Chapter 8

Conclusions,
Perspectives,
Summary
1. Conclusions

In the foregoing chapters an account is given of molecular dynamics simulations performed on several cell surface receptors. These are proteins, which are present on the outside of cells and enable them to detect the presence of chemical or physical stimuli in the surroundings. One of the central questions remaining to be elucidated in biochemistry/pharmacology is how the inside of the cell is made aware of the stimulation of the parts of the receptors on the outside of the cell. This work was aimed at understanding some of the mechanisms involved in signalling.

The main body of the work focused on two receptor-ligand complexes, namely the TRAIL-Death Receptor 5 complex and the Erythropoietin-Erythropoietin Receptor complex. The first of these is involved in the immune response against tumours, and is of interest as an anti-cancer agent. The second is involved in the production of red blood cells and is an important therapeutic against anaemia, which is formally a shortage of oxygen carriage capacity of the blood.

Both of these complexes involve multimeric receptors, consisting of a number of identical subunits; three in the case of the Death Receptor and two in the case of the Erythropoietin Receptor. Besides, in both cases, the subunits of the receptor are bound to the intracellular domains through a single linker, suggesting that the mechanism of action either involves the coming together of the subunits upon binding of the ligand, or a change in the relative orientation.

The results obtained from the simulations, suggested that in the case of the Death Receptor 5, the subunits are likely to form aggregates in the absence of the ligand, TRAIL. In addition, binding of the ligand diminished the motility, suggesting freezing as another factor in receptor activation. Further study also showed that it is likely that the complexes formed after binding of ligand may form larger networks, which could play a role in signalling. In the case of the Erythropoietin Receptor the results suggested that the relative orientation, notably the rotational relationship, between the membrane bound domains was critical for the activity of the receptor. The simulations have led to a new model regarding the activation of the receptor. Besides, the simulations allowed discrimination between the states of the receptor induced by an active ligand (EMP1) and an inactive ligand (EMP33).

The results mentioned above mainly concern the practical application of molecular dynamics. However, during this work several contributions were also made concerning the methodology. In particular, it is shown that simulations of proteins or other macromolecules can be made roughly twice as efficient by choosing an appropriate simulation cell. Next to that, methods are presented to make robust assessments of the influence of external factors on proteins in simulation, using statistical theory.
Before taking a brief look into the future, it is necessary to reflect on the work performed. Although they can be very convincing, it should be borne in mind that simulations are not real. They are what we expect to happen in reality based on a set assumptions derived from what can be measured from real systems. However, there are many discrepancies between simulations and reality. Illustrations of this are the force field used (see Chapter 4) and the use of a limited volume simulation system (see also Chapter 4). In addition, simulations are commonly performed using an artificial grid, such that the system resembles a state in between solution and a crystal. E.g. in Chapter 5 the properties of the grid were employed to investigate the formation of a 2D crystal, but the bias added to the system restricts the generality of conclusions drawn. In general, it is good to be encouraged by results obtained, but one should always take care with the conclusions drawn from them and separate those which are solid enough to be written down publicly and those which can only be used to generate and attenuate hypotheses as loose ends for further research.

2. Perspectives

Cell surface receptors are the main targets for pharmacological intervention. In past times, the development of drugs was largely a matter of chance, of trial and error. Nowadays, drug design is more and more based on rational processes. The field concerned with the development of new drugs is called medicinal chemistry. The most common practice in medicinal chemistry is to search through fast libraries of chemical compounds to identify one or more compounds which have affinity (bind to) and/or activity (stimulate) a certain receptor or other protein. The compounds are then chemically modified and the effects of these modifications are used to suggest further modifications. This cycle is repeated until, ideally, a ligand emerges which has the desired effect on the receptor and is specific enough not to affect the function of other proteins, which would lead to side effects. The usual metaphor is that of a lock (the receptor) and a key (the ligand). Starting with a passkey or a set of passkeys, one tries to deduce the elements of the keys responsible for opening the lock desired. If the lock is considered at all, then only to see how the key fits in the lock as it is (in the locked position). In other words, the target protein is usually regarded a more or less rigid structure used to see whether a certain ligand could fit, disregarding the effect the ligand should invoke. Ideally, it should be enough to have a lock to derive the requirements for a good key. If disassembled the lock reveals its mechanisms, the gears and clicks. From these it can be deduced what the mechanism of the lock is and thus what properties a key should have to open it. In terms of proteins and drugs, reality is more complex. But also in that case, knowing the mechanisms underlying receptor action will prove invaluable for the design of drugs, speeding up the process of development, increasing the selectivity and reducing the costs involved.

The work described in this thesis is a step towards protein based drug design, in which the mechanisms of activation, the dynamics of the target protein, is taken into account. In particular the framework of statistical analysis for molecular dynamics provides a number of tools which will allow narrowing the gap between conventional methods for rational drug design and the dynamical features of the target proteins.

3. Summary
3.1 Chapter 2: Data Processing and Analysis of Results using Statistical Methods

In this chapter a framework is presented for statistical methods to be applied to data obtained from molecular dynamics simulations. Some key elements and mathematical backgrounds of statistical theory are reviewed. Several tests and methods are discussed in relation to molecular dynamics. These methods are mainly concerned with two aspects. The first aspect is the comparison of simulation results obtained from different simulations. The second aspect is the use of methods based on principal component analysis for the investigation of mechanical properties of proteins, with emphasis on mechanical interactions between subunits in multimeric systems. In this context, several new methods are presented, based on principal component analysis, but intended for the study of interacting systems. In addition to the two main topics, a few words are spent on data reduction in molecular dynamics. In particular, a method is presented to reduce a structure to a limited set of principal coordinates, describing the relative orientations of domains.

3.2 Chapter 3: The Near-Densest Lattice Packing

Molecular dynamics simulations are computationally demanding, especially when used to investigate large systems, such as the cytokine – receptor complexes studied here. However, the solute usually accounts for only ~10% of the number of atoms in the simulations system, the rest being occupied by solvent. In this chapter a method is presented to reduce the amount of solvent in a simulation system. This is done by calculating the near-densest lattice packing (NDLP) of the solute, given a minimal distance between the neighbouring copies. It is shown that this leads to a reduction of the volume of the simulation system of ~55% on average, compared to that of a rectangular box, and results in an increase in the simulation speed of approximately 120%.

3.3 Chapter 4: Comparison of the Effect of an External Condition on the Simulation Outcomes

The comparison of simulations is non-trivial because of the chaotic nature of the processes and the complex data sets. At the start of this work there were no robust methods available for such comparisons. Two methods were developed, based on statistical tests for the comparison of multiple sets of data, notable ANOVA and MANOVA. The first method is based on comparing the sampling of the conformational space, whereas the second method uses a number of properties derived from the trajectories and averaged over a given time window. These methods were initially derived and applied for the assessment of the influence of the type of simulation cell on the dynamics observed. It was found that the box type used can have a small, but statistically significant effect. Besides, a rectangular box type and a truncated octahedron were found to be more prone to yield results different from that obtained in other box types than the rhombic dodecahedron or the NDLP box. A second comparison of simulations was performed to assess the effect of changes in the force field parameters on the simulation outcome. A modification of the statistical model was made to allow comparison across many different proteins. It was found that the older force field set (GROMOS96 43a1) was different from both newer sets (GROMOS96 53a5 and 53a6), whereas the two newer sets did not yield statistically significant differences on the time scales used. The differences observed
could be traced back to the presence of charged residues in the proteins, using multiple regression analysis.

3.4 Chapter 5: Characterization of Interactions between Death Receptor 5 and its Ligand TRAIL

The Tumour Necrosis Factor (TNF) – Related Apoptosis – Inducing Ligand (TRAIL) and its receptors have received much attention during the past decade for their involvement in the selective eradication of cancer cells by activation of the cell suicide (apoptosis) machinery. In this chapter, an account is given of a series of simulations performed to gain insight in the mechanisms involved in TRAIL mediated activation of the Death Receptor 5, one of four cell-surface receptors for TRAIL. The results show that the receptor is likely self-associate in the absence of TRAIL, suggesting a rearrangement of receptor subunits or a conformational change during activation. It is also shown that binding of TRAIL leads to a dramatic decrease in the motility of the receptor subunits, suggesting freezing as a factor in the activation. In addition, a number of simulations were performed to assess the propensity of both the TRAIL – Death Receptor 5 and the related TNFb - TNF Receptor 1 to form a higher order network. It was found that both systems formed networks in the simulations. The setup of the simulations likely biased the outcomes, but interestingly the networks obtained were different. While the TRAIL – Death Receptor 5 complex formed a densely packed network, the TNFb - TNF Receptor 1 complex formed a looser, open arrangement.

3.5 Chapter 6: Determinants of Activity in the Erythropoietin Receptor Studied with Molecular Dynamics Simulations

The Erythropoietin (EPO) Receptor (EPOR) is involved in the production of red blood cells and is an important target for anti-anaemic therapy, although EPO is probably best known for its misuse as a performance enhancing substance. The aim of the work laid down in this chapter was to gain understanding in the mechanism of activation of the receptor and in the difference between binding of EPO and binding of a series of agonistic and antagonistic ligands, known as EPO Mimetic Peptides (EMPs). A particular question concerned the difference between binding of an agonistic peptide (EMP1) and an antagonistic one (EMP33). The crystal structures of the EPOR in complex with each of these peptides have been made available, but these can not explain the difference in activity. The molecular dynamics simulations of the EPOR in complex with EPO, in complex with each of a series of EMPs with different activities and as an unbound receptor showed several things. First, EPO imposes a distinct orientation of the membrane bound domains, but the orientation obtained in the simulations is different from that observed in the crystal structures. The orientation obtained from the unbound receptor dimer is completely different from the EPO bound orientation. Binding of EMP33 results in an orientation which is distinct from the orientation obtained with EMP1 or any other more or less active EMP, providing an explanation for the difference in activity based on the conformation of the EPOR. Finally, binding of EMP1 or other agonistic EMPs yields orientations of the membrane bound domains which are different from the orientation obtained with EPO, providing an explanation for the orders of magnitude difference in activity.
3.6 Chapter 7: Calcium binding to the Purple Membrane

Bacteriorhodopsin is a light-sensitive receptor found in certain species of archaea (proto-bacteria). The aim of the work laid down in this chapter was to identify possible binding sites for calcium inside or around bacteriorhodopsin and to investigate the stability of a number of previously identified putative binding sites. For this project a model of bacteriorhodopsin was set up, using the available information concerning the natural matrix of bacteriorhodopsin, the purple membrane. Four simulations were performed with calcium ions placed at a number of previously suggested binding sites and three simulations in which calcium ions were placed in the extracellular solvent, using different concentrations. The simulations showed that two of the putative binding sites, in the core of bacteriorhodopsin (the Schiff base region) and at a more external location (the proton release group) could accommodate a calcium ion without leading to serious distortions of the structure of bacteriorhodopsin. However, the simulations suggested that these two sites could not be occupied simultaneously. The second series of simulations, in which calcium ions were initially distributed in the extracellular solvent, suggested a further two binding sites on the bacteriorhodopsin – lipid interface.