Setting the base for modelling of a

Ion parametrization and membrane protein modelling

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Abstract

Metal ions are of vital function in biological systems. To penetrate cells, they have to be transported by membrane proteins. One type of these proteins is called [redacted] few members of this protein family have been thoroughly studied and structurally determined, whereas the workings of other members are shrouded in mystery. Knowing the structure and the ion transportation pathway of a transport protein is necessary to understand better how it functions.

Molecular Dynamics (MD) simulations can be used to provide dynamic information about the interactions between the transported ions and the transporter protein. To use this method, it is necessary to have well derived force field parameters for the ions and a starting structure for the protein. This thesis sets the groundwork for further examination of a [redacted] specific [redacted] called [redacted] by aiming for using the [redacted] X-ray crystallography structure to model and simulate the protein inserted into a membrane, and by validating and deriving force field parameters Co(II) and Zn(II). The final aim is to perform simulations of the protein together with Co(II) and Zn(II) ions and compare their transportation pathway through [redacted] with EXAFS data.

This master thesis has focused first on testing and validating available Zn(II) and Co(II) ion Lennard-Jones parameters, as published by Stote & Karplus in 1995 and Babu & Lim in 2005, against experimental structural, kinetic and thermodynamic properties of the ions in water solution. Investigation showed that the parameters from Stote & Karplus and Babu & Lim for Zn(II) and Co(II) do not capture kinetic and thermodynamic properties. Due do observed deficiencies in these parameters, new parameters for both ion species were developed to better capture their experimental properties in solution. The newly derived parameters capture kinetic and thermodynamic properties, but have some deviation ($\approx 10\%$) from experimental values concerning ion-water distances.

Homology modelling of [redacted] was performed, after which the protein was inserted into a membrane and the system was equilibrated. Modelling resulted in a model unfit for use because of the large structural deviation from the [redacted] X-ray crystallography structure from the [redacted] Because of differences in hydrophobic depth between the membrane lipids and the protein, an aspartic acid residue is embedded in the membrane. Equilibration simulations show evidence of membrane deformation, possibly because of the hydrophobic mismatch and the position of the aspartic acid residue. The protein-membrane system was equilibrated in terms of pressure, temperature and potential – but the structural deviation of the protein indicates that more simulation time might be necessary.

Keywords: Molecular dynamics simulations, Lennard-Jones parameters, divalent cations [redacted] protein membrane simulation
## Contents

1. Introduction

2. Methods
   - Molecular Dynamics simulation
     - Thermodynamic ensembles, periodic boundary conditions, thermostats and barostats
     - Potential energy surfaces and force fields
     - Performing an MD simulation
     - Simulation set up for salt solution
     - Simulation set up for single ion in solution
   - Analysis of salt solution
     - Radial Distribution Function
     - Solvation free energy
     - Potential of Mean Force
   - Ion interaction parameters
   - Re-parametrization approach
   - Protein modelling and simulation
     - Homology modelling
     - Protein membrane insertion
     - Simulation set up for protein-membrane equilibration

3. Structural, thermodynamic and kinetic characterization of Co(II) and Zn(II) in aqueous solution
   - Babu & Lim (2005) and Stote & Karplus (1995) parameters
     - Structural characteristics
     - Ion-water exchange
     - Thermodynamic characteristics
     - Discussion
   - New parameters
     - Parametrization
     - Thermodynamic characteristics
     - Structural characteristics
     - Ion-water exchange
     - Discussion

4. Protein modelling and simulation
   - Homology modelling and insertion into membrane
   - Equilibration
   - Discussion
   - Conclusion
   - Future work

Bibliography

Appendix
1. Introduction

Inorganic ions are vital for biological function, participating in, for example, enzymatic activity and reactions (Anastassopoulou & Theophanides, 1995). The divalent metal cations cobalt, zinc and copper are examples of these elements. Divalent cobalt ions (Co(II)) are a constituent of vitamin B12; necessary for many functions, some of which are synthesis of bone proteins, creation of the amino acid methionine and formation of erythrocytes. A deficiency of cobalt can lead to neuropsychiatric abnormalities (weakness, memory loss, personality changes), decreased osteoblast activity and pernicious anemia (Gerard & Derrickson, 2009). Divalent zinc ions (Zn(II)) are contained in several enzymes and also catalytic and co-catalytic proteins such as carbonic anhydrase and superoxide dismutase. Further, transcription factors called zinc fingers use the ions to stabilize their fold (Lim, et al., 2005).

To maintain concentrations, ions have to be able to move in and out of the cell. Because of their charge, ions are not able to diffuse through the cell membrane and instead rely on active or passive transport by transporter proteins in the cell membrane. In passive transport, ions are moved along their concentration gradients through ion channels, requiring no additional energy
X-ray crystallography is a high-resolution fine structure technique, in which a three-dimensional molecular structure is obtained by crystallizing the protein of interest. The order of magnitude of the X-ray wavelength makes it possible to resolve small structures in proteins. The method provides structural information of the conformational state the protein was crystallized in. A drawback to this method is thus that it does not capture the many different possible conformations of the protein (Smyth & Martin, 2000) and thus its dynamics. Furthermore, the crystallization can be a tedious task and the result does not necessarily reflect the characteristics of the protein in its true physiological environment (Acharya & Lloyd, 2005).

EXAFS provides interactional information. Its advantage lies in being element selective and having high sensitivity to detect the coordination number and chemical identity of nearest neighbours. This provides a route for understanding how the ions interact with specific protein residues (Wong, 1986). However, the method fails to differentiate between amino acids that contain the same components in their side chains. This is the case for the sulfur in cysteine and methionine as well as the double bonded oxygen in aspartic and glutamic acid. The coordination number also has an error of +/- 1. Another disadvantage is that the modality requires a tunable X-ray source, usually a synchrotron, which can be hard to attain.

While these both methods can provide structural information, they do not capture the conformational behavior that are a large part of the protein’s functionality. Any ligand binding, or other complex formations, induce a conformational change in the protein (Hospital, et al., 2015). These changes are sometimes minuscule and can be hard to detect using experimental methods. The transport proteins ion selectivity is largely dominated by thermodynamic
properties in the form of energetic barriers (Varma, et al., 2011) which cannot be captured by EXAFS or X-ray crystallography. Only a limited number of properties of biological systems are thus measurable by experimental techniques.

Molecular Dynamics (MD) simulations is a method that has become increasingly popular with the fast rise of computing power in the last years, and that can provide complementary data to experimental information. Atomistic MD provides an atomistic description of molecules, as well as averages, distributions and time series of any definable quantity (van Gunsteren, et al., 2006) at time scales that can be of the same order as those of biological processes (Hospital, et al., 2015). The technique also allows for studying environments that are hard to examine in real life and predicting properties of molecules under these conditions (van Gunsteren, et al., 2006). Experimental structures, obtained from for example the previously mentioned techniques, are used to build an initial model for the simulation. Experimental data can then be used for comparing the simulated results.

When studying the selectivity and structure of proteins such as MD simulations allows for tracking the ions on their way to and in the protein. This can reveal the exact transportation pathway of the ion through the protein, offering explicit information about what residues aids in the transportation and in what way. Furthermore, the full 3D structure of the protein can be studied before, during and after the transportation to give further information.

The accuracy of simulations relies on correct derivation of the force fields that govern intramolecular interactions and describe the potential $U$ of the system. A simple functional form of a force field contains four terms, three of which accounts for the deviation of bonds from their equilibrium values. The fourth term describes non-bonded interactions, containing electrostatic interactions and van der Waals interactions. While there are more advanced models that contain other terms, these four factors are key components of all force fields. Force fields are also distinguished by how they are parametrized. Not all force fields will allow representation of all types of molecules, and the parameters are not necessarily interchangeable between force fields (Leach, 2001).

Metal ions in solution can be described by a non-bonded model in which Coulomb and van der Waals forces are used to define the interactions between the metal ions and surrounding particles (Li, et al., 2014). The goal when parametrizing solvated ions is to capture the structure which includes the coordination number (CN) and the distance to the first hydration shell $d(m-Ow)$. The thermodynamic property of solvation free energy, $\Delta G_{sol}$, should also be as close to experimental values as possible. One would also like to capture the kinetics of the ion, in the form of the rate constant $k$ and the mean residence time of ion-water contact $\tau$ (water exchange).

Figure 2. A visualization of the coordination shell of Co(II) (green).
Table 1. Experimental structural, kinetic and thermodynamic properties of Co(II) and Zn(II).

<table>
<thead>
<tr>
<th>Ion m(II)</th>
<th>CN</th>
<th>d(m-Ow) (nm)</th>
<th>ΔG&lt;sub&gt;sol&lt;/sub&gt; (kJ/mol)</th>
<th>k (s&lt;sup&gt;-1&lt;/sup&gt;)&lt;sup&gt;1,3&lt;/sup&gt;</th>
<th>τ (s)&lt;sup&gt;1,3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co</td>
<td>6</td>
<td>0.208-0.212</td>
<td>-1915.017</td>
<td>1.1 × 10&lt;sup&gt;6&lt;/sup&gt;</td>
<td>8.8 × 10&lt;sup&gt;-7&lt;/sup&gt;</td>
</tr>
<tr>
<td>Zn</td>
<td>6</td>
<td>0.205</td>
<td>-1955.180</td>
<td>&gt; 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>&lt; 10&lt;sup&gt;-7&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Ohtaki & Radnai (1993), <sup>2</sup>Marcus (1994), <sup>3</sup>Helm & Merbach (2005)

In this thesis, a set of available force field parameters for Co(II) and Zn(II) were trialed and tested against experimental data revealing disadvantages of the parameters. New parameters for both ions were derived that capture the structural and dynamical properties presented in experimental data. The aim of this work was to validate already published parameters for Co(II) and Zn(II) and to derive novel parameters that capture structural, thermodynamic and dynamical properties of the ions in aqueous solution. In case of deficiencies in the tested parameters, new parameters for both ion species will be derived. Doing so paves the way for further study and simulation of transport mechanism and selectivity. The final aim is to perform simulations of the protein together with Co(II) and Zn(II) ions and compare their transportation pathway through with EXAFS data.
2. Methods

Molecular Dynamics simulation

New configurations are found in Molecular Dynamics (MD) simulations by integrating Newton’s laws of motion. Solving the differential equation \( \frac{d^2x_i}{dt^2} = \frac{F_{xi}}{m_i} \) results in a trajectory that provides information of how the particle \( i \) with mass \( m_i \) changes position \( x_i \) as it is acted on by the force \( F_{xi} \). The force \( F_{xi} \) can further be defined as the negative derivative of the potential \( U(r) \) of the system.

Due to its deterministic nature, the future state of a system can be predicted from its current state. Experimental data is used to create a first model for the simulation, containing the initial positions of the particles. The initial velocities are generated randomly through a Maxwell-Boltzmann distribution that depends on the user-provided end temperature of the system and masses of the particles.

Verlet or Leap frog algorithms and integrators can be used to generate new configurations from the initial model. The Verlet algorithm uses the positions and accelerations at time \( t \), and the previous positions at \( t-\Delta t \) to calculate the coming position at \( t+\Delta t \):

\[
r(t + \Delta t) = 2r(t) - r(t - \Delta t) + a(t)\Delta t^2
\]

Clearly there is no specific velocity term in the above equation, which is a disadvantage for the Verlet algorithm. Velocities can only be calculated after the position at the next step is found. Further, the method may suffer from a lack of precision as the third term is negligible compared to the other two.

Leap frog is a development of the Verlet algorithm:

\[
r(t + \Delta t) = r(t) + v(t + \frac{1}{2} \Delta t)\Delta t
\]

\[
v(t + \frac{1}{2} \Delta t) = v(t - \frac{1}{2} \Delta t) + a(t)\Delta t
\]

The velocity at time \( t \) is obtained by,

\[
v(t) = \frac{1}{2} \left( v(t + \frac{1}{2} \Delta t) + v(t - \frac{1}{2} \Delta t) \right)
\]

Compared to Verlet, Leap frog contains velocity terms and all terms are of the same size. The main disadvantage is that velocity and position are not synchronized.

MD simulations can be used to provide an atomistic description of a system. By starting from an initial configuration and letting the system evolve in time, statistical mechanics together with a correctly defined force field and suitable boundary conditions allows macroscopic properties of the system to be inferred from the microscopic simulations.
Thermodynamic ensembles, periodic boundary conditions, thermostats and barostats

A statistical ensemble provides a probability distribution for the state of a system by comprising a large number of computer-generated copies of a system, where each duplicate represents a state in which the real system can occupy. The probability distribution is produced by considering all copies of the system at all times (Gibbs, 1902). Thermodynamic ensembles are statistical ensembles that fulfills certain requirements, such as statistical equilibrium, and are used to find thermodynamic system properties using either classical- or quantum mechanics (Kittel & Kroemer, 1980).

A thermodynamic ensemble with constant number of particles $N$, volume $V$ and temperature $T$ is called the canonical ensemble or $NVT$ ensemble. Isothermic-isobaric ensembles have a constant number of particles $N$, temperature $T$ and pressure $P$: $NTP$ ensemble. A simulation performed with a constant number of particles $N$, volume $V$ and energy $E$ uses the micro canonical ($NVE$) ensemble (Leach, 2001).

Periodic boundary conditions (PBC) are commonly used in MD simulations. The simulation is conducted in a user defined box, around which there are an infinite number of identical boxes with the same content. A particle that leaves the simulation box through the right side will be replaced by an image particle from the left-hand side. This ensures that the number of particles is always kept constant. Particles in an PBC MD simulation are influenced by particles from all boxes (Bekker, 1996). While this approach simplifies simulations and reduces boundary effects, it does not remove the boundaries but simply shifts them to infinity.

The pressure and temperature are controlled by using couplings in the form of barostats and thermostats. These are designed to make sure that the simulation samples from the correct ensemble by altering the temperature or pressure so that the average value of the quantities are correct with an accepted size of fluctuations. In simulations, the temperature is calculated from the total kinetic energy of the system (Leach, 2001). In this thesis, the Berendsen (Berendsen, et al., 1984), Velocity-rescaling (Bussi, et al., 2007) and Nosé-Hoover thermostats (Nosé, 1984) (Hoover, 1985) have been used. The Parrinello-Rahman (Parrinello & Rahman, 1981) and Berendsen barostats (Berendsen, et al., 1984) are also implemented.

The Berendsen thermostat changes the temperature by mimicking a coupling to an external heat bath with reference temperature $T_0$. Deviations from the reference value are reduced exponentially and according to

$$\frac{dT}{dt} = \frac{T_0 - T}{\tau}$$

where $\tau$ is a time constant. The strength of the coupling can be changed based on the needs of the user and the thermostat subdueds kinetic energy fluctuations. This means there in an error associated with the thermostat that scales with $\frac{1}{N}$, where $N$ is the number of particles in the system. For large systems, the error will thus be insignificant but the distribution of the kinetic energy will be affected. The Velocity-rescaling thermostat is in principle a Berendsen thermostat, but with an added term that ensures that the distribution of the kinetic energy is correct (Abraham, et al., 2018).

While the Berendsen thermostat is efficient in making the system reach the reference
temperature, it does not reproduce the correct canonical ensemble, which the Nosé-Hoover thermostat does. This is done by adding terms to the Hamiltonian of the system: one term for the coupled heat bath and one friction term. The latter adjusts the speed of the particles until the system reach the reference temperature (Marenduzzo, 2009).

The Berendsen barostat changes the pressure by altering the lengths of the box vectors and the coordinates with a scaling matrix every \( n \) steps. Similar to the thermostat, fluctuations are decreasing as

\[
\frac{dP}{dt} = \frac{P_0 - P}{\tau}.
\]

As with the thermostat, this barostat does not reproduce a true NPT ensemble although the average pressure is correct. The Parrinello-Rahman pressure coupling is the "Nosé-Hoover" of the barostats and does reproduce the correct ensemble by altering the Hamiltonian of the system (Abraham, et al., 2018).

One can expect that there will be significantly more fluctuations in the pressure than in the temperature, due to the virial part of pressure. How much the pressure oscillates depends on how many particles the simulated system contains and which barostat is used (Leach, 2001).

**Potential energy surfaces and force fields**

While molecular modelling could in principle be handled as a quantum mechanical problem and generated by use of such methods, it would be time consuming and complicated. Instead the force field method is used, in which the motion of electrons is ignored. By using the Born-Oppenheimer approximation, the energy is calculated as a function depending only on the nuclear coordinates, \( U(\mathbf{r}) \). Any movement, be it positional, rotational or vibrational, will most likely result in an energy change (Leach, 2001).

The force field is the expression that describes the potential \( U \) of the system with regards to the positions \( \mathbf{r} \) of its \( N \) atoms. One example of a force field functional form is,

\[
U(\mathbf{r}^N) = \sum_{\text{bonds}} \frac{k_i}{2} (l_i - l_{i0})^2 + \sum_{\text{angles}} \frac{k_i}{2} (\theta_i - \theta_{i0})^2 + \sum_{\text{torsions}} \frac{U_n}{2} (1 + \cos(n\omega - \gamma)) \\
+ \sum_{i=1}^{N} \sum_{j=i+1}^{N} \left( 4\varepsilon_{ij} \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right) + \frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}}
\]

The first term accounts for the bonded interaction between two atoms and the second is a sum of all valence angles in the molecule. Both terms are modelled by a harmonic potential. The third term describes a torsional potential that describes how the energy changes as a bond rotates. Non-bonded interactions are described by the last term and contains a Lennard-Jones potential that accounts for the van der Waals-interactions and a Coulomb potential for electrostatic interactions. A force field is not solely defined by its functional form, but also by the its parameters (values of the constants in the formula above). Furthermore, two force fields with the same functional form may give results that are comparable in accuracy (Leach, 2001).

The long-range electrostatics, or non-bonded interactions, consists of long-range van der Waals- and electrostatic interactions. Handling of the non-bonded interactions is among the most time-consuming portion of molecular dynamics (Leach, 2001). Ideally, all non-bonded
interactions between all atoms should be calculated which is not trivial for most systems. To simplify the calculations a cut-off distance is introduced, beyond which all non-bonded interactions are ignored. While this is computationally more efficient, one has to pay attention to the fact that using a cut-off distance can affect the simulation to be more imprecise (Field, 2007). van der Waals and electrostatic interactions between atoms separate by three bonds, also known as 1,4 atoms, are considered differently than other non-bonded interactions as they contribute to the rotational barrier and torsional potential around the central bond (Leach, 2001).

Another technique to deal with non-bonded interactions is the Particle Mesh Ewald (PME) method, in which a mesh or grid is introduced into the simulation cell. The electrostatic potential at each grid point is found by creating a representation of the charge distribution on the mesh and then solving the Poisson equation using a Fast Fourier Transform (FFT). The potentials at all mesh points are then transferred to the atoms in the simulation box and all associated energies can be calculated (Field, 2007).

To visualize the potential $U(r)$, changes in the energy of a system in the form of a new configuration, can be regarded as movements on a potential energy surface. Any system will strive to be in a configuration that leads to the lowest energy possible, represented as local and global minima on the potential energy surface. At these points, the system is in a more stable state. While the global minimum does correspond to the configuration of lowest energy, it is not always the most populated one depending on its shape. A deep and narrow minimum can be less populated than a broad minimum of higher energy, as the vibrational energy levels are less accessible in the deep minimum (Leach, 2001).

For a system containing $N$ atoms, each point on the potential energy surface can be considered as a point in the $6N$-dimensional phase space. If all points could be sampled the partition function, containing all the information of the system at thermal equilibrium, could be calculated independent of the starting configuration. Therefore, the goal of any simulation is to let the system evolve over sufficient amount of time, allowing it to pass through all possible configurations. To get reliable values, enough of phase space must be sampled (Leach, 2001).

**Performing an MD simulation**

Molecular simulations can generally be performed in three steps: energy minimization (EM), equilibration and production run using molecular dynamics.

Performing an EM allows the system to enter a low energy configuration, removing steric clashes or unsuitable geometry. The goal is not to find the configuration of lowest possible energy but to relax the system. Most systems then undergo two equilibrations using the NVT and NPT ensembles respectively, to ensure that temperature, pressure and volume are converging to the set values. After EM and equilibration, the production run is performed.

In this thesis, all simulations have been performed using GROMACS (available at http://www.gromacs.org) version 5.1.4 and the force field CHARM36 for proteins (Best, et al., 2012) available at http://mackerell.umaryland.edu/charmm_ff.shtml.

**Simulation set up for salt solution**

A 9 nm cubic box was solvated with 23891 TIP3P (Jorgensen, et al., 1983) water molecules and equilibrated to a temperature of 298 K and 1 atm pressure. 129 of the solvent water molecules were replaced with 43 metal ions $M$(II) (Co(II), Zn(II)) and 86 Cl(I) counter ions
resulting in $[\text{M(II)Cl(I)}] = 0.1$ M. The run time of the simulation was 100 ns. The first 10 ns was removed to discard initial data when the system was not equilibrated.

Periodic boundary conditions were used in all directions. Long-range interactions were handled using PME and a cut-off distance of 1.2 Å. The pressure and temperature was controlled using the Parrinello-Rahman barostat and velocity rescaling thermostat. A time step of 2 fs was used, and the Leap-frog integrator and algorithm was used.

**Simulation set up for single ion in solution**

A 3 nm cubic box containing 877 TIP3P (Jorgensen, et al., 1983) water molecules. Simulations were run in which one of the water molecules were replaced with Co(II) or Zn(II). Reference pressure, temperature was set to 1 atm and 298 K respectively. The simulation run time was 200 ns using Leap-frog integrator and algorithm.

**Analysis of salt solution**

**Radial Distribution Function**

The Radial Distribution Function (RDF), also known in statistical mechanics as the pair correlation function, calculates the probability $g(r)$ of finding an atom, or molecule, a distance $r$ from a reference atom/molecule compared to the ideal gas distribution. The function has a characteristic look for elements in different phases; for a crystal, the RDF has several sharp peaks that are related to the lattice structure. For a liquid, the RDF has a few peaks for small $r$ and converges to a constant value of one for longer distances.

RDFs of the metal ions in relation to the chloride counter ions and the oxygen of water, Ow, were collected to investigate the coordination number, distance to the first hydration shell and behavior of the metal ions. The coordination number is the number of water molecules in the immediate vicinity of the ion. The distance to these water molecules then constitute the distance to the first hydration shell (Clementi, 1976). The salt solution simulation set up was used.

The coordination number (CN) can be found by integrating the RDF $g(r)$ from 0 to a distance $r'$ from the particle of interest,

$$CN = 4\pi \rho \int_0^{r'} g(r)r^2 \, dr$$

where $\rho$ is the number of particles per unit volume (Leach, 2001).

**Solvation free energy**

The free energy is one of the most important quantities in thermodynamics as it describes the tendency of the system to react and associate (Kollman, 1993) and is given by the Gibbs function for an NPT ensemble. However, some difficulties occur when attempting to obtain the free energy from MD simulations, especially in the case of liquids. For the free energy, the main contribution does not come from the low energy configurations, but from the high ones, of which there are not many in a liquid. As MD simulations are built to explore the low energy regions of phase space, the important high energy regions adding to the free energy will be left untouched resulting in an inaccurate free energy value (Leach, 2001).
Solvation is defined as the transfer of a solute particle from a fixed position in an ideal gas to a fixed position in the solvent (Marcus, 1994). For a solute, such as an ion, to be solvated, the total energy of the system must be reduced through multiple steps that affect both enthalpy and entropy. A cavity in the solvent must be formed to accommodate the solute which is both enthalpically and entropically unfavorable as solvent-solvent interactions are decreased and solvent ordering increased. The solute must then be separated from the bulk to enter the cavity, thus decreasing solute-solute interactions and increasing enthalpy. As the solute enters the cavity, new interactions are formed with the solvent and the enthalpy becomes more favorable. The complete solvation then results in increased entropy (Anslyn & Dougherty, 2006).

Consequently, the solvation free energy takes into account both enthalpic and entropic hydration effects. Only the sum of the solvation free energies of neutral combinations of ions are possible to acquire by experimental techniques. Hence, the solvation free energy of single ions is presented as relative solvation free energies by setting the solvation free energy of an arbitrary ion to zero (Lewis, et al., 1961). Usually, the arbitrary ion is the proton, meaning that calculation of its absolute solvation free energy is paramount to obtaining correct values. However, the solvation free energy of the proton cannot be measure directly and so poses as a large source of error in relative solvation free energy calculations (Bryantsev, et al., 2008). In the case of Zn(II) and Co(II), the relative solvation free energy of one ion to another is therefore more accurate.

**Thermodynamic Perturbation**

One approach to calculate the relative solvation free energy is to consider the free energy difference between two different states A and B where the energy difference between the states is path independent. There are three possible methods to do this: thermodynamic perturbation, thermodynamic integration and slow growth. In this thesis, thermodynamic perturbation is used, which can be described as a thermodynamic cycle. Such a cycle, as seen in fig. 3, describes a sequence of linked thermodynamic processes that involve transfer of energy and work in and out of the system, eventually returning the system to its initial state (Cengel & Boles, 2002).

![Thermodynamic cycle of solute M and N.](image.png)

*Figure 3. Thermodynamic cycle of solute M and N.*
It then follows that,

\[-\Delta G_{\text{water}}^{M \rightarrow N} = \Delta G_{N}^{\text{pol}} - \Delta G_{M}^{\text{pol}}\]

Consider the states A with the Hamiltonian $H_A$ and state B with $H_B$. The free energy difference between the two states in ensemble $C$ can then be expressed as,

\[\Delta G_{A \rightarrow B} = -k_B T \ln \langle e^{-H_B - H_A/k_B T} \rangle_C\]

This holds if states A and B only differ from each other in a trivial way. If the differences are more complex, a coupling parameter $\lambda \in \mathbb{R} | 0 \leq \lambda \leq 1$ can be used, which is the case for both thermodynamic perturbation and integration. As the value of the coupling parameter changes a transformation of states occur. $\lambda$ equal to zero corresponds to state A with the Hamiltonian $H_A$; $\lambda$ equal to one means that state A has been transformed to state B with the corresponding Hamiltonian $H_B$. The Hamiltonian depends on the coupling parameter as,

\[H(\lambda) = \lambda H_B + (1 - \lambda) H_A\]

The coupling parameter changes by $d\lambda$ for which the free energy is calculated. The difference in the Hamiltonian for the transformation,

\[\Delta H' = H_{A+d\lambda} - H_{\lambda}\]

allows for the calculation of the free energy difference between states A and B as a function of the coupling parameter. In thermodynamic perturbation, the energy difference is calculated as,

\[\Delta G_{A \rightarrow B} = \sum_{\lambda=0}^{1} -k_B T \ln \langle e^{-\Delta H'/k_B T} \rangle_{\lambda}\]

**Bennett Acceptance Ratio**

The basis of Bennett Acceptance Ratio (BAR) (Bennett, 1976) is that for a configuration $q$ at two different states A and B there is a pathway connecting the two potentials of each state and a potential energy difference of $\Delta U_{AB}(q)$. Assuming that both A and B share the same configurational space, but their energies are different because of some variable, for example the Lennard-Jones parameters in the case of ions, there is a relationship between the distributions of potential energy differences of states sampled from A and states sampled from B (Bennett, 1976). The free energy from going from state A to state B is then calculated as,

\[\Delta G_{A \rightarrow B} = k_B T \sum_{i=1}^{N-1} \ln \left( \frac{f(U_{\lambda_i} - U_{\lambda_i+1} + C)_{\lambda_i+1}}{f(U_{\lambda_i+1} - U_{\lambda_i} - C)_{\lambda_i}} + C \right)\]

where $\lambda_1$ is state A and $\lambda_{N-1}$ state B, $f(\lambda)$ is the Fermi function and C an energy offset related to states A and B (Bruckner & Boresch, 2011).
Relative solvation free energy simulation set up

The relative hydration free energy $\Delta G_{M_A(I) \rightarrow M_B(II)}^{(1)}$ was calculated using the single ion in solution simulation set up, in relation to Mg(II) and two sets of Ca(II) parameters. The evaluation was done using BAR. For the validation of already existing parameters, a lambda step size of 0.2 was used, equaling 6 lambda points, after checking the convergence of the relative solvation free energy simulation. This was done by running the same relative solvation free energy simulation three times with different lambda step sizes, until their values converged with an error of $\pm$ 2 kJ/mol. In deriving the new parameters, 6 lambda points proved to not be enough. Instead, a lambda step size of 0.1 was used, yielding 11 lambda points. The simulation run time was 1 ns for each lambda in both cases.

The Berendsen temperature and pressure couplings were used and set to reference temperature of 300 K and 1 atm respectively. Periodic boundary conditions were implemented in all directions and the non-bonded interactions were handled using PME and a cut-off distance of 1.2 Å.

Potential of Mean Force

The Potential of Mean Force (PMF) describes how the free energy changes as a function of a reaction coordinate, such as the separation between two atoms. The energy surface along the coordinate constitutes the PMF. For a solvent system, the PMF contains both solvent effects and the interaction between solutes along the coordinate. Fig. 4 shows the PMFs for four cations.

![Graph showing PMFs for different ions](image)

*Figure 4. Potential of mean force between ion and a water oxygen for all investigated ions. The data for Mg$^{2+}$ have been obtained with umbrella sampling. Simulations performed using the CHARMM27 force field for ions and TIP3P as water model (Allnér, et al., 2012).*

The point of highest energy corresponds to the transition state of the process and so the energy barrier $\Delta G^\dagger$ can be found. From this, one can calculate the rate constant $k$ and the mean residence time $\tau$ of water-ion contact, also known as the water exchange,

$$k = Ae^{-\Delta G^\dagger/RT}$$

$$\tau = \frac{1}{k}$$
The constant $A$ is equal to the average speed with which the activated complex crosses the barrier and is related to the frequency at which the system oscillates at its minima (McQuarrie & Simon, 1997),

$$A = \sqrt{\frac{E^*}{2\pi m^*}}$$

$E^*$ is the second derivative of the global minima of the PMF at the corresponding stationary point and was obtained by fitting the minima with a fourth order polynomial. $m^*$ is the reduced mass of the atom pair.

The PMF can be calculated by its relation to the RDF $g(r)$,

$$PMF(r) = -k_BT \ln g(r) + C$$

This approach can cause large errors if not enough of phase space is sampled in the simulation. The more advanced technique employs umbrella sampling to circumvent the sampling problem, in which the force field is adjusted so that unfavorable states are sampled (Leach, 2001).

**Ion-water exchange as a function of time**

The rate constant $k$ and mean residence time $\tau$ of water-ion contact can also be calculated by tracking the changes of the coordination number in the first hydration shell as a function of time. An exchange event is defined when the distance to the first hydration shell crosses a threshold distance. The rate constant for the exchange of one water molecule can then be found by dividing the number of exchanges per second by the average coordination number (Villa, et al., 2009).

In this thesis, the threshold distance for Zn(II) was set to 2.5 Å and 2.6 Å for Co(II). The same single ion in solution simulation set up was used, with the exception of the simulation run time. To be sure to capture exchange events for both ions the run time was set to 200 ns.

**Ion interaction parameters**

Non-bonded interactions dominate for solvated ions and they are thus parametrized in terms of Lennard-Jones parameters. The Lennard-Jones potential between two atoms $i, j$ at a distance $r_{ij}$ can be written in several forms,

$$U_{ij}(r_{ij}) = 4\epsilon_{ij} \left( \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right) = \epsilon_{ij} \left( \left( \frac{R_{min,ij}}{r_{ij}} \right)^{12} - 2 \left( \frac{R_{min,ij}}{r_{ij}} \right)^{6} \right) = \frac{A}{r_{ij}^{12}} - \frac{B}{r_{ij}^{6}}$$

where $\sigma$ is the distance to the point where the potential is zero, $\epsilon$ is the depth of the potential well and $R_{min}$ is the distance where the potential has its minimum.
It is common to use Lorentz-Berthelot combination rules to obtain the parameters between two atom types $i$ and $j$. $\sigma_{ij}$ is given as the arithmetic mean and $\varepsilon_{ij}$ as the geometric mean,

$$\sigma_{ij} = \frac{1}{2}(\sigma_i + \sigma_j)$$

$$\varepsilon_{ij} = \sqrt{\varepsilon_i \varepsilon_j}$$

The above rules are most effective when dealing with similar atoms, but has the disadvantage of overestimating $\varepsilon_{ij}$, giving the potential an exaggerated well depth that influence the interactions with other particles.

To change parameters from the first form of the Lennard-Jones potential to the second version, one can use the following relations,

$$\sigma_i = 2\left(\frac{R_{\text{min},i}}{2} \frac{1}{\sqrt{2}}\right)$$

$$R_{\text{min},ij} = \frac{R_{\text{min},i} + R_{\text{min},j}}{2}$$

Table 2 below summarizes all parameter sets used in this work, with their corresponding Lennard-Jones potentials in fig. 6. The implementation of the parameters in the CHARMM36 force field for proteins can be found in appendix A1.
Table 2. The implemented and used Lennard-Jones parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Co(II)</th>
<th>Zn(II)</th>
<th>Mg(II)</th>
<th>Ca(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set A</td>
<td>$\epsilon_{ii}$(kJ/mol)</td>
<td>1.8786</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\sigma_{ii}$(nm)</td>
<td></td>
<td>0.24006426</td>
<td></td>
</tr>
<tr>
<td>Set B</td>
<td>$\epsilon_{ii}$(kJ/mol)</td>
<td>1.046</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\sigma_{ii}$(nm)</td>
<td>0.194216</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Set C</td>
<td>$\epsilon_{ii}$(kJ/mol)</td>
<td>0.758</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\sigma_{ii}$(nm)</td>
<td>0.210056</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Set D</td>
<td>$\epsilon_{ii}$(kJ/mol)</td>
<td>0.001234</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\sigma_{ii}$(nm)</td>
<td>0.277</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Set E</td>
<td>$\epsilon_{ii}$(kJ/mol)</td>
<td></td>
<td></td>
<td>2.338830</td>
</tr>
<tr>
<td></td>
<td>$\sigma_{ii}$(nm)</td>
<td></td>
<td></td>
<td>0.226453</td>
</tr>
</tbody>
</table>


Figure 6. Lennard-Jones potentials for all implemented parameters.

Set A (Åqvist, 1990) was fitted against the solvation free energy and the right structural properties. The SPC (Berendsen, et al., 1981) water model was used. No consideration was taken to the dynamical properties of the ions.

The Zn(II) parameters in set B were derived by fitting an ab initio Zn(II)-water potential energy surface to obtain the position and depth of the Lennard-Jones potential. The parameters were then altered to capture the coordination numbers and distances to the first and second solvation shell in TIP3P (Jorgensen, et al., 1983) water. As with set A, dynamical properties were not considered in the derivation.

Set C parameters were derived by performing free energy perturbation simulations in TIP3P (Jorgensen, et al., 1983) water and calculating the solvation free energy relative to the Zn(II) parameters from Stote & Karplus (1995). The authors state that the limitations in their
parameters, that are of consequence for Co(II), is that they reflect only structural and thermodynamic properties of the ions. No dynamic processes have been taken into account, such a water exchange. A further consideration not stated by the authors is that the parameters have not been tested with any counter ions. Any shortcomings in the set B Zn(II) parameters are also likely to transfer to the set C Co(II) parameters.

Set D parameters were developed in TIP3P (Jorgensen, et al., 1983) water. They were derived by using the default CHARMM27 (MacKerell Jr., et al., 2001) Mg(II) parameters acted as a starting point, and was adjusted by altering the repulsive term of the Lennard-Jones potential. RDFs and PMFs were used to fit the parameters to capture both structural and dynamical characteristics of the ion. The solvation free energy relative to the default parameters were calculated as validation.

For set E, a three-pronged approach was used to derive the parameters in TIP3P (Jorgensen, et al., 1983) water, by reproducing the experimental solvation free energy, monitoring structural characteristics via the RDF and taking dynamical properties into account. The authors state that while it is possible to reproduce the correct solvation free energy and coordination number with the same parameters with ease, the limitation of the method lies in also acquiring the right distance to the first solvation shell.

Re-parametrisation approach

New Co(II) and Zn(II) parameters were derived accounting for structural, thermodynamic and kinetic properties using the strategy and the results of Mamakulov & Schwierz (2018), illustrated in fig. 7. The absolute solvation free energy was plotted against different values of the Lennard-Jones parameters $\sigma_{10}$ and $\varepsilon_{10}$, where O is the TIP3P (Jorgensen, et al., 1983) oxygen atom, with Cl(I) as counter ion.

Figure 7. Re-parametrisation approach. Parameter combinations were selected by choosing the Lennard-Jones parameters that reproduce the correct absolute solvation free energy of the ion. The graphs show the Lennard-Jones parameters plotted as a function of each other, as well as the absolute solvation free energy.

16
The accurate solvation free energies for both ions, that differ by only a few kJ/mol, should be found using three possible Lennard-Jones parameter combinations either directly or through adjustment. To convert the parameters to the Lennard-Jones parameters between two cations $ii$, the Lorentz-Berthelot combination rules were applied in conjunction with the Lennard-Jones parameters for the TIP3P (Jorgensen, et al., 1983) oxygen: $\sigma_0 = 0.315061 \text{ nm}, \epsilon_0 = 0.6364 \text{ kJ/mol}$.

This yielded three converted parameter combinations that in theory should reproduce the correct solvation free energy:

\[
\begin{align*}
\{ & \sigma_{ii} = 0.14939 \text{ nm} \\
& \epsilon_{ii} = 0.27718 \text{ kJ/mol} \\
& \sigma_{ii} = 0.20494 \text{ nm} \\
& \epsilon_{ii} = 0.07605 \text{ kJ/mol} \\
& \sigma_{ii} = 0.26493 \text{ nm} \\
& \epsilon_{ii} = 0.00063 \text{ kJ/mol}
\end{align*}
\]

All tried combinations can be found in the appendix.

The new parameters were then tested by studying the RDFs and calculating the water exchange. Set E parameters (Mamatkulov & Schwierz, 2018) were selected for the calculation of the relative solvation free energy as they were derived using the same water model of TIP3P (Jorgensen, et al., 1983) as in this work and because they also considered dynamical properties of the ions, in contrast to the set A (Åqvist, 1990) parameters.

## Protein modelling and simulation

### Homology modelling

structure as an initial model for molecular dynamics simulations, missing residues and amino acid side chains had to be added using homology modelling.

In homology modelling, one uses the primary structure of structurally determined protein as a template to deduce the unknown structure of a target protein. The method relies on the fact that the amino acid sequence of a protein decides its 3D structure. Furthermore, two sequences are almost guaranteed to fold into similar structures if the length of the sequences are long enough and the percentage of identical amino acids is sufficiently high (Krieger, et al., 2003). When performing homology modelling, it is also useful to consider the functions of both the target and template protein. As the 3D structure of a protein decides its function, and the structure is decided by its amino acid sequence (Alberts, et al., 2002), it is wise to choose a template that has similar biological function as the target.

The Protein Data Bank (PDB) (Berman, et al., 2000) houses information about experimentally determined protein structures. For an unknown structure with sequence A (the target), the first step in homology modelling is to compare A with all other sequences in the PDB. The comparison itself can be done using, for example, the Basic Local Alignment Search Tool
(BLAST) (Altschul, et al., 1990) that provides the stored sequences that aligns with A with varied percentages. The modeler then chooses a sequence B (the template) that aligns with A based on the alignment percentage and other variables. The model of A is then found by mutating the residues that differ between A and B and validated by investigating if bond lengths and angles are within reason and the energy of the model (Krieger, et al., 2003).

Homology modelling is a multi-step process that requires evaluation and decisions at each stage. One can never be certain that the correct choices are made between many options that can appear to be almost indistinguishable, meaning that one can never be certain that the arrived at model is true (Krieger, et al., 2003). Furthermore, there are many error sources in homology modelling stemming from a low alignment match between the target and the template (Sánchez & Sali, 1997) one has to consider. The accuracy of the homology model can be assessed by structural comparison with the experimentally known structure. This is usually done by calculating the Root Mean Square Deviation (RMSD) between the homology model and the experimental structure, superimposed on each other, and the number of structurally aligned residues (Zemla, 2003).

In this thesis, the homology modelling was performed using the software Modeller (Eswar, et al., 2006). Structural comparison between models was done using Chimera (Pettersen, et al., 2004) and the built-in function MatchMaker (Meng, et al., 2006). The homology modelling was

Five homolog models were produced using Modeller. The model chosen for further analysis was selected by its Discrete Optimized Protein Energy (DOPE) (Shen & Sali, 2006) number, which was the lowest between all five models.

**Protein membrane insertion**

Protein structures found by experimental means, such as X-ray crystallography, might contain low resolution information about a few of the membrane lipids surrounding the protein but not about the complete lipid bilayer environment. This poses a problem as studies have found that the lipid molecules take part in the regulation of the structure and function of membrane proteins (Lee, 2009). To capture this effect, and also to assure that the protein is in an environment similar to its native one, protein structures that are to be simulated through MD must be inserted into a membrane with a suitable lipid composition.

To simulate a membrane protein, the first step is to orient the protein in the membrane to find its correct position (Stansfeld & Sansom, 2011). One way to do this is by using a tool such as the Orientation of Proteins in Membranes (OPM) database (Lomize, et al., 2012) that provides information about the residues that likely are embedded in the membrane. These residues can then be used as a target for the protein position, in conjunction with comparison with the known position in the membrane of other proteins with similar structure and function.
embedded in a membrane using CHARMM-GUI (Jo, et al., 2008). The system was set to a size of 120 Å and the membrane composition 3:1 POPE and POPG. A standard concentration of 0.15 M NaCl was used, together with a water thickness of 30 Å. The final system contained 342 POPE chains, 114 POPG chains, 61087 TIP3P (Jorgensen, et al., 1983) water molecules, 298 Na(I) and 166 Cl(I).

Simulation set up for protein-membrane equilibration
The system was equilibrated using the output files from CHARMM-GUI (Jo, et al., 2008), consisting of energy minimization, six rounds of equilibration and a production run.

The energy minimization was performed for 5600 steps using the steepest descent method. This algorithm works by slowly shifting the coordinates of the atoms to move the system closer and closer to the minimum point by moving along the gradient of the system using line search and Lagrange multipliers (Leach, 2001). Two rounds of NVT-equilibrations were done for 25 ps using a time step of 1 fs. The Berendsen thermostat was used to equilibrate the temperature to 310 K. As the pressure is harder to equilibrate, four rounds of NTP equilibrations were completed using the Berendsen barostat set to 1 atm. The simulation time was 25 ps for the first and second rounds, with a time step of 1 fs and 2 fs respectively. Simulation time and the time step was equal in equilibrations two and three. The fourth round of equilibration had a time step of 2 fs and 40 ns of simulation time. The production run was simulated for 46 ns, using the Nosé-Hoover thermostat and Parrinello-Rahman barostat.

POPE, POPG and the protein was constrained initially and then fully released during the last equilibrations. Particle Mesh Ewald (PME) and a cut-off distance of 1.2 Å was used for all simulations. The progress of the equilibration and production run was monitored by following the temperature, pressure and potential of the system. In the production run, the Root Mean Square Deviation (RMSD) of the protein was used to analyze if the system was sufficiently equilibrated. Successful equilibration means that pressure and temperature has converged to the set values, the potential of the system is negative and stable and the RMSD of the protein has plateaued.
3. Structural, thermodynamic and kinetic characterization of Co(II) and Zn(II) in aqueous solution

Babu & Lim (2005) and Stote & Karplus (1995) parameters

Structural characteristics
Two 0.1 M ZnCl₂ and CoCl₂ solution simulations have been used to investigate the structural properties of the ion-water system using set B (Stote & Karplus, 1995) for Zn(II) and set C (Babu & Lim, 2005) for Co(II) as parameters. Radial Distribution Functions (RDFs) were calculated to examine the solvated structure of the two ion species, in terms of coordination numbers (CN) and distances to the first solvation shell.

![Radial Distribution Function g(r)](image)

Figure 8 and 9. Radial distribution functions for Zn(II)-Ow and Co(II)-Ow, their solvation shells and coordination numbers.

As seen in fig. 8 and 9, the coordination numbers are 6 for both ion species with similar distances to the first hydration shells; 2.12 Å for Co(II) and 2.08 Å for Zn(II). The coordination numbers are in agreement with experimental values and the distances lie within the experimental ranges for both ions, summarized in table 3.

Table 3. Experimental structural properties of Co(II) and Zn(II) obtained by X-ray diffraction and EXAFS data.

<table>
<thead>
<tr>
<th>Ion</th>
<th>M(II)</th>
<th>CN¹</th>
<th>d(M-Ow)¹ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co</td>
<td>6</td>
<td>0.208-0.212</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>6</td>
<td>0.205</td>
<td></td>
</tr>
</tbody>
</table>

¹Ohtaki & Radnai (1993)
Fig. 10 shows that the chloride ions are moving around somewhat freely, seen by the blurriness of the lines, there is also some unexpected ordering. Furthermore, it appears that there is a chloride in the second solvation shell of both ions.

**Ion-water exchange**

Two approaches have been implemented to calculate the water exchange. In the first approach, the water exchange was calculated by tracking the coordination number of the ions as a function of time. An exchange event was defined when the distance to the first solvation shell crossed a threshold distance, set to 2.6 Å for Co(II) and 2.5 Å for Zn(II). The number of events divided by the total simulation time and the number of water molecules in the coordination shell, gives the exchange rate of one water molecule.

The Co(II) parameters exhibit exchange with a rate constant $k$ in the order of magnitude of $10^9$ s$^{-1}$ and a residence time $\tau$ of $10^{-9}$ s. The set B parameters (Stote & Karplus, 1995) show an exchange on the order of $10^7$ s$^{-1}$ with a residence time of $10^{-7}$ s. Exchange rates for both ion species differ from the experimental values summarized in table 4. The set C parameters (Babu
& Lim, 2005) overestimates the rate constant with a factor of 10^3 whereas the set B Zn(II) parameters (Stote & Karplus, 1995) slightly underestimates the exchange.

Table 4. Experimental values for the exchange rate and residence time between water and Co(II) and Zn(II).

<table>
<thead>
<tr>
<th>Ion M(II)</th>
<th>k (s^-1)^1,2</th>
<th>τ (s)^1,2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co</td>
<td>1.1 × 10^6</td>
<td>8.8 × 10^-7</td>
</tr>
<tr>
<td>Zn</td>
<td>&gt; 10^7</td>
<td>&lt; 10^-7</td>
</tr>
</tbody>
</table>


In the second approach, the exchange was calculated by inverting the RDFs for Co(II)-Ow and Zn(II)-Ow to obtain the Potential of Mean Force (PMF) for both ion species. From the PMF, the energy barrier between the metal ion and the oxygen of water can be found, which can then be used to calculate the exchange rate and residence time of the water molecules.

![PMF graphs](image)

Figure 13 and 14. The PMFs between the metal ion and the oxygen atom obtained by RDF inversion. Zn(II) left, Co(II) right.

After obtaining the PMFs, values of kinetic constants were calculated and are summarized in table 5.

Table 5. Calculated values for the energy barrier, exchange rate and residence time of ion-water contact, by RDF inversion.

<table>
<thead>
<tr>
<th>Ion M(II)</th>
<th>ΔG^+ (kJ/mol)</th>
<th>k (s^-1)</th>
<th>τ (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>∼ 13</td>
<td>∼ 1.52 × 10^{13}</td>
<td>∼ 6.58 × 10^{-14}</td>
</tr>
<tr>
<td>Co</td>
<td>∼ 17</td>
<td>∼ 3.00 × 10^{12}</td>
<td>∼ 3.3 × 10^{-13}</td>
</tr>
</tbody>
</table>

The simulation, sampling was insufficient to produce reliable PMFs. The lack of sampling caused certain points of the RDF to be zero, making the corresponding points in the PMFs approach infinity as a result of the relation between the RDF and PMF. As a consequence, the approach did not yield relevant or accurate values of the investigated constants; calculated values are roughly double that of experimental ones.
**Thermodynamic characteristics**

Thermodynamic characteristics are captured in the form of the solvation free energy. For both Co(II) and Zn(II), using set B (Stote & Karplus, 1995) and set C (Babu & Lim, 2005) parameters, the relative solvation free energy, $\Delta G_{rel.}$ was calculated relative to Ca(II) set A (Äqvist, 1990) and set E (Mamatkulov & Schwierz, 2018) and Mg(II) set D (Allnér, et al., 2012). The calculations were performed using thermodynamic perturbation and a total of 11 lambda points.

The results presented in table 6 shows that the set B Zn(II) (Stote & Karplus, 1995) and set C Co(II) (Babu & Lim, 2005) parameters were unable to reproduce experimental relative solvation free energies relative to set D Mg(II) parameters parameters (Allnér, et al., 2012). The experimental trend between Co(II) and Zn(II), however, is conserved as the relative solvation free energy for Co(II) is more favorable than for Zn(II).

<table>
<thead>
<tr>
<th>$M(II) \rightarrow N(II)$</th>
<th>Parameters</th>
<th>$\Delta G_{rel.}$ (kJ/mol)</th>
<th>$\Delta G_{rel.experimental}$ (kJ/mol)$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn → Ca</td>
<td>Set A</td>
<td>194.61±0.59</td>
<td>408.36</td>
</tr>
<tr>
<td></td>
<td>Set E</td>
<td>164.75±0.83</td>
<td></td>
</tr>
<tr>
<td>Zn → Mg</td>
<td>Set D</td>
<td>27.90±0.08</td>
<td>125.10</td>
</tr>
<tr>
<td>Co → Ca</td>
<td>Set A</td>
<td>155.17±0.82</td>
<td>410.00</td>
</tr>
<tr>
<td></td>
<td>Set E</td>
<td>122.44±0.88</td>
<td></td>
</tr>
<tr>
<td>Co → Mg</td>
<td>Set D</td>
<td>-69.41±0.08</td>
<td>84.93</td>
</tr>
</tbody>
</table>

$^1$Marcus (1994).

**Discussion**

Set B Zn(II) (Stote & Karplus, 1995) and set C Co(II) (Babu & Lim, 2005) parameters reproduce correct coordination numbers and distances to the first solvation shell for both ion species, as seen in the RDFs.

Attempting to obtain the water exchange by means of Radial Distribution Function (RDF) inversion proved to be ill-advised due to the inadequate sampling not unusually seen in the case of liquids. The insufficient sampling caused the calculated kinetic constants to be almost double that of experimental values. As the method did not perform well for the set B (Stote & Karplus, 1995) and set C (Babu & Lim, 2005) parameters, the procedure was not performed for the newly derived ion parameters and the exchange was studied by tracking the coordination number over time.

Doing so showed that the the set B (Stote & Karplus, 1995) parameters for zinc underestimates the water exchange rate constant. This means that the energy barrier for the parameter set is larger compared to experimental data, indicating that interactions with set B Zn(II) will be stronger than expected and that the mean residence time of ion-water contact is exaggerated. Indeed, this might serve as an explanation concerning the clustering of counter ions. The set B Zn(II) parameters will likely hold strong interactions with water, counter ions and other molecules. The set C (Babu & Lim, 2005) Co(II) parameters overestimates the exchange rate, suggesting that the parameters reproduce an energy barrier smaller than that reported by experimental techniques, resulting in faster water exchange.
When calculating the solvation free energy relative to set D Mg(II) (Allnér, et al., 2012) parameters, not only did the values not agree with the experimental trend they were also off by almost 100 kJ/mol for Zn(II) and 150 kJ/mol for Co(II). The discrepancy between the relative solvation free energy trend in this work compared to the experimental trend can be attributed in some part to the set B parameters (Stote & Karplus, 1995). These parameters reproduce a solvation free energy of Zn(II) that is less favorable than the solvation free energy of Mg(II) suggesting that the trend in this work will be inherently wrong depending on set B parameters. As the set C (Babu & Lim, 2005) parameters were derived using set B parameters to calculate the relative solvation free energy, it is clear that the shortcomings of the set B parameters, when it comes to reproduce solvation free energy, will also transfer to the set C parameters.

Two different ion species were selected for calculation of the relative solvation free energy: Mg(II) and Ca(II), where the latter was used for the final calculations. This is because of the difficulties surrounding Mg(II), which is a difficult ion to parameterize because of its strong interactions with water and slow water exchange (Allnér, et al., 2012). Further, its small size means that more complex effects must be taken into account. Thus, it is not trivial to obtain parameters that work well for this particular cation, meaning that calculation of solvation free energy relative to Mg(II) can pose problems on different fronts. For this reason, the relative solvation free energy calculations were also done with Ca(II), using two different sets of parameters. The relative solvation free energy calculations using Ca(II) instead of Mg(II) should be less complicated as Ca(II) is of similar size to both Zn(II) and Co(II) while having interactions of less noteworthy size. As seen by the Lennard-Jones potential for Ca(II), the well depth indicating the strength of interaction with water is significantly less deep than the potential for Mg(II).

However, neither the Zn(II) nor Co(II) parameter sets reproduce the right relative solvation free energy in conjunction with the set A (Åqvist, 1990) and set E (Mamatkulov & Schweirz, 2018) parameters. As both sets A and E were parameterized to reproduce the experimental Ca(II) solvation free energy, the inability to produce the correct relative solvation free energy likely lies in some deficiency in the Zn(II) and Co(II) parameters.

An explanation for the shortcomings of the set B Zn(II) (Stote & Karplus, 1995) and set C Co(II) (Babu & Lim, 2005) parameters could be that neither are parameterized using counter ions. Further, no particular effort was taken to capture the dynamical properties of the ions in the form of water exchange. Although both sets do display exchange, it is either exaggerated or understated. This would suggest that although the parameters work reasonably well for themselves, they do not perform well in the presence of other ions. This can pose a problem when simulating complex biological systems such as proteins, where there are many different species of ions and molecules present.
New parameters

Parametrization

The new set of parameters for Zn(II) and Co(II) were derived by altering the Lennard-Jones potential to find solvation free energies relative to Ca(II), for both ion species, that are similar to experimental values. 60 relative solvation free energy calculations were performed to find the new parameters, summarized in the appendix. The parameters were further adjusted to capture structural and kinetic characteristics, in the form of coordination numbers (CN), distances to the first solvation shell and water exchange. Table 7 shows the new sets of parameters and fig. 15 their Lennard-Jones potentials.

Table 7. The newly derived set of parameters for Zn(II) and Co(II).

<table>
<thead>
<tr>
<th>Ion M(II)</th>
<th>$\varepsilon_{ii}$ (kJ/mol)</th>
<th>$\sigma_{ii}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn</td>
<td>0.25</td>
<td>0.14600</td>
</tr>
<tr>
<td>Co</td>
<td>0.25</td>
<td>0.14325</td>
</tr>
</tbody>
</table>

Figure 15. The Lennard-Jones potential for the new set of Co(II) and Zn(II) parameters, as presented in table 7.

Thermodynamic characteristics

The relative solvation free energy for the newly derived parameters for Co(II) and Zn(II), summarized in table 10, were calculated relative to set E Ca(II) (Mamatkulov & Schwierz, 2018) parameters, using thermodynamic perturbation with a total of 11 lambda points.

Table 10. Calculated relative solvation free energies for the new sets of parameters.

<table>
<thead>
<tr>
<th>M(II) $\rightarrow$ N(II)</th>
<th>$\Delta G_{rel}$ (kJ/mol)</th>
<th>$\Delta G_{rel,experimental}$ (kJ/mol)$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn $\rightarrow$ Ca</td>
<td>405.20±0.47</td>
<td>408.36</td>
</tr>
<tr>
<td>Co $\rightarrow$ Ca</td>
<td>410.75±0.95</td>
<td>410.00</td>
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</table>

$^1$Marcus (1994)

Calculated relative solvation free energies in this work differ from experimental values approximately 1 % for Zn(II) and 0.04 % for Co(II).
Structural characteristics
Two 0.1 M ZnCl₂ and CoCl₂ solution simulations have been used to investigate the structural characteristics displayed by the new sets of parameters. RDFs were calculated to examine the solvated structure of the two ion species, in terms of coordination numbers and distances to the first solvation shell.

![Radial Distribution Function g(r)](image)

Figure 16 and 17. RDFs for Zn(II)-Ow and Co(II)-Ow, coordination numbers and first and second solvation shells.

The newly derived set of parameters display the experimentally determined coordination number of six for both ions. For the new set of parameters, the distances are 0.188 nm for Zn(II) and 0.190 nm for Co(II). Compared to structural characteristics determined by experimental methods, found in table 8, the distances are off by approximately 10 % for both ions.

Table 8. Experimental structural properties of Co(II) and Zn(II) obtained by X-ray diffraction and EXAFS data.

<table>
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<th>Ion M(II)</th>
<th>CN¹</th>
<th>d(M-Ow) nm</th>
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<tr>
<td>Co</td>
<td>6</td>
<td>0.208-0.212</td>
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<tr>
<td>Zn</td>
<td>6</td>
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</table>

¹Ohtaki & Radaii (1993)

Ion-water exchange
The water exchange was calculated by tracking the coordination number of the ions as a function of time. An exchange event was defined when the distance to the first solvation shell crossed a threshold distance, set to 2.6 Å for Co(II) and 2.5 Å for Zn(II). The number of events divided by the total simulation time and the number of water molecules in the coordination shell, gives the exchange rate of one water molecule.
Figure 18 and 19. Changes in coordination number as a function of time for Co(II) and Zn(II) respectively.

The rate constant $k$ and the mean residence time of water-ion contact for the new set of parameters was calculated using the figures above and are summarized in table 9.

Table 9. Calculated rate constants and mean residence times of water-ion contact for the new set of parameters.

<table>
<thead>
<tr>
<th>Ion</th>
<th>$k$ (s$^{-1}$)</th>
<th>$\tau$ (s)</th>
<th>$k_{experimental}$ (s$^{-1}$)$^{1,2}$</th>
<th>$\tau_{experimental}$ (s)$^{1,2}$</th>
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<td>$2.6 \times 10^7$</td>
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<td>$&gt; 10^7$</td>
<td>$&lt; 10^{-7}$</td>
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$^{1}$Ohtaki & Radnai (1993), $^{2}$Helm & Merbach (2005).

For the newly derived parameters, the exchange rates, rate constants and mean residence times are of the same order of magnitude as experimental values indicating that they capture the dynamic properties of the ions well.

Discussion
The re-parametrization reproduces the correct relative solvation free energy and coordination number. The distances to the first solvation shell are off by 10% for Co(II) and 8% for Zn(II). Some of this error can of course be attributed to the parametrization approach itself: by examining the derived parameters for other ion types by Mamakulov & Schwierz (2018), an error is found for the distance to the first solvation shell for the majority of the ions, which then should be expected for other parameters derived by the same method. As stated in the methods section, the authors themselves highlights this as the major limitation to their method.

The relative solvation free energy differs from experimental values by roughly 1% and 0.04% for Zn(II) and Co(II) respectively, which is well within the acceptable range. Dynamic properties of the ions are also reflected well in the new set of parameters, that exhibit a water exchange of the same order of magnitude for both ion species.

Further testing and optimization of the parameters can be undertaken to perhaps improve the distances to the first solvation shell, resulting in more efficient parameters. However, as the new sets of parameters capture the kinetic and thermodynamic properties of the ions well, and the structural characteristics within reason, they can pave the way for more accurate simulations in which Co(II) and Zn(II) ions are present.
4. Protein modelling and simulation

Homology modelling and insertion into membrane

conjunction with data from the Orientation of Proteins (OPM) database (Lomize, et al., 2012). As seen in fig. 20 the transmembrane residues of the calculated by the OPM database agree with the OPM predicted transmembrane residues of

While the transmembrane residues suggested by the OPM database for realistic when compared to homolog, there is a hydrophobic mismatch between the hydrophobic thickness of the lipid types used in the membrane (28.3 Å), and the predicted hydrophobic thickness of calculated by the OPM database (Lomize, et al., 2012). Due to this, a part of non-transmembrane residues of were inserted into the membrane which resulted in an aspartic acid residue being positioned in the outskirts of the lipid bilayer.
Equilibration

The protein-membrane system was equilibrated using equilibration output files generated by CHARMM-GUI (Jo, et al., 2008). The system underwent energy minimization, two rounds of NVT-equilibrations, four rounds of NTP-equilibrations and a production run.

![Graphs showing temperature and pressure over time](image)

Figure 22. The temperature and pressure of the protein-membrane system after equilibration.

As seen in fig. 22 above, the temperature and pressure is equilibrated to set values. As expected, the fluctuations in the pressure is far greater than the fluctuations in temperature.
Figure 23. The potential energy of the system after production run, and the RMSD of the protein backbone.

The potential energy of the system is negative and converged, indicating that the system is stable. The RMSD however has not yet plateaued (fig. 23), suggesting that the system could benefit from more simulation time.

The difference in hydrophobic depth between the lipids and the protein, suggests that some rearrangement of the lipids can be expected. As seen in fig. 24, there does seem to be some shift of the lipids as to accommodate the hydrophobic mismatch and the aspartic acid residue inserted into the membrane as a result of it.
The protein-membrane system is equilibrated when considering temperature, pressure and potential. However, the RMSD of the protein suggests that the system could benefit from more simulation time.

As a result of the hydrophobic mismatch between the bilayer thickness and the protein, an aspartic acid residue was inserted into the membrane. Because of hydrophilic nature of aspartic acid, the energy penalty for placing such a residue in a hydrophobic environment is large and the amino acid is typically not found embedded in membranes (Barnes, 2007).

However, there is some experimental evidence of hydrophilic residues, such as arginine, in the lipid bilayer. The effect that follows is that the membrane is considerably deformed to accommodate for the arginine, by maintaining strong interactions between the arginine, water and lipid head groups (Hristova & Wimley, 2011). There specifically are indications that arginine can be inserted into the membrane for proteins in the form of ion channels and voltage gated ion channels (Schow, et al., 2011). The simulated protein-membrane system does indeed show some evidence of membrane deformation around the site of the aspartic acid residue. One can speculate that a longer simulation, or using a larger membrane, would make the deformation effect more prominent. Another explanation for the deformation could be that an aspartic acid residue in an hydrophobic environment can go from a deprotonated state to a protonated one. This can cause issues in the simulation as there is no parametrization for a protonated state aspartic acid residue in the commonly used force fields.
Conclusion

Investigation and evaluation of the structural, kinetic and thermodynamic characteristics of the set B Zn(II) (Stote & Karplus, 1995) and set C Co(II) (Babu & Lim, 2005) parameters have shown that both parameter sets have deficiencies when considering kinetic and thermodynamic properties. Both sets capture experimental structural properties of both ion species, but neither set can reproduce correct solvation free energies relative to set A Ca(II) (Äqvist, 1990), set E Ca(II) (Mamatkulov & Schwierz, 2018) or set D Mg(II) (Allnér, et al., 2012) parameters. Further, the set B (Stote & Karplus, 1995) parameters overestimate the kinetic characteristics by a factor of $10^3$ s$^{-1}$, whereas the set C (Babu & Lim, 2005) parameters underestimates by a factor of roughly 10 s$^{-1}$. These faults in both parameter sets can be explained by the lack of testing with other ions, and that less thorough was put into capturing thermodynamic and kinetic properties. This mean that there will be issues when conducting simulations together with other types of ions.

The newly derived set of parameters for Co(II) and Zn(II) capture experimental kinetic and thermodynamic properties, as well as most of the structural characteristics. One shortage of the parameters is that they do not fully capture the experimental distances to the first hydration shells. This can be explained by the method used to derive the parameters, published by Mamatkulov & Schwierz (2018). As the new parameters have been tested with counter ions, it is plausible to expect them to perform better than set B (Stote & Karplus, 1995) and set C (Babu & Lim, 2005) in the presence of other ion species.

The protein-membrane system is equilibrated when considering temperature, pressure and potential. The RMSD of the protein suggests more simulation time could be necessary. A number of aligned residues. Because of the lipid types used, and the hydrophobic thickness of the protein, a hydrophobic mismatch was inevitable when the protein was inserted into the membrane. This caused an aspartic acid residue to be embedded into the membrane. The simulation showed some evidence for lipid deformation, possibly to accommodate for the hydrophobic mismatch and the aspartic acid residue.

Future work

In future work, further testing and adjustment of the new parameters could lead to a distance to the first hydration shells that is closer to experimental values. The parameters could then be
Bibliography


Jo, S., Kim, T., Iyer, V. & Im, W., 2008. CHARMM-GUI: a web-based graphical user


Appendix

A.1

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Figure 1. Implementation of all sets of parameters in GROMACS used in this work in the nonbonded.itp file of the CHARMM36 force field for proteins.

A.2

Table 1. Summary of all tested combinations of Lennard-Jones parameters and their corresponding relative solvation free energy, relative to Ca(II) set E (Mamatkulov & Schwierz, 2018).

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<tr>
<th>$\varepsilon$ (kJ/mol)</th>
<th>$\sigma$ (nm)</th>
<th>$\Delta G_{rel}$ (kJ/mol)</th>
<th>$\lambda$ points</th>
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