynamics under investigation (in the case of our

[7] The *Pawula* theorem (see [74]) merely states that the Kramers-Moyal expansion may stop after either the first or the second order but if it does not stop after the second term it has to take into account an infinite number of terms

system of reacting substances this would be the integration of the macroscopic rate equations). For simplicity, only the case of a single variable $n$ will be treated. Later a multi-substance chemical system will be considered again. $n$ will have a mean value of order $\Omega$ and will have a spread around this mean of order $\sqrt{\Omega}$, that is, it will behave like

$$n = \Omega\phi(t) + \sqrt{\Omega}y, \tag{A.26}$$

where $y$ is the (scaled) deviation of $n$ of the deterministic behavior. Expressing the time derivative of $P$ through the derivatives of $\hat{P}(y,t)$

$$\partial_t P(n,t) = -\dot{\phi}(t)\sqrt{\Omega}\frac{\partial \hat{P}}{\partial y} + \frac{\partial \hat{P}}{\partial t} \tag{A.27}$$

the Kramers-Moyal expansion (A.23) becomes

$$\frac{\partial \hat{P}}{\partial t} - \dot{\phi}(t)\sqrt{\Omega}\frac{\partial \hat{P}}{\partial y} = \sum_{k=1}\frac{(-1)^k}{k!}\Omega^{-k/2}\left(\frac{\partial}{\partial y}\right)^k\left[\alpha_k(\Omega\phi + \Omega^{1/2}y)\hat{P}(y)\right]. \tag{A.28}$$

Assume, that the jump rates $t(m|n)$ depend in such a way on the size parameter $\Omega$ that one may write

$$t(m|n) = f(\Omega)\sum_j \Omega^{-j}\psi_j\left(\frac{n}{\Omega}, m\right), \tag{A.29}$$

where $f(\Omega)$ is a function describing the dependence of the system's evolution time on its size. One then arrives at

$$\frac{\partial \hat{P}}{\partial t} - \dot{\phi}(t)\sqrt{\Omega}\frac{\partial \hat{P}}{\partial y} = \tag{A.30}$$
$$f(\Omega)\sum_{k=1}\sum_{j=0}\frac{(-1)^k}{k!}\Omega^{-(j+k/2)}\left(\frac{\partial}{\partial y}\right)^k\left[\tilde{\alpha}_{kj}(\phi + \Omega^{-1/2}y)\hat{P}(y)\right],$$

where the jump moments $\tilde{\alpha}_{kj}$ are given by

$$\tilde{\alpha}_{kj}(y) = \sum_m m^k\psi_j(y,m). \tag{A.31}$$

Rescaling time by

$$\tau = \Omega^{-1}f(\Omega)\,t \tag{A.32}$$

removes the overall factor $f(\Omega)$. Finally, assuming small noise one may expand $\tilde{\alpha}_{kj}(\phi + \Omega^{-1/2}y)$ around $\tilde{\alpha}_{kj}(\phi)$ to obtain

$$\frac{\partial \hat{P}}{\partial \tau} - \frac{d\phi}{d\tau}(\tau)\sqrt{\Omega}\frac{\partial \hat{P}}{\partial y} = \tag{A.33}$$
$$\sum_{k=1}\sum_{j=0}\frac{(-1)^k}{k!}\Omega^{-(j+k/2)+1}\left(\frac{\partial}{\partial y}\right)^k \hat{P}(y)\left[\tilde{\alpha}_{kj}(\phi) + y\Omega^{-1/2}\tilde{\alpha}'_{kj}(\phi)\right.$$
$$\left. + \frac{y^2}{2!}\Omega^{-1}\tilde{\alpha}''_{kj}(\phi) + \ldots\right].$$

The term of order $\sqrt{\Omega}$ will vanish if

$$\frac{d\phi}{d\tau} = \tilde{\alpha}_{10}(\phi), \tag{A.34}$$

which is just the assumed behavior of $\phi$ as can be seen by multiplying the ME (A.21) by $\mathbf{n}$ and summing over $\mathbf{n}$.

The term independent of $\Omega$ is

$$\frac{\partial \hat{P}}{\partial \tau} = -\tilde{\alpha}'_{10}(\phi)\frac{\partial}{\partial y}y\hat{P} + \frac{1}{2}\frac{\partial^2}{\partial y^2}\tilde{\alpha}_{20}(\phi)\hat{P}. \tag{A.35}$$

Now, the FPE for the system of chemical reactants ($X_1$, $X_2$,..., $X_N$) with particle numbers $\mathbf{n}$=($n_1$, $n_2$,...,$n_N$) from above (A.1.1) will be derived. It will be shown that the second order truncation of the Kramers-Moyal expansion is justified in systems with small noise: performing the same variable transformations $\mathbf{n} \mapsto (\phi, \mathbf{y})$ as above one obtains an FPE equivalent to (A.35).

Let ${k^+}_j$ be the reaction rate of the $j$-th reaction $\mathbf{n} \mapsto \mathbf{n} + \mathbf{v}_j$ and ${k^-}_j$ the rate of the inverse reaction. Let ${m^{+/-}}_{ij}$ be the number of molecules of substance $i$ involved in the forward/backward $j$-th reaction:

$$\begin{aligned} {m^+}_{ij} &= \Theta(-\nu_{ij})|\nu_{ij}| \\ {m^-}_{ij} &= \Theta(\nu_{ij})|\nu_{ij}|. \end{aligned}$$

Here, $\Theta(x)$ is the Heavyside function

$$\Theta(x) = \begin{cases} 1 & \text{if } x \geq 0 \\ 0 & \text{else .} \end{cases}$$

The Master Equation of the system becomes

$$\partial_t P(\mathbf{n},t) = \sum_{j=1}^{R} \Big( \big[ {t^-}_j(\mathbf{n}+\mathbf{v}_j) P(\mathbf{n}+\mathbf{v}_j,t) - {t^+}_j(\mathbf{n}) P(\mathbf{n},t) \big] + \big[ {t^+}_j(\mathbf{n}-\mathbf{v}_j) P(\mathbf{n}-\mathbf{v}_j,t) - {t^-}_j(\mathbf{n}) P(\mathbf{n},t) \big] \Big) , \qquad \text{(A.36)}$$

where the rate constants ${t^+}_a$ and ${t^-}_a$ of the $a$-th reaction are defined as

$$\begin{aligned} {t^+}_a(\mathbf{n}) &= {k^+}_j \prod_i^N \frac{n_i!}{(n_i - {m^+}_{ia})!} \\ {t^-}_a(\mathbf{n}) &= {k^-}_j \prod_i^N \frac{n_i!}{(n_i - {m^-}_{ia})!} . \end{aligned} \qquad \text{(A.37)}$$

The factors $(\cdot)_i$ appearing in the expression for the rate constants ${t^+}_a$ $({t^-}_a)$ are the numbers of ways of choosing a combination of ${m^+}_{ia}$ $({m^-}_{ia})$ molecules out of a number of $n_i$.

The Kramers-Moyal expansion of the ME above is

$$\partial_t P(\mathbf{n},t) = \sum_{a=1}^{R} \sum_{k=0} \left( \frac{(\mathbf{v}_a)^k}{k!} \left( \frac{\partial}{\partial \mathbf{n}} \right)^k \big[ {t^-}_a(\mathbf{n}) P(\mathbf{n},t) \big] + \frac{(-\mathbf{v}_a)^k}{k!} \left( \frac{\partial}{\partial \mathbf{n}} \right)^k \big[ {t^+}_a(\mathbf{n}) P(\mathbf{n},t) \big] \right) . \qquad \text{(A.38)}$$

Switching from the extensive variable $\mathbf{n}$ to the intensive variable $\mathbf{x}$

$$\mathbf{x} = \frac{\mathbf{n}}{\Omega}, \qquad \text{(A.39)}$$

will result in rate constants ${\tau^{+/-}}_a$, obeying the scaling relation

$${\tau^{+/-}}_a = \frac{{t^{+/-}}_a}{\Omega}, \qquad \text{(A.40)}$$

which states that the discrete rate constants $t^{+/-}$ are extensive quantities. With this transformation the Kramers-Moyal expansion to second order reads

$$\partial_t p(\mathbf{x}) = -\sum_{i=1}^{N} \frac{\partial}{\partial x_i} A_i(\mathbf{x}) p(\mathbf{x}) + \frac{1}{2\Omega} \sum_{i=1}^{N} \sum_{j=1}^{N} \frac{\partial^2}{\partial x_i \partial x_j} B_{ij}(\mathbf{x}) p(\mathbf{x}). \qquad \text{(A.41)}$$

where

$$A_i = \sum_{a=1}^{R} \nu_{ia} \left( {\tau^+}_a - {\tau^-}_a \right) \tag{A.42}$$

$$B_{ij} = \sum_{a=1}^{R} \nu_{ia}\nu_{ja} \left( {\tau^-}_a + {\tau^+}_a \right) . \tag{A.43}$$

$\nu_{ij}$ is the $i$-th entry in $\mathbf{v}_j$ (see A.9) and $p(\mathbf{x})$ is the probability density of the intensive variable $\mathbf{x}$.
Now, a variable transformation like (A.26) will be performed by introducing the new variable $\mathbf{y}$ through

$$\mathbf{y} = \frac{\mathbf{x} - \phi(t)}{\epsilon}, \tag{A.44}$$

where, again, $\phi(t)$ is the solution of the deterministic part of the system's dynamics and $\epsilon = 1/\sqrt{\Omega}$. Expressing the time derivative of $p$ through the derivatives of $\hat{p}(\mathbf{y}, t)$

$$\partial_t p(\mathbf{x}, t) = \frac{\partial \hat{p}}{\partial t} - \frac{\dot{\phi}(t)}{\epsilon} \sum_{i=1}^{N} \frac{\partial \hat{p}}{\partial y_i} \tag{A.45}$$

the truncated Kramers-Moyal expansion becomes

$$\begin{aligned} \partial_t \hat{p}(\mathbf{y}) = &- \sum_{i=1}^{N} \frac{\partial}{\partial y_i} \left[ \frac{A_i(\phi(t) + \epsilon\mathbf{y}) - A_i(\phi(t))}{\epsilon} \hat{p} \right] \\ &+ \frac{1}{2} \sum_{i=1}^{N} \sum_{j=1}^{N} \frac{\partial^2}{\partial y_i \partial y_j} \left[ B_{ij}(\phi(t) + \epsilon\mathbf{y}) \hat{p} \right] . \end{aligned} \tag{A.46}$$

Assuming small noise, $A_i(\phi(t) + \epsilon\mathbf{y})$ may be expanded around $\phi(t)$:

$$A_i(\phi(t) + \epsilon\mathbf{y}) = \sum_{k=0} \frac{(\epsilon\mathbf{y})^k}{k!} \left[ \left( \frac{\partial}{\partial \mathbf{x}} \right)^k A_i(\mathbf{x}) \right]_{\mathbf{x}=\phi} . \tag{A.47}$$

Expanding $B_{ij}$ similarly and keeping only terms to 0-th order in $\epsilon$ we are finally left with

$$\partial_t \hat{p}(\mathbf{y}) = - \sum_{i=1}^{N} \frac{\partial}{\partial y_i} \left[ {A'}_i(\phi(t), \mathbf{y}) \hat{p}(\mathbf{y}) \right] + \frac{1}{2} \sum_{i=1}^{N} \sum_{j=1}^{N} \frac{\partial^2}{\partial y_i \partial y_j} \left[ B_{ij}(\phi(t)) \hat{p}(\mathbf{y}) \right] , \tag{A.48}$$

where

$$A'_i(\phi(t), \mathbf{y}) = \sum_{j=1}^{N} y_j \left[ \frac{\partial}{\partial x_j} A_i(\mathbf{x}) \right]_{\mathbf{x}=\phi} . \tag{A.49}$$

A typical problem in SIMMUNE is the binding of certain interleukins to receptors situated in the membrane of a cell. As an example, $\mathbf{A}'$ and $\mathbf{B}$ will now be determined for a system consisting of a receptor $R$, an interleukin $L$ and the bound receptor state $R:L \equiv B$ with concentrations ($r$, $l$, $b$) and one type of reaction

$$R + L \overset{k^{+/-}}{\rightleftharpoons} B \tag{A.50}$$

will be considered. The rate constants $t^+$ and $t^-$ are

$$\begin{aligned} t^+ &= k^+ r l \, , \\ t^- &= k^- b \, . \end{aligned}$$

The concentration of the interleukin $L$ is assumed to be large enough to be left unchanged by the reaction. The stoichiometric vector of the reaction is $\mathbf{v} = (-1, 1)$. The components of $\mathbf{A}$ are thus

$$\begin{aligned} A_1 &= -(k^+ r l - k^- b) \, , \\ A_2 &= -A_1 \, . \end{aligned}$$

So the components of $\mathbf{A}'$ are

$$\begin{aligned} A'_1 &= -(k^+ y_1 \phi_l - k^- y_2) \, , \\ A'_2 &= -A'_1 \, , \end{aligned}$$

where $\phi(t)$ is – as in the section above – a solution of the deterministic dynamics in the system.
The components of $\mathbf{B}$ are

$$\begin{aligned} B_{11} &= k^+ \phi_r \phi_l + k^- \phi_b \, , \\ B_{12} &= B_{21} = -B_{11} \, , \\ B_{22} &= B_{11} \, . \end{aligned}$$

(A.48) is the promised equivalent of (A.35). It is a FPE that relates the probability density of the fluctuations $\mathbf{y}$ of $\mathbf{x}$ to the deterministic path $\phi(t)$. It can however only for very simple chemical systems be solved analytically. As a basis for numerical approaches the corresponding stochastic differential equation may be used – which will be explained in the next section where the assertion that the *drift* part of the FPE represents the deterministic dynamics of the system will be illustrated with the above example chemical system.

**Stochastic differential equations**

Stochastic differential equations were introduced for the first time when brownian motion was investigated mathematically by Einstein and Langevin in the first decade of the 20th century. The *Langevin* equation describing a particle's brownian motion is

$$\frac{dx}{dt} = a(x,t) + b(x,t)\xi(t), \tag{A.51}$$

where $x$ is the position of the particle $a(x,t)$ is a function describing friction and $b(x,t)\xi(t)$ the term responsible for the particle's random motion wherein $\xi(t)$ is a rapidly fluctuating random quantity. In brownian motion, the random jumps of the particle are caused by uncorrelated, directionally unbiased collisions with the solvent's molecules. The mathematically idealized formulation of the properties of $\xi(t)$ would be to state that $\xi(t)$ has a vanishing autocorrelation time:

$$\langle \xi(t), \xi(t') \rangle = \delta(t - t'), \tag{A.52}$$

and, since $\xi(t)$ should contain no drift component (which would rather be in $a(x,t)$)

$$\langle \xi(t) \rangle = 0. \tag{A.53}$$

We know that in brownian motion the increment of the length of the path due to random collisions is proportional to $\sqrt{dt}$ for the time increment $dt$. The difference equation corresponding to A.51 hence is

$$x(t + dt) = x(t) + a(x,t)dt + b'(x,t)\sqrt{dt}. \tag{A.54}$$

The relation between $b(x,t)$ and $b'(x,t)$ will become clear below.

Looking at

$$\frac{x(t + dt) - x(t)}{dt} = a(x,t) + \frac{b'(x,t)}{\sqrt{dt}} \tag{A.55}$$

it is obvious that $x(t)$ is continuous, but not differentiable, since the limit $dt \to 0$ for the above equation does not exist.
It can be shown (see, for example, [35]) that for a variable $X$ the dynamics of which is described by a continuous markov process the fluctuation component of the increment $X(t + dt) - X(t)$ is proportional to $N(t)\sqrt{dt}$ where $N(t)$ is a unit gaussian random variable. Denoting the distribution from

which $N(t)$ is drawn by $\mathbf{N}(0,1)$ (a gaussian distribution with mean 0 and variance 1) we may write

$$\frac{N(t)}{\sqrt{dt}} = \left[\frac{\mathbf{N}(0,1)}{\sqrt{dt}}\right] = \left[\mathbf{N}(0,1/dt)\right], \tag{A.56}$$

where the square brackets mean 'sample from'.
Using the definition of a *gaussian white noise process*[8] $g_w$

$$g_w = \lim_{dt\to 0} \mathbf{N}(0,1/dt) \tag{A.57}$$

one may now formally write the *white noise Langevin equation*

$$\frac{dx}{dt} = a(x,t) + b(x,t)g_w. \tag{A.58}$$

Note, that (A.58) is not a conventional differential equation and that one needs a prescription of how to calculate the increment $dx$. One may write the increment as

$$dx = \int_t^{t+dt} a(x,t')dt' + \int_t^{t+dt} b(x,t')dW(t'). \tag{A.59}$$

Here, $dW(t)$ is a so-called *Wiener increment*, the increment $w(t+dt) - w(t)$ of a *Wiener process* $w(t)$ which is characterized by its transition probability (density)

$$p(w,t|w',t') = \frac{1}{\sqrt{2\pi(t-t')}} \exp\left(-\frac{(w-w')^2}{2(t-t')}\right). \tag{A.60}$$

(A.59) contains, however, some ambiguity as to when one should evaluate the integrants. One approach is to evaluate them at the beginning of the time interval $dt$, thus at $t$. This approach is called the *Ito* interpretation. In contrast, the *Stratonovich* interpretation is to evaluate them at the intermediate point $\overline{x} = (x(t) + x(t+dt))/2$ which leads to an implicit expression for $dt$.
I will not go into the details of how to integrate stochastic differential equations here. They can be found in, e.g., [49], [61], or [42].

[8] a random process $\xi(t)$ with $< \xi(t), \xi(t') >= \delta(t-t')$ is called *white* noise since the fourier transform of the two-time correlation

$$F(\omega) = \int d\tau < \xi(t), \xi(t+\tau)) > e^{i\omega\tau} = 1$$

is independent of the frequency $\omega$.

It is worthwile mentioning that for each sequence of Wiener increments one obtains a different trajectory $x(t)$. Therefore, one often refers to the *simulation* of a stochastic differential equation rather than to its *solution*.

Now, (A.48) and the example system from the end of last section will be considered again. With

$$\tilde{\mathbf{A}} = \begin{pmatrix} -k^+ l & k^- \\ k^+ l & -k^- \end{pmatrix}$$

one can write (A.48) for our receptor-ligand system as

$$\partial_t \hat{p}(\mathbf{y}) = -\sum_{i=1}^{2} \frac{\partial}{\partial y_i} \left[ \left[ \tilde{\mathbf{A}}(l(t))\mathbf{y} \right]_i \hat{p}(\mathbf{y}) \right] + \frac{1}{2} \sum_{i=1}^{2} \sum_{j=1}^{2} \frac{\partial^2}{\partial y_i \partial y_j} \left[ B_{ij}(\phi(t), l(t)) \hat{p}(\mathbf{y}) \right] \ , \tag{A.61}$$

where $y_{1/2}$ are the fluctuations of the concentrations of R and B.
It can be shown that equation (A.61) above is equivalent to the stochastic differential equation

$$d\mathbf{y} = \tilde{\mathbf{A}}(l(t))\mathbf{y}dt + \sqrt{\mathbf{B}(\phi(t), l(t))} d\mathbf{W}. \tag{A.62}$$

Here, $d\mathbf{W}$ is the increment of a two-variable Wiener process

$$\mathbf{w}(t) = (w_1(t), w_2(t))$$

with the transition probability density

$$p(\mathbf{w}, t|\mathbf{w}', t') = \frac{1}{2\pi(t-t')} \exp\left( -\frac{(\mathbf{w}-\mathbf{w}')^2}{2(t-t')} \right). \tag{A.63}$$

The square root $\sqrt{\mathbf{B}}$ in (A.62) is to be understood such that

$$\sqrt{\mathbf{B}} \sqrt{\mathbf{B}}^T = \mathbf{B}.$$

Since that part of the increment of $\mathbf{y}$ that is proportional to $dt$ describes the deterministic part of the system's motion it should be possible to recover the deterministic reaction kinetics of the example chemical system from the last section. And replacing $\mathbf{y}$ by $(r, b)^T$ we find

$$\dot{r} = -k^+ \cdot r \cdot l + k^- \cdot b \tag{A.64}$$

$$\dot{b} = k^+ \cdot r \cdot l - k^- \cdot b \tag{A.65}$$

which are the deterministic equations for the time derivatives of $r$ and $b$.

## A.2 Simulation parameters

### A.2.1 Numerical parameters for reaction-kinetics simulations from 3.3

| figure | $p_{infect}$ | $p_{kill}$ | $p_{resp}$ | $s$ | $d_I$ | $d_K$ | $d_C$ | $I_0$ | $K_0$ | $C_0$ |
|---|---|---|---|---|---|---|---|---|---|---|
| Fig. 3.1 | 0.3 | 0.5 | 0.1 | 0.01 | 0.01 | 0.01 | 0.01 | 0.1 | 0.1 | 1.0 |
| Fig. 3.2 | 0.3 | 1.0 | 0.8 | 0.01 | 0.01 | 0.01 | 0.01 | 0.1 | 0.1 | 1.0 |

### A.2.2 Cell parameters from 8.1.2

Here, the cell parameters like mechanisms and intracellular transports are given in the format produced by the SIMMUNE modeler and parsed by the SIMMUNE simulator:

```
{
        description = "chemotacting leukocyte"
        matrix = "YES"
        lifespan = 1.000200e+05
        speed = 2.000000e+01
        InternalCompartments = (FActinCompartment)
        InnerTransports = {
                Messenger_From_CK1 = {
                        From = "Surface"
                        To = "FActinCompartment"
                        StartComplex = "CK1R_CK1"
                        EndComplex = "ActinB"
                        loss = 0.000e+00
                        ratio = 1.000e+00
                        rate = 1.000e+00
                        delay = 3.000e+00
                        production = "YES"
                        target_removal = "NO"
                }
                Messenger_From_CK2 = {
                        From = "Surface"
                        To = "FActinCompartment"
                        StartComplex = "CK2R_CK2"
                        EndComplex = "ActinB"
                        loss = 0.000e+00
                        ratio = 1.000e+00
```

```
        rate = 1.000e+00
        delay = 3.000e+00
        production = "YES"
        target_removal = "NO"
}
Actin = {
        From = "FActinCompartment"
        To = "UnderSurface"
        StartComplex = "Actin"
        EndComplex = "Actin"
        loss = 0.000e+00
        ratio = 3.000e-01
        rate = 1.000e+00
        delay = 1.000e+00
        target_removal = "NO"
}
Actin2 = {
        From = "UnderSurface"
        To = "FActinCompartment"
        StartComplex = "Actin"
        EndComplex = "Actin"
        loss = 0.000e+00
        ratio = 3.000e-01
        rate = 1.000e+00
        delay = 1.000e+00
        production = "NO"
        target_removal = "NO"
}
CK1R_Recycling = {
        From = "Surface"
        To = "Surface"
        StartComplex = "CK1R_CK1"
        EndComplex = "CK1R"
        loss = 0.000e+00
        ratio = 5.000e-02
        rate = 1.000e+00
        delay = 6.000e+02
        production = "NO"
        target_removal = "NO"
}
```

```
                CK2R_Recycling = {
                        From = "Surface"
                        To = "Surface"
                        StartComplex = "CK2R_CK2"
                        EndComplex = "CK2R"
                        loss = 0.000e+00
                        ratio = 5.000e-02
                        rate = 1.000e+00
                        delay = 6.000e+02
                        production = "NO"
                        target_removal = "NO"
                }
        }
        mechanisms = {
 actin_init = "1?][;!produceIntracellularMolecule:Actin*1.000e+04;"
 movement = "1?[];!moveAlongInternalGradOf:ActinB_Actin*FActinCompartment;"
 init = "1?][;!displayMembraneMolecule:CK1R*EVERY_SIDE*1.000e+04;
         !displayMembraneMolecule:CK2R*EVERY_SIDE*1.000e+04;"
        }
}
```

## A.3 Index of special terms

Here, a list of special terms and the chapter and page where they were introduced is given:


- Adaptive autonomous agent: chapter 5.1, page 38
- Antibody: chapter 2.4.1, page 16
- Antigen: chapter 2.2, page 14
- Antigen presenting cell, APC: chapter 2.4.3, page 19
- B-cell: chapter 2.2, page 14
- Cellular automaton, CA: chapter 3.5.2, page 30
- Clonal selection: chapter 2.5, page 19
- Complement: chapter 2.2, page 14
- Cytokines: chapter 2.2, page 14
- Dendritic cell: chapter 2.2, page 14
- Expert system: chapter 5.1, page 37
- Idiotypic network: chapter 2.9, page 23
- Immnunoglobulines: chapter 2.4.1, page 16
- Interleukins: chapter 2.2, page 14
- Isotype (of an antibody): chapter 2.4.1, page 17
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## Concluding remarks

In the introduction to this report I mentioned the biologists' longing for simple, comprehensible models as seem to govern physics.
On the other side of the fence: A famous physicist[9] once told an anecdote exemplifying his doubt about the biologists' conviction that in order to understand a biological system to a reasonable degree one has to know a lot of details about it.
Trying to develop a computer program that should be able to simulate some aspects of immune system behavior I surely didn't want to ignore Ockam's razor[10]. Numerous discussions with *real* immunologists have, however, taught me how difficult it is to make sensible use of this razor in models of complex biological systems. I am convinced that the complexity of a model should be determined by the specialists working on the model's subject. This is what SIMMUNE offers: a simple way to create models and simulations as complex as the subject of investigation requires.

*In-silico biology*, the simulation of biological processes on the computer, is still a rather young discipline. Since it is not as commercially rewarding on short term as *bioinformatics* which deals mainly with the automated analysis of genomic data, it will have to mostly rely on the academic world to offer it the structure it needs to develop.
Judging from my own experience, I think that physics should be an important part of future university programs for theoretical biology/in-silico biology. Knowledge about statistical physics, dynamical systems, multiscale methods and other subjects one usually only learns about during physics courses is an invaluable helper in tackling the intricacies of complex systems from biology, too.

[9] R.P. Feynman
[10] *entia non sunt multiplicanda sine necessitate*

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Open my eyes that I may see
the wonders in your law.

Psalm 119, verse 18