Chapter 2

MD Startup

This chapter describes several molecular dynamics tricks, which are employed in the later molecular dynamics simulations. These tricks are needed to keep the molecular dynamics simulations reliable, and not too time consuming.

The calcium ion, present near the active site of phospholipase A2, is the source of a strong polarizing field, which enhances the interactions with its protein ligands. This increased interaction due to polarization by the calcium ion, is not included in conventional molecular dynamics simulations. Because the calcium ion, in these simulations, is located in a stable environment, the nonbonded interaction parameters are changed to incorporate this polarization effect. Simulations show that the changed force field parameters result in a stable calcium environment within the protein, while the conventional simulation parameters cause a severe distortion of the calcium binding loop of the protein.

For the case of substrate binding, the environment of the calcium ion is changing during the binding process, and a dynamic polarization model is needed to take into account the changing interactions. This problem is solved by using atomic polarizability, a method using non-mutual polarization of the ligands of the calcium ion. This method is only used for the ligating oxygen, phosphate and carbon atoms of the phospholipid substrate molecule.

In order to obtain a reliable starting conformation for use in later molecular dynamics simulations, phospholipase A2 has to be specifically oriented with respect to the monolayer for successful binding of the protein. Because the time scale of present molecular dynamics simulations is far too short to scan all degrees of freedom, the nonbonded interaction energy between phospholipase A2 and a DDPC monolayer is calculated as a function of protein orientation, using a rigid protein and monolayer,
and excluding water molecules. The result of these docking simulations is an energy surface that describes the interaction of protein with the monolayer as a function of protein orientation. One of the three minima of this energy surface is much wider than the others, and thus more favorable. The protein orientation in this minimum agrees with the expected orientation with the active site cleft facing the membrane surface.
2.1 Interaction of calcium and PLA$_2$

2.1.1 Introduction

Calcium binding by proteins plays several roles in biochemistry, such as in transport carriers in cell membranes, in storage reservoirs or in enzymes.\textsuperscript{56} The function of the calcium ion in PLA$_2$ is a very important one. It serves as a structural,\textsuperscript{10} a catalytic,\textsuperscript{22} and a substrate binding factor.\textsuperscript{57,58} Metal ions like calcium have a coordinating effect on their ligands. Due to the strong electrical field of calcium ions in proteins, they have a stabilizing effect on protein structures.\textsuperscript{59} In native PLA$_2$, the calcium ion is coordinated by five protein ligand oxygen atoms and two water molecules (see figure 2.1). During the binding of a phospholipid substrate molecule, the phosphate group and the carbonyl oxygen of the sn-2 ester bond become a ligand of the calcium ion and replace two water molecules. After a substrate molecule is bound to the active site, the calcium ion polarizes the sn-2 carbonyl oxygen bond, favoring a nucleophilic attack by the active site water molecule.

Due to the strong electrical field of the calcium ion, the ligands of calcium become polarized, and become tightly bound to the calcium ion.\textsuperscript{59} In conventional molecular dynamics simulations polarization is not included. This leads to an underestimation of the interactions of calcium with its protein ligands.\textsuperscript{60}

![Figure 2.1: Schematic presentation of the calcium ion and its ligands as it is found in the X-ray structure of PLA$_2$.](image)

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2.1 Interaction of calcium and PLA$_2$
2.1 Interaction of calcium and PLA$_2$

<table>
<thead>
<tr>
<th>Oxygen Ligand</th>
<th>GROMOS-87$^{66}$</th>
<th>Shiratori$^{65}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$E_{\text{min}}$ ($\text{kcal/mol}$)</td>
<td>$R_{\text{min}}$ (Å)</td>
</tr>
<tr>
<td>Water</td>
<td>-0.137</td>
<td>3.35</td>
</tr>
<tr>
<td>Carboxyl</td>
<td>-0.105</td>
<td>3.46</td>
</tr>
<tr>
<td>Carbonyl</td>
<td>-0.171</td>
<td>3.19</td>
</tr>
</tbody>
</table>

Table 2.1: Nonbonded parameters of standard GROMOS$^{66}$ and the same parameters as calculated by Shiratori.$^{65}$ $R_{\text{min}}$ is the Ca-ligand distance at the energy minimum $E_{\text{min}}$.

The most elegant way to solve this problem is to include polarizability into molecular dynamics force fields.$^{61}$ However since polarizability is not available in present molecular dynamics force fields, other methods have to be used to take into account the altered calcium ligand interactions.$^{62,63}$

Assuming that the environment of the calcium ion is not changing within the time scale of the molecular dynamics simulation, several methods can solve the polarization problem. For example, the calcium ion can be virtually bound to its direct ligands by introducing harmonic potentials.$^{64}$

The solution used here is an application of the method of Shiratori.$^{65}$ Shiratori fitted the nonbonded interaction energy parameters (for the Lennard-Jones and Coulomb interaction) to a calculated energy profile. This energy profile was generated by ab-initio quantum mechanics calculations. In table 2.1 the properties of the interaction energy profile with the modified parameters of Shiratori are compared to those obtained with GROMOS-87$^{66}$ using the SPC$^{67}$ water model. In our simulations we modified the charges on the carbonyl and carboxyl oxygens and the repulsive parameters to the values calculated by Shiratori (See table 2.3). The charge of water oxygen was not changed to his value of -0.870, because that would alter the coulomb interactions of all water oxygen atoms in the simulation. Since a majority of the oxygen atoms is not close to the calcium ion, the charge of the water oxygen atom is kept at its original value of -0.820.

2.1.2 Methods

All simulations and analyses were performed with the GROMACS$^{68}$ software package, and the GROMOS-87$^{69-71}$ force field with modified interaction parameters between carbon and water oxygen (OW). The modified Carbon-OW parameters are given in table 2.2.

The structure of PLA$_2$ was extracted from Protein Databank$^{72}$(pdb) entry 1p2p.$^{10}$
2.1 Interaction of calcium and PLA<sub>2</sub>

<table>
<thead>
<tr>
<th>Interaction</th>
<th>GROMOS-87 Force Field</th>
<th>Modified Carbon - OW parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C12</td>
<td>C6</td>
</tr>
<tr>
<td>CH&lt;sub&gt;3&lt;/sub&gt; - O</td>
<td>4.489107e-06</td>
<td>4.866675e-03</td>
</tr>
<tr>
<td>CH&lt;sub&gt;2&lt;/sub&gt; - O</td>
<td>5.165685e-06</td>
<td>4.902118e-03</td>
</tr>
<tr>
<td></td>
<td>C12</td>
<td>C6</td>
</tr>
<tr>
<td></td>
<td>8.242733e-06</td>
<td>4.804129e-03</td>
</tr>
<tr>
<td></td>
<td>9.558380e-06</td>
<td>4.856962e-03</td>
</tr>
</tbody>
</table>

Table 2.2: C12 and C6 parameters of the Lennard-Jones interaction between carbon and water oxygen. All simulations were performed using the modified parameters. Units: for C12: kJ mol<sup>-1</sup> nm<sup>-<sub>12</sub></sup> for C6: kJ mol<sup>-1</sup> nm<sup>-6</sup>.

The five crystal water molecules from the pdb structure were used for the starting configuration of the MD calculations. After all polar hydrogen (aromatic hydrogen atoms are not included) atoms were added, the protein was centered in a box with dimensions 5.48 × 6.46 × 4.32 nm. The box was filled with 4193 water molecules. In order to compare the effect of the altered nonbonded parameters, two sets of simulation parameters were used. The first set is the ‘Old Parameters set’ (OP) which is the standard forcefield which was described at the beginning of this section. The second parameter set, ‘New Parameters set’ (NP), is the same forcefield, with the nonbonded calcium interactions between calcium and its protein ligands changed to the values calculated by Shiratori.<sup>65</sup> The charges of the oxygen atoms O Tyr28, O Gly30, O Gly32, O<sub>±</sub> Asp49, and O<sub>±</sub> Asp49 (see figure 2.1 on page 24) were changed to the calculated values of Shiratori.<sup>65</sup> The charges of the carbon atoms in the same charge group of the oxygen atoms were also changed to retain the original unit charge of the involved charge group. The modified parameters are listed in table 2.3.

2.1.3 Simulations

Both systems were energy minimized using steepest descents energy minimization, and subsequently submitted for 100 ps of NPT MD. Temperature was kept constant at 300 K with a coupling constant τ<sub>T</sub> of 0.1 ps. Pressure was kept constant at 1.0 × 10<sup>5</sup> Pa with a coupling constant τ<sub>P</sub> of 0.5 ps. A twin-range cut-off was used with a value of 1.0 nm for the Lennard-Jones and Coulomb interactions computed at every step, and a value of 1.8 nm for the Coulomb interactions evaluated every 10 steps. The neighbour list was updated every 10 steps. A summary of the simulation parameters is listed in table 2.4. Each 0.5 ps the conformation of protein and water was stored for analysis. The following software packages, apart from GROMACS, were used for analysis and the creation of images: Molscript,<sup>73</sup> and Xmgr.<sup>74</sup>
2.1 Interaction of calcium and PLAP

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Charge (C)</th>
<th>C12</th>
<th>C6</th>
<th>Charge (C)</th>
<th>C12</th>
<th>C6</th>
</tr>
</thead>
<tbody>
<tr>
<td>125Ca Tyr28 Cα</td>
<td>0.380</td>
<td>0.12962E-05</td>
<td>0.15337E-02</td>
<td>0.764</td>
<td>0.12962E-05</td>
<td>0.15337E-02</td>
</tr>
<tr>
<td>125Ca Tyr28 O</td>
<td>-0.380</td>
<td>7.93900e-07</td>
<td>1.50800e-03</td>
<td>-0.764</td>
<td>7.07562e-07</td>
<td>1.94118e-03</td>
</tr>
<tr>
<td>125Ca Gly30 Cα</td>
<td>0.380</td>
<td>0.12962E-05</td>
<td>0.15337E-02</td>
<td>0.764</td>
<td>0.12962E-05</td>
<td>0.15337E-02</td>
</tr>
<tr>
<td>125Ca Gly30 O</td>
<td>-0.380</td>
<td>7.93900e-07</td>
<td>1.50800e-03</td>
<td>-0.764</td>
<td>7.07562e-07</td>
<td>1.94118e-03</td>
</tr>
<tr>
<td>125Ca Gly32 Cα</td>
<td>0.380</td>
<td>0.12962E-05</td>
<td>0.15337E-02</td>
<td>0.764</td>
<td>0.12962E-05</td>
<td>0.15337E-02</td>
</tr>
<tr>
<td>125Ca Gly32 O</td>
<td>-0.380</td>
<td>7.93900e-07</td>
<td>1.50800e-03</td>
<td>-0.764</td>
<td>7.07562e-07</td>
<td>1.94118e-03</td>
</tr>
<tr>
<td>125Ca Asp49 CGα</td>
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<td>0.12962E-05</td>
<td>0.15337E-02</td>
<td>0.988</td>
<td>0.12962E-05</td>
<td>0.15337E-02</td>
</tr>
<tr>
<td>125Ca Asp49 OD1</td>
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<td>1.29900e-06</td>
<td>1.50800e-03</td>
<td>-0.994</td>
<td>1.43960e-06</td>
<td>3.94952e-03</td>
</tr>
<tr>
<td>125Ca Asp49 OD2</td>
<td>-0.635</td>
<td>1.29900e-06</td>
<td>1.50800e-03</td>
<td>-0.994</td>
<td>1.43960e-06</td>
<td>3.94952e-03</td>
</tr>
<tr>
<td>125Ca Water OW</td>
<td>-0.820</td>
<td>1.14500e-06</td>
<td>1.62200e-03</td>
<td>-0.820</td>
<td>1.53500e-06</td>
<td>2.85916e-03</td>
</tr>
</tbody>
</table>

Table 2.3: Force field parameters describing the interaction between the calcium ion and its ligands, used in the simulations. The GROMOS force field is the GROMOS-87 force field with the changed Carbon-OW parameters, while the modified GROMOS force field is the same force field, with the changed parameters of Shiratori listed in Table 2.1. The charges given in the table are the charges located on the protein atoms, not on the calcium ion. The nonbonded interactions of the carbon atoms were not changed, while the charges of the carbon atoms were changed in order to keep the total charge of the charge group equal to the value in the GROMOS-87 force field. The charge of water oxygen was not changed to -0.870, which was calculated by Shiratori, because that would alter the Coulomb interactions of all water oxygen atoms, which are mainly not interacting with the calcium ion.

<table>
<thead>
<tr>
<th>Simulation</th>
<th>Old Parameters (OP)</th>
<th>New Parameters (NP)</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simulation length</td>
<td>100</td>
<td>100</td>
<td>ps</td>
</tr>
<tr>
<td>Time step (Δt)</td>
<td>2</td>
<td>2</td>
<td>fs</td>
</tr>
<tr>
<td>Pressure (p)</td>
<td>1.0 x 10^5</td>
<td>1.0 x 10^5</td>
<td>Pa</td>
</tr>
<tr>
<td>Pressure coupling constant (τ_p)</td>
<td>0.5</td>
<td>0.5</td>
<td>ps</td>
</tr>
<tr>
<td>Temperature (T)</td>
<td>300</td>
<td>300</td>
<td>K</td>
</tr>
<tr>
<td>Temperature coupling constant (τ_T)</td>
<td>0.1</td>
<td>0.1</td>
<td>ps</td>
</tr>
<tr>
<td>Twin range cut-off</td>
<td>1.0/1.8</td>
<td>1.0/1.8</td>
<td>nm</td>
</tr>
<tr>
<td>Neighbourlist update</td>
<td>20</td>
<td>20</td>
<td>fs</td>
</tr>
</tbody>
</table>

Table 2.4: MD Simulation parameters of the 'New Parameter' NP and 'Old Parameter' OP simulations. Both simulations were conducted using constant pressure and constant temperature (NPT).
2.1 Interaction of calcium and PLA$_2$

2.1.4 Results

In order to compare for both parameters sets the distortion from the original starting structure, which is the energy minimized X-ray structure,$^{10}$ the Root Mean Square Deviation (RMSD) of the calcium binding group is plotted versus time in figure 2.2. The calcium binding group is defined as the calcium atom, and its five protein ligands (O Tyr28, O Gly30, O Gly32, O Asp49, and O Asp49). The RMSD at time $t$ was calculated using the formula:

$$\text{RMSD}(t) = \left[ \frac{1}{N} \sum_{i=1}^{N} [r_i(t) - r_i(0)]^2 \right]^{\frac{1}{2}}$$

where $r_i(t)$ is the position of atom $i$ at time $t$, and $N$ is the number of atoms (in this case 6). The RMSD is calculated after the calcium binding group is root least squares fitted to the conformation of the calcium binding group at $t = 0$. As can be seen quite clearly from figure 2.2, the RMSD in the simulation using the new parameters is constant, while the RMSD of the simulation using the old parameters quickly increases, indicating that the conformation of the calcium binding group is moving away from its original conformation in the original X-ray structure.

The behavior of water near the calcium ion is monitored by calculating the number of water molecules coordinating the calcium ion versus time. The results for the NP and OP simulation are given in figure 2.3. A water molecule is counted as coordinating when the distance between calcium and the water oxygen atom is less than 0.35 nm. This value is based on the distance between calcium and oxygen as found in the X-ray structure of PLA$_2$. The number of water molecules coordinating the calcium ion is rapidly increasing during the OP simulation, reaching a total number of six after 50 ps. During the NP simulation, there is no fluctuation, and the number of coordinating water molecules remains stable at a value of 2, which is exactly the same as in the X-ray structure of PLA$_2$. $^{10}$

In exactly the same way, the number of protein ligand atoms of the calcium ion is calculated. A protein atom is defined as coordinating the calcium ion, when the distance between any protein atom and calcium is less than 0.35 nm. Only the five original ligating oxygen atoms were used in the analysis. The results for the NP and OP simulations are given in figure 2.4. In the NP simulation, the number of ligands remains stable at the expected value of five. Already at the start of the OP simulation, the calcium ion is losing its ligands. After 50 ps only two of the original five protein ligands are left.

The structure of the calcium binding loop is displayed in figure 2.5. The ribbon structures represent the conformation of the calcium binding loop (ribbon structure)
2.1 Interaction of calcium and PLA$_2$

Figure 2.2: Root Mean Square Deviation (RMSD) of the calcium binding group, compared to the starting structure. The calcium binding group is defined as the calcium ion, and the five protein ligand oxygen atoms (O Tyr28, O Gly30, O Gly32, O61 Asp49, and O62 Asp49). The RMSD of the NP simulation is plotted with a solid line, while the RMSD of the OP simulation is plotted using a dashed line.

Figure 2.3: Number of water ligands of calcium in PLA$_2$ during the OP simulation (dashed line) and the NP simulation (solid line).
2.1 Interaction of calcium and PLA$_2$

and the calcium ion (sphere) at 100 ps of the OP (left picture) and 100 ps of the NP simulation (right picture). As can be seen in both pictures, there is a big difference between both structures. At the end of the OP simulation, the calcium ion has left the calcium binding loop and entered the water region. Due to the departure of the calcium ion, the original calcium binding region is not constrained anymore, and has a distorted conformation. The picture at the end of the NP simulation is not different from that at the start. The calcium ion is still in its binding pocket, and the surrounding protein structure is still firmly attached to the calcium ion.

2.1.5 Discussion and conclusions

Charged metal ions in proteins are a source of trouble in molecular dynamics simulations. Because polarizability is not included in the forcefields, the interactions between an ion and its protein ligands is weaker than it should be, because the ligands are not polarized by the ion. The result of this error is that the metal ion moves away from its original site, and moves into a more favorable region, which is in this case the water region. The original binding site is destabilized by the departure of the metal ion, resulting in distortion of the protein conformation. This is clearly displayed in the OP simulation, where the calcium ion is located in a binding pocket of phospholipase A$_2$. During the simulation the calcium ion rapidly leaves the binding...
2.1 Interaction of calcium and PLA₂

New calcium parameters (NP)
Old calcium parameters (OP)

![Figure 2.5: Ribbon presentation of the region around the calcium ion in the active site at 100 ps during the NP (right) and the OP (left) simulation. The calcium ion is presented by the dark sphere. The ribbon and the coil in the left picture are distorted. ]

site, and dissolves in water, which has a more favorable interaction with the metal ion compared to the protein ligands.

The optimal way to overcome this problem is to introduce polarizability into the forcefield. Since it is still not possible to include an efficient polarizable model in the forcefield, this is not an option.

The method used in our simulation is based on the assumption that the environment of the calcium ion remains basically the same. There is some fluctuation in the ligands of the ion, but the ion does not leave the binding site and the ligands remain bound throughout the complete simulation. On the assumption that conformation is static, the nonbonded forcefield parameters describing the calcium ligand interactions were changed taking into account the polarizing effect of the calcium ion. The parameters used here were calculated by Shiratori. The charge of water oxygen was not changed to the value calculated by Shiratori but kept at its original value of 0.820 because the interactions of the water molecules in the simulation would be distorted if the interactions involving any water oxygen atom would change.

In the NP simulation, the picture is different from the OP simulation. Due to the modified parameters, the calcium ion remains tightly bound to the calcium binding region of the protein. The protein conformation is not distorted and there is no tendency visible of water molecules moving towards the calcium ion. The modification of the calcium binding interactions is thus a good method to keep the calcium binding region stable throughout a molecular dynamics simulation. In later simulations (described in chapters 3 and 4), which are extended for more than 1 ns, the calcium binding site also proves to be stable, and the calcium ion remains tightly bound to the protein ligands.

It should be noticed that it is also possible to constrain the calcium ion in the cal-
2.1 Interaction of calcium and PLA₂

cmium binding loop by adding several harmonic constraints between calcium and its ligands. The difference between this method, and the method used in our simulations, is small. The stability of the calcium binding loops is not thought to change dramatically when using the other method.

A serious problem can arise when the environment of the calcium ion is not constant anymore. The environment of the calcium ion can change when for example a phospholipid molecule binds to the active site. Two oxygen atoms of the phospholipid replace the two water molecules, which are normally bound to the calcium ion (see figure 2.1 on page 24). The effect for the protein ligands is small, but the interaction between calcium and the two phospholipid oxygen atoms is not realistic due to the polarizing field of calcium ion. This problem is solved by introducing atomic polarizability for the phospholipid oxygen atoms, and this will be discussed in the following section of this chapter.
2.2 Atomic polarizability

That divalent ions in proteins can give rise to severe forcefield problems in molecular dynamics simulations has already been shown in the previous section. The method presented here is another method to solve the polarizability problem related to the presence of the calcium ion. The problem is caused by the following situation. When a phospholipid is binding to the active site, two water molecules will be replaced by two phospholipid oxygen atoms. These two oxygen atoms, schematically displayed in figure 2.6, become more polarized as their distance towards the calcium ion decreases. The method used in the previous section assumes the ligands to be stable and will therefore not work. Here we present a different method based on atomic polarizability. In this way the most important polarizability effects of substrate binding can be taken care of.

2.2.1 Non-mutual polarization

Non-mutual polarization was successfully used by Linssen\textsuperscript{75} in order to keep a chloride ion constrained in a hydrophobic environment of haloalkane dehalogenase.\textsuperscript{76} Non-mutual polarization adds an extra force to approximate the effect of polarization. The basis of the method of non-mutual polarization is the distinction of two types of particles: polarizable, and non-polarizable. In non-mutual polarization, non-polarizable atoms which are ions (in our case only the calcium ion) polarize the
2.2 Atomic polarizability

polarizable atoms, i.e. the oxygen atoms of the phospholipid. There is no induced polarization between atoms.

The induced dipole moment $\mu_i$ on a polarizable particle $i$ at position $r_i$ in the field $E_j$ caused by a polarizing particle $j$ at position $r_j$ is given as:

$$\mu_i = \alpha_i E_j$$

where $\alpha_i$ is the polarizability (assumed to be isotropic) of particle $i$. $E_j$ is given as:

$$E_j = \frac{f q_j}{r^3} r$$

with $f = 1/4\pi\epsilon_0$, $r = r_i - r_j$, $r = |r|$ and $q_j$ is the charge of particle $j$, the source of the electric field.

The induced dipole moment $\mu_i$ on a polarizable particle $i$ at position $r_i$ in the field $E_j$ caused by a polarizing particle $j$ at position $r_j$ is given as:

$$\mu_i = \alpha_i E_j$$

where $\alpha_i$ is the polarizability (assumed to be isotropic) of particle $i$. $E_j$ is given as:

$$E_j = \frac{f q_j}{r^3} r$$

with $f = 1/4\pi\epsilon_0$, $r = r_i - r_j$, $r = |r|$ and $q_j$ is the charge of particle $j$, the source of the electric field.

The induced dipole moment $\mu_i$ on a polarizable particle $i$ at position $r_i$ in the field $E_j$ caused by a polarizing particle $j$ at position $r_j$ is given as:

$$\mu_i = \alpha_i E_j$$

where $\alpha_i$ is the polarizability (assumed to be isotropic) of particle $i$. $E_j$ is given as:

$$E_j = \frac{f q_j}{r^3} r$$

with $f = 1/4\pi\epsilon_0$, $r = r_i - r_j$, $r = |r|$ and $q_j$ is the charge of particle $j$, the source of the electric field.

The total contribution of polarization to the potential energy is:

$$V_{tot, pol} = -\frac{1}{2} \alpha_i \int \frac{q_j^2}{r^4} dr$$

The contribution to the force exerted on particle $i$ is now given as:

$$F_{pol,i}(r) = -2\alpha_i \int \frac{q_j^2}{r^5} dr$$

and the contribution to the force on particle $j$ is:

$$F_{pol,j}(r) = -F_{pol,i}(r)$$

If there is no mutual polarization, the contributions to the force as well as to the potential energy are pair additive with respect to different polarizable atoms. The forces are not pair additive to polarizing atoms, but since in our application only one polarizing atom (the calcium ion) is present, this is of no consequence. This non-mutual polarisation method is used in chapter 4 where the binding of a phospholipid molecule to the active site of PLA$_2$ is described. The charge groups that are treated as polarizable are the carbonyl and phosphate groups of the phospholipid molecule that binds in the active site. The interaction of the protein ligands with the calcium ion in these simulations is that described in the previous section. The atomic polarizabilities used in our simulations are given in table 2.5 and were taken from Applequist et. al.\textsuperscript{77} and the Handbook of Chemistry and Physics.\textsuperscript{78}
Table 2.5: Atomic polarizabilities $\alpha^*$ used for the non-mutual polarizability model. Note that $\alpha^* = \alpha f$ is used in the tabulated values.

<table>
<thead>
<tr>
<th>atom-type</th>
<th>$\alpha^*$ (Å$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (carbonyl)</td>
<td>1.027</td>
</tr>
<tr>
<td>O (carbonyl)</td>
<td>0.841</td>
</tr>
<tr>
<td>P</td>
<td>3.63</td>
</tr>
</tbody>
</table>

2.3 Docking of phospholipase $A_2$

2.3.1 Introduction

In this section we describe the docking of phospholipase $A_2$ (PLA$_2$) on a monolayer surface. PLA$_2$ must be properly placed on a monolayer surface in order to generate a reasonable starting conformation to use in subsequent molecular dynamics simulations of PLA$_2$ in such a monolayer environment.

It is of course possible to orient PLA$_2$ on the basis of visual inspection and chemical intuition: The active site entrance is surrounded by a ring of hydrophobic residues and it seems logical to orient that face of the molecule towards the monolayer. However, we wish to approach the orientation on binding to the monolayer as a process driven by the interactions between enzyme and monolayer and to investigate if the proper orientation can be generated without introducing a subjective bias.

The protein and the phospholipid surface represent two objects which can bind in orientations that can not be evaluated within the time scale of a conventional molecular dynamics simulation. Therefore we have to reduce the number of degrees of freedom in the simulation in order to make it possible that all remaining degrees of freedom can be searched.

2.3.2 Theory

In order to reduce the number of degrees of freedom, the protein and the monolayer are treated as rigid bodies, reducing the degrees of freedom to six. This reduces to a number of five by removing the rotation around the axis perpendicular to the monolayer surface. In figure 2.7 the protein is schematically displayed together with the two rotational degrees of freedom ($\varphi$ and $\theta$). These five degrees of freedom can be split up into two rotational and three translational degrees of freedom.

As the protein and the monolayer are treated as rigid bodies, the molecular dynamics energy function can also be simplified by removing all bonded interactions. The
energy function \( V \) is now built up from the Lennard-Jones and the Coulomb energies.

\[
V = \sum_{i,j} \left( S(r_{ij}) \frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}} + \frac{C_{12}}{r_{ij}^6} - \frac{C_6}{r_{ij}^6} \right)
\]

(2.1)

Solvent is not included in the simulations. Therefore an additional screening function \( S \) is included in the Coulomb term of the energy function. This screening function is defined as:

\[
S(r_{ij}) = e^{-C r_{ij}}
\]

where \( C \) is a constant. This type of screening is expected as a result of the presence of an electrolyte solution; then \( C \) is the inverse Debye length. In the actual simulations the effect of \( C \) was tested for several values. Because no significant changes of the results were observed upon changing \( C \), a value of 0 was chosen for the presented results. The screening function is thus equal to 1.

In order to find the optimal orientation, the energy is calculated as a function of protein orientation, defined by the two rotational degrees of freedom. This can also easily be plotted on a map-like graph.

### 2.3.3 Methods and simulations

All simulations were performed using the GROMACS \(^{68} \) software package. The structure of phospholipase A\(_2\) was extracted from Protein Databank (pdb) \(^{72} \) entry 1p2p.\(^{10} \) All crystal water molecules were removed, and hydrogen atoms were added to the protein structure. The conformation of the protein was subsequently energy minimized using a steepest descents energy minimization method. The monolayer conformation was extracted from an equilibrated 1,2-diacyl-sn-glycerol-3-phosphocholine (DDPC) monolayer simulation.\(^{79} \)

The interaction between protein and monolayer was scanned over the five degrees of freedom. 960 protein orientations, evenly distributed over a sphere, were used for the rotational degrees of freedom. For each orientation the protein was oriented with respect to the monolayer surface in such a way that the vector \( \mathbf{N} \perp \), defined by the angles \( \varphi \) and \( \theta \), is perpendicular to the monolayer surface. This is schematically displayed in figure 2.7.

For each protein orientation the interaction between protein and monolayer was calculated at 100 points on the monolayer surface. For each of these 96000 points,
the protein was moved in a direction perpendicular to the monolayer surface, from within the monolayer to a distance from the monolayer where the interaction energy between protein and monolayer is zero, and the lowest interaction energy found was stored. The following software packages, apart from GROMACS, were used for analysis and the creation of images: Gnuplot,80 Molscript,73 and Xmgr.74

2.3.4 Results

Figure 2.8 gives a typical plot of the interaction between protein and monolayer during the movement of the protein along the axis perpendicular to the monolayer surface (z-direction). The orientation of the protein and the translation parallel to the monolayer plane are fixed. The interaction energy in the graph is normal for a non-bonded interaction. Below $-0.3$ nm the interaction energy rapidly increases as a result of close contacts between protein and monolayer. When the protein is moved away from the monolayer, the interaction energy flattens to zero.

Figures 2.9, 2.10, and 2.11 show the minimum potential interaction energy of a specific protein orientation, plotted as a function of the location of the protein with respect to the monolayer surface. All figures show a large fluctuation in the calculated potential energy.

The minimum interaction energy for each protein orientation is plotted against protein orientation with respect to the DDPC monolayer in figure 2.12. In this plot, there are three regions which show a favorable interaction of the protein with the
Figure 2.8: Example of the interaction energy between protein and monolayer as a function of distance between protein and monolayer. Similar curves were generated for 960 orientations and 100 positions of the protein with respect to the monolayer.

Figure 2.9: Minimum potential energy interaction as a function of location on the monolayer surface for protein orientation $((\varphi, \theta))=(125^\circ, 55^\circ)$. This corresponds to location 1 in figure 2.12.
2.3 Docking of phospholipase A₂

Figure 2.10: Minimum potential energy interaction as a function of location on the monolayer surface for protein orientation \((\varphi, \beta)=(255^\circ, 75^\circ)\). This corresponds to location 2 in figure 2.12.

Figure 2.11: Minimum potential energy interaction as a function of location on the monolayer surface for protein orientation \((\varphi, \beta)=(280^\circ, 55^\circ)\). This corresponds to location 3 in figure 2.12.
2.3 Docking of phospholipase A$_2$

Figure 2.12: Contour plot of the potential energy as a function of the orientation of PLA$_2$ with respect to the DDPC monolayer. $\varphi$ is the longitude or azimuth angle, $\theta$ is the lattitude. The poles are represented by $\theta = \pm 90^\circ$. 
monolayer. These three regions are the following $\varphi, \theta$ combinations: 1 \((125^\circ, 55^\circ)\); 2 \((255^\circ, 75^\circ)\); and 3 \((280^\circ, -55^\circ)\). Each of these regions is marked with the corresponding number in figure 2.12. Of each of these orientations, a conformation of the protein and monolayer is plotted in figures 2.13, 2.14 and 2.15 respectively. Note that only in figure 2.13 the entrance of the hydrophobic cleft of PLA$_2$ is oriented towards the monolayer surface. The difference between the three regions is that several low energy subregions are grouped around region 1. This is not the case for regions 2 and 3. When examining the data used to generate figure 2.12, it seems that the regions 2 and 3 are the result of a limited number of low energy interactions, while the region 1 is a result of more low energy interactions. Thus, we conclude that region 1 gives the most favorable orientation.

Figure 2.13: Configuration of protein and monolayer for protein orientation 
$(\varphi, \theta)=(125^\circ, 55^\circ)$. This corresponds to location 1 in figure 2.12.
2.3 Docking of phospholipase A$_2$

Figure 2.14: Configuration of protein and monolayer for protein orientation $(\phi, \theta) = (255^\circ, 75^\circ)$. This corresponds to location 2 in figure 2.12.

Figure 2.15: Configuration of protein and monolayer for protein orientation $(\phi, \theta) = (280^\circ, -55^\circ)$. This corresponds to location 3 in figure 2.12.


2.3 Docking of phospholipase A₂

2.3.5 Discussion and conclusions

Phospholipase A₂ was docked on a DDPC monolayer in order to create a starting conformation for subsequent molecular dynamics simulations. The basis of the docking method is the reduction of the number of degrees of freedom in the system. The forcefield was built up from the nonbonded interaction energy functions, i.e. the Lennard-Jones and Coulomb interaction function. Solvent is not treated explicitly in the simulations. Therefore an additional exponential shielding function was added to the Coulomb potential in order to simulate the shielding by water. During the setup of the final docking simulation, the shielding function was set to a value of 1 because there was no significant change seen in the output of the docking program. This is rather surprising, because solvent interactions are expected to be very important when a protein is binding to a monolayer surface.

The docking simulations lead to three protein orientations which have a favorable interaction with the monolayer surface. These three regions are equally low in energy and one of these energy minima consists of four low energy regions spread around the selected value. This suggests that this minimum is overall more favorable than the other minima. Further inspection of the simulation data also suggests that the two other minima are built up from fewer points in orientational space than the grouped minimum. The chance that the grouped minimum is the correct orientation is thus higher.

Visual inspection of the three docked protein structures suggests that the structure from region 1 has the most favorable orientation with respect to the monolayer. This structure has most of its surface in close contact with the monolayer, while the other two structures just have interactions with a small part of the protein. The contact area between the selected structure and the monolayer is very large compared to that of the other two structures, the latter looking like standing on a tip.

The protein is oriented with the entrance of the hydrophobic cleft towards the phospholipid surface. This strongly supports the validity of this protein conformation because a substrate molecule located in the monolayer binds to the active site by moving through the hydrophobic cleft. This conformation is the one chosen for the subsequent molecular dynamics simulations.

The docking method as described here seems to work quite well for this system. The main question is then if it will also work for other systems. It is likely that the validity of the method depends on the type of objects being docked onto each other. Two large surfaces involving many interactions may give reliable results, but surfaces with small contact areas will be more sensitive to the details of the interactions included in the docking method. Specifically, the incorrect treatment of water may cause artefacts in such cases.
The reduction of the number of degrees of freedom allows the docking procedure to use a complete systematic search of the available configuration space. All the possible conformations will thus be scanned. The method of presenting the results in a globe-like map gives a picture which looks very informative. However, it must be noted that we have here the same problem as encountered in imaging the globe on a map. The region closer to the poles ($\theta = \pm 90^\circ$) is enlarged, while the regions around the equator are presented on a much smaller scale. In judging the area we must be aware of the fact that the size of the spot is thus dependent on its latitude. However, the three minima found here, are all located on approximately the same latitude, and thus their sizes can be compared without taking this effect into account.