Molecular dynamics of sense and sensibility in processing and analysis of data
Wassenaar, Tsjerk Andrys

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Chapter 5

Characterization of Interactions between Death Receptor 5 and its Ligand TRAIL
1 Introduction

1.1 Tumour Necrosis Factor

In the late 19th century, it was observed that patients recovering from bacterial infections or intentionally injected with mixtures of bacterial toxins showed spontaneous regression of certain tumours[1, 2]. Later, it was shown that bacterial endotoxins can induce hemorrhagic necrosis of certain tumours transplanted in guinea pigs[3] and mice[4]. These effects were at the time thought to result from endotoxin-induced hypotension leading to a decrease in the blood-flow to the tumour, and subsequent ischemia[5].

The existence of a direct mechanism for the induction of apoptosis or programmed cell death in cells first became apparent in 1975 when the Tumour Necrosis Factor (TNF) was extracted by Carswell and colleagues from the serum of mice infected with Bacillus Calmette-Guerin – a gram-negative bacterium[6]. They found that the isolated compound was sufficient to induce hemorrhagic necrosis in tumours in the absence of bacterial endotoxins. They hypothesized that the endotoxins would induce the release of TNF from activated macrophages.

TNF was the first member of a large family of proteins to be discovered, the TNF superfamily. Many of these proteins play important roles in maintaining homeostasis. For a review on the TNF superfamily, see reference [7]. Many members of this family are involved in programmed cell death or sustaining cell life. The members of the TNF superfamily belong to a larger class of proteins called cytokines, which are involved in a manifold of regulatory mechanisms, notably in the immune system.

The involvement of TNF in hemorrhagic necrosis and the physiological importance of the members of the TNF superfamily in general, have led to extensive research, aimed at understanding the functions and how their properties could be exploited for therapeutic intervention. In particular, TNF and several other members of the family have been, and still are, the subject of intense study as anti-tumour therapies.

Ten years after the discovery of TNF, it was shown that its action was the result of binding to membrane bound receptors, of which two different types exist[8]. As was the case for TNF, the receptors were found to be members of a larger family, the TNF-Receptor (TNFR) superfamily. Two features shared by both the TNF and TNFR superfamily are conserved structure and a high degree of promiscuity. Many of the proteins from the TNF superfamily bind to a number of different members of the TNFR superfamily and some members of the latter bind to a number of different ligands from the TNF superfamily.
Figure 5.1: Structural features of the TRAIL – DR5 and the TNFβ – TNF-R1 complex. A. The TRAIL – DR5 complex (PDB ID 1D4V[44]). Structural elements of TRAIL are coloured yellow (β-sheet) and red (α-helix). The AA”-loop is highlighted in magenta. Structural elements of the receptor subunits (β-sheet) are coloured blue. Cystine bridges are shown as sticks. B. Top view of the TRAIL – DR5 complex (i.e. looking down to the cell surface), highlighting the trimeric structure. Colouring is as outlined above. C. The zinc-finger domain. View inside the TRAIL trimer. The zinc finger domain is shown as spheres. The structure of the domain was modelled from the structure from Hymowitz et al. (PDB ID 1D0G)[43]. Note that Mongkolsapaya et al.[44] did not resolve a zinc ion, whereas Cha et al. resolved a zinc-finger domain, but not the ligating chloride ion[45]. D. Modularity in the Death Receptor 5 (shown on the left) and in the TNF Receptor 1 (p55; shown on the right). Modules are indicated by colours. The DR5 N1 domain is shown in green. A1 domains are shown in orange/red/magenta and B2 domains are shown in different shades of blue. E. The structure of the TNFβ – TNF-R1 complex (from PDB ID 1TNR[76]). The colours are chosen consistent with the colours used in the TRAIL – DR5 complex. F. Top view of the TNFβ – TNF-R1 complex. The image shows the trimeric structure of the complex, as well as the absence of a long extended loop, such as the AA”-loop in 1KAIL.
1.2 TRAIL: Tumour - necrosis – factor (TNF) Related Apoptosis - Inducing Ligand

With regard to the possible therapeutic use of TNF, the enthusiasm was dampened when it was found that the systemic levels of TNF needed for a tumoricidal effect lead to severe apoptosis in healthy cells[9]. Another promising member of the family, CD95L or FasL, suffered from the same problem. The hearts and hopes of some were raised again, when in 1995 a new member of the TNF family was identified, the TNF-Related Apoptosis-Inducing Ligand, or TRAIL[10, 11]. This protein was found to induce apoptosis selectively in a number of carcinogenic cell lines[10-13], whereas healthy cells remained largely unaffected[14]. Later, TRAIL was also shown to induce apoptosis in HIV infected T-cells[15].

TRAIL is expressed on the surface of activated T-cells as a homotrimeric type II protein, i.e. bound to the cell membrane through the N-terminal. Each subunit consists of 281 amino acids and has a mass of 32.5 kDa. It can be cleaved by proteases into a soluble form with a molecular weight of around 24 kDa[16, 17]. The structure of TRAIL is shown in Figure 5.1, where some characteristic features are highlighted. The topology of TRAIL is characterized by a β-jellyroll structure[18]. The three subunits are covalently bound through a zinc-finger domain in the core of the protein, formed by three adjacent cysteine residues. It has been demonstrated that the presence of a zinc ion in TRAIL is necessary for the stability and activity of the protein[19]. TRAIL is structurally distinct from the majority of the TNF superfamily members due to the presence of a large extended loop on the side of each subunit, the AA’’-loop.

1.3 TRAIL receptors

TRAIL appeared to be among the most promiscuous members of the TNF superfamily, having a total of five associated receptors, four of which exclusively bind TRAIL. Of these receptors, two have direct access to the cells apoptotic machinery through a so-called death-domain. These are the Death Receptor 4 (DR4)[20] and the Death Receptor 5 (DR5, also known as Apo2, TRAIL-R2, TRICK-2 and KILLER)[21-28]. The other three receptors lack a functional death domain and are thought to be decoy receptors regulating TRAIL mediated apoptosis, by sequestering TRAIL and thus preventing it from activating the death receptors. Two of the decoy receptors are membrane bound, namely Decoy Receptor 1 (DcR1, also known as TRAIL-R3 or TRID)[21, 24, 26, 29] and Decoy Receptor 2 (DcR2, also known as TRAIL-R4 or TRUNDD)[30-32]. The first of these decoy receptors completely lacks an intracellular domain and is tethered to the membrane through binding to a GPI anchor. DcR2 contains a truncated death domain, incapable of mediating apoptosis, but is reported to be capable of activating NF-κB[30, 33], thus promoting cell survival. These membrane-bound decoy receptors were found to be present on normal cells, while their expression is reduced in certain malignant cell types, accounting for a possible mechanism of selective targeting of tumour cells by TRAIL. The third decoy receptor is the soluble member of the TNFR superfamily osteoprotegerin (OPG), which is involved in osteoclastogenesis (bone production)[34, 35].

The TRAIL receptors, like the other members of the superfamily, are typically trimeric type I membrane proteins, with the exception of OPG. The activation of these receptors was previously believed to be triggered by the attraction and interfacing of three receptor subunits mediated by
interaction with the trimeric ligand. This was assumed to lead to the alignment and subsequent interaction of the intracellular domains, which in turn gives rise to the formation of the intracellular signalling complex by recruitment of downstream effector proteins. In the case of the death receptors, the signalling complex is termed the Death Inducing Signalling Complex or DISC, the formation of which leads to activation of caspases (notably caspase 8), leading to apoptosis[36, 37]. The pathway of TRAIL mediated apoptosis is shown in Figure 5.2.

Recent evidence has shown that the TRAIL receptors have a propensity to form homo- and heterodimers in the absence of TRAIL[38]. Similar results have been obtained for the TNF receptors I (p55) and II (p75)[39], Fas[40, 41] and CD40[39]. These findings have led to the suggestion that mere cross-linking of receptor subunits is not enough for activation. Rather, the ligand either induces a specific conformational change or reorientation of the pre-associated receptor, or the pre-associated receptors form a higher order network upon binding by TRAIL, as was proposed for the TNF – TNFR complex by Naismith et al[42].

Figure 5.2: Signalling pathways in TRAIL mediated apoptosis. Binding of TRAIL to Death Receptor 4 or 5 leads to recruitment and activation of FADD and subsequently of procaspase 8 and 10 into the Death Inducing Signalling Complex (DISC; pathway on the left). Activated procaspase 8/10 undergoes auto cleavage, followed by dimerization. In so-called type I cells, activated caspase 8 is sufficient for triggering apoptosis, whereas in type II cells, the intrinsic apoptotic pathway, involving release of cytochrome C from the mitochondria, has to be activated first, by cleavage of another protein, called Bid. Caspase 8/10 then activates downstream effectors of apoptosis, notably caspases 9 and 3. Negative modulation of the caspase cascade involves cFLIP and IAP, whereas release of Smac/Diablo has a negative regulatory effect on IAP, thus promoting the cascade. As shown on the right side, activation of Death Receptors 4 and 5 by TRAIL also leads to activation of TRADD and subsequently of TRAF2. This protein activates two pathways, leading to activation of NF-κB and of JNK, promoting life. The overall effect of TRAIL binding is the result of a complex balancing between the different signals.

1.4 The structures of TRAIL and the Death Receptor 5

The structures of TRAIL[18] and the TRAIL-DR5 complex[43-45] have been solved by X-ray crystallography. The structures of the DR5 and the TRAIL – DR5 complex are also shown in Figure 5.1, in which some of their most characteristic features have been highlighted. The three available structures of the TRAIL – DR5 complex are in close agreement with regards to the orientation of the receptor and its interactions with TRAIL. However, the structure solved by Cha et al.[45]
suggests a tighter interaction between the receptor and the AA’-loop of TRAIL than the structures of Hymowitz et al.[43] and Mongkolsapaya et al.[44]. The C-termini of the receptor, which are attached to the cell surface, are separated by 5.4 nm in all three structures. It is interesting to note that a similar distance of 5.2 nm separates the binding sites on TRAF2 (TNF Receptor Associated Factor)[46], which is a trimeric adaptor protein involved in the initiation of signalling in certain members of the TNF-R superfamily, albeit not for DR4 and DR5. The death receptors DR4 and DR5 have a high degree of homology. Their extracellular domains are characterized by the presence of two cysteine rich domains (CRD), which have a characteristic cysteine knot topology. Each of the cysteine rich domains consists of two distinct modules, termed A1 and B2, according to the nomenclature introduced by Naismith et al.[47]. In addition, the N-terminal domain consists of a small, truncated domain, termed N1, which is characteristic of the TRAIL receptors[48]. In Figure 5.1 the different modules have been indicated with different colours. Due to the presence of many cysteine bridges, the structures are very rigid and conserved throughout the receptor family. As an example, in Figure 5.1 an overlay of the TNF-R1 with the TRAIL-bound DR5 is shown. Apparently, the high selectivity for specific ligands is the result of differences in the contact residues and the number of cysteine-rich domains, rather than in the overall structure.

1.5 Reverse signalling

Several groups have demonstrated that receptor binding by members from the TNF superfamily not only mediates a response in the receiving cell, but also gives rise to a signal in the cell hosting the ligand. These members include mTNF (membrane bound TNF), CD40L, FasL, OX40L, CD30L, LIGHT, TRANCE, CD27L (CD70), CD137L and TRAIL[49]. In particular, the cross-linking of TRAIL by plate-bound DR4-Fc fusion protein led to enhanced proliferation of the host T-cell and increased the production of Interferon γ (IFN-γ) under certain conditions[49]. In addition, Herr et al. demonstrated that the binding of TRAIL to its target receptors leads to increased expression of TRAIL, thus causing auto-amplification of the apoptotic signal in cell-to-cell contact[50]. Reverse signalling in the TNF superfamily was recently reviewed by Eissner et al.[51].

1.6 Aim of the study

In this chapter, the results of several projects concerning TRAIL and the Death Receptor 5 are presented. The general aim was to understand and characterize TRAIL mediated DR5 activation through a series of molecular dynamics simulations. To this end, simulations were performed on the TRAIL – DR5 complex, the unliganded DR5 trimer and the unliganded DR5 monomer. In particular, changes in the structure and the motility of DR5 induced by the interaction with TRAIL were investigated.

In addition, several simulations were performed to investigate the propensity of TRAIL – DR5 as well as TNFβ – TNF-R1 complexes to form higher order complexes (networks) and make some inferences on the nature of these networks. For this part of the study, the system constructed such that two receptor ligand complexes would form a regular, loose arrangement under periodic boundary conditions.
Figure 5.3: DR5 motility and decomposition of the mean square deviation (MSD). A. Distribution plots showing the contributions of rigid body mean square deviations (dotted lines) and residual fluctuations (dashed lines) to the total mean square deviation (solid lines). The colours indicate the different simulations. The results for the unbound receptor monomer are shown in green, results for the unliganded receptor trimers are shown in blue and results obtained from the TRAIL – DR5 complex are shown in orange. B. Stereo view giving an impression of the range of conformations sampled in the simulations of the free receptor monomer. All structures were aligned by performing a least squares fit on the N-terminal (CRD1) domain. C. Stereo view giving an impression of the range of conformations sampled in the simulations of the unliganded receptor trimer. All structures were aligned by performing a least squares fit on the N-terminal (CRD1) domain. D. Stereo view giving an impression of the range of conformations sampled in the simulations of the Death Receptor 5 in complex with TRAIL. All structures were aligned by performing a least squares fit on the N-terminal (CRD1) domain.

Figure 5.4: Changes in collective motions in DR5 due to self-association and TRAIL-binding. A. Projections of frames onto the first two eigenvectors obtained from principal component analysis. Projections of the simulations of the unliganded receptor monomer are shown in green, projections of the simulations of the unliganded receptor trimer are shown in blue and projections of the simulation of DR5 in complex with TRAIL are shown in orange. Prior to principal component analysis all frames were aligned by performing a least squares fit using the N-terminal domain (CRD1) as a reference. The projections shown in green can be regarded reference points. The projections of the unliganded receptor trimer show more sampling in extreme regions, whereas sampling is restricted if the receptor is bound to TRAIL. B. Boxplots and density estimates of sampling along the first eigenvector. Colours are as defined above. The insets illustrate the loadings of atoms with the first principal component obtained from the simulations of 1. the unliganded receptor monomer, 2. the unliganded receptor trimer and 3. the DR5 in complex with TRAIL. C. Boxplots and density estimates of sampling along the second eigenvector. Colours are as defined above. The insets illustrate the loadings of atoms with the second principal component obtained from the simulations of 1. the unliganded receptor monomer, 2. the unliganded receptor trimer and 3. the DR5 in complex with TRAIL.
Table 5.1: List of simulations

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<td>Unliganded receptor trimer</td>
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<td>Double TRAIL complex</td>
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Table 5.2: Eigenvalues and their cumulative sums for the first twelve eigenvectors. Eigenvectors and values were obtained per set by decomposition of the covariance matrices of concatenated trajectories of the monomers in solution, the receptor subunits in the simulations of the free receptor trimer and the receptor subunits in the simulation of the TRAIL-DR5 complex, respectively. Note that in each case the eigenvectors are arranged in order according to magnitude. A given index does not necessarily correspond to the same eigenvector in the different systems.

<table>
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2 Results

The simulations performed for this Chapter are summarized in Table 5.1. The numbers in the table refer to the different simulation systems described in the methodology section. In total, 19 simulations were performed with a total simulated time of 400 ns.

2.1 Characterization of the extracellular binding domain of Death Receptor 5

The results for this section are presented in Table 5.2 and Figures 5.3 - 5.7. Table 5.2 summarizes the results of principal component analysis performed on the Death Receptor in complex with TRAIL (system 1), as part of the unliganded receptor trimer (system 2) and as a free monomer in solution (system 3). In this table the eigenvalues and the cumulative sums of the eigenvalues corresponding to the first twelve eigenvectors are given. Figure 5.3 shows the results of the decomposition of the total mean square displacement (MSD) of the Death Receptor 5 observed in the simulation into rigid
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body motions and residual contributions, according to the method introduced in Chapter 2.8. In addition, the insets in this figure give an indication of the range of conformations sampled during the simulations. Figure 5.4 shows the results of more detailed investigation of motions along the first two eigenvectors obtained with principal component analysis. In Figure 5.5 a series of images is shown, summarizing the sequence of events in the simulations of the unliganded receptor trimer. The interfaces formed in these simulations are shown in more detail in Figure 5.6, which gives the contributions of individual residues. In addition, this figure shows the contributions of individual residues of the receptor subunits to the formation of interfaces with TRAIL and between adjacent TRAIL-DR5 complexes in a simulation allowing the formation of a higher order complex (network). The changes in the TRAIL-DR5 complex in a simulation starting from the crystal structure are illustrated in Figure 5.7, which shows the RMSD and a stereo image of the initial and final structures obtained.

**Figure 5.5: Self-association and C-terminal positions of Death Receptor 5 during the first 5 ns of simulations.** The three panels show stereo views summarizing the events in the first 5 nanoseconds of simulation of the three simulations of the unliganded receptor trimer. The snapshots are taken with intervals of 1 ns. All three figures show a rapid collapse of the receptor trimer to a trimeric aggregate.
In figures where simulations of the free receptor monomer, the unliganded receptor trimer and the Death Receptor 5 in complex with TRAIL are compared, the results are coloured consistently with green, blue and orange, respectively. In other figures these colours are used to indicate the different subunits in the trimeric systems of the Death Receptor 5 or TRAIL.

### 2.1.1 The receptor monomer unbound

Simulations of the unbound receptor monomer (extracellular ligand binding domain) were performed to estimate the conformational space accessible in the absence of ligand or interacting receptor subunits. Additionally, these simulations were used to gain insight into the mechanics of the receptor subunits. In the following sections, these simulations are used as a frame of reference. In total, ten simulations of the unbound receptor monomer in solution were performed, each 20 ns in length, giving a total of 200 ns. The decomposition of the MSD, given in Figure 5.3A, shows that the greater part of the motility of the Death Receptor 5 is explained by rigid body motions (dotted line). The contribution of local or residual fluctuations (dashed line) is small. The most important contributions come from the hinge-bending motions of CRD1 relative to CRD2, around the hinge point formed by Gln138.

Principal component analysis revealed that the first two components capture most of the total fluctuation (81%). These components correspond to the hinge bending motions. The third component is complementary to the first two and describes the deviation from linearity associated with these hinge-bending motions. The fourth component captures a twisting motion of CRD2 relative to CRD1, while the fifth and higher order components describe more local fluctuations. The projections of the trajectories onto the first two eigenvectors are given in Figure 5.4. These projections reveal an almost circular distribution, without showing clear regions of higher density.

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**Figure 5.6: Contributions of Death Receptor 5 to interface surface areas.** A, B and C. Contributions of residues of the Death Receptor 5 to interfaces between receptor subunits in the simulations of the unliganded receptor trimers. Contributions are averaged over the last nanosecond of simulation. The different colours used in a panel indicate the contributions of the different receptor subunits. D. Contributions of residues to interfaces between Death Receptor 5 and TRAIL. Contributions are averaged over the last nanosecond of simulation. The different colours indicate the contributions of the different receptor subunits. E and F. Contributions of residues of Death Receptor 5 to receptor – receptor interfaces between TRAIL – DR5 complexes forming a network in simulation. Each panel shows the contributions to the interfaces of one of the complexes. Different colours are used to indicate the different receptor subunits within a TRAIL – DR5 complex.
The principal component analysis was complemented with DynDom analysis[52], which allows the investigation of domain rotations and hinge axes, when two different structures of a molecule are given. Applied to the extreme projections of all trajectories of the free receptor monomer in solution onto the first two eigenvectors, this revealed that the total rotation around the hinge point was 49 and 39 degrees respectively. The twisting motion described by the fourth principal component yielded a total rotation of 81 degrees.

2.1.2 The unliganded receptor trimer

Simulations of the unliganded receptor trimer, starting from the configuration as given in the crystal structure (1D0G) from which TRAIL was removed, were performed to get an impression of the behaviour of the receptor trimer in the absence of TRAIL. The main purpose was to gain insight in the state of the unliganded receptor trimer, regarding both the conformation and the dynamics. Three simulations of 20 ns were performed on the unliganded receptor trimer. All three simulations showed a similar sequence of events in the first stages of the simulations, but established different conformations at the end of the simulations. For each simulation, snapshots from the first five nanoseconds of the trajectory and the final structure obtained are given in Figure 5.5. From this figure it can be seen that the receptor subunits swiftly aggregate, apparently forming a trimeric complex. In the simulations, two of the receptor subunits rapidly interact with each other. The third subunit follows slightly later. The final distances between the Val179 Cα atoms, taken as indicators of the C-terminal positions, vary and suggest the formation of an asymmetric complex. The final complex obtained in each of the simulations was not well ordered.

The burial of solvent exposed surface is often correlated with the free energy of association[53-56]. For that reason the changes in the exposed surface were investigated. The aggregation was accompanied by a loss of solvent accessible surface. The size of the area buried by the interactions ranged from 2.5 to 14.4 nm², with a ratio of hydrophobic to hydrophilic surface area buried between 1.2 and 1.5. Figures 5.6A-C show the contributions of residues to interfaces formed in the simulations of the unliganded receptor trimers. Different colours are used to identify the different interfaces. From these figures it can be seen that the residues involved in the interactions between DR5 subunits in the different simulations of the unliganded receptor trimer are spread over the sequence of the subunits. However, one stretch of residues, from 80 to 95, is consistently observed to be involved in the interactions for two of the subunits in each simulation. This region is indicated by the shaded area in the figure.

To investigate the possible changes in the motility of the receptor, the total mean square deviation was decomposed into contributions due to rigid body motions and due to residual fluctuations. This decomposition is shown in blue in Figure 5.3A. From this figure it is clear that the contribution of rigid body motions (dotted line) is decreased with respect to the free receptor monomer in solution, although an additional peak is observed at high values, corresponding to the formation of bent conformations. In contrast, the distribution of the residual MSD (dashed line) has broadened and the mean of the distribution has shifted to higher values.

Following the decomposition of the mean square deviation, the results from principal component analysis were compared to the results obtained for the free receptor monomer in solution. In Figure 5.4, the projections of the receptor conformations of the unliganded receptor trimer on the first two components obtained from the simulations of the free receptor monomer are shown in blue. From Figure 5.4A, which shows the projections on the plane spanned by these components, it can be seen that the sampling is concentrated in the central region as well as on the edges. The sampling of more
extreme regions is also reflected in the larger eigenvalues obtained for the first two components (see Table 5.2) and in the visualizations of the loadings (insets in blue in Figures 5.4B and 5.4C). Again, the principal component analysis was complemented with DynDom analysis using the extreme projections of the trajectories on selected eigenvectors. This revealed that with respect to the first two components the total angle between the extremes was 55 and 44 degrees, respectively. The total rotation between the extreme projections associated with the twisting motion described by the fourth component was found to be 91 degrees.

2.1.3 The TRAIL – DR5 complex

To assess the influence of TRAIL on the state of the DR5 trimer, one simulation of 35 ns was performed on the TRAIL – DR5 complex, starting from the crystal structure. The time evolution of the system during the simulation is illustrated in Figure 5.7, which shows the root mean square deviation (RMSD) with respect to the starting structure (solid line) and with respect to the time averaged structure obtained from the last nanosecond of the simulation (34 to 35 ns, dashed line). Also shown in this figure is a cross-eyed stereo view of the starting structure and the final structure obtained from the simulation (Figure 5.7B), focusing on the C-termini of the receptor. The RMSD with respect to the starting structure is levels off after 4 ns to a value of approximately 0.4 nm. In contrast, the RMSD with respect to the final conformation reveals a steady, almost linear decrease from 0.4 nm at the start to 0.08 nm at the end of the simulation. The changes in the complex are dominated by a rearrangement of the loops of TRAIL and a change in the orientation of the C-terminal domains of the receptor subunits, which fold around TRAIL. This can also be seen from the initial and final structures shown in Figure 5.7B. These images show that, during the simulations, the C-terminal domains move closer together, and pack more tightly. This change is accompanied by a decrease in the distances between the C-termini, measured between the Val179 Cα atoms, from 5.4 nm in the crystal structure to 2.0 nm at the end of the simulation. Note that, in contrast to the simulations of the unliganded receptor trimers, the threefold symmetry between the C-terminal domains is retained.

The changes in the TRAIL – DR5 complex result in a total decrease of the solvent accessible surface area of 26.4 nm². This change is mainly due to rearrangements in the loop regions of TRAIL. The contributions of the residues of the receptor subunits to the TRAIL – DR5 interfaces (Figure 5.6D) show that there is overlap with the region found involved in self-association on CRD2. However, the main contribution of CRD2 to the TRAIL – DR5 interface involves residues which lie more towards the N-terminus (residues 74-80).

The motility of the receptor subunit in the TRAIL – DR5 complex is decreased substantially compared to that observed for the free receptor monomer in solution. This can be seen, for example in Figure 5.3D, which shows a representative sample of structures from the simulation, superimposed using a least-squares fit on the membrane distal domain (CRD1). The decomposition of the MSD, shown in orange lines in Figure 5.3A, reveals that the difference with respect to the free receptor monomer is due to a restriction of rigid body motions, rather than of the residual fluctuations, which show similar distributions. The restriction of the conformational freedom can also be seen in Figure 5.4, where the results of the TRAIL bound receptor are shown in orange. The projections on the first two eigenvectors are localized in comparison with those of the free receptor monomers and the unliganded receptor trimers. Further analysis with DynDom on the extreme projections of the trajectory on the first two components revealed that the total angle between the extremes was 26 and
20 degrees, respectively. The total rotation between the extreme projections associated with the twisting motion described by the fourth component was found to be 43 degrees.

2.2 On the formation of higher order networks

The results for this section are given in Figures 5.8 and 5.9. Figure 5.8 shows the formation of a network of TRAIL-DR5 complexes and the formation of a network of TNFβ-TNFR1 complexes. In Figure 5.9 the formation of interfaces in the simulation of two TRAIL-DR5 complexes is shown. In addition, that figure shows the contributions of individual residues of TRAIL to interfaces in the TRAIL-DR5 complex, between adjacent TRAIL-DR5 complexes and between two TRAIL trimers forming a dimer in simulation.

2.2.1 Network formation by TRAIL – DR5 complexes

It has been suggested that TRAIL mediated DR5 activation involves the formation of a higher order complex or network. To investigate this possibility, a simulation was performed to assess the propensity of TRAIL – DR5 complexes to aggregate and form such a network. Figure 5.8A shows a sequence of stereographic images that summarizes the events in this simulation. In Figure 5.8B a sequence of stereographic images is shown, that summarizes the events in a simulation with a similar setup, but with two TNFβ-TNFR1 complexes. That simulation was performed as a control to get an indication to which degree the simulation setup would bias the results. In the simulation of the TRAIL – DR5 complexes, these form a higher order complex, resulting in the contraction of the system. The distances between the centres of mass are initially around 7.3 nm in all directions. At the end of the simulation these distances

Figure 5.7: Root mean square deviation and conformational change in the TRAIL – DR5 complex. A. Root mean square deviation (RMSD) of the TRAIL – DR5 complex in simulation relative to the crystal structure (solid line) and relative to the average structure obtained from the last nanosecond of simulation. The RMSD from the crystal structure shows a steep increase to a plateau value, whereas the RMSD from the average structure from the end of the simulation shows a steady decrease. B. Stereo view of the TRAIL – DR5 complex as seen from the side of the cell membrane, showing the C-terminal regions of the DR5 (cartoon representation) folded around TRAIL (surface representation). The receptor configuration shown in white is obtained from the end of the simulation. For reference, the receptor configuration as seen in the crystal structure is shown in grey. During the simulation, the C-terminal regions of the Death Receptor 5 fold closer around TRAIL.
have decreased to values between 5.6 – 5.8 nm. The surface spanned by the lattice vectors corresponding to the cell membrane decreases 38%, from 130 nm$^2$ at the start of the simulation to 80 nm$^2$ at the end. Similar interactions are observed at all three independent interfaces. These involve the binding of the AA''-loop of one TRAIL – DR5 complex in the groove formed by the AA''-loop of the opposite TRAIL and the associated receptor subunit. During the simulation a total area of 55 nm$^2$ was desolvated, with the contributions of the three interaction sites equal to 21.4, 13.2 and 20.3 nm$^2$.

**Figure 5.8: Network formation of TRAIL – DR5 and TNF$_\beta$ – TNF-R1 complexes.** Left side: Snapshots (cross eyed stereo) from the first 10 ns of simulation of two TRAIL – DR5 complexes, showing the formation of a higher order network. Snapshots were taken at 0.0, 1.0, 3.0, 6.0 and 10.0 ns. Right side: Snapshots (cross eyed stereo) from the first 10 ns of simulation of two TNF$_\beta$ – TNF-R1 complexes. Snapshots were taken at 0.0, 1.0, 3.0, 6.0 and 10.0 ns.
2.2.2 Network formation by TNF$_\beta$ – TNFR1 complexes

As mentioned, a simulation of two TNF$_\beta$ – TNFR1 complexes was performed, starting from a similar setup as the simulation of two TRAIL – DR5 complexes, to assess the influence of the starting arrangement on the final structure formed. A sequence of images giving a visual summary of the events in this simulation is given in Figure 5.8B. From this figure it is clear that a network is formed again, but in contrast to the compact network observed for the TRAIL – DR5 supercomplex, the one obtained from the simulation of TNF$_\beta$ – TNFR1 is open.

The interactions observed are not perfectly symmetrical. The three interaction sites do not reveal consistent interaction interfaces. However, the interactions appear to involve the membrane distal regions previously identified as the pre-ligand association domain[57]. One interaction appears to involve the membrane proximal domains, causing a tilt of the two independent TNF$_\beta$ – TNFR1 complexes. The surface of the interfaces areas formed by the interactions are significantly smaller than in the case of the TRAIL – DR5 complex, measuring 8.2, 11.9, and 7.9 nm$^2$ for the three interfaces.

2.2.3 The formation of dimers by TRAIL

The simulation of two TRAIL – DR5 complexes showed the formation of a network in which the AA"-loop played an important contribution. This raised the question whether TRAIL, starting from a similar arrangement, would be able to establish a network by itself in simulation. Figure 5.9 shows the sequence of images summarizing the simulation of two TRAIL units, which were allowed to interact over the periodic boundaries. The figure shows that during the simulation an asymmetric dimer was formed. The area of the interfacial surface at the end of the simulation is about 6.5 nm$^2$, which is comparable to the size of the surface between the TRAIL trimers at all three interfaces in the simulation of the TRAIL – DR5 supercomplex.

![Figure 5.9: Dimerization of TRAIL.](image)

Snapshots from the simulation of two TRAIL trimers in a hexagonal prism simulation cell. N-termini were constrained to a plane. Snapshots were taken with an interval of 4 ns. The sequence shows the formation of a dimer of trimers, oriented with a ~90° angle.
3 Discussion

3.1 TRAIL mediated DR5 activation

3.1.1 The Death Receptor monomer free in solution

The results obtained from the simulations of the free receptor monomer in solution suggest that the receptor is primarily a mechanical construct, as illustrated by the decomposition of the mean square deviation. The greater part of the motility is explained by rigid body motions of the CRD1 domain relative to the CRD2 domain. These domains appear to move independently, except for a flexible hinge region connecting them. Note, the presence of a flexion point was already suggested previously by Jones who made a comparison of the ligand bound structures of the Death Receptor 5 and the TNF receptor 1[58]. The specificity for the ligand apparently arises from the topology of the binding surfaces alone. Given the similarities between the different receptors of the TNF receptor superfamily, it is likely that this is a common feature of this family of receptors.

To identify the characteristic motions of the receptor, principal component analysis was performed. The results of this analysis confirmed the importance of hinge-bending motions as well as of twisting motions. The projection of the configurations on the first two eigenvectors showed that the sampling of the conformational subspace defined by these motions was almost complete. This demonstrated both the freedom in inter domain motions and the fact that equilibrium is more easily attained in the case of rigid bodies which are connected by a flexible linker.

3.1.2 Self association of death receptor five in the absence of TRAIL

The simulations performed on the trimeric Death Receptor 5, starting from the configuration as observed in the crystal structures, but with TRAIL removed from the complex, suggest that the receptor has a tendency to self-associate. Besides, these simulations suggest that the process of self-association is mediated by interactions between the membrane proximal domains (CRD2). The aggregation was associated with a loss of both hydrophilic and hydrophobic solvent accessible surface, where the desolvated hydrophobic surface area was consistently larger than the desolvated hydrophilic surface area. Given that the change in free energy due to the desolvation of a hydrophobic surface outweighs the penalty for desolvation of a hydrophilic surface of equal size[53-56], the observed interactions are expected to be energetically favourable.

In each simulation of the unliganded receptor trimer two subunits primarily interact through the region formed by residues 80 to 95. The role of this region in the process of self-association might be verified by mutagenesis studies. Note that the structures obtained at the end of the simulations were unordered and differed between the simulations, indicating that equilibrium was not reached.

The rapid aggregation of the subunits observed in the simulations is in agreement with the recently reported propensity of the Death Receptor 5 to homo-oligomerize[38]. Based on Surface Plasmon Resonance (SPR) studies, Lee et al. suggested the formation of dimers, whereas our results suggest the formation of trimers. This is not necessarily a contradiction. In the simulations, the binding of two subunits is stronger than the binding of a third subunit, suggesting the receptor might preferentially form dimers. Second, SPR might not detect the formation of trimer species at lower concentrations. If the receptors are pre-associated, this implies that activity must depend on the precise orientation or relative position of the extracellular binding domains. The action of TRAIL could involve cross-linking of receptor dimers, according to the previously stated expanding network hypothesis[59, 60], or the
rearrangement of receptor dimers to form trimers. In both cases, the rearrangement of the extracellular domains will lead to a repositioning of the cytosolic domains, in turn causing activation of the associated effector proteins (FADD).

Apart from the rearrangement of the receptor subunits, it is also possible that a change in conformation and/or motility is involved in the activation or maintaining the inactive state of the receptor. To investigate this possibility, principal component analysis was performed on the Death Receptor 5 as a free monomer in solution, as part of an unliganded trimer and in complex with TRAIL. Comparing the results obtained from principal component analysis performed on the receptor subunits in the trimeric aggregate to those obtained from the receptor monomers free in solution (Figure 5.4), several things are worth noting. First, the projections of the trajectories of each subunit on the first two eigenvectors obtained from the free monomer suggest that, in the case of the receptor trimer, there is more sampling in extreme regions, which are not readily accessible to the receptor monomer. This is also reflected by the results obtained from DynDom performed on the most extreme projections on these eigenvectors. In the trimer a larger total angle around the hinge axis is sampled, suggesting that a larger volume of conformational space is accessible. Given that these extreme regions were not or only marginally sampled by the free receptor monomers, it is suggested that in the case of the unliganded receptor trimer the association can effect the bending of the domains and promote the adoption of a more kinked conformation. While caution must always be used when interpreting results from simulations, it is worth noting that Lee et al. have suggested that the self-association could involves a conformational change in the receptor subunits, based on the fact that the on-rate they observed in the SPR experiment was smaller than the off-rate. Such a conformational change could well involve bending of the membrane distal (CRD1) domains, which would be in line with the results obtained here. However, to verify this hypothesis, a much more extensive investigation than performed here would be required.

3.1.3 The TRAIL – DR5 complex in silico

The simulation of TRAIL in conjunction with DR5 demonstrates the overall stability of the receptor – ligand complex. In addition, the simulation shows that there is little or no specific interaction between the AA”-loop and the receptor subunits, in contrast to the model postulated by Cha et al.[45] In fact, attempts to remodel the loop to bring it in agreement with their proposed orientation, in close contact with the receptor, failed and led to disruption of the geometry of the complex (data not shown). In this regard, the simulation provides support for the models proposed by Hymowitz et al.[43] and Mongkolsapaya et al.[44] over the model of Cha et al.

The results obtained in this study also show distinct deviations from all models, most notably in regard to the position of the C-terminal domains of the receptor subunits. Given that these domains are directly linked to the membrane spanning α-helices their precise arrangement is likely to be involved in the activation of the receptor. The crystal structures all show a distance between the C-termini of 5.4 nm. As noted previously this corresponds well with the distance observed between the binding sites in the crystal structure of TRAF2 (TNF Receptor Associated Factor 2)[58]. However, the crystallization process may have given rise to crystal packing forces. The simulation shows that the C-terminal domains of the receptor fold closer around TRAIL, reducing the distances between the C-termini to approximately 2 nm. These changes occur progressively over the length of the simulation, as reflected in the RMSD with respect to the average structure obtained from the last ns, shown in Figure 5.7. This suggests that, in the crystal structure packing effects result in the separation of the C-terminal domains. Note that the relaxation of the receptor – ligand complex in simulation is slow on the timescale of the simulation. It takes about 25 ns before the distances between the
C-termini converge to their final value. Such long relaxation times can have consequences for the interpretation of results obtained from short simulations of large complexes. In particular, the collective motions arising from the relaxation processes can be mistaken for functional modes. Whether full relaxation has been reached can be determined by inspection of the cosine content of the principal components[61].

3.1.4 TRAIL mediated DR5 activation

Taken together, the simulations of the free receptor monomer in solution, the unliganded receptor trimer and the TRAIL-DR5 complex suggest two factors that may contribute to the mechanism of TRAIL mediated DR5 activation. First, that the receptor resides on the cell surface as a preformed complex, most likely a dimer or trimer, with the ligand binding domains exposed. Second, that the binding of TRAIL results in the rearrangement of the receptor subunits, imposing threefold symmetry on the resulting complex. Third, that TRAIL inhibits the motility of the receptor subunits, almost completely restricting inter domain motions. The resulting constrained arrangement of the C-termini will lead to the stabilization of a trimeric state of the intracellular death domains, leading to activation.

3.2 Network formation by the TRAIL-DR5 and TNF-TNFR complexes

As was mentioned in the introduction, it has been proposed that the activation of the DR5 also requires the formation of higher order complexes or networks. It has been demonstrated experimentally that binding of TRAIL to the DR5 is followed by migration of the complex to so-called lipid rafts, leading to a high concentration of receptor – ligand complexes. It was also shown that this process is important for activation of the Death Receptor. In particular, it was recently demonstrated that exposure of colon carcinoma cells to resveratrol (3,5,4’-trihydroxystilbene) causes redistribution of DR4 and DR5 into lipid rafts, leading to sensitization of these cells to TRAIL induced apoptosis[62]. Lipid rafts are believed to be specialized domains in the cell membrane, which are rich in ceramide and cholesterol functioning as compartments for the concentration of receptors and associated proteins. The concentration of TRAIL-DR5 complexes into lipid rafts could stimulate the formation of a higher order network. To investigate the propensity of the TRAIL-DR5 complex to form such networks, a simulation was performed starting from two TRAIL-DR5 complexes which were allowed to interact over the periodic boundaries on all sides, thus mimicking an infinite TRAIL-DR5 lattice.

In the simulation the system swiftly contracts to form a compact network (Figure 5.8). Furthermore, it is seen that the extended AA”-loop of TRAIL binds in the groove formed by the opposite AA”-loop and the associated receptor. This is observed on all three independent interaction surfaces. Note, that the grooves to the left and right of the AA”-loop are not equal and there are two distinct ways in which the association can occur. Both are observed in the simulation. Further analysis shows that the association involves TRAIL – TRAIL, TRAIL – DR5 and DR5 – DR5 interactions between the facing complexes. At two interaction sites these interactions contribute equally. The size of the surface buried by each interaction is about 6.5 nm², yielding a total interface surface of 20 nm². At the third interaction site, the two DR5 subunits in close proximity are not in a
position allowing interaction. The surfaces buried by the other two interactions, TRAIL – TRAIL and TRAIL – DR5, are both about 6.5 nm$^2$, which is equal to the corresponding interactions at the other two sides. The total area of that interface surface was thus 13 nm$^2$.

Although the proposed network is a possible arrangement, it is also clear that the simulation setup may have biased the results. The use of only two independent TRAIL – DR5 complexes in the simulation cell promotes the formation of a network and the initial spatial arrangement may have favoured the interactions observed. This said, the distance of 2 nm between any two units at the start of the simulation, not including the AA”-loop, should have been sufficient to allow reorientation of the complexes in an alternative arrangement, such as a so-called extended network, if that were more favourable. In addition, the fact that similar interactions are observed at all interfaces, suggests a certain degree of specificity. Nevertheless, it will be of interest to investigate whether a similar network is formed when starting from a different initial arrangement or with more independent units in a simulation cell. The computational cost associated with the size of such a system might be decreased by using a coarse-grained protein force field, which should be well applicable to investigate protein – protein interactions involving the interfacing of more or less static surfaces.

As a control for the simulation of the TRAIL – DR5 superstructure, a simulation was started from a similar starting configuration of the TNF$\beta$ – TNF-R1 complex. The results from this simulation show a completely different picture. The resulting configuration is established by interactions between the receptor subunits only. The TNF$\beta$ trimers do not interact either with each other or with receptor units from the neighbouring complex. In fact, the complex obtained seems to correspond well to the ‚extended network hypothesis‘ proposed by Naismith et al.[60]. Interestingly, the results suggest that two distinct interactions could contribute to the formation of the network. These interactions correspond to the interactions observed by Naismith et al. in the parallel form of the crystal structure of the unliganded TNF-R1[60]. The first of these interactions involves the membrane distal domain, which was previously identified as a pre-ligand association domain (PLAD)[39]. The other interaction involves the membrane proximal domain.

Again, it would be of interest to investigate the specificity of the observed interactions by starting from different initial arrangement, possibly using more independent units in a simulation cell.

### 3.3 TRAIL acting as a receptor: reverse signalling

As was mentioned in the introduction, the binding of cell bound TRAIL to the DR5 not only gives rise to a signal in the receiving cell, but also leads to increased activity in the host cell. The mechanism of this so-called reverse signalling is poorly understood. In the case of TRAIL the process of reverse signalling is particularly enigmatic, since TRAIL has only a short intracellular domain with no apparent functionality.

There are several mechanisms possible by which TRAIL could mediate such signals. Signalling could, in principle, be related to the precise orientation of the N-termini of the extracellular domain of TRAIL. However, the overall rigidity of TRAIL observed in all simulations does not show any indication of variability in the positions of the N-termini which could differentiate between activity and inactivity. A more plausible explanation for reverse signalling by TRAIL would be the arrangement of the intracellular domains resulting from the formation of a TRAIL – DR5 network. This would cause the intracellular domains to adopt a specific relative orientation, which could function as a recognition site for intracellular effector molecules. Given the contraction and network formation observed in the simulation of the TRAIL – DR5 superstructure, it is likely that such an arrangement of the intracellular domains would be formed.
To account for an off-state, it would then be necessary that the membrane bound TRAIL does not form such a network by itself. Given the important role of the AA''-loop in the formation of the network, the inability of TRAIL to form a network by itself needed to be verified. To investigate this possibility, a simulation of two TRAIL trimers was performed, starting from the same configuration as for the TRAIL – DR5 supercomplex. To mimic attachment to the host cell surface, the N-termini were constrained to a plane. Interestingly, the simulation shows the formation of a dimer of TRAIL trimers being formed. The formation of this dimer is mediated primarily by interactions between the two AA''-loops, establishing a total interfacial surface area of 6.5 nm$^2$, suggesting that the interaction is energetically favourable. It is interesting that the two TRAIL trimers are found to adopt a 90° relative orientation despite the constraints put on the N-termini. It is however difficult to assess the significance of this based on one simulation. Nonetheless, given the simulation results it seems unlikely that TRAIL forms a network in the absence of DR5.

If the formation of a network structure were required for reverse signalling by TRAIL, the formation of dimers could provide a means to inhibit this process. For this reason it would be interesting to assess the propensity of TRAIL to dimerize in vitro.

4 Conclusions

This chapter contains the results obtained from a series of simulations of the TRAIL – DR5 system as well as from a simulation of the TNF$\beta$ – TNFR1 complex. The aim of these simulations was to gain insight in the mechanisms involved in the activation of the receptor by investigating the differences between the bound and unbound states.

The simulations provide evidence that the Death Receptor five self-associates in the absence of TRAIL through interactions of the membrane-proximal (CRD2) domains. Though the self-association of these receptors has become well established over the past few years, the domains responsible for this process have not yet been experimentally identified. In addition, our results support the suggestion that higher order complexes of TRAIL and the DR5 could form after binding of TRAIL to its receptor. The formation of such networks could account for the almost binary on/off effect that TRAIL exerts when initiating apoptosis. The loss of motility of the Death Receptor after binding to TRAIL could presumably contribute to the formation of a highly ordered intracellular arrangement of the death domains.

The model for the activation of DR5 by TRAIL suggested by the simulations is that the unbound receptor resides at the cell surface as open preformed dimers or possibly trimers, stabilized by interactions between the CRD2 domains. The distance and relative orientation between the C-termini of the extracellular part of the receptor inhibit the formation of an active trimeric cytoplasmic death domain. Binding of TRAIL leads to a repositioning of the receptor subunits, enforcing threefold symmetry, as well as restricting the degrees of freedom of the extracellular domains, stabilizing the active state of the death complex. The TRAIL bound receptor may then further associate to form an active network arrangement. In the case of membrane bound TRAIL, the formation of such a network can also lead to a specific orientation of the intracellular domains of TRAIL, which could mediate reverse signalling.
5 Methodology

5.1 Simulations

5.1.1 General

Unless explicitly stated otherwise, simulations were performed with the Gromacs package for molecular simulations version 3.2.1[63-65] in conjunction with the GROMOS 43a2[66, 67] united atom force field. Water was modelled explicitly using the simple point charge (SPC) model[68]. The protonation state of ionizable groups was chosen to correspond to pH 7.0. Ions (sodium or chloride) were added to neutralize the system and to reach a physiological salt concentration (0.15 M). Non bonded interactions were evaluated using a twin range cut off of 0.9 and 1.4 nm. Interactions within the shorter range cut off were evaluated at every step whereas interactions within the longer range cut off were evaluated every 10 steps. To correct for the neglect of electrostatic interactions beyond the longer range cut off, a Reaction Field (RF) correction[69] was used with $\epsilon_{RF} = 78.0$. In all simulations the system was kept at a constant temperature of 300K by applying a Berendsen thermostat[70]. Protein and solvent molecules were independently coupled to the heat bath with a coupling time of 0.1 ps. The pressure was kept constant by anisotropic weak coupling to a reference pressure of 1 bar using a Berendsen barostat[70] with a coupling time of 1.0 ps and a compressibility of 4.6×10^{-5} bar^{-1}. The time step used for the integration of the equations of motion was 0.002 ps. The bond lengths and angle of the water molecules were constrained using the SETTLE algorithm[71]. Bond lengths within the protein were constrained using the LINCS algorithm[72]. Starting velocities were randomly assigned from a Maxwellian distribution with different random seeds for each of the simulations. For all systems energy minimization in vacuum was performed to remove bad contacts within the protein. After solvation energy minimization was performed again to remove bad contact situation between the solvent and the protein. Prior to the production runs, short (10 ps) were performed with position restraints on all heavy atoms, followed by 10 ps equilibration of unrestrained molecular dynamics. The production runs had a length of 20 ns. The starting structures of the simulation systems are shown in Figure 5.10.

5.1.2 The TRAIL – DR5 complex

There are three structures available of the TRAIL – DR5 complex, 1D4V[44], 1D0G[43] and 1DU3[45], which were solved independently by different groups. The starting structure for the simulations (setup 1) was mainly based on the crystal structure 1D4V. This structure contains a 1:1 complex, which was used to construct the biologically functional 3:3 complex. The reason for using 1D4V was the fact that this structure was the only one in which the AA"-loop of TRAIL was resolved. A zinc and a chloride ion were introduced in the core of TRAIL, ligated by the three cysteine residues according to the configuration found in structure 1D0G. Though not resolved in 1D4V, the presence of a zinc ion has been shown to be vital for the structure and activity of TRAIL. The TRAIL – DR5 complex was placed in an NDLP box, determined according to the method given in Chapter 3. The resulting unit cell had a volume of 630 nm^3. The protein was solvated with ~17k water molecules, bringing the total amount of atoms at ~60k. A rhombic dodecahedron box with the same minimal distance between periodic images would have yielded a volume of 1530 nm^3 and a total of ~150k atoms (Chapter 3).

To describe the interactions between the Zn(II) and the protein, the GROMOS 43a2 force field was extended to include parameters for the so-called zinc-finger domain. These parameters were taken...
Figure 5.10: Simulation systems. A. TRAIL-DR5 complex in an NDLP box. B. Unliganded DR5 trimer in a rectangular box. C. Unliganded DR5 monomer in a rhombic dodecahedron box. D. Two TRAIL – DR5 complexes in a hexagonal prism box. E. Two TNFβ – TNF-R1 complexes in a hexagonal prism box. F. Two TRAIL trimers in a hexagonal prism box.

Figure 5.11: The topology of the zinc-finger domain of TRAIL. Residues are indicated by boxes. Charge groups are indicated by dashed lines. Atom names refer to the names used in Table 5.3. Bonds, angles and dihedrals are listed in Table 5.3.
Interactions between Death Receptor 5 and its Ligand TRAIL from literature[73] and are given in Table 5.3. The simulation model and the zinc-finger domain are shown in Figure 5.10A and Figure 5.11, respectively. Simulations were performed using a modified version of Gromacs, in which the roto-translational constraint algorithm of Amadei et al.[74] was implemented. This routine was necessary to keep periodic images from direct interactions, as described in Chapter 3. This simulation was performed at constant volume (NVT) with the bonds within the protein constrained using the SHAKE[75] algorithm. The production run of the TRAIL-DR5 complex had a length of 35 ns.

5.1.3 The unbound DR5 trimer

The starting structure for simulations of the unbound trimer (setup 2) was taken from structure 1D0G. From this structure, TRAIL was manually removed. Missing residues and atoms in the receptor subunits were modelled according to the configuration in the other subunits where possible. Otherwise, they were modelled according to the configuration in structure 1DU3. The three receptor subunits were solvated in a rectangular box of dimensions 9.6 nm × 9.8 nm × 9.8 nm, giving a unit cell volume of 920 nm³. The model was solvated with ~29k water molecules, bringing the total number of atoms to ~90k. The starting model is shown in Figure 5.10B. In the simulations the C-termini of (the extracellular domains of) the receptor subunits were constrained to the xy-plane to mimic the attachment to the membrane. Three simulations were performed, each 20 ns in length.

5.1.4 Unbound DR5 monomers

Individual receptor subunits (setup 3) were taken from each of the available crystal structures, giving a total of ten starting structures. Note, the ten starting structures correspond to the ten configurations found in the crystal structures: one in the model of Mongkolsapaya et al., three in the model of Hymowitz et al. and six in the model of Cha et al. (assymetric unit). Again, where necessary, missing residues and atoms were modelled according to the configuration as found in one of the other monomers. Each of the monomers was solvated in a rhombic dodecahedron box with a volume of 800 nm³ with ~26k water molecules, giving a total system size of ~80k atoms. The starting model is shown in Figure 5.10C. The free receptor monomers were simulated for 20 ns.

5.1.5 The TRAIL – DR5 supercomplex

The starting configuration for the simulation of a TRAIL – DR5 superstructure (setup 4) was formed from two 3:3 TRAIL-DR5 complexes (setup 1). These were oriented such that the sides were facing each other with an overall distance of 2 nm, with the AA”-loops reaching out to a mutual distance of 0.5 nm. The unit cell was a hexagonal prism, of which the lattice vectors in the plane of the two receptor – ligand complexes were chosen such, that the infinite periodic simulation system would form a regular grid of more or less equally spaced complexes. The third vector was chosen perpendicular to this plane with a length of 9.44 nm, to assure that the distance between periodic images in that direction would be at least 2 nm. The volume of the unit cell was 1260 nm³. The system was solvated with ~33k water molecules, to yield a total number of ~116k atoms. The model is shown in Figure 5.130D. The production run for the simulation of the TRAIL – DR5 supercomplex
had a length of 30 ns.

5.1.6 The TRAIL supercomplex

The starting structure for the simulation was based on setup 4, from which the receptor subunits were removed. The unit cell definition of that system was used for the simulations, such that the starting configuration consisted of a more or less regular arrangement of TRAIL molecules, facing each other with the AA'-loops. The system was solvated with ~36k water molecules, yielding a total system size of ~120k atoms. The model is shown in Figure 5.10E. To mimic attachment to the membrane of the host cell, the N-termini were constrained to the xy-plane. The production run of the system had a length of 20 ns.

5.1.7 The TNFβ – TNFR p55 supercomplex

The simulation system of the TNFβ – TNFR p55 supercomplex was constructed in a manner similar to setup 1. The starting structure for a single receptor – ligand complex was taken from PDB structure 1TNR[76]. Two of these complexes were orientated such that the sides were facing each other with an approximate distance of 2 nm. A hexagonal prism unit cell was built following the procedure used for setup 1. The volume of the unit cell was 1150 nm³ and solvation with ~29k water molecules yielded a total system size of ~105k atoms. The simulation system is shown in Figure 5.10F. The system was used for a production run of 20 ns.

5.2 Analysis

5.2.1 Solvent accessible surface area (SASA) and interface surface area (ISA)

The solvent accessible surface area was determined according to the method of Eisenhaber[77] et al., with a solvent probe radius of 0.14 nm. Atoms were classified as hydrophobic if the absolute charge was less than 0.2e. The size of the interface surface area between two subunits A and B was

\[
ISA_{AB} = SASA_A + SASA_B - SASA_{AB}
\]

(5.1)

determined according to

where \(SASA_{AB}\) is the solvent accessible surface of the complex AB and \(SASA_A\) and \(SASA_B\) are the total surface areas of A and B, respectively, determined as the solvent accessible surface area of the subunit in the given conformation as if it were not in complex.

5.2.2 Rigid body and residual mean square displacement

The total mean square displacement of all atoms obtained after performing a least-squares fit on the reference structure taken from the crystal structure (1D0G) was decomposed into a residual part and a part due to pure rigid body motions of the CRD1 and CRD2 domains, according to the method described in Chapter 2.8.3.
Table 5.3: Force field parameters for the Zinc-finger domain in TRAIL for use with the GROMOS96 43a2 united atom force field

A. Atom types, charges and charge groups

<table>
<thead>
<tr>
<th>Atom</th>
<th>Type</th>
<th>Residue</th>
<th>Charge</th>
<th>Chargegroup</th>
</tr>
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<tbody>
<tr>
<td>Zn</td>
<td>ZN2+</td>
<td>ZFD</td>
<td>0.49</td>
<td>0</td>
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<tr>
<td>Cl</td>
<td>CL-</td>
<td>ZFD</td>
<td>-0.75</td>
<td>0</td>
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<tr>
<td>S1</td>
<td>S</td>
<td>ZFD</td>
<td>-0.58</td>
<td>0</td>
</tr>
<tr>
<td>S2</td>
<td>S</td>
<td>ZFD</td>
<td>-0.58</td>
<td>0</td>
</tr>
<tr>
<td>S3</td>
<td>S</td>
<td>ZFD</td>
<td>-0.58</td>
<td>0</td>
</tr>
<tr>
<td>N</td>
<td>N</td>
<td>CSX</td>
<td>-0.28</td>
<td>1</td>
</tr>
<tr>
<td>H</td>
<td>H</td>
<td>CSX</td>
<td>0.28</td>
<td>1</td>
</tr>
<tr>
<td>CA</td>
<td>CH</td>
<td>CSX</td>
<td>0.00</td>
<td>2</td>
</tr>
<tr>
<td>CB</td>
<td>CH2</td>
<td>CSX</td>
<td>0.00</td>
<td>2</td>
</tr>
<tr>
<td>C</td>
<td>C</td>
<td>CSX</td>
<td>0.38</td>
<td>3</td>
</tr>
<tr>
<td>O</td>
<td>O</td>
<td>CSX</td>
<td>-0.38</td>
<td>3</td>
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</table>

B. Bond parameters

<table>
<thead>
<tr>
<th>Bond</th>
<th>Atoms</th>
<th>(b_0) (nm)</th>
<th>(k_b) (kJ mol(^{-1}) nm(^{-2}))</th>
<th>Type(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Zn – Cl</td>
<td>0.235</td>
<td>2.00e+05</td>
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<tr>
<td>2</td>
<td>Zn – S</td>
<td>0.238</td>
<td>2.00e+05</td>
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<tr>
<td>3</td>
<td>S – CB</td>
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<td>5.62e+06</td>
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<tr>
<td>4</td>
<td>CB – CA</td>
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<td>7.15e+06</td>
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<tr>
<td>5</td>
<td>CA – N</td>
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<td>8.71e+06</td>
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<tr>
<td>6</td>
<td>N – H</td>
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<td>1.87e+07</td>
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<tr>
<td>7</td>
<td>CA – C</td>
<td>0.153</td>
<td>7.15e+06</td>
<td>26</td>
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<tr>
<td>8</td>
<td>C – O</td>
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</table>

C. Angle parameters

<table>
<thead>
<tr>
<th>Angle</th>
<th>Atoms</th>
<th>(\theta) (°)</th>
<th>(k_\theta) (kJ mol(^{-1}) deg(^{-1}))</th>
<th>Type(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Cl–Zn–S</td>
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<tr>
<td>b</td>
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<td>c</td>
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<tr>
<td>d</td>
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<td>e</td>
<td>CB–CA–N</td>
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<td>520</td>
<td>12</td>
</tr>
<tr>
<td>f</td>
<td>CB–CA–C</td>
<td>109.5</td>
<td>520</td>
<td>12</td>
</tr>
<tr>
<td>g</td>
<td>CA–N–H</td>
<td>115.0</td>
<td>460</td>
<td>17</td>
</tr>
<tr>
<td>h</td>
<td>N–CA–C</td>
<td>109.5</td>
<td>520</td>
<td>12</td>
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<tr>
<td>i</td>
<td>CA–C–O</td>
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D. Dihedral parameters

<table>
<thead>
<tr>
<th>Dihedral</th>
<th>Atoms</th>
<th>(\phi) (°)</th>
<th>(k_\phi) (kJ mol(^{-1}) deg(^{-1}))</th>
<th>Mult.</th>
<th>Type(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Cl–Zn–S–CB</td>
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<td>0.00</td>
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<tr>
<td>B</td>
<td>S–Zn–S–CB</td>
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<td>0.00</td>
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<td>C</td>
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<td>D</td>
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<td>5.92</td>
<td>3</td>
<td>17</td>
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</tbody>
</table>

\(^a\) Corresponding atom type definition in GROMOS96 43a2 force field
\(^b\) Corresponding bond type definition in GROMOS96 43a2 force field
\(^c\) Corresponding angle type definition in GROMOS96 43a2 force field
\(^d\) Corresponding dihedral angle type definition in GROMOS96 43a2 force field
\(^e\) No corresponding type definition available
6 References

22. Chaudhary, P.M., *et al.*, *Death receptor 5, a new member of the TNFR family, and DR4 induce FADD-