Simulation of transport through OmpF and OmpC channels

by

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The development and progress of a nation is according to the measure and degree of that nation’s scientific attainments. Through this means its greatness is continually increased, and day by day the welfare and prosperity of its people are assured.

Abdu’l-Bahá
Abstract

The outer membrane porins F and C (OmpF and OmpC) are major pores in the cell membrane of the Gram-negative bacterium *Escherichia coli*. They are considered the main pathways for ions and molecules through the membrane. Using the crystal structures, it is possible to study OmpF and OmpC in computer simulations. In this thesis, the ion conductance through these nano pores is simulated in all-atom molecular dynamics. Although the amino acid sequences of both pores are similar, their conductance is different. The temperature dependence of the conductance is calculated for different salt concentrations. Good agreement is seen in the comparison between simulations and experiments. The advantage of molecular dynamics simulations is that they allow a deeper view on the molecular interaction leading to the macroscopic observation. Ion pathways can be followed, and the interaction of ions with certain residues can be observed. Both pores, OmpF and OmpC, have a charged constricted area in the middle of the pore. The behavior of the pore can be changed by mutating key residues in this constriction zone. The effect of mutations on the transport of ions is investigated and compared to the results obtained from wild type OmpF. Also here, experimental measurements show good agreement with the simulations.

Furthermore, porins are the main pathways of antibiotics into the cell. The translocation of antibiotics through membrane is not yet understood in all details. During translocation, a blocking of pores through antibiotics is observed. Here, blocking of porins by the beta lactam antibiotic ampicillin is simulated, as well as, translocation of ampicillin through OmpF.
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Chapter 1

Introduction

Biological cells have a clear boundary of their environment. This boundary is built up by the cell membrane, which is the major protection barrier from the environment. The osmotic pressure in the inner and outer part of the cell is preserved by the cell membranes [1]. All interactions of a cell with its environment occur through the membrane. Therefore, membranes are of high interest in research [2–4]. The main pathways for molecules and ions through the membrane are pores and carrier proteins. Pores are transmembrane proteins which act as channel. They are passive channels, i.e., they only allow transport down the concentration gradient in contrast to many carrier proteins [1,5].

Drugs have to pass the cell membrane to have an influence on the cells [6,7]. One basic strategy of drug resistance is to prevent drugs entering the cell [1] or staying in the cell. This can be achieved by actively extruding drugs out of the cell [8] or by blocking the entrance of drugs into the cell through alteration of the membrane permeability [9]. Drugs can be actively extruded, e.g., by increasing the number of carrier proteins that pumps drug out of the cell. The blocking of the entrance of drugs can be achieved, e.g., by altering pores so that drugs cannot translocate anymore through them. The increasing drug resistance leads to the necessity for new drugs [10,11]. Predictions show that without new antibiotics, soon there will be no effective treatment against multi drug resistant bacteria and that infections cannot become treatable again, as in the preantibiotic area [12,13]. The knowledge of the interactions with the membrane during translocation is crucial for new drug design [14]. The experiments and simulations of the passage of ions through the pore help to get a deeper understanding of the translocation mechanism. One important property of pores is the ion conductance which can be determined in black
lipid membrane (BLM) experiments and molecular dynamic (MD) simulations. The ion conductance of the pores can be used to indirectly detect antibiotic translocation [15] or as a probe for the opening degree of channels, such as TolC [16–18].

This thesis show the results of ion conductance and antibiotic translocation and blocking simulations of two pores OmpF and OmpC and is structured as follows: In the next chapter, a general presentation of methods to determine the ion current is given. MD simulation, which are used in this thesis, but also other methods, which are used by various groups to simulate ion conductance, are presented. The method how to calculate the ion current from the simulation results is shown as well. Also, the experimental BLM method is presented, which is used by our collaborators to measure ion currents and conductances.

A general introduction into biological channels is presented in chapter 3 before an overview of the two pores used in this work, OmpF and OmpC, is presented. The setup of the system for the simulations is shown as well. After these two introductory chapters, the obtained results will be presented in the followed chapters. The results of bulk water simulations are presented in chapter 4. Bulk water simulations are simple simulations which allow to conclude on the limits of the simulations. Here, bulk water simulations are also used as a benchmark for the conductance. In chapter 5, the ion conductance of wild type (WT) OmpF and its mutants are shown in experiments and simulations. The reported atomistic details provide a view on aspects which are not accessible in experiments. The OmpC ion conductance is presented in chapter 6. In addition, comparisons of the OmpC and OmpF results are shown, which highlight main differences and similarities. The simulation of antibiotic translocation through OmpF and pore blocking by antibiotics are presented in chapter 7. At the end, the main conclusions and outlooks are presented in chapter 8. Additional work performed during the PhD phase is shown in the appendix.
Chapter 2

Methods

This chapter introduces the methods employed in this work and other common methods for the calculation of ion conductance. The first two sections present continuum models and Brownian dynamics (BD), two common methods to simulate ion conductance through channels used by other research groups. In this work MD simulations are used. An introduction to MD simulations is presented in section 3. In section 4, the methods to calculate the current are presented. To complete the introduction, the experimental methods are also shown at the end of this chapter.

2.1 Continuum Methods

Continuum calculations are methods that can be used in a wide range of simulations. The main concept is to approximate the simulated system by a continuum with specified properties. This yields a system with a few continues elements, instead of a high number of single atoms. A decrease in the computational need is the effect of this approximation on one side. On the other side, it is not possible to obtain details on molecular level - only information of the continuum is provided.

In the field of ion transport through a channel, one can use the Nernst-Planck equation \[ J_\alpha(R) = -D_\alpha(R) \left( \nabla C_\alpha(R) + \frac{C_\alpha(R)}{k_B T} \nabla V^\text{eff}_\alpha(R) \right) \] (2.1)

with the flux density \( J_\alpha \), the position \( R \), the concentration \( C_\alpha \), and the effective potential \( V^\text{eff}_\alpha \) of the ion type \( \alpha \). For the case of equilibrium without net flux
CHAPTER 2. METHODS

One can use the non-linear Poisson-Boltzmann continuum theory \[20,21\]

\[
\nabla[\epsilon(R)\nabla\phi(R)] = -4\pi \left( \rho_P(R) + \sum_{\alpha} C_{\alpha}^{bulk} e^{-U_{core}(R)/k_BT} e^{-q_{\alpha}\phi(R)/k_BT} \right) \tag{2.2}
\]

with the space dependent dielectric constant \(\epsilon(R)\), the charge density of the channel \(\rho_P(R)\), the ion charge \(q_{\alpha}\), the bulk concentration of the ion \(C_{\alpha}^{bulk}\), the electrostatic potential \(\phi(R)\), and the repulsive core potential \(U_{core}(R)\). The Poisson-Boltzmann equation is a special case of the Poisson equation

\[
\nabla[\epsilon(R)\nabla\phi(R)] = -4\pi \left( \rho_P(R) + \sum_{\alpha} C_{\alpha}(R) \right) \tag{2.3}
\]

with the concentration \(C_{\alpha}(R)\). The ion current is calculated by solving the two coupled differential equations, the Poisson equation Eq. 2.3 and the Nernst-Planck equation Eq. 2.1, in a self-consistent manner \[22\]. This mean-field theory is called Poisson-Nerst-Planck (PNP).

The parameters for the calculations are obtained empirically. Ions and water are treated as continuous material. Also, the channel is approximated in form and charge distribution. These approximation do not allow to analyze the movement of single ions and molecules. Therefore, this method is of limited use to get a deeper understanding of antibiotics interaction with channels, but also to take a deeper look of the ion interaction with the channel.

Im and Roux \[23\] and Miedema et al. \[24,25\], for example, have performed PNP simulations to calculate ion currents through membrane.

2.2 Brownian Dynamics

In BD simulations not every atom is treated as a continuum as considered in the previous model. The equation of motion in a BD simulation is obtained from the Langevin equation \[26\]

\[
m_i \frac{d^2}{dt^2} R_i(t) = F_i(t, R) - m_i\gamma_i \dot{R}_i(t) - \xi_i(t) \tag{2.4}
\]

with the stochastic or random forces \(\xi_i\), the systematic forces \(F_i\) and the friction forces \(m_i\gamma_i \dot{R}_i\). \(m_i\) is the mass, \(\dot{R}_i(t)\) the velocity of the \(i\)th particle and \(\gamma_i\) the reciprocal of the relaxation time of the system. In this approach the electrostatic field \(q_i\mathbf{E}(R)\) with the charge of the ion \(q_i\) and the electric field \(\mathbf{E}\)
2.3. MOLECULAR DYNAMIC SIMULATIONS

are included in the systematic force $F_i$. The electric field is calculated by the Poisson equation (Eq. 2.3). The interaction with water is not included in the systematic, but in the friction and stochastic force. These two are connected with each other via the fluctuation-dissipation theorem [27]

$$m_i \gamma_i = \frac{1}{2k_BT} \int_{-\infty}^{\infty} \langle \xi_i(0) \xi_i(t) \rangle \, dt$$  \hspace{1cm} (2.5)

with the temperature $T$, the Boltzmann constant $k_B$ and the average of the force $\langle \xi_i(0) \xi_i(t) \rangle$. As one can see, the BD approach is not a pure deterministic method. It also contains stochastic parts. In the case of ion conductance through channels the ions and channel are calculated explicitly while the solvent is calculated implicitly with random forces and free energy profiles [23]. One suggested algorithm for calculating velocity and position of ions for one time step is the following [27,28]:

1. Calculate the electrostatic potential acting on the ion by solving the Poisson equation (Eq. 2.3).

2. With the result the systematic force $F_i$ and the time derivative of the systematic force can be calculated.

3. Sample the random force $\xi_i$.

4. The position and the velocity of the ion is calculated by solving the Langevin equation (Eq. 2.4).

These steps are repeated for all ions and for the required simulation time. Im et al. [29] employed a Grand Canonical Monte Carlo BD, where ions are destroyed and created during the simulation. BD simulations for calculating ion currents have been performed, e.g., by Allen et al. [26], by Im and Roux [23], and by Phale and group members [30,31].

2.3 Molecular Dynamic Simulations

Classical MD simulations are calculations, used to simulate mainly solid state, liquids, and biological systems. They are based on Newton’s equation of motion

$$m_i \frac{d^2 \mathbf{R}_i(t)}{dt^2} = F_i(t) = -\nabla V(\mathbf{R}_i(t))$$  \hspace{1cm} (2.6)
CHAPTER 2. METHODS

with the force $F_i$ and the potential $V$ of the atom $i$ \[32\]. Compared to
continuum simulations like Poisson-Nernst-Planck theory and to BD, one obtains
more details, e.g., the trajectory of each atom can be observed using all-atom
MD. The main disadvantage is the higher computational cost which make sim-
ulations of larger systems and for longer time scales not feasible compared to
the previous models. That is why MD simulations have stricter limits com-
pared to the continuum modeling and to BD. BD simulations are 4 to 5 orders
of magnitude faster than all-atom MD simulations \[33\]. Usual MD simulation
times are 10-100 ns. The limit nowadays is in the 10 $\mu$s area \[34\] for small
systems. For comparison in Fig. 2.1 the timescale of some processes in the
molecular biology is shown. The number of particles is limited to few mil-
ions of atoms \[35\] nowadays. One way to speed up simulations is to decrease
the number of degrees of freedom \[36\]. This approach is called coarse-grained
MD \[37\].

The initial positions of the atoms can be obtained by X-ray or nuclear mag-
netic resonance crystallography, but also simulations with other placement of
atoms are possible. Initial velocities can be distributed randomly on a Maxwell
distribution. The calculation of the forces and the integration of the equation
of motion of the atoms are deterministic. Damping process, e.g., for the pres-
sure conservation and the temperature thermostat, include random factors.
In NpT ensembles (constant number of atoms, pressure, and temperature),
pressure control can be gained by the Nose-Hoover method, which rescale the
volume at each time step \[38\], and temperature can be controlled by Langevin
dynamics, in which the velocities of the atoms are rescaled \[39\]. The inte-
gration of the equation of motion is calculated, e.g., with the velocity Verlet
scheme \[39\].

The forces acting on atoms in MD simulations are calculated using force
fields, such as CHARMM27 \[40\] and AMBER94 \[41\]. The force fields include
the parameters for different atom types. The parametrization of the force fields
are performed with quantum calculations or by fitting to experimental values
\[42, 43\]. With the parameters and the positions of the neighboring atoms,
the force acting on an atom can be calculated. The resulting forces can be
sorted as bonded and non-bonded forces. In Fig. 2.2 a scheme of the different
bonded and non-bonded terms is shown. Bonded forces occur between atoms
with covalent bonds and are short range forces. A common approximation
is to split the force into a bond, an angle, as well as proper and improper
dihedral parts. The bonds, angles and improper dihedrals are approximated by
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<table>
<thead>
<tr>
<th>$10^{-15}$</th>
<th>$10^{-12}$</th>
<th>$10^{-9}$</th>
<th>$10^{-6}$</th>
<th>$10^{-3}$</th>
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<td>fs</td>
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<td>s</td>
<td>1000 s</td>
</tr>
</tbody>
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- Bond vibration
- DNA twisting
- Water dynamics
- Helix-Coil transition
- Lipid exchange via diffusion
- Lipid-Protein binding
- Protein folding

Figure 2.1: Timescale in molecular biology.

harmonic potentials, which are computationally simple. Proper dihedrals, or so called torsion angles, are approximated, for example, with cosine functions. In total the bonded potential for a system with $N$ atoms is given by

$$V^{\text{bonded}}(\mathbf{R}_1, \ldots, \mathbf{R}_N) = \sum_{\text{bonds}} \frac{k_l}{2} (l - l_0)^2 + \sum_{\text{angles}} \frac{k_\Theta}{2} (\Theta - \Theta_0)^2$$
$$+ \sum_{\text{impr.}} \frac{k_\omega}{2} (\omega - \omega_0)^2 + \sum_{\text{torsion}} \frac{k_\varphi}{2} (1 + \cos(n_\varphi + \delta)).$$ (2.7)

with the bond lengths $l$, the reference bond length $l_0$, the angle between two bonds $\Theta$, the reference angle $\Theta_0$, the angles $\omega$ and $\varphi$, the reference angle $\omega_0$, and the spring constants $k_l$, $k_\Theta$, $k_\omega$, and $k_\varphi$.

The non-bonded part consists of van der Waals and electrostatic forces. These are long-range forces. Especially the electrostatic force, with the simple 1/distance decrease, not only influences neighboring atoms. The van der Waals forces are approximated by the Lennard Jones (6,12) potential. Therefore, the total non-bonded potential for the atom $i$ can be written as

$$V^{\text{nonbonded}}(\mathbf{R}_1, \ldots, \mathbf{R}_N) = \sum_{j=1, j \neq i}^N \left( \frac{q_i q_j}{4 \pi \varepsilon_0 l_{ij}} + 4 \varepsilon_{ij} \left[ \left( \frac{\sigma_{ij}}{l_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{l_{ij}} \right)^6 \right] \right).$$ (2.8)

with the charges $q_i$ and $q_j$, the vacuum permittivity $\varepsilon_0$, the distance between two atoms $l_{ij}$ and the Lennard Jones parameters $\sigma_{ij}$ and $\varepsilon_{ij}$. The calculation
of the relative position of all atoms is very time-consuming. That is why cutoff distances are used. To avoid sharp cutoffs, which can lead to artifacts, i.e., in the energy conservation, one introduces also a switching distance to smooth the transition. The electrostatic force is calculated with algorithms, such as the particle mesh Ewald method. In the particle mesh Ewald method the electrostatic force is divided into a short range and a long range part. To increase the calculation time, the short range part is calculated in the real space and the long range term in the Fourier space. The computational scaling with the particle mesh Ewald method is $O(N \log N)$ with $N$ atoms, while the direct calculation of Coulomb’s law scales with $O(N^2)$.

Classical MD simulations are based on Newton’s law and are, therefore, not designed to simulate chemical reactions and transitions between electronic states. These processes are not included in the model used in MD simulations. For such purposes, quantum calculations are appropriate [44]. There are different simulations based on quantum mechanics mostly divided in \textit{ab initio} and \textit{semi empirical} or \textit{empirical} methods. \textit{Ab initio} calculations do not have empirical results as basic parameters for the calculations. Hückel method and Hartree-Fock are examples for quantum mechanical methods [45, 46]. Excitation of electronic states can be simulated with wave packet propagation where the time-dependent Schrödinger equation is solved for each time step. Furthermore, with the time-dependent perturbation theory one can include interactions with laser pulses into the simulations. In the Appendix, an application of a wave packet calculation to simulate excitation of iodine molecules with femtosecond pulses is shown. These methods use approximations for the calculation, e.g., the Born-Oppenheimer approximation and they are more accurate than MD simulations, but need much more computational time. That is why these kind of calculations have not been used yet for systems with several thousand atoms, such as biological pores. The combination of classical simulations and quantum calculations, so called \textit{quantum mechanics/ molecular mechanics} (QM/MM) simulations, are used for simulations of larger systems with a limited number of reactions or excitations of electronic states, but also for simulations of hole and proton transfer [47, 48]. In QM/MM, quantum calculations are executed locally, at the positions where reactions happens, while the rest of the system is treated classically [49]. For example, one can perform QM/MM to explore potential energy surfaces [50]. Phatak et al. performed QM/MM simulations to find proton storage [51] and to simulate proton transfer [52] in bacteriorhodopsin. Kubar et al. simulated hole transfer in DNA
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Figure 2.2: The different bonded and non-bonded parts of the force fields.
with QM/MM methods [53].

## 2.4 Computation of the Current

Due to the atomic details of MD simulations, the pathways of ions and molecules through the pore can be tracked. The ionic current in a system of the length $L_z$ can be determined by summing up the movement of the charges projected on $z$ axis via [54,55]

$$I(t) = \frac{1}{L_z} \sum_{i=1}^{N} q_i \frac{z_i(t + \Delta t) - z_i(t)}{\Delta t}.$$  \hspace{1cm} (2.9)

The results are independent of the time step $\Delta t$. One crucial point which should not be forgotten while calculating the current is the drift of the center of mass. To take this drift into account the relative movements of charges are considered for the current calculation instead of the absolute movements.

Currents can also be calculated by summing the charge of ions $j(t)$ that cross a virtual plane in a certain time interval $\Delta t$:

$$I(t) = \sum_{j(t)} \frac{q_j(t)}{\Delta t}$$ \hspace{1cm} (2.10)

The results are rather similar to the one of Eq. 2.9, but can lead to higher standard deviations for the finite duration of one trajectory. The advantage of the method with Eq. 2.10 is that it gives a handle to calculate the error of the current value by assuming a Poisson distribution for permeation events [56]. The underlying physical assumption for this Poisson distribution is the statistical independence of the individual ion crossing. The error of a single trajectory is then calculated using $\pm I/\sqrt{N_c}$ where $N_c$ is the number of crossing events.

Furthermore, the cation and anion currents are compared to each other. The ratio of the cation and anion current is calculated to obtain a measure for the selectivity. This ratio is not directly comparable with the experimentally measured selectivity, but it provide a computationally inexpensive result, which allows statements about pore properties.
2.5 Experimental Methods

Experimental measurements are performed in the so called patch-clamp or in the BLM setup. In the patch-clamp method, a porin within a membrane is clamped with a micropipette. Then the current through the pipette and the pore is observed [57,58].

BLM is an \textit{in vitro} measurement. This work aimed to simulate BLM experiments performed by the Winterhalter group at Jacobs University. In these BLM experiments, a chamber is separated into two parts via a 25 $\mu$m thick Teflon foil with a small aperture up to 50 $\mu$m diameter. Adding a solution with lipids into the chamber will lead to a planar lipid bilayer building in the hole [59]. Pores, added on one side, inject into the bilayer. After a stable membrane is built, the solute in the chambers can be changed. The new solution is usually a water and salt solvent. Molecules, such as antibiotics, are put into the solvent if their translocation has to be measured as well. Electrodes in the two chambers are used to apply an electric field [2]. If a voltage is applied across the lipid bilayer and implicitly across the nanopore, an ion current is detected. The measured resulting current is in the 100 pA range. The sampling frequency of the measurement is in the 10 kHz range. Evaporation for high temperatures is reduced either by using a thin layer of mineral oil on the surface of the solvent or by covering the cuvette. Measurements show that oil on top of the buffer does not have a significant effect on the results. In Fig. 2.3, a scheme of a BLM setup is shown. Antibiotic translocation can be measured by the blocking of the ionic current, which leads to a partial reduction in the measured current. If one monomer is blocked, the current is reduced by 1/3 as shown in Fig. 2.4 (first and second row). With larger antibiotic concentration the blocking events and also the probability of two monomers being blocked at the same time increases (Fig. 2.4 last row).
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Figure 2.3: BLM setup. From the Winterhalter group.

Figure 2.4: The measured current and blockage of monomers. From [60].
Chapter 3

Bacterial Outer Membrane

In this chapter, membranes in general and biological channels in particular are presented. Highly interesting for this work are the pores OmpF and OmpC, which are described in detail. Also the setup of the systems used in this thesis is presented below.

3.1 General Introduction to Biological Channels

Depending on the cell type, the structure of membranes are different. Gram-negative bacteria, such as *Escherichia coli* (*E. coli*), possess two cell membranes, the inner and the outer membrane, separated by the periplasmic space in between. In general, membranes mainly consist of proteins, a lipid bilayer and saccharides [1,61].

Lipids consist of a hydrophilic head group and a hydrophobic tale. In the lipid bilayer the hydrophobic tales of two lipids are facing each other while the hydrophilic head groups are in contact with the environment. The non-polar inner part of this bilayer prevents the translocation of ions and big polar molecules while the head groups ensure solubility in water under physiological conditions.

Membrane proteins have a huge variety of functions. One function, some trans-membrane proteins are full-filling, is to build a channel. Such channels are, e.g., porins. Porins are passive, pore-forming proteins in the outer membrane of Gram-negative bacteria [62,63]. They are used for the translocation of molecules and ions through the outer membrane [6]. In Fig. 3.1, a scheme
of the outer and inner membrane including membrane proteins is shown.

The composition of lipid and protein depends on the cell function. For example, the membrane of intestinal villi cells has more membrane proteins and are more permeable than myelin cells, which act as electrical insulation for nerve cells [61]. The myelin membrane is therefore consisting of thick non-permeable lipids. Nerve cells have ion channels to produce an ion gradient. These channels are highly sensitive pores. It is remarkable that they do not select only between cations and anions, but also discriminate different elements. One particular example is the K$^+$ channel. This channel permeates K$^+$ at least 10,000 times more than the very similar Na$^+$ ions [65]. The structure of K$^+$ channels shows negatively charged residues at the entrance of the pore blocking anions to enter the pore and attracting cations. Within the channel, some rigid residues are facing carbonyl oxygens to the pore volume [66–68]. This is at the most constricted area in the channel. In this area the channel is not wide enough for an ion including the surrounding waters to pass. Therefore, the ions have to interact with the carbonyl oxygens instead of water molecules to pass the pore. The distance of the rigid carbonyl oxygens match with the radius of the K$^+$ hydration shell [65]. The smaller Na$^+$ would need a smaller distance to the oxygen atoms. For the passing of this selectivity filter, Na$^+$ needs much more energy to break the interaction with the surrounding water and to interact with the non-fitting pore. Because of that, K$^+$ permeates more
3.2 Introduction to OmpF and OmpC

In this thesis, the focus will be on the outer membrane protein OmpF and OmpC. These two porins belong to the major porins of E. Coli. The OmpF-OmpC balance is highly regulated by different genetic control systems while OmpC, for example, is expressed at high osmotic stress. OmpF and OmpC belong to the general (non-specific) porins [63]. The transport is governed mostly by diffusion, facilitated by concentration gradients or internal binding sites [69]. Regulation of the passage is obtained by the limited size of the pore and by the charged residues which interact with the molecules passing the pore. These lead to a sieve like behavior.

Each monomer of the OmpF and OmpC trimers consists of 16 anti-parallel $\beta$-sheets forming a rigid $\beta$-barrel. The $\beta$-sheets are connected with each other via 8 loops at the extracellular side and 8 turns at the periplasmic side. Loop L2 connects the monomers with each other and loop L3 is folded inside the pore. Loop L3 builds the constriction zone in the middle of the porin and is responsible for the hour-glass shape of the pore. An OmpF monomer has, in total, 340 and an OmpC monomer 346 residues [70,71].

OmpF is about 5 nm long. The inner diameter of the pore is about 3 nm and is narrowed to 1 nm in the constriction zone. There one has 3 positively charged residues, R42, R82, and R132, on one side as well as two negatively charged residues, D113 and E117, on the other side, located on loop L3. This constellation of charged residues leads to a high electric field, which can orient water molecules [72,73] and separates the ion fluxes along the surface of the pores [30,72,74,75]. Performing point mutations may cause structural changes [76,77] that have a strong influence on the ion flux and selectivity [25,31,78]. For example, recently, Miedema and group member created a Ca$^{2+}$ selective OmpF channel by point mutating charged residues in the constriction zone [24,25,79]. In Fig. 3.2, an OmpF trimer is shown with highlighted constriction zone. The pore is, in total, negatively charged. The basis for computer simulations of OmpF was set with the crystal structure by Cowan et al. in 1992 [70,80,81]. OmpF is used as a pathway into the cell by molecules up to 600 Dalton [82], i.e., $\beta$-lactam antibiotics. Yamashita et al. [83] provided a crystal structure of OmpF with 1.6 Å resolution. The difference of the two structures $\Omega_1$ and $\Omega_2$ of the same systems are calculated with the root mean
square deviation (RMSD) which is equal

$$\text{RMSD}(\Omega_1, \Omega_2) = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (R_{1,i} - R_{2,i})^2}$$

(3.1)

with the position $R_{j,i}$ of the atom $i$ in the structure $j$ and the total number of atoms $N$. The RMSD between the structure of Yamashita et al. and the structure provided by Cowan et al. with 2.4 Å resolution is only 0.26 Å. To keep consistency of the simulations, the older structure of Cowan et al. is used also in the latest simulations.

OmpF is used in a wide range of research areas, in contrast to OmpC, although OmpC has a lot in common with OmpF. The sequence of both pores are 60% identical [71]. However, some charged residues differ. These small changes result in a quite different behavior from many perspectives, as cation vs. anion selectivity and channel conductance [62, 69, 71, 84, 85]. OmpC is slightly more cation selective and the charge of each monomer is higher than in OmpF. In Fig. 3.3, the outer loops of an OmpF and OmpC monomer are shown. One can see that there is a difference between the extracellular entrance of both pores. OmpF and OmpC differ in the length of the loops. OmpC has a much longer L4 loop (14 residues more) while OmpF has longer L1 and L6 loops (5 residues more each). In OmpC, the key residues in the constriction zone are the negative residues: D105, E109, and the positive residues: R37, R74, R124 [71]. Because of the only very recent availability of the crystal structure by Baslé et al. in 2006 [71], OmpC is not that widely used in computer simulations as OmpF. Investigating the two pores, OmpF and OmpC, can bring a deeper understanding of the basic mechanisms of pore kinetics and transport, because of their high homology combined with the differences in their physical and transport properties.

### 3.3 OmpF and OmpC in Research

Patch clamp experiments with OmpF and OmpC were performed, e.g., by the groups of Delcour [87–89] and of Pagés [90]. Rosenbusch and group members [31, 77, 78] performed experiments with different mutants to determine the influence of the loops L3 and L2 as well as the charged residues in the constriction zone. Miedema et al. performed measurements on modified porins to change the selectivity [24, 25, 91]. BLM experiments to determine ion conductance of channels and the translocation of substrates like antibiotics were
Figure 3.2: Top view of the OmpF trimer and side view of two of the monomers with highlighted constriction zone (red: negatively charged residues D113 and E117, blue: positively charged residues R42, R82, and R132, as well as orange: loop L3). The figure was generated using VMD [86].
performed by Winterhalter and group members [2,60,85,92,93]. Research focus has also been directed to properties such as pH sensitivity [94]. The size of the pores plays an important role in selectivity [91], as do specific binding sites for uptake of antibiotics [7], polyamines (polyamine blocking) [62,84,95,96] or other molecules.

Another interesting aspect is the experimentally observed subconductance of OmpC and voltage gating in OmpF [62,84,94]. Subconductance and voltage gating are the processes of a lower conductance state for several ms. These processes start, respectively end with a rapid decrease and increase of the ionic current. In Fig. 3.4 the experimentally measured current including subconductance states is shown. Although, gating is sometimes also refer as subconductance, one should note that subconductance states clearly differ from gating, as they are much smaller in amplitude and occur at lower voltages as well. Subconductance is observed in measurements performed at 50 mV of applied voltage, whereas gating is observed above 200-250 mV. These cannot be reproduced by MD simulations, as these effects are - with ms timescale - clearly out off the simulation time frame these days. For OmpC and OmpF, transitions between different conductance levels have been reported [97], OmpA has been shown to have two open states with different conductance values, between which the dominant one is being determined by the temperature [98], while OmpG traces have also shown conductance "flickering" and gating eliminated by mutations [99]. Although these effects are well observed, the explanation of these processes on molecular level is at the beginning [100].

It is essential to understand the relation between structure and mechanism
in the transport through these porins. The mesoscopic dimension and the complexity of the channel render any exact model on the ion conductance difficult to formulate and, thus, impossible to solve analytically or numerically. Only approximative methods are currently available. A possible way to determine the qualitative behavior of the ion conductance is by solving the Poisson-Nernst-Planck equations with an appropriate charge distribution [102]. One step going towards particle-based theories of ion conductance are BD simulations [22, 30, 31], as described in chapter 2.2.

In general, MD simulations are performed to get an atomistic view and more details than continuous models. All-atom MD simulations of ion conductance through porins were not feasible in the past, because of the lack of computer power. Back in 1998, simulations of a system with an OmpF trimer took 6 months for 1 ns [4], while nowadays, less than 1 day is needed. In early works, single ion permeation through OmpF monomers were simulated [103] and the structure of OmpF were studied [81]. Robertson and Tieleman simulated the pore dynamics and behavior of water molecules [74] and translocation of molecules like alanine and methylglucose through OmpF channels [73]. Im and Roux ran MD simulation to calculate diffusion and permeation properties of OmpF porins [72]. They also compared MD with BD and continuum electrodiffusion theory [23]. Ceccarelli and co-workers [93, 104] simulated the
translocation of antibiotics through OmpF using metadynamics. Malek and Maghari took a closer look on the orientation of molecules during translocation through OmpF [105]. The recent improvements in computational performance and the development of new algorithms allowed large-scale MD simulations of biological pores [4, 22, 106, 107] and enabled simulation of properties such as channel conductance [108], e.g., simulation of ion conductance through α-hermolysin and MscS are performed by Schulten and group members [55, 56].

3.4 Setup of the System

For a complete picture of ion transport and to benchmark the agreement between theory and experiment, the bulk conductivity was simulated. Therefore, cubic water boxes, with side lengths of 4.2 nm, were simulated, which had potassium ($\text{K}^+$) and chloride ($\text{Cl}^-$) ions as salt. These system sizes are large enough for the purpose of this study, as was tested previously [55]. Within these boxes, roughly 2500 water molecules are present. To obtain, e.g., a 1 molar (M) KCl solution, 45 $\text{K}^+$ and 45 $\text{Cl}^-$ ions were added. KCl is chosen as salt, because the $\text{K}^+$ and the $\text{Cl}^-$ ions have almost the same mass. Therefore, their mobilities are approximately equal.

The structure of OmpF or OmpC were used to built a trimer out of monomers. OmpF structure was crystallized by Cowan et al. [80] (Protein Data Bank code 2OMF) and the OmpC structure by Basilé et al. [71] (Protein Data Bank code 2J1N). Afterwards, the trimer was embedded into a palmitoyl-oleoyl-glycero-phosphathidylethanolamine (POPE) lipid bilayer, which was constructed from preequilibrated patches using VMD [86]. Phosphathidylethanolamine is the major lipid head group in bacterial membranes [1]. For comparisons, also simulations with palmitoyl-oleoyl-glycero-phosphatidylcholine (POPC) were performed, and they did not show significant changes to the simulations with POPE. Simulations with AMBER force field are all performed with POPC, because of the missing POPE parameters. The membrane was put in the $x$-$y$ plane, centered in $z$ direction, and the simulation box was chosen to have a hexagonal profile in the $x$-$y$ plane.

In a first series of simulations by Prof. Kleinekathöfer, the side length of the hexagon was set to 73 Å. This system contained about 112,000 atoms. To reduce the computational cost, I used a smaller system. The smaller size did not influence the accuracy of the results. The length of the smaller hexagon, which contained roughly 85,000 atoms, was 63 Å in $x$-$y$ direction. For both
shown in chapters D127, D312, E296, and 5 and 6 -1 0 0 discussed in 5 -1 0 0 discussed in 5 -1 -1 -1 5 and 7 -1 -1 0

Table 3.1: The protonation states of OmpF used in this work. The first one is suggested by Im and Roux [72], while the last one is suggested by Varma and Jakobsson [109].

system sizes, the height of the system was set to 80 Å (z direction). To obtain a 1 M KCl solution having about 15,000 water molecules (small setup), about 520 ions (potassium and chloride) had to be added. To neutralize the highly charged pore, additionally, 30 potassium counter ions were added. In Fig. 3.5, the simulated system with OmpF is shown.

It is not possible to directly simulate different pH values in MD simulation. Classical MD simulations do not provide the possibility to simulate unbounded protons. Also the number of water molecules is too small so that possible H$_3$O$^+$ to H$_2$O ratios are far away from the biologically interesting pH values, i.e., one H$_3$O$^+$ molecule within 15,000 water molecules is equal to a pH of less than 3. In simulations with amino acids, one can simulate the effect of different pH values by the protonation states of amino acids. The protonation states shown in Tab. 3.1 are used in the simulations with OmpF. The main differences in these states are the two residues D312 and E296. These two residues are close to each other and interact. Varma and Jakobsson [109] showed that these two can share a proton and suggest, because of the strong interaction, to protonate E296. For the ion conductance simulations with OmpC, none of the amino acids were protonated. The equivalent residues to D312 and E296 in OmpF are D299 and D315 in OmpC. Also for OmpC, it is suggested that these two residues share one proton [71]. Following this suggestion, in the OmpC simulations with ampicillin, only D299 was protonated.

3.5 Simulation Procedure

In the calculations, the ionic conductance through OmpF is determined following the general method described in [55]. The MD simulations described below
Figure 3.5: The simulated hexagonal system with water (red lines), ions (red and blue spheres), lipids (cyan lines) and OmpF (cyan ribbons).
have been performed using NAMD 2.6 [110] together with the GAFF94 (general AMBER force field) [41] or CHARMM27 [40] force field and the TIP3P water model [111]. Periodic boundary conditions are employed and the electrostatics are calculated with the particle mesh Ewald method. The van der Waals potentials are evaluated with a cutoff of 12 Å and a switching distance of 10 Å. The non-bonded forces were evaluated every 2 fs and the electrostatic forces every 4 fs. Bond constraints are not applied to give more flexibility to the hydrogen bonds, and therefore, the time step is set to 1 fs in NAMD as, e.g., also in Refs. [55,56]. Temperature control is gained by Langevin dynamics, which acts on a selection of atoms. For the bulk simulation, this selection contained only the oxygen atoms of the water molecules with a damping constant of 0.2 ps\(^{-1}\), whereas in the membrane simulations, only the heavy atoms of the lipids were selected with a damping constant of 1.0 ps\(^{-1}\), following the procedure in [55].

To keep the center of mass of the box fixed during the simulations, restraints are applied. Different atom types were constrained in several simulations to observe the influence of restraints on the results. Restraints were applied on the backbone atoms of the β-sheet residues, on the backbone atoms of three β-sheets and on heavy atoms of lipids with a distance of at least 10 Å to the porin. Although, the flexible parts of the porin were free to move, applying restraints on the β-sheets seems to have an influence on the results. Because of that, restraints are not applied on the porin in simulations afterwards. Since the error bars are high, it is not possible to make precise conclusions on the influence of restraints on the results.

In contrast to standard MD simulations, ion conductance calculations require application of an external electric field. In the simplest approach, the electric field is assumed to be homogeneous, and the voltage drops linearly over the simulation box [54–56,112], but the validity of this approximation needs to be further investigated [22,113,114]. For example, Roux [115] recently reported an extensive investigation of this problem and confirmed the validity of the constant external electric field approximation, even though, it adds a force to all ions, regardless of their positions, which is unphysical. An electrical field is induced by charge inbalance between the two side of the membranes in biological system. Charge inbalance in the simulations here, with one membrane and periodic boundary condition, will not work, because the periodic boundary conditions connects the two side of the membrane directly with each other so that the charge inbalance will be balanced quickly. More precise and
CHAPTER 3. BACTERIAL OUTER MEMBRANE

computationally more demanding methods to simulate external electric fields were suggested. Sachs et al. [116] used a twin bilayer system to create, explicitly, two water phases with different ion concentrations. Delemotte et al. [113] added isolating layers to a bilayer system, which enable to work with different ion concentration on the two side of the bilayer. In this work, a homogeneous external field $E$ is applied in $z$ direction proportional to the voltage $V$,

$$E = \frac{V}{L_z}$$

with the system length in $z$ direction $L_z$. The field was applied using the build-in function of NAMD 2.6 [110]. Additionally, one can try to introduce corrections for the inaccurate field. Böckmann et al. [114] suggested to rescale the electric field, because of the incorrect voltage drop over the membrane. This cannot be applied here. The high ion concentration, as it is used here, corrects the voltage drop over the membrane, and therefore, the system is not equivalent to the one studied by Böckmann et al. [114].

The system was equilibrated for 1 ns in a NpT ensemble, followed by a 1 ns equilibration run in a NVT ensemble with an electrical field corresponding to an applied voltage of 1 V. For the simulations of the current-voltage ($I - V$) characteristics, different voltages ranging from -1 V to 1 V were applied. Since the system is periodic, ions that leave the simulation box will reenter from the opposite side of the simulation box. This prevents the system from getting partially charged. For the bulk simulations, trajectories of 3-4 ns with an applied external field were produced and then analyzed. For these simulations, a voltage of 0.4 V was applied, which corresponds to the same electric field as in the pore simulations. In the case of membrane simulations, the trajectories have a length of 10 ns.

While in experiment a voltage of ±1 V applied for a few µs destroys the membrane, in simulations a smaller voltage leads to poor statistics which have to be compensated by longer simulation times. Higher voltages ($\geq \pm 2$ V) destroy the membrane even in simulations. In experiments, 50 mV is applied. Low voltages allow to use the same membrane in a complete series of measurement without destroying it.
Chapter 4

Modeling of Conductivity in Bulk Water

One general goal is to understand the effect of the channel wall on the ion permeation. Subsequently, the results have to be compared to bulk conductivity data. Therefore, as a first step the concentration and temperature dependence of KCl in bulk water is modeled starting with a similar approach as previously reported in Ref. [55]. As a control on the underlying interactions, the temperature dependence was calculated and compared to the experimental values. The temperature dependence from 2 to 90 °C of the bulk conductivity is shown in Fig. 4.1. Modeling and experimental results are given for the five different KCl concentrations 0.3, 0.5, 0.8, 1.0, and 1.5 M. The agreement between experiment and theory are best for the 0.3, 0.5, and 0.8 M concentrations with only a slight difference between the experimental and theoretical slopes. Larger differences become visible at 1.0 M and more pronounced for 1.5 M KCl. Higher temperatures cause larger deviations. It is not too surprising that the best agreement between experimental and theoretical data is in the temperature range between 20 to 40 °C since the employed force field parameters were fitted temperature-independently to data obtained roughly at room temperature.

To elucidate the agreements and disagreements in more detail, the concentration dependence of the conductivity is shown in Fig. 4.2 at a fixed temperature of 37 °C. Obviously, the agreement between experimental and theoretical data is very good for concentrations below 1 M. For concentrations above 1 M, the deviations increase rapidly, since the theoretical conductivity saturates while the experimental data show a rather linear behavior in the
Figure 4.1: Bulk conductivity versus temperature for different salt concentrations in experiment (solid lines) and simulation (dashed lines) for 0.3 M (green), 0.5 M (magenta), 0.8 M (blue), 1.0 M (red), and 1.5 M (black) KCl.
studied concentration regime up to 4 M. Hence, we limit our simulations to the range 0.3 M to 1 M KCl solutions. For the bulk concentration dependence, a theoretical model is given by the Kohlrausch law [117]. Unfortunately, this theoretical approach is only applicable at very low salt concentrations. One can see in Fig. 4.2 that the Kohlrausch law is for concentration as small as 0.3 M KCl not accurate and results even in negative conductance values for salt concentrations above 2.5 M.

Test calculations with AMBER force fields (GAFF 94) were performed and showed heavy clustering of the K$^+$ and Cl$^-$ ions. This behavior has also been reported recently by Chen and Pappu [118] and by Auffinger et al. [119] together with some proposed enhancement. The problem with the original AMBER force fields are inaccurate van der Waals parameters for the potassium ions. These parameters were improved to obtain more realistic results [118]. Using these improved AMBER parameters, clustering is prevented and, as shown in Fig. 4.2, similar results for the bulk conductivity compared to those obtained with the CHARMM force fields can be achieved. In Fig. 4.3, the ion clustering with the standard AMBER force field is shown and compared to CHARMM force fields.

Figure 4.2: Bulk conductivity versus ion concentration at a fixed temperature of 37 °C for experiment, theory (Kohlrausch's law), and MD simulation with CHARMM and improved AMBER force fields.
Figure 4.3: K\(^+\) (ocher) and Cl\(^-\) (cyan) clustering in bulk simulations for AMBER (left) and CHARMM (right) force fields. With the standard AMBER force field, ions tend to build clusters whereas, with the CHARMM force field, they move freely.
Chapter 5

Conductance through wild type OmpF and its mutants

In this chapter the results of the ion current through OmpF are presented. The all-atom MD simulations of WT OmpF are compared with BLM experiments in the first section. Atomic details could be obtained by the MD simulations. Some of these details, such as, ion path ways and ion pairing are presented in section 2. Mutations generated in the constriction zone can change the conductance and the selectivity of the porin, as shown in section 3. A discussion of the results is given in the last section.

5.1 Conductance of Wild Type OmpF

Each simulation for the OmpF system covered about 10 ns. After an equilibration period, the external electric field was applied (see chapter 3) to calculate the current through the OmpF trimer. In Fig. 5.1, the cumulative current is displayed and from its slope the current is deduced, which is equivalent to the use of Eq. 2.9. Furthermore, the conductance is calculated as the ratio of current and applied voltage.

In Fig. 5.2, typical ion currents for a single trimeric OmpF channel in 1 M (molar) KCl are shown for a transmembrane voltage of 50 mV and different temperatures. These traces are obtained in experiments by the Winterhalter group. As expected for bulk ion solution, increasing the temperature from 0°C to 90°C increases the channel conductance. It is interesting to note that OmpF shows voltage dependent channel closure. The higher the voltage, the
Figure 5.1: Cumulative current through OmpF trimer. From the slope of the cumulative current the current is deduced.

more gating occurs. As can be seen in Fig. 5.2, at high temperatures gating is enhanced.

In Fig. 5.3, the measured experimental conductance are compared with modeling. Using 0.5 M and 1 M KCl solutions as electrolytes, an external voltage of 1 V was applied for simulations whereas the BLM experiments were performed at 50 mV. Since the conductance is shown, experiment and simulation are directly comparable, assuming a linear current-voltage ($I - V$) characteristic (see below). The experiments show a rather linear behavior of conductance versus temperature for both salt concentrations. Nevertheless, the increase is slightly faster than linear. Compared to the experiments, the calculated curves show more fluctuations, which are due to the uncertainty in the simulation data as discussed below. The general trend of the slope is rather similar to the experimental one, but the absolute values are too small.

By repeating measurements the error for the experimental measurement is obtained, which is within the symbol size in the figures. To get an handle on the errors attached with the MD simulations, two different kinds of error analysis are performed. Assuming independent permeation events (see chapter
5.1. CONDUCTANCE OF WILD TYPE OMPF

Figure 5.2: Representative single channel traces obtained at different temperatures for wild type OmpF in 1 M KCl solution at pH 7.5 and a transmembrane potential of 50 mV. From [120].

Figure 5.3: Conductance versus temperature for simulations with 1 V and experiments with 50 mV applied voltage for two different salt concentration. For the 1 M KCl solution also simulation results are shown which are corrected for the bulk deficiencies.
2), the error in the current can be estimated as \( \pm I/\sqrt{N_c} \). This leads to an error of 13 % at maximum. As a second method, an error analysis was performed by repeating the simulations for identical configurations several times and calculating the standard deviation. For 0.5 M at 80 °C as well as 90 °C, the simulations were repeated four times and for 23 °C, nine times. Because of the large computational costs, the error analysis is performed only for these temperatures, and not for all the temperatures shown in Fig. 5.3. The relative error obtained using this method were 20 %, 24 %, and 14 % for 23, 80, and 90 °C, respectively. Especially at 80 °C, it can clearly be seen that the error obtained using the latter method is much larger than the first approach, which implied uncorrelated permeation events. This is a clear indicator of the fact that the ion transport through the pore is a highly correlated process. By inspecting the trajectories more closely, it can be seen that some of the ions are trapped for long times intervals- several ns- for example, at the corresponding counter charges at the inner channel surface especially near the constriction zone. This can result in a significant current fluctuation. In the subsequent discussions of results, it will be assumed that the error in the current is 20 %.

The simulation parameters and results are also presented in Tab. 5.1.

In a further step, this data is corrected by introducing a temperature-dependent correction factor deduced from the ratio of simulated and experimental bulk conductivity. A similar correction was introduced in Ref. [56] for the voltage dependence. This corrects for some of the force-field deficiencies that already occur in the bulk simulations. The results for the 1 M concentration including correction are shown in Fig. 5.3. One can see that with the correction the slope of the experimental and simulated conductance are quite similar. The correction is not applied here for the 0.5 M simulation, since, in that case the correction factor is close to unity.

Fig. 5.4 shows cuts of the electrostatic potential map for ±1 V in the y-z plane through the center of one of the pores. As previously suggested for \( \alpha \)-hemolysin [55] and the MScS channel [56, 112], these plots have been obtained by averaging the electrostatic field of all atoms as well as the external electrostatic field over a complete trajectory. Therefore, these reflect an overlay of external field, membrane and protein charges and averaged ion positions. The pore is in the middle of these cuts and the ions are driven by the electrical field through this pore. The resulting electric field is of course influenced by the pore and by the lipids. In Fig. 5.4 the lipids are located at \( z \) positions between ±20 Å and have a large positive potential difference between bulk and lipid.
5.1. CONDUCTANCE OF WILD TYPE OMPF

As one can see, the lipids constitute an electrostatic barrier while the potential in the water regions above and below the membrane is more or less constant. The gradient of the membrane potential and especially the absence of peaks in the headgroup area, is typical for CHARMM force fields [121]. Basically, there is no potential change in the water above and below the membrane, but only within the membrane and protein region.

In Fig. 5.3, the experimental and theoretical conductance data are compared assuming a linear $I - V$ characteristic. The $I - V$ curve of OmpF in a 1 M KCl solution is shown in Fig. 5.5. Within the experimentally available voltages an almost linear relation is obtained. The theoretical curve is nonlinear. At large voltage, the slope of the $I - V$ curve is in reasonable agreement with the slope from the experimental data, while at low voltages in the simu-

| $T$ [°C] | $[\text{KCl}]$ | $t_S$ [ns] | $N_c$ | $G$ [nS] | $|\Delta G|$ | $|\Delta G/G|$ |
|---|---|---|---|---|---|---|
| 2 | 0.5 | 10.0 | 67 | 1.06 | 0.13 | 13 |
| 12 | 0.5 | 10.6 | 100 | 1.51 | 0.15 | 10 |
| 22 | 0.5 | 10.0 | 87 | 1.39 | 0.15 | 11 |
| 37 | 0.5 | 10.0 | 114 | 1.82 | 0.17 | 10 |
| 57 | 0.5 | 10.7 | 156 | 2.34 | 0.19 | 9 |
| 72 | 0.5 | 10.5 | 199 | 3.03 | 0.22 | 8 |
| 80 | 0.5 | 10.0 | 208 | 3.32 | 0.23 | 7 |
| 90 | 0.5 | 10.0 | 229 | 3.56 | 0.24 | 13 |
| 2 | 1.0 | 12.9 | 118 | 1.46 | 0.14 | 10 |
| 12 | 1.0 | 10.0 | 96 | 1.54 | 0.16 | 11 |
| 22 | 1.0 | 12.2 | 189 | 2.49 | 0.18 | 8 |
| 37 | 1.0 | 12.4 | 311 | 4.02 | 0.23 | 6 |
| 47 | 1.0 | 10.8 | 313 | 4.63 | 0.27 | 6 |
| 57 | 1.0 | 10.4 | 313 | 4.80 | 0.28 | 6 |
| 72 | 1.0 | 10.6 | 386 | 5.9 | 0.3 | 6 |
| 80 | 1.0 | 10.0 | 340 | 5.5 | 0.3 | 6 |
| 90 | 1.0 | 10.0 | 433 | 6.9 | 0.4 | 5 |

Table 5.1: Details and results of the simulations including temperature $T$, simulation time $t_S$, crossing events $N_c$, conductance $G$ and the error of the conductance $|\Delta G| = |G/\sqrt{N_c}|$. 
Figure 5.4: Electrostatic potential maps for $V = \pm 1$ V in the $y-z$ plane (as shown on the left hand side) at an $x$ position corresponding to the middle of one of the three pores (as shown in the top panel). The graphs have been calculated using a modified “PMEPot” plugin for VMD [55,56].

Simulations, the current is much smaller than in experiment. This might be due to several reasons, including the protonation states of some channel residues that are more important at smaller external fields and the bad statistics in the MD results due to the finite simulation time of 10 ns.

Different protonation states have been used to get a better idea of their influence. The residues with unknown protonation states are D127, D312, and E296. While in most simulations presented in this thesis D312 and E296 are neutralized, also simulations with additional D127 neutralized and with none of the three neutralized have been performed. Because of the high error range of the MD simulations the results do not allow a statement about the protonation state of the residue and the conductance of OmpF. Recently also calculations with only E296 protonated were performed. Simulations with this protonation states in conjunction with AMBER force fields with improved ion parameters have shown surprisingly good agreement with experiment as shown in Fig. 5.6. The same setup was also used with CHARMM force fields, but there the results did not show the same agreement as before (also shown in Fig. 5.6). The reason for this could be due to the use of AMBER force field instead of CHARMM for the equilibration. Further investigations are required.
5.1. **CONDUCTANCE OF WILD TYPE OMPF**

**Figure 5.5:** $I-V$ curve for experiment and simulation at room temperature.

**Figure 5.6:** Conductance versus temperature for simulations with 1 V and experiments with 50 mV applied voltage and 0.5 M KCl. The simulated systems have only E296 protonated.
5.2 Atomic Details

The dynamics of ions in the pore have been analyzed. The ion pathway and ion pairing are presented in the following. Using other models, it was reported before [30, 72] that positive and negative ions have distinct pathways on the surface of the constriction zone, i.e., the two ion types are separated. This could also be confirmed with MD simulation as shown in Fig. 5.7.

The ion binding to certain residues within the channel, has no clear dependence on temperature although at low temperatures there seems to be a prolonged binding time. The number of ions inside the channel is, basically, temperature independent, as can be seen in Fig. 5.8. For low ion concentrations, such as 0.1 M KCl, mainly potassium ions are in the channel and these are close to the channel surfaces and none in the middle of the channel whereas for 1 M KCl the surface transport is saturated and the still distinct ion densities are extended towards the center of the channel. This increases the possibility for ion pair formation.

Due to the confinement, the ion pairing may influence the conductance. The atomistic details of the MD simulations allow to investigate this further. Ions of opposite charge being at distances below 4.1 Å are counted as pairs. To quantify their concentration inside the channel a region of around 12 Å length in the constriction zone was analyzed. Figs. 5.9 A and C show the increase in ion pairs for increasing ion concentrations. At 0.5 M the number of pairs in the channel is about the same than in the bulk, whereas at 1 M KCl their concentration roughly doubles in the bulk and triples in the channel. The number of ion pairs seems to be rather temperature independent, whereas, see
5.3. **OMPF MUTANTS**

Starting with the WT configuration, the ion flux is perturbed by introducing mutations in the constriction zone. In order to understand the influence of the charged residues, five different combinations were mutated. The generated mutants are:

- NQAAA (this notation indicates the final residue configuration): all...
charged residues in the constriction zone were neutralized - D113N, E117Q, R42A, R82A and R132A

- NQRRR: all negatively charged residue in the constriction zone were neutralized - D113N and E117Q

- DEAAA: all positively charged residue in the constriction zone were neutralized - R42A, R82A and R132A

- RRRRR+EE: the negatively charged residues in the constriction zone were mutated into positively charged, two additionally positively charged residues at the extracellular vestibule were mutated into negatively charged residues - D113R, E117R, R167E and R168E

- DEERE: two positively charged residues in the constriction zone were mutated into negatively charged residues - R42E and R132E

The mutation RRRRR+EE has the same net charge as the WT, but the positions of the charged residues have been altered. An increased Ca$^{2+}$ over
5.3. **OMPF MUTANTS**

Cl$^-$ selectivity has been introduced with the mutation DEERE [24]. In the NQRRR, the RRRRR+EE, and the DEERE mutations the mutated amino acids have approximately the same size as those in the WT, i.e., the size of the constriction zone is approximately preserved, but with some local rearrangements due to charge modifications. Since alanine is smaller than arginine, the size is changed as well for the mutants NQAAA and DEAAA. In the simulations, the WT system was equilibrated in a NpT ensemble, then the mutated variant was equilibrated. The applied voltage simulations lasted for 10 ns and the stability of the systems was checked by observing the RMSD. In addition, the mutants NQRRR and DEAAA were created experimentally using point mutagenesis and their KCl conductance and selectivity were measured.

In the cases of NQAAA, the neutral constriction zone, the current is approximately the same as that of the WT as shown in Tab. 5.2. Since most conductance values have been determined by a single trajectory, the uncertainty is rather high, and therefore all conductance values within a range of ±20% are assumed the same. Mutating only the negatively charged residues (NQRRR) leads to a decrease of the conductance by almost a factor of two. Also in the experiments a decrease of the conductance was observed. In the RRRRR+EE mutation, this effect is even enhanced. On the other hand, mutating the positively charged residues (DEAAA) will lead to an increased conductance, as it does for the DEERE mutation. Also for the DEAAA mutant, a higher conductance was measured in experiment, though, the increase was not as large as in the simulations.

A second important quantity is the selectivity. Usually the selectivity of ion channels is characterized in the framework of the Goldman-Hodgkin-Katz voltage equation [122] defined by the permeability ratio of two ion sorts. It is well known [62] that WT OmpF is slightly cation selective, and in agreement with this the current ratio $I_{K^+}/I_{Cl^-}$ has a value of 1.2 (see Tab. 5.2). This value nicely agrees with the value earlier obtained by PNP and Brownian dynamics simulations by Im and Roux [23]. The latter authors also extracted the selectivity from their simulations either by determining the current ratio at zero applied voltage or the reversal potential. Both are not easily possible for the present MD simulations. Using some approximations and special considerations for the case of OmpF, Roux and Im [23] even showed the approximate equivalence between permeability and current ratios. To estimate the selectivity for mutants we assume the current ratio of 1.2 to correspond to a selectivity of 3.5 as measured for the WT. Subsequently, we multiply the
CHAPTER 5. CONDUCTANCE THROUGH OMPF

Acronym mutated residues \( G_{MD} \) [nS] \( G_{exp} \) [nS] \( I_{K^+}/I_{Cl^-} \) \( S_{exp} \) \( S_{est} \)
---
WT - \( 3.0 \) \( 4.0 \) \( 1.2 \) \( 3.5 \pm 0.2 \) \( 3.5 \)
NQAAA neg. & pos. neutralized \( 3.4 \) \( 3.6 \) \( 10.5 \)
NQRRR negative neutralized \( 1.9 \) \( 1.8 \) \( 0.7 \) \( 2.9 \pm 0.2 \) \( 2.0 \)
DEAAA positive neutralized \( 4.7 \) \( 4.2 \) \( 4.5 \) \( 14.1 \pm 1.4 \) \( 13.1 \)
RRRRR+EE neg. to pos. & pos. to neg. \( 1.2 \) \( 0.1 \) \( 0.3 \)
DEERE pos. to neg. \( 4.1 \) \( 5.6 \) \( 16.3 \)

Table 5.2: Conductance \( G \) from MD simulation and from experiment, the theoretical ratio of the partial currents \( I_{K^+}/I_{Cl^-} \), the selectivity \( S \) from experiment, and the estimated selectivity from simulation at 22 °C for 1 M KCl WT and for different mutants. The second column shows which charges in the constriction zone have been neutralized or changed in sign (RRRRR+EE and DEERE).

current ratios of the mutants by the corresponding factor of 3.5/1.2 to obtain an estimate for the selectivity. This estimate is denoted \( S_{est} \) in Tab. 5.2, and for the WT OmpF it is, by definition, equal to the experimental value.

Concerning the selectivity, there is a significant change in the current ratio in almost all studied mutations. As expected, removing negative charges (NQRRR) leads to more anions than cation passing the pore. Removing positive charges in the constriction zone (DEAAA and DEERE) has the opposite effect. Already the single mutation R82Q has a clearly visible effect beyond the numerical uncertainties (not shown in the table). When all positive residues in the constriction zone are mutated into neutral ones (DEAAA), the ratio \( I_{K^+}/I_{Cl^-} \) is about four times as large as for the WT. Interestingly the result for neutralizing all charges (NQAAA) is quite similar to that of only neutralizing the positive charges (DEAAA). In Fig. 5.10, the selectivity and the conductance are shown as function of the charged residues in the constriction zone. Interestingly, one finds an almost linear dependence of the conductance on the net charge in the constriction zone. But also the specific arrangement of the charges plays an important role and, for example, the conductance of the DEERE mutant is smaller than that of the DEAAA mutant. The arrangement of the charges in the constriction zone has also an important influence on the selectivity which, for example, increases by a factor of three when going from the WT to the NQAAA mutant. In the latter mutant, there are no charges within the constriction zone, whereas in the WT, five charges result in a net
5.4 Discussion

In the case of the OmpF simulations, the theoretical conductance-temperature curve shows some fluctuations that clearly display the limitations of the current MD simulations caused by the heavy numerical calculations involved. In the best case, one would do an extensive sampling, i.e., run the same simulation...
over and over again with different initial positions and velocities possible for the experimental setup. This is computationally not feasible, as, already the determination of one conductance value needs a 10 ns simulations of the full system. To estimate the error, three values were computed several times, and from this an error of about 20 % was estimated for the current values. This error is, of course, much larger than in the bulk simulations, because the ions now have to pass through the three narrow pores of the trimer and therefore, the ion counting statistics become much worse. Therefore, it is also problematic to perform calculations with low salt concentrations, such as 100 mM, since then the number of ions in the system is reduced then by a factor of ten and longer simulation times would be needed for a decent statistical measure.

Several coarse-grained simulations especially Brownian dynamics simulation and continuum models, such as the Nernst-Planck formalism [23, 24, 123, 124], for OmpF have been performed. As an advantage, such calculations do not suffer from the sampling problem to that extent as the present MD simulations do. However, these theories involve several more or less severe approximations. One can, of course, try to determine the parameters for these simplified formalisms from all-atoms simulations as performed by Im and Roux [72] and others [56, 112]. Such a hierarchy of models is certainly needed at some point [125], but is also not without its problems. Diffusion constants, for example, are normally needed in such models but would have to be fitted temperature-dependent for a study as the present one. Also ion-ion interaction or the hydration shells are much more difficult, or even impossible, to handle. Molecular details, such as ion pairing, do need an all-atom representation to enable to catch the most important facts. Using applied-field MD, it could be shown that there is an enhanced number of ion pairs in the channel with decreasing lifetime at higher temperatures. From this atomic detail, it was proposed that this higher probability of breaking ion pairs at larger temperatures does lead to an increase in charge carriers, which in turn leads to an increase in conductance. Both, surface conductance and ion pairing, contribute to ion transport and are modulated by the structure of the mesoscopic channel. This study shows that one needs to look at atomic level to understand differences in bulk and channel conductance. It opens certainly a large field of investigations for natural and artificial pores and their design to fulfill certain properties.

The conductance and the selectivity of a nanopore can be engineered using
mutations. The present results of the simulations and the experimental data show good qualitative agreement. The measured values of the reversal potential and therefore the selectivity for WT OmpF are similar to those previously reported in the literature [85,92,126,127]. The value for the reversal potential, in the case of the mutant DEAAA, is in good agreement with experimental data by Miedema et al. [24], and the difference in the pH value between these studies supports data by Aguilella-Arzo et al. suggesting a plateau for the reversal potential in the range of pH 6-8 [124]. For the NQRRR mutant, it should be mentioned that a decrease in the cationic selectivity was also reported for CaCl$_2$ [128]. Applying Brownian Dynamics simulations, Phale et al. [31] have shown that in the case of this mutant in a NaCl solution, the ion conductance is reduced by a factor of two compared to the WT, and that the selectivity ($P_{Na}/P_{Cl}$) is changing from 4.5 (WT) to 1.0 (NQRRR). Furthermore, Phale et al. [31] studied the same fivefold mutation as in Tab. 5.2 (NQAAA) and observed an increase of the selectivity from 4.5 (WT) to 12.3 (NQAAA), which is in good agreement with the threefold increased ratio $I_K/I_{Cl}$ observed for KCl in the present investigation. Using Brownian Dynamics and PNP approaches, conductance and selectivity for mutants not studied here were obtained [23,124], which support our main conclusions.

Using Brownian dynamics, Schirmer and Phale [30] as well as Im and Roux [23] analyzed the average ion density in the channel. They found two separate pathways for the chlorine and potassium ions while passing the pore. As expected, the positive potassium ions tend to be located close to negative residues at the inner pore surface, whereas the negative chlorine ions are near to the positive residues, as also found in the present study and displayed in Fig. 5.11. If one compares the iso surfaces of the ion density of the mutated pores with those of the WT, it can be seen that the densities change considerably. For the mutation NQAAA, the chloride ion density is drastically reduced close to the constriction zone. This is in accordance with the enlarged ratio $I_K/I_{Cl}$ and the experimental measured selectivity as given in Tab. 5.2. So the change in selectivity can be nicely correlated with the iso density plots. For the third case in Fig. 5.11, the mutation NQRRR, one can observed a reduced potassium iso density, while the chloride density looks similar to that of the WT. Consequently and in accordance with Tab. 5.2, not only the potassium selectivity is changed into that of a chloride, but also the total current is also reduced.
Figure 5.11: Iso density surfaces of the Cl$^-$ (left) and K$^+$ densities (right) averaged over the full trajectory. The top row displays the WT, the middle row the protein with all bases in the constriction zone mutated to neutral ones (DEAAA) and the bottom row with all acidic residues to neutral ones (NQRRR). The shown iso surfaces in all sub-figures have all the same iso value. In addition, the charged/mutated residues in the constriction zone are highlighted.
Chapter 6

Temperature-dependent Behavior of OmpC versus OmpF Conductance

In this chapter the results of the all-atom MD simulations and BLM experiments with OmpC are presented. The temperature-dependent ion conductance of OmpC is compared to its structural homologue OmpF at different KCl concentrations in the first section. In the second section the pore structures of OmpF and OmpC in simulations are compared, while the ion densities and ion pathways are compared in section 3. Further results are presented in section 4.

6.1 Conductance in Experiment and Simulations

For the OmpC simulations the system is built up with the same procedure as described for OmpF. The hexagonal side length is 63 Å and the thickness is approximately 80 Å. Overall, the system contains approximately 88,000 atoms including 16,000 water molecules. All residues of the protein are assumed to stay in their normal protonation state. First, the temperature dependence of OmpC in setups of different KCl concentration has been studied and compared with BLM experiments. In Fig. 6.1 the conductance is shown for four different KCl molarities and, as expected, the pore conductance increases linearly with temperature and with salt concentration. The experimental results are in a very good agreement with simulations at low KCl concentration and low
Figure 6.1: Temperature dependence of OmpC conductance for different salt concentrations in experiment and simulation. To guide the eye, also the linear interpolations are shown. Panel a) presents experimental and computational results in rather good agreement for low salt concentrations while panel b) shows results for high KCl concentrations with increasing deviations between theory and experiment. The value of the buffer pH was 7.5.

temperature, while the agreement decreases with increasing salt concentration and growing temperature. Still, overall agreements are to be considered good, as experimental and simulation data shows strong deviance at high salt concentration and temperature, even for the simple system of a water box with ions, as this has been shown in chapter 4. It is interesting to note that the agreement is significantly better for OmpC compared to OmpF, as shown in Fig. 6.2. The observed increase of the conductance with the salt concentration shows no saturation effects at the studied concentrations. Doubling the salt concentration from 0.5 M to 1.0 M leads to an almost doubled conductance, while in OmpF doubling from 0.5 M to 1.0 M leads to an increase less than two times.

In order to elucidate the effect of the channel, the temperature effect of the bulk conductivity and the remaining effect of the channel is separated. To this end, the channel conductance is normalized by using the ratio of the pore conductance over the bulk conductivity. In case of OmpC, this ratio depends less on the salt concentration than in case of OmpF, as shown in Fig. 6.3. While for OmpF, a change with different salt concentrations is observed, there is almost no change with 0.5 and 1.0 M for OmpC. This is in accordance with the concentration dependence of the conductance of OmpC and OmpF.
6.2. PORE STRUCTURE

Figure 6.2: Comparison of conductance for OmpC and OmpF in measurement and simulation. Panel a) shows the comparison at 0.5 M KCl, while b) presents data for 1 M KCl. The pH value was set to 7.5.

mentioned above. The results lead to the conclusion that the ion transport is significantly different for both pores, OmpC and OmpF, which is caused by minor electrostatic differences in the structure of the pore lumen.

In simulation, on average, OmpC is found to have a smaller ratio of the pore conductance over the bulk conductivity, but the difference between the pores seems less pronounced compared to experiment. Linear fits show a increase of the ratio with increasing temperature. An error of ±20% was estimated for the OmpF calculations and similar error will also hold for the OmpC calculations. Therefore more precise conclusions from the simulations are impossible at this time.

6.2 Pore Structure

In Fig. 6.4, the pore area is shown as a function of the position along the channel axis for OmpF and OmpC. The OmpF profile is in agreement with the one reported earlier by Im and Roux [72]. One can see that the constriction zone, i.e., the narrowest part of the channel, for OmpC is roughly 10 Å long, while for OmpF its length is only about 5 Å. Concerning the charges both porins are very similar in the z range from 0 to 5 Å. As only differences, OmpC has the negatively charged residue D18, instead of the neutral V18 in OmpF, the neutral residues W72 and N119 instead of the positively charged K80 and the negatively charged D127, as well as, the positively charged residue K317
Figure 6.3: Conductance over bulk conductivity for OmpC and OmpF in a) measurements and b) simulation. The difference in the ratios between OmpF at 0.5 M and 1 M KCl and the same concentrations in OmpC is to be noted in a).

instead of the neutral I314 in OmpF. Responsible for the longer constriction zone in OmpC might be the positively charged residue K317, which attracts the negatively charged residues on the L3 loop E109 and D113. The L3 loop of OmpF is folded less into the volume of the pore partly because of the missing positively charged residue corresponding to K317. In Fig. 6.5, these residues are shown for OmpC and equivalent for OmpF. Note the different folding of the L3 loop. The negatively charged residue D113 in OmpC has to be counted as a key residue within the constriction zone, while its counter part in OmpF, D121, is not counted as part of the constriction zone.

In the extended constriction zone in OmpC (z range from 5 to 10 Å), there is a composition of two positively charged residues R92 and R124 and two negatively charged residues D118 and E66. E66 is on the L2 loop of the neighboring monomer. In OmpF this complex is not in the 5 Å long constriction zone anymore, where it has, in addition to R100, R132, D126, and E71, a positively charged residue K80 and a negatively charged residue D127. While K80 is directly exposed to the pore volume, D127 is in the background on loop L3. These complexes are highlighted in Fig. 6.5 and Fig. 6.6. Overall both complexes are neutral, but the positive charges in OmpF are more exposed to the pore volume, and have, therefore, more effect on the ion conductance. In conclusion, the additional negatively charged residue D113 and the less exposed positive charges explain the larger cation selectivity
Figure 6.4: Cross-section area of the OmpC and the OmpF pore as a function of the channel position $z$ averaged over 10 ns trajectories. The $\beta$-barrel extends from $z$ values of -17 to 15 Å.

of OmpC compared to OmpF. In Fig. 6.7 the cumulative current for the potassium and chloride ion is shown for OmpF and OmpC. Notice the higher selectivity of the OmpC porin.

6.3 Ion Densities and Ion Pathways

To elucidate the surface attraction of the ions within the pore, the number of ions within 3 Å of the pore surface was compared to the total number of ions in the pore. As shown in Fig. 6.8, about 50% of all ions are found in this region confirming the importance of surface effects. This claim is supported by the experimental pore conductance showing no apparent saturation in a concentration range from 0.3 M to 1.5 M (data not shown). Comparing the ratio of ions at the surface found in OmpC with that in OmpF one observes a significantly higher ratio for the former pore, as seen in Fig. 6.9. The ratios correspond to the average ratio of the volume within 3 Å from the pore walls to total volume. For these calculations only the ions in the $\beta$-barrel region were considered, i.e., ions within $z > -17$ Å and $z < 16$ Å. For both pores the ratio of ions at the surface over all ions is smaller for higher salt concentrations and growing with temperature. The temperature dependence is slightly stronger in OmpC than in OmpF, however, this lies within the error range of the present simulations.

Further analyzing the MD simulation results reveals that the ratio of the
Figure 6.5: The inner pore area of OmpC and OmpF. Highlighted are negatively charged residues (red), positively charged residues (blue), neutral residues with charged equivalent in the other pore (white), and the L3 loop (purple). Different colors is also given to the extended constriction zone and its counter part in OmpF (z range from 5 to 10 Å, lime) and to the L2 loop from the neighboring monomer (orange).
6.3. ION DENSITIES AND ION PATHWAYS

Figure 6.6: Some charged residues in the z range from 5 to 10 Å of OmpC and OmpF. Red highlighted are negatively charged residues, blue are positively charged residues. The L3 loop is highlighted purple and the L2 loop from neighboring monomer is shown in orange. Also neutral residues with their charged equivalent in the other pore are highlighted (white).

Figure 6.7: Cumulative current through the OmpF and OmpC trimers for the potassium and chloride ions. The ratio of the resulting currents is used as an indication for the selectivity.
Figure 6.8: Calculated number of ions within 3 Å distance from the surface relative to the total number of ions in OmpC. An average value of roughly 0.5 can be noted.

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Figure 6.9: Same as in Fig. 6.8 but shown as comparison between OmpC and OmpF at 0.5 M KCl and 1 M KCl, respectively. Note the stronger temperature dependence for OmpC compared to OmpF.

Figure 6.10: Iso surfaces of ion density for simulations with 22 °C at 0.5 M KCl. a) Cl$^-$ density in OmpF, b) Cl$^-$ density in OmpC, c) K$^+$ density in OmpF and d) K$^+$ density in OmpC. All iso surfaces represent the same density value.
6.4 Free Energy Profile, Residue Fluctuation and Water Dipole Moments

The free energy profile allows to estimate the work needed to translocate an ion through the porin. It shows where the main transport barriers exist but also where the wells, which attract ions, are located. For multi-ion systems the one-dimensional profiles are calculated as

\[ W_\alpha(z) = -k_B T \ln(\rho_\alpha(z)/\rho_{\alpha}^{\text{bulk}}) \]  

(6.1)

with the Boltzmann factor \( k_B \), the ion density \( \rho_\alpha(z) \) of ion sort \( \alpha \) at position \( z \) and the bulk density \( \rho_{\alpha}^{\text{bulk}} \). The obtained energy profiles reveal the change of the free energy of the ions in dependence of the \( z \) position along the channel axis. The free energy in the bulk is used as reference energy. Previously, Im and Roux calculated the free energy profile [23] based on the Brownian dynamics approach. Here, the all-atom MD simulations provide more atomic details, but with the drawback of a poor statistic, i.e., larger possible errors. The results for OmpC and OmpF are displayed in Fig. 6.11. It is interesting to note that single ions are trapped next to charged residues in the pores. Such events are long lived and have, partially based on the poor statistics, a strong influence on the outcome of the calculation. The free energy profiles reveal energy barriers for the chloride ions but also wells for potassium ions. The attractive region for the K\(^+\) ions is between energy barriers - smaller ones for OmpC and a high energy barrier for OmpF. These formation is the reason for the higher potassium current through OmpC. The attraction is caused by negative charges in the constriction zone which trap positive K\(^+\) ions. While the barrier in the chloride profile is wider for OmpF, OmpC has a higher peak at \( z \) values around 15 Å which lead to low chloride conductance.

In contrast to OmpF, subconductance is clearly seen in experiments with OmpC. Measurements show a temperature dependence of the subconductance and almost no pH dependence [101], which is an indication that pore dynamic is involved in the subconductance. Therefore, the fluctuation of the residues of OmpF and OmpC are compared with each other. The study of the fluctuation reveals that the extra cellular loops L4, L5 and L7, the periplasmic turns T1 and T4 and the inner loop L3 have the largest fluctuations for OmpC. Typical fluctuation patterns of the residues are shown in Fig. 6.12. Other simulations have shown other peak heights but the same positions, e.g., the residues with the largest fluctuations, are the same in all simulations, as shown
Figure 6.11: Free energy profiles for OmpC and OmpF for simulations with 0.5 M KCl at 22 °C with a bin size long the channel axis of 2 Å. The energy values are compared to the bulk free energy (z< -25 Å and z>25 Å).
in Fig. 6.12 for two temperatures. Concerning the comparison between OmpC and OmpF, the L4 loop fluctuates considerably more in OmpC independent of salt concentration and temperature. A look at the sequences reveals that in OmpC the L4 loop has 14 residues more than in OmpF and is therefore much longer [71]. This extended length could be a reason for larger fluctuations and also for the occurrence of the subconductance states. In OmpF, the high fluctuation of the L6 loop was also observed by others [72, 129]. Note that the fluctuation for OmpF is higher for 22°C than for 57°C. Maybe, the high fluctuation is the reason for the low conductance in the simulation at 22°C (see chapter 5).

It was observed that water molecules are oriented in the OmpF pore [72]. The orientation of water molecules are due to the electric field of the charged residues in the pore. As expected also in the OmpC pore water molecules orient. Fig. 6.13a shows absolute value of the water dipole moment in depen-
dency of the $z$ coordinate. This results are obtained from the simulation with 0.5 M KCl at 22 °C. In the constriction zone, the absolute value of the water dipole moment is highest. The dipole moment is oriented in the constriction zone in the $x$ and $y$ direction as shown in Fig. 6.13b. Note that the orientation is changing a lot with the $z$ position of the water in the pore and that the $z$ orientation of the water dipoles is negligible compared to the $x$ and $y$ orientation. The influence of the applied electric field is rather small, since there is almost no potential drop in the bulk, as shown in the previous chapter, and therefore, the $z$ orientation of the dipole is not stronger in the bulk than the other orientations.

Figure 6.13: The water dipole moment (arbitrary units) in dependence of the $z$ position. a) the absolute value and b) the orientation in the $x$, $y$, and $z$ direction.
Chapter 7

Ampicillin in OmpF and OmpC

Channels, such as OmpF, are not only interesting membrane proteins concerning ion transport, but maybe much more important concerning the translocation of substrate molecules and especially of antibiotics [60, 93]. This is a field of intensive investigations both experimentally [7, 60] and theoretically [73, 104, 130, 131]. In BLM experiments, the translocation of molecules is indirectly measured monitoring the blockage of the channel and the resulting drop in ion conductance. For a complete simulation of the experimental setup, a combined study of ion transport and substrate translocation is needed.

One kind of simulations, presented here, are simulations with antibiotics placed into the constriction zone. With this simulation the blocking is simulated. Another point, we are interested in, is to get a molecular view on the translocation. Because this translocation is in the ms time region, it is not feasible to simulate it with classical MD simulations. One way to overcome this barrier is to use metadynamics [132]. In metadynamics, the simulations are speedup by adding artificial Gaussian shaped potentials to the potential energy surface of the system. These Gaussians are added to the valleys in the potential so that the system is leaded out of them. Using this method, potential barriers can be overcome in shorter timescales. Such kind of simulations are performed, e.g., by Ceccarelli et al. [93, 104, 133].

Here, we try to speed up the translocation by adding directed forces to the antibiotics to steer them through the pore. This method is called steered molecular dynamics (SMD) [134]. As antibiotic, the β-lactam ampicillin is used in the following. Fig. 7.1 shows the structure of ampicillin. Because of the missing parametrization of antibiotics in the CHARMM force fields, the AMBER94 force field are used. The previously mentioned improvements for
the ions are also used here. A harmonic force is applied on the center of mass of the ampicillin molecule. The harmonic potential moves with time forcing the ampicillin molecule to follow it. Important parameters are the force constant, the velocity with which the harmonic force moves and the selection of atoms the force acts on.

A special interest is the free energy profile of ampicillin in OmpF. Different ways to calculate the free energy are developed, i.e., umbrella sampling [135, 136] or the Jarzynski equality [136–139]. Kosztin et al. [140] suggest to steer molecules in forward and time reverse (backward) direction to get the rare paths of the free energy. This will lead to free energy profiles with less sampling. But even using this method one needs a large number of simulations. Not each simulation could be used for the calculation. Only the one which correspond to the same free energy profile can be used. But the free energy profile of the translocation depends on many parameters. For example, it is shown that for the translocation of moxafloxaxin through OmpF, there are several free energy values for the same $z$ position of the antibiotic [133]. The free energy is a multi dimensional surface. Even slight changes in the translocation, i.e., different angles of the antibiotic to the porin, can result in different profiles. Therefore, it is important to introduce constraints to the translocation process. In some preliminary simulations the translocation was enhanced by a pull in $z$ direction without any further constraints. The best pulling strategy has not been worked out yet. Therefore free energy profiles cannot be shown here. The parameters used so far do not show any energy minimums, i.e., any binding sites in the pore.

The results of two SMD simulations are presented in the following. In both simulations one ampicillin molecule is steered through one monomer of a OmpF trimer. The steering velocity is in one simulation 2.5 m/s and in the other one 1.25 m/s. Force Constants are set in both simulation to 191.2 kcal/mol/Å$^2$ - the ampicillin molecules are pulled with very high velocities and forces. Using these parameters, the ampicillin molecules are translocating without delay through the porins, as shown in Fig. 7.2. The analysis of different bond angles showed that the angels do not change a lot, i.e., the initial structure of the ampicillin molecule is stable during the pulling process. Also, the absolute value of the force is not changing a lot. Only around the constriction zone, a short shift of the applied force is observed. Fig. 7.3 shows the average force applied on the ampicillin molecules. In Fig. 7.4 the numbers of OmpF atoms within 6 Å of the ampicillin molecules are shown. One can see that these
numbers have their maximums in the constriction zone, which was expected. Equal results are also obtained by Robertson and Tielemans with alanine and sugar through OmpF [73]. Robertson and Tieleman pulled with a velocity of 5 m/s. This speed could not be used with ampicillin, because in that case the simulation become unstable, i.e., some atoms starts to move faster than the allowed limit of the simulation program.

Figure 7.1: The molecular structure of ampicillin. The 2D structure is taken from [64].
Figure 7.2: The $z$ positions of the ampicillin molecules in dependence of time for two different steering velocities.

Figure 7.3: Average of the SMD forces on the ampicillin molecules in dependence of the $z$ position. In the inlays the original SMD forces in dependence of the $z$ position are shown. Colors are the same as in 7.2.
7.1. CURRENT BLOCKING BY AMPICILLIN

Figure 7.4: The number of OmpF atoms within 6 Å of the ampicillin molecules in dependence of the $z$ positions. Colors are the same as in 7.2.

7.1 Current Blocking by Ampicillin

The initial structures of the complete systems for these simulations were provided by our collaborators, the Ceccarelli group from Cagliari, Italy. They provided us with equilibrated OmpC and OmpF structures in a rhombus shape with POPC as lipid. One ampicillin molecule is placed into the constriction zone of one monomer, the two other monomers are not blocked, as shown in Fig. 7.5. The position of the ampicillin was estimated based on ampicillin translocation simulation with metadynamics. Additionally, simulations with an OmpF mutant, R132A, are in work. Because the position of the ampicillin in the constriction zone could not be determined for this mutant, we only have the simulation without antibiotic. For the simulation in this chapter OmpF has only E296 and OmpC has D299 protonated. The system was equilibrated for an extended time (20 ns) by our collaborators to ensure stability.

Beginning from these structures, we performed the following: For the conductance simulations, additional potassium and chloride ions were added. The salt concentration was increased to 0.5 M. After adding the salt, the system was minimized and equilibrated again to ensure equilibrium of the new system. The production runs were performed with 1 V applied voltage. To hinder antibiotic movement, harmonic restraints were applied to the antibiotic. Therefore, the constraints function of NAMD is used with the force constant set to 1 kcal/(mol Å$^2$) on the center of mass of the ampicillin molecule.

In case of the R132A mutant, BLM experiments did not show blocking
of the ion current, suggesting ampicillin cannot to translocate through this mutant whereas metadynamic simulations show clear translocation. One hypothesis is that although the antibiotic is in the constricted zone, there is enough space for ions to pass. Alanine is smaller than Arginine, therefore, the constricted zone is larger in the case of the R132A mutant. Because of the missing position of ampicillin inside the constriction zone of the R132A mutant, it is not possible to validate this hypothesis at this time.

The simulations with antibiotics show a lowering of the conductance in the case of WT OmpF. Tab. 7.1 shows the conductance results for simulations with 0.5 M KCl at 22 °C. The average conductance of OmpF with one ampicillin in the constriction zone over the four simulations is $(1.94 \pm 0.16)$ nS (8 %). Compared to the one without ampicillin (2.26 nS), we have a reduction of 14 %. If the conductance of each monomer would be independent from each other and one monomer would be totally blocked we would expect a decrease of 33 %. As one can see the conductance shows high variance. Because of the high error range, one should be careful with general statements. Therefore, one should repeat a simulation several times and average the outcome to get more reliable results. This is computationally very time consuming. Most probably, the poor statistics are responsible for the results of the conductance of OmpC, where the blocked porin has the same conductance as the free one.

Figure 7.5: Ampicillin in the constriction zone of OmpF. The key residues are highlighted. The residue and antibiotic atoms with strong interaction with each other are shown by spheres.
7.1. CURRENT BLOCKING BY AMPICILLIN

(shown in Tab. 7.1). More simulations are needed to ensure reliable results.

| Configuration       | $t_S [\text{ns}]$ | $N_c [\text{nS}]$ | $G [\text{nS}]$ | $|\Delta G|$ | $|\Delta G/G|$ |
|---------------------|-------------------|------------------|----------------|--------------|--------------|
| OmpF R132A, no AMP  | 10                | 206              | 3.30           | 0.23         | 7            |
| OmpF WT, no AMP     | 9.4               | 132              | 2.26           | 0.20         | 9            |
| OmpF WT, AMP        | 10                | 133              | 2.13           | 0.19         | 9            |
| OmpF WT, AMP        | 10                | 122              | 1.85           | 0.18         | 10           |
| OmpF WT, AMP        | 17                | 194              | 1.78           | 0.14         | 8            |
| OmpF WT, AMP        | 17                | 206              | 2.04           | 0.14         | 7            |
| OmpC WT, no AMP     | 10                | 73               | 1.2            | 0.14         | 12           |
| OmpC WT, AMP        | 10                | 73               | 1.2            | 0.14         | 12           |

Table 7.1: Details and results of the simulations including simulation time $t_S$, crossing events $N_c$, conductance $G$ and the error of the conductance $|\Delta G| = |G/\sqrt{N_c}|$ for simulations with 0.5 M KCl at 22 °C.
Chapter 8
Conclusions and Outlook

The main goal of this PhD was to enable a molecular view on the translocation processes through the OmpF and OmpC porins. Therefore, all-atom MD simulations were performed. As a benchmark for the simulations, bulk water simulations were performed and compared to experiments. Simulations and experiments in the temperature range from 2 to 90 °C were performed. It could be shown that for temperatures around room temperature the agreement between simulation and experiment is the best. The reason for this could be the force fields which were acquired around room temperature. Another important property next to the temperature dependency is the concentration dependency. For low salt concentrations up to 1 M KCl experiments and simulations have the same conductivity. For higher salt concentrations the conductivity of the simulations shows a clear saturation while the experimental conductivities increase linearly.

This finding showed an upper limit for salt concentration in simulation with the pore. The lower limit is set by the statistics. Low salt concentration leads to less crossing events by ions. Therefore, only simulations with 0.3 to 1 M KCl were performed. The simulations and experiments with OmpF and OmpC have shown the same effects observed with bulk water, i.e., with increasing salt concentration and with increasing temperatures the discrepancy between simulated and experimental conductances increases. At low salt concentrations and temperatures around room temperature the conductance in simulation and experiment agree. In the case of OmpC we have a better agreement than in OmpF. There is no proved explanation for this. One might speculate that this is due to the interaction of the ions. As shown in Fig. 6.10, the density of potassium and chloride in OmpF is more or less equal, but in OmpC, there is a
higher potassium density and a lower chloride density. Higher salt concentra-
tion do not only have influence on the potassium concentration but also on the
chloride concentration in the pore, which leads to more interaction between
chloride and potassium. This could be the reason why for 0.8 and 1.0 M KCl
the simulated OmpC conductance has less agreement with experiment than
with 0.3 and 0.5 M.

Another aspect is that the pore conductance increases faster with temper-
ate than the bulk conductivity. The analysis at the molecular level reveals
that ions tend to build more ion pairs in the pore than in the bulk for higher
salt concentrations while for lower salt concentrations the ion pairing ratio
is approximately the same for bulk and pore. The life time of the ion pairs
is higher for the pore than for bulk, but the life time is decreasing faster in
the pore than in the bulk with increasing temperatures. This is one reason
why the pore conductance is increasing faster with temperature than the bulk
conductivity.

Comparison of the pore area of OmpF and OmpC have shown that the
constriction zone of OmpC is longer. It is believed that this is due to one
positively charged residue in OmpC with a neutral counter part in OmpF.
The positive residue attracts negatively charged residues on the L3 loop which
fold more into the pore volume. Also, the different selectivities have their
reasons in differences in charged residues. Ion pathways are in agreement with
the selectivity differences.

Understanding and modifying the details of ion transport through the pores
would make it possible to alter the function of them. Recently, for example, it
has been reported that OmpF can be made Ca\textsuperscript{2+} selective by a few mutations
in the constriction zone [25], and that the charge selectivity in \(\alpha\)-hemolysin
has been modified by using noncovalent molecular adapters [141]. It has been
shown here, numerically as well as experimentally, that altering the charge of
only one residue within the constriction zone can have a clear effect on the
transport properties of the channel, such as conductance and selectivity.

As OmpC and OmpF are rather similar, mutation studies involving the
neutralization of a charged residue in one pore and the neutralization of its
equivalent residue in the other one, might present itself to be an interesting
approach in the study of conductance and ion pathway. Furthermore, despite
the homology of OmpC and OmpF, not all charged residues have equivalent
residues in the other porin. Mutating these residues, e.g., in OmpC will show
their influence on conductance and selectivity. One basic question that could
be solved this way is if the number and the position of the charged residues is responsible for the different behavior of OmpC and OmpF. Residues that are exposed to the pore lumen are more favorable for mutations in this respect.

Several points can certainly be improved to get an even better agreement with experiments. There is some uncertainty in the protonation states of some of the residues, especially important are those in the constriction zone [72,109,129,142]. Different protonation states can result in different conductance of the porin. Therefore, it is important to do further studies on the protonation states of the porin. Furthermore, it is assumed that simulations with improved water models and polarizable force fields would improve the results. In addition, the role of polarization effects could be significant [143,144].

The questions presented above are basic ones and might lead to the biologically most relevant problem: understanding the transport of certain substrates (as polyamines or antibiotics) through the pores and, thus, at a later time, being able to predict these computationally as well. Simulations of the blocking of the pore by ampicillin have shown that the blocking did not have a decrease of 33 %, but the high variance of the simulation results show that further studies are necessary. Also, simulations of the translocation of ampicillin were preformed. Here, the simulations are at the very beginning. Further simulations could lead to results which are comparable to experiments. The obtained molecular view lead to a deeper understanding of the translocation process.

One main drawback of the all-atom MD approach are the high computational time and costs needed for the simulations. This drawback vanishes with increasing computational power. For example, the available computer cluster at this time allows more simulations in parallel than the one used at the beginning of this work in 2006. Also, the calculation time is decreased, because of increased CPU clock and efficiency. Simulations with 80,000 to 100,000 atoms are performed on 16 to 32 CPU cores. The used clusters offer up to 8 cores per node. With increasing number of cores per CPU, it will be soon possible to calculate similar simulations on a single node. This will avoid the time consuming network communication between the nodes and should result in much faster simulations. Furthermore, computations of MD on graphics processing units (GPU) of modern graphic cards becomes interesting [145–147]. It is possible to reach a speedup of more than 700 times with one graphic card compared to single CPU calculations [148]. Further enhancement can be achieved by more efficient software, e.g., the recently available NAMD 2.7 is noticeably faster than NAMD 2.6 (5 to 10 % in bulk water simulation; on one node- 8 cores).
Overall, molecular dynamic simulation will become an even more powerful tool for studies at atomic scale.


Appendix: Wave Packet Calculations

Although this topic is not related to the main topic of my PhD the following publication is written during my PhD and is therefore attached here. Using wave packet calculations it is possible to simulate the interaction of simple molecules with laser pulses. Here, the interaction of iodine molecules with fs laser pulses is simulated. The aim is to get comparable results as in experiments by the Materny group at Jacobs University and observe the behavior of the electronic states.

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Shaping femtosecond coherent anti-Stokes Raman spectra using optimal control theory

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Optimal control theory is used to tailor laser pulses which enhance a femtosecond time-resolved coherent anti-Stokes Raman scattering (fs-CARS) spectrum in a certain frequency range. For this aim the optimal control theory has to be applied to a target state distributed in time. Explicit control mechanisms are given for shaping either the Stokes or the probe pulse in the four-wave mixing process. A simple molecule for which highly accurate potential energy surfaces are available, namely molecular iodine, is used to test the procedure. This approach of controlling vibrational motion and delivering higher intensities to certain frequency ranges might also be important for the improvement of CARS microscopy.

I. Introduction

Nonlinear spectroscopy is still a rapidly developing field of research.1,2 Especially the possibilities of laser pulses on the femto- and even attosecond time scales lead to new and improved techniques in connection with coherent control schemes. One of these nonlinear spectroscopic approaches is femtosecond time-resolved coherent anti-Stokes Raman scattering (fs-CARS).3 Time-resolved CARS is theoretically well studied starting with early investigations4,5 to present-day comparisons with experiments for simple systems.6–15 Recently CARS spectroscopy has been extended to CARS microscopy.16 This technique holds great promise for future developments since it is a technique for non-invasive, label-free high resolution, fast, three-dimensional microscopy. Implementing femtosecond pulse techniques in order to shape the spectral components, i.e. the vibrational motion, in CARS microscopy can result in high peak intensity for a given laser pulse energy. In ref. 17 this is theoretically attempted using chirped-pulse adiabatic control while in the present contribution optimal control theory is applied.

In CARS a pump laser excites the sample and a Stokes laser de-excites it back to the electronic ground state. Therefore the vibrational excited state in the electronic ground state is the result of the interaction of these two fields with the molecule. In a next step the probe laser excites the sample again and an anti-Stokes signal is emitted. This scenario can be implemented using monochromatic laser fields or femtosecond pulses leading to fs-CARS. Depending on the delay times between the pulses in fs-CARS, it is possible to investigate the dynamics on ground and excited potential energy surfaces (PESs). In order to simulate the data obtained in experiments one usually performs wave packet calculations. In most of these simulations a perturbative expansion of the wave function is used to calculate the third-order polarization7,8,12,14,15 though a non-perturbative treatment is also possible.2,13 The non-perturbative approaches are more CPU-time consuming and using them together with control algorithms is not so straightforward. From the polarization in the time domain one can then determine nonlinear signals or spectra. In addition, some of these investigations include the effect of the rotational dynamics. The vibrational coherence has, for example, been included in the studies of time-gated, frequency-resolved CARS.18–20

To get signals with high spectral resolution one usually applies CARS with monochromatic lasers. Recently femtosecond laser pulses have also been used to enhance nonlinear signals in regions where spectral resolution is demanded. This is done despite the fact that femtosecond pulses have a broad spectral width and excite several modes coherently when these modes lie within this spectral range. But with closed-loop coherent control techniques, experimental groups21–26 were able to design laser pulses to such an extent that only certain modes in a molecule are excited. In this way certain frequency ranges of a CARS spectrum can be enhanced while others are suppressed.

First approaches to coherent control of molecular dynamics were based on intuitive schemes like the Brumer–Shapiro scheme in the frequency domain27 or the pump-dump scheme of Tannor, Kosloff and Rice in the time domain.28 The control algorithms by Rabitz and coworkers29 as well as the Krotov algorithm30 give complete freedom to the laser pulses to be shaped. Recently the optimal control formalism was extended to handle also target states distributed in time.31–34 With this formalism frequency dispersed transient absorption signals were enhanced in predefined frequency ranges.32,33 In the present contribution we combine this approach with the calculation of fs-CARS spectra in order to tailor these. This is achieved by either shaping the probe or the Stokes pulse. In addition, the shaped laser pulse can be limited to a certain fluence and to a certain frequency range.33,35 These techniques will be employed below. The fluence of the laser pulse has to be limited to ensure that the strength of the laser pulse is within the validity range of perturbation theory while the limitation to certain frequency...
ranges allows us to ensure that CARS processes and not other excitation processes are the outcome of the control algorithm.

In the next section the perturbation theory for the calculation of fs-CARS signals is reviewed and the optimal control formalism for the spectra is developed. The results will be presented in Section III. Optimal control theory is used with a test target, i.e. the control of a wave packet on the excited PES using three laser pulses, in the first subsection of Section III. In the second part of that section, CARS spectra are controlled by optimizing the probe or the Stokes pulse. A summary and conclusions will be given in the last section. Throughout the paper the Planck constant $\hbar$ is set to unity.

II. Theory

A. Perturbation theory and spectra

To simulate fs-CARS spectra as well as other nonlinear spectra the third-order polarization $P^{(3)}$ is of fundamental importance. In the time domain the time-dependent Schrödinger equation

$$i \frac{\partial}{\partial t} |\Psi(t)\rangle = (H - \mu E(t))|\Psi(t)\rangle$$

(2.1)

for the vibrational wave function $|\Psi(t)\rangle$ has to be solved to obtain $P^{(3)}(t)$. The laser field is denoted $E(t)$ and may be a sum of several fields $E_i(t)$. The dipole moment $\mu$ is assumed to be constant, i.e. the Franck-Condon approximation is invoked. Since we focus on resonant CARS processes in the following, a ground and an excited state are taken into account. For the example of the iodine molecules treated below, these are the ground and an excited state $|X^1\Sigma_u^+\rangle$ and first excited $B^1\Pi_u$ PES (see Fig. 1). The parameters of the corresponding Morse potentials $V_M$ are given in ref. 36 and 37. These states are chosen to yield results comparable with previous investigations.12 As the initial state we always use the lowest vibrational level of the electronic ground state.

In the following the wave function $|\Psi(t)\rangle$ is expanded in a perturbation series

$$|\Psi(t)\rangle = \sum_m |\Psi^{(m)}(t)\rangle$$

(2.2)

where the perturbation order is labeled by the superscript $m$. By an iterative scheme which is discrete in time one can determine the wave functions which are perturbed by the electric field $E(t)$.7,38

$$|\Psi^{(m+1)}(t + \Delta t)\rangle = U(\Delta t)|\Psi^{(m+1)}(t)\rangle + i\Delta t \mu E(t + \Delta t)|\Psi^{(m)}(t + \Delta t)\rangle.$$  (2.3)

The time evolution operator $U(\Delta t) = \exp(-iH\Delta t)$ propagates $|\Psi^{(m+1)}(t)\rangle$ to $|\Psi^{(m+1)}(t + \Delta t)\rangle$ without any laser field. In the following the wave vector, and therefore the direction of the pump pulse, is denoted by $k_{pu}$, of the Stokes pulse by $k_{St}$ and that of the probe pulse by $k_{pr}$. One has to note that, for example, the first-order wave function has contributions from all different laser pulses:

$$|\Psi^{(1)}(t)\rangle = |\Psi^{(1)}(k_{pu}, t)\rangle + |\Psi^{(1)}(k_{St}, t)\rangle + |\Psi^{(1)}(k_{pr}, t)\rangle.$$  (2.4)

Higher-order wave functions have contributions from all possible combinations of the different laser pulses.

Fig. 1 Ground and first excited PES of the iodine dimer and the excitation scheme for a CARS experiment; $a_0$ denotes the Bohr radius.

The lowest perturbation order in the polarization $P(t) = \langle \Psi(t)|\mu|\Psi(t)\rangle$ to which all three laser pulses—pump, Stokes and probe—contribute is the third order $P^{(3)}(t)$ and from this order the spatial dependence of the fields can also be determined. The polarization in anti-Stokes direction $k_{as} = k_{pu} - k_{St} + k_{pr}$ is given by 7,39

$$P^{(3)}(k_{as} - k_{St} + k_{pr}, t) = 2\text{Re}\langle \Psi^{(3)}(t)|\mu|\Psi^{(3)}(k_{pu} - k_{St} + k_{pr}, t)\rangle + \langle \Psi^{(3)}(t)|\mu|\Psi^{(3)}(k_{pu} - k_{St} + k_{pr}, t)\rangle$$

(2.5)

For positive time delays, i.e. the probe pulse occurs after the Stokes pulse, the second term vanishes while for negative time delays the first and the fourth term are zero.7,39 Below we concentrate on scenarios with positive time delays and assume that there is no time delay between pump and Stokes pulse.

In experiments the polarization $P^{(3)}(t)$ is not detected directly but its time integral

$$S = \int_{-\infty}^{t_e} dt |P^{(3)}(t)|^2$$

(2.6)

is measured. The upper limit $t_e$ of this integral is defined by the integration interval of the photon detector. Of course one should always bear in mind that the signal $S$ depends on the time delays between the different pulses. Another experimentally accessible quantity is the spectrally dispersed transient CARS spectrum:10,40

$$S(\omega) = |P^{(3)}(\omega)|^2$$

(2.7)

which is nothing else than the squared absolute value of the Fourier transform $\mathcal{F}$ of $P^{(3)}(t)$

$$P^{(3)}(\omega) = \int_{-\infty}^{\infty} dt P^{(3)}(t) e^{i\omega t} \equiv \mathcal{F} P^{(3)}(t).$$

(2.8)

For later convenience we rewrite $S(\omega)$ in the form

$$S(\omega) = \left(\int dt P^{(3)}(t) \cos(\omega t)\right)^2 + \left(\int dt P^{(3)}(t) \sin(\omega t)\right)^2.$$  (2.9)

The calculation and control of the fs-CARS spectrum $S(\omega)$ is the main topic of this contribution.
Above, only the vibrational dynamics were discussed. To include rotations in the dynamics an additional term \( V_{\text{rot}} \) has to be added to the above-mentioned Morse potentials \( V_M \):

\[
V_{\text{eff}}(l) = V_M + V_{\text{rot}}(l) = V_M + \frac{j(j+1)}{2mR^2} \tag{2.10}
\]

where \( R \) is the internuclear distance. For each rotational quantum number \( j \) there is a different potential and therefore one needs to propagate a wave function \( \Psi_j \) for each \( j \). Starting with rotational quantum number \( j \) the dipole selection rules lead to states with \( j \pm 1 \) after an electronic transition. This yields a coupling of the wave functions with different \( j \) (see Appendix for the coupled equations). For the iodine system at usual experimental temperatures of about 100 °C the distribution of the rotational states has a maximum close to \( j = 60 \) and a full width at half maximum (FWHM) of 95. Including so many coupled wave functions in the simulations naturally requires a lot of computational time. As an example, we compare in Fig. 2 the spectra of a scenario with a linearly chirped pump and unchirped Stokes and probe pulses. Between pump and Stokes pulses there is no time delay, but a delay of 600 fs occurs between the Stokes and probe pulses. All Gaussian pulses have a length (FWHM) of 100 fs. The frequency of the Stokes pulse is 15 640 cm\(^{-1} \) (\( \lambda = 639 \text{ nm} \)) and that of the probe pulse 16 908 cm\(^{-1} \) (\( \lambda = 591 \text{ nm} \)). The linear chirp of the pump pulse is such that the frequency is 15 640 cm\(^{-1} \) (\( \lambda = 639 \text{ nm} \)) while passing the first half maximum and 16 908 cm\(^{-1} \) (\( \lambda = 591 \text{ nm} \)) while passing the second half maximum. Replacing the Morse potential by the effective potential [see eqn (2.10)] leads to a small shift in the spectral half maximum. Replacing the Morse potential by the effective potential, \( V_{\text{eff}} \), and a full width at half maximum (FWHM) of 95

In CARS simulations without control it could be seen that rotations do not have an influence on the peak position of the CARS signal obtained from eqn (2.6). Just the peak intensity is changed.

**B. Control**

In the following we describe how nonlinear spectra can be controlled via optimal control theory. Since we want to shape one of the laser pulses \( E_i \) and since the spectra are calculated within perturbation theory, the fluence, i.e. the integrated strength, of this laser pulse \( E_i \) has to be restricted. To achieve this we choose a control functional of the form\(^{33,35} \)

\[
J(E_i) = J_0(E_i) - \lambda_p \left( \frac{1}{2} \int dt \frac{E_i^2(t)}{s(t)} - I_0 \right). \tag{2.11}
\]

Here \( J_0(E_i) \) is the molecular property or observable to be optimized which will be detailed below. The second term constrains the fluence of the control field to a value \( I_0 \). The function \( s(t) \) guarantees smooth switching on and of the pulse. Moreover, one can avoid overlapping laser fields using this function which is important for the perturbative treatment of the CARS signals. Furthermore, the penalty factor \( \lambda_p \) is determined in each step of the iterative algorithm following the arguments in ref. 35 to be

\[
\lambda_p^{i+1} = \sqrt{\frac{\int dt (\mathcal{F}(\mathcal{F}^{-1}[f(0)\mathcal{F}[E_i^0(t)]]))^2}{I_0}}. \tag{2.12}
\]

Here \( \mathcal{F}^0(t) \) describes a spectrally constrained laser field\(^{12} \)

\[
\mathcal{F}^0(t) = \mathcal{F}^{-1}[f(0)\mathcal{F}[E_i^0(t)]] \tag{2.13}
\]

with the filter function \( f(\omega) \) to avoid pulses with very high or low frequencies. If one does not want to apply any spectral constraint one can replace \( E_i^0 \) by \( E_i^0 \) in eqn (2.12). The determination of \( E_i^0 \) at each step of the iteration is described below.

The property we want to control is a frequency spectrum, either globally or in a certain frequency range. Such a control goal is certainly not local in time and therefore in a first step we use a version of the optimal control scheme developed for target states distributed in time\(^{31–34} \). In general a corresponding control functional with control operator \( O(t) \) can be written as

\[
J_d(E_i) = \int dt \langle \Psi(t, E_i) | O(t) \Psi(t, E_i) \rangle. \tag{2.14}
\]

In ref. 31–33 Kaiser and May developed a formalism for the optimal control of absorption spectra which is based on an

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Fig. 2 CARS spectrum of an iodine molecule neglecting all rotational effects, i.e. using only one wave function together with \( V_M \) (dotted), just including the effective potential, i.e. using only one wave function together with \( V_{\text{eff}} \) (dashed), and using 5 coupled wave functions with \( V_{\text{eff}} \) (solid).
exact treatment of the time-evolution operator. Here we will use the above-mentioned perturbative treatment. Time-local control goals are a special case of these generalized target functions. If we want, for example, to shape wave packets to have the form $|\Psi_{\text{tar}}\rangle$ at the final time $t_f$, one can use

$$O(t) = \delta(t - t_f)|\Psi_{\text{tar}}\rangle\langle\Psi_{\text{tar}}|.$$  

(1.15)

This will be used as a test case below.

To determine the control pulse we have to maximize the control field with respect to the control pulse $E_J$:

$$\frac{\delta \mathcal{I}(E_J)}{\delta E_J} = 0.$$  

(1.16)

Using the above definitions of $\mathcal{I}(E_J)$ and $J_0(E_J)$ yields

$$E_J(t) = \frac{2\psi(t)}{k_p^2} \text{Re} \int \text{d}\tau \langle \Psi(\tau)| O(\tau) \frac{\delta \Psi(\tau)}{\delta E_J(t)} \rangle.$$  

(1.17)

In a next step the wave function $|\Psi(t)\rangle$ is replaced by its perturbation expansion (2.2). One has to keep in mind that each of these perturbation orders consists of contributions from the different laser pulses as detailed in eqn (2.4) for the first-order wave function. Within perturbation theory the temporal order of the laser pulses is important. To make notation simpler we assume that the pump pulse comes first, the Stokes pulse is applied at the same time as the pump pulse or later, and finally the probe pulse occurs. In experiments with negative time delay, for example, the probe pulse is first. The results below can also be applied to these cases after a proper renaming of the pulses.

1. Control of a time-distributed target state with the probe pulse. Let us start by controlling the probe pulse and keeping the pump and Stokes pulses fixed. For this case the ket vector in eqn (2.17) can be written as a sum of those terms contributing to the wave function which depend on the probe pulse

$$\frac{\delta \Psi(\tau)}{\delta E_p(t)} + \frac{\delta \Psi^{(1)}(k_{p_1} - k_{p_2}; \tau)}{\delta E_p(t)} + \frac{\delta \Psi^{(2)}(k_{p_2} - k_{p_3}; \tau)}{\delta E_p(t)} + \frac{\delta \Psi^{(3)}(k_{p_3} - k_{p_4}; \tau)}{\delta E_p(t)}.$$  

(1.18)

A detailed look at the first term on the right-hand side of eqn (1.18) yields

$$\frac{\delta \Psi^{(1)}(k_{p_2}; \tau)}{\delta E_p(t)} = -i \frac{\delta}{\delta E_p(t)} \int_0^\tau \text{d}t' e^{iH(t' - \tau)} \mu E_p(t') |\Psi^{(0)}(t')\rangle.$$  

(1.19)

This equation is very similar to the one obtained from an exact propagation of the wave function (see Appendix of ref. 31). So one proceeds in the same way as in eq. 31 in order to calculate the integral in eqn (2.17):

$$\int \text{d}t \langle \Psi(\tau)| O(\tau) \frac{\delta \Psi(k_{p_2}; \tau)}{\delta E_p(t)} \rangle = \langle \zeta(\tau)| |\Psi(0)(t)\rangle$$  

(2.20)

with the auxiliary function $|\zeta(\tau)\rangle$ which propagates according to

$$\frac{\partial |\zeta(\tau)\rangle}{\partial \tau} = -iH |\zeta(\tau)\rangle - O(t)|\Psi(\tau)\rangle.$$  

(2.21)

Here $|\Psi(\tau)\rangle$ has to be calculated as the sum of the different perturbation orders. The other terms in eqn (2.18) can be treated in a similar fashion leading to an expression for the controlled probe pulse:

$$E_p(t) = \frac{2\psi(t)}{k_p^2} \text{Re} \langle \zeta(t)| \mu |\Psi^{(0)}(t)\rangle + \langle \zeta(t)| k_{p_3} - k_{p_2}; \tau \rangle + \langle \zeta(t)| k_{p_4} - k_{p_3}; \tau \rangle + \langle \zeta(t)| k_{p_5} - k_{p_4}; \tau \rangle.$$  

(2.22)

Using this expression time-local control targets (like shaping a wave packet at a certain moment in time) and time-nonlocal targets (like maximizing the intensity of a whole spectrum) can be achieved. To determine the control field, one first propagates $|\Psi(t)\rangle$ from $t = 0$ to $t = t_f$ with an initial guess for the laser field. Then one propagates $|\Psi(t)\rangle$ and $|\zeta(t)\rangle$ backwards in time and calculates the laser field. This procedure is repeated iteratively. The time evolution within the perturbative treatment (2.3) is performed using the split operator method.

2. Control of CARS spectra with the probe pulse. To be able to increase the amplitude of certain frequency regions in the CARS spectrum we have to modify the control goal because of the terms involved in the expressions for $P^{(3)}(t)$.

The target functional is now given by

$$J_0(E_J) = \int_{\omega_{k_p} - \omega_{k_{p_1}}/2}^{\omega_{k_p} + \omega_{k_{p_1}}/2} \text{d}\omega |P(\omega)|^2.$$  

(2.23)

Taking the functional derivative of the integrand with respect to the control field $E_J$ yields

$$\frac{\delta}{\delta E_J(t)} |P(\omega)|^2 = 2\int \text{d}t P^{(3)}(t) \cos(\omega t) \frac{\delta}{\delta E_J(t)} \left( \int \text{d}t P^{(3)}(t) \cos(\omega t) \right) + 2\int \text{d}t P^{(3)}(t) \sin(\omega t) \frac{\delta}{\delta E_J(t)} \left( \int \text{d}t P^{(3)}(t) \sin(\omega t) \right).$$  

(2.24)

Since we concentrate on positive time delays, only the three terms contribute to $P^{(3)}(t)$ in eqn (2.5). Similar to the results above, for controlling the probe pulse we get

$$\frac{\delta}{\delta E_p(t)} \int \text{d}t \cos(\omega t) \langle |\Psi^{(0)}(t)\rangle |\mu |\Psi^{(3)}(k_{p_2} - k_{p_1}) \rangle = \langle \zeta(t) | \mu |\Psi^{(3)}(k_{p_3} - k_{p_1}, t) \rangle$$  

(2.25)
with
\[
\frac{\partial |Z_2(t)\rangle}{\partial t} = -iH|Z_2(t)\rangle - i\mu|\Psi(0)(t)\rangle \cos(\omega t),
\] (2.26)
and
\[
\frac{\delta}{\delta E_{pr}(t)} \int dt \cos(\omega t)\langle \Psi(2)(k_{St} - k_{pu}, t) | \mu | \Psi(1)(k_{pu}, t) \rangle
= \langle Z_2(t) | \mu | \Psi(0)(t) \rangle
\] (2.27)
with
\[
\frac{\partial |Z_2(t)\rangle}{\partial t} = -iH|Z_2(t)\rangle - i\mu|\Psi(2)(k_{St} - k_{pu}, t)\rangle \cos(\omega t),
\] (2.28)
as well as
\[
\frac{\delta}{\delta E_{pr}(t)} \int dt \cos(\omega t)\langle \Psi(2)(k_{St} - k_{pu}, t) | \mu | \Psi(1)(k_{pu}, t) \rangle
= \langle Z_3(t) | \mu | \Psi(1)(k_{St}, t) \rangle
\] (2.29)
with
\[
\frac{\partial |Z_3(t)\rangle}{\partial t} = -iH|Z_3(t)\rangle - i\mu|\Psi(1)(k_{pu}, t)\rangle \cos(\omega t).
\] (2.30)
If we want to control the spectra, for example enhance them in the range \(\omega_0 \pm \Delta \omega\) by controlling the probe pulse, the final expression is given by
\[
E_{pr}(t) = \frac{2\pi(t)}{\lambda_p} \Re \int_{\omega_0 - \Delta \omega}^{\omega_0 + \Delta \omega} d\omega \left( \int dt P(3) \cos(\omega t) \right)
\times \left( \langle Z_1(t) | \mu | \Psi(1)(k_{pu} - k_{St}, t) \rangle + \langle Z_2(t) | \mu | \Psi(0)(t) \rangle \right)
+ \langle Z_3(t) | \mu | \Psi(1)(k_{St}, t) \rangle + \left( \int dt P(3) \sin(\omega t) \right)
\times \left( \langle Z_4(t) | \mu | \Psi(2)(k_{pu} - k_{St}, t) \rangle + \langle Z_5(t) | \mu | \Psi(0)(t) \rangle \right)
+ \langle Z_6(t) | \mu | \Psi(1)(k_{St}, t) \rangle
\] (2.31)
where \(|Z_4(t)\rangle, |Z_5(t)\rangle\) and \(|Z_6(t)\rangle\) equal \(|\tilde{Z}_4(t)\rangle, |\tilde{Z}_5(t)\rangle\) and \(|\tilde{Z}_6(t)\rangle\) but with sine instead of cosine functions in the respective differentials eqn (2.26), (2.28) and (2.30). Therefore, in addition to the differential equations for the various perturbation orders of \(|\Psi(t)\rangle\) with different combinations of the laser pulses one gets six inhomogeneous differential equations, which also can be solved by propagating \(|\Psi(t)\rangle\) and \(|Z(t)\rangle\) forward and backward in time. The integral over \(\omega\) in eqn (2.31) is performed below in a discretized version.

3. Control of CARS spectra with the Stokes pulse.
To control the Stokes pulse instead of the probe pulse one has to take the functional derivative (2.24) with respect to \(E_{St}\). But since now the derivative is taken with respect to the second field the resulting equations are more involved. Instead of having auxiliary functions \(|\tilde{Z}_j\rangle\) one has a two-level hierarchy of such equations. The optimized Stokes field is given by
\[
E_{St}(t) = \frac{2\pi(t)}{\lambda_p} \Re \int_{\omega_0 - \Delta \omega}^{\omega_0 + \Delta \omega} d\omega \left( \int dt P(3) \cos(\omega t) \right)
\times \left( \langle Z_1(t) | \mu | \Psi(1)(k_{pu}, t) \rangle \right)
+ \langle Z_3(t) | \mu | \Psi(1)(k_{pu}, t) \rangle
+ \left( \int dt P(3) \sin(\omega t) \right)
\times \left( \langle Z_4(t) | \mu | \Psi(2)(k_{pu} - k_{St}, t) \rangle + \langle Z_5(t) | \mu | \Psi(0)(t) \rangle \right)
+ \langle Z_6(t) | \mu | \Psi(1)(k_{St}, t) \rangle
\] (2.32)
with
\[
\frac{\partial |Z_1(t)\rangle}{\partial t} = -iH|Z_1(t)\rangle - i\mu E_{pr}|Z_1(t)\rangle
\] (2.33)
\[
\frac{\partial |Z_2(t)\rangle}{\partial t} = -iH|Z_2(t)\rangle - i\mu E_{pr}|Z_2(t)\rangle
\] (2.34)
\[
\frac{\partial |Z_3(t)\rangle}{\partial t} = -iH|Z_3(t)\rangle - i\mu E_{pr}|Z_3(t)\rangle
\] (2.35)
and
\[
\frac{\partial |\tilde{Z}_1(t)\rangle}{\partial t} = -iH|\tilde{Z}_1(t)\rangle - i\mu|\Psi(0)(t)\rangle \cos(\omega t),
\] (2.36)
\[
\frac{\partial |\tilde{Z}_2(t)\rangle}{\partial t} = -iH|\tilde{Z}_2(t)\rangle - i\mu|\Psi(1)(k_{pu}, t)\rangle \cos(\omega t),
\] (2.37)
\[
\frac{\partial |\tilde{Z}_3(t)\rangle}{\partial t} = -iH|\tilde{Z}_3(t)\rangle - i\mu|\Psi(1)(k_{pu}, t)\rangle \cos(\omega t),
\] (2.38)
where again \(|\tilde{Z}_4(t)\rangle, |\tilde{Z}_5(t)\rangle, \) and \(|\tilde{Z}_6(t)\rangle\) have sine instead of cosine functions in the differential equations equaling eqn (2.36), (2.37) and (2.38) respectively. So the control of the Stokes pulse leads to more coupled differential equations than the shaping of the probe pulse. The control of the pump pulse leads to even more complicated equations. In particular, some of the integrals cannot be calculated by the trick of auxiliary differential equations. Therefore we restrict ourselves here to the shaping of probe and Stokes pulses.

III. Results
A. Wave packet control
In this first application we want to test the formalism developed above for a simple goal, namely the shaping of a wave packet in the upper electronic state using three pulses. This target could of course be reached using just one pulse but here it serves as a test of the three-pulse approach. The control goal is to get a Gaussian wave packet centered at \(x = 5.4 \, \text{a}_0\) on the excited state (cf. Fig. 1) at final time \(t_f = 800\, \text{fs}\) by shaping the probe pulse. The pump and the Stokes pulses have a Gaussian shape with a maximum at 200 fs (no delay time between pump and Stokes) and a FWHM of 100 fs. The pump pulse has the frequency \(\omega_p = 18\, 675\, \text{cm}^{-1}\).
(\( \lambda = 535 \text{ nm} \)) and the Stokes pulse \( \omega = 17\,140 \text{ cm}^{-1} (\lambda = 583 \text{ nm}) \). As an initial probe we use a Gaussian pulse, centered at 800 fs, i.e. with 600 fs time delay, and the same frequency as the pump pulse. As smoothing function \( s(t) \) we use a sine function which takes 10% of the propagation time to increase from 0 to 1 at the beginning and the same time to decrease from 1 to 0 at the end. In between these times the smoothing function is set to unity. In this way we also ensure that there is always a delay between the Stokes and the probe pulse. In Fig. 3 the wave function up to the third order for the unshaped initial pulse and for the shaped pulse is shown. In addition, the (scaled) control goal is displayed to emphasize how accurately the control goal is achieved. The absolute value of the resulting wave packet is of course small since it is created within a third-order process.

### B. Control of CARS spectra with the probe pulse

The above control scenario of shaping a wave packet in a third-order process serves as a good test for our algorithm but is not easily accessible in experiments. In the next step we come to the control of fs-CARS spectra which can be measured experimentally. Our goal is to increase the amplitude of the spectrum in a predefined frequency interval. The parameters of pump and Stokes pulses are the same as in the previous subsection, as are those of the unshaped Gaussian probe pulse. Using the Gaussian laser pulse one gets a broad Gaussian-shaped CARS spectrum with a maximum at 20\,110 \text{ cm}^{-1}, a FWHM of 407 \text{ cm}^{-1} and an enhanced high-frequency tail. In the first scenario the goal is to increase the amplitude of the spectrum in the frequency range 20\,410 \pm 50 \text{ cm}^{-1}, i.e. for frequencies that are significantly larger than the maximum of the spectrum with the Gaussian pulse. In Fig. 4 the spectra using the unshaped Gaussian and the shaped probe pulse are shown. In addition the interval of the target frequencies is marked. In this target frequency region the spectrum certainly increases while an obvious oscillatory pattern arises. The frequency of this pattern of 200 \text{ cm}^{-1} correlates with the energy difference between two vibrational levels. A splitting of the spectra into vibrational modes is also observed for the unshaped pulse if the integration time \( t_I \) is increased.

The power spectrum of the shaped probe pulse is displayed in the upper panel of Fig. 5. It is determined via

\[
F_\epsilon(\omega, t) = \int_{t-I/2}^{t+I/2} dt' W(t'-t, \tau) E(t') e^{-i\omega t'} \tag{3.39}
\]

with the electric field \( E(t') \) and the Blackman window

\[
W(t'-t, \tau) = 0.42 - 0.5 \cos \left( \frac{2\pi(t'-t)}{\tau} \right) - 0.08 \cos \left( \frac{4\pi(t'-t)}{\tau} \right) \tag{3.40}
\]

with the time resolution \( \tau \) which is limited from below by the time step of the wave packet propagation. The power spectra give similar information concerning the laser pulse as the experimentally used frequency-resolved optical gating (FROG) traces do.

As one can see in Fig. 5 the shaped probe pulse clearly consists of two parts in the time domain which are about 160 fs apart. It also shows an oscillatory behavior on the frequency scale. The time between the two peaks in the shaped probe pulse is approximately equal to the cycle duration \( T = 168 \text{ fs} \) of the second-order wave packet, i.e. the wave packet after the pump and Stokes pulses, on the ground state. Each time the wave function is in the correct position the probe pulse is non-vanishing. Shaped control pulses with more than one peak, so-called pulse trains, were observed experimentally by Dudovich et al.\textsuperscript{46}

In the next step we want to increase the amplitude of the spectrum in a frequency range below the maximum of the unshaped Gaussian pulse to show that the algorithm works in both regions. For this aim we choose the target region 20\,010
\( \pm 20 \text{ cm}^{-1} \). The result is also shown in Fig. 4. Obviously the control algorithm is successful, but one observes again the oscillatory pattern in the frequency domain. The power spectrum of the corresponding shaped pulse is displayed in the lower panel of Fig. 5. This time the larger part of the laser energy goes into the first of the two sub-pulses which are again about 160 fs apart. For the previous scenario this was the other way round. In further calculations (not shown) it was observed that the ratio of the amplitudes of the two sub-pulses depends on the frequency interval in which the CARS spectrum should be enhanced.

C. Control of CARS spectra with the Stokes pulse

Now we keep the pump and the probe pulses fixed and shape the Stokes pulse. The pump pulse has the frequency 20 020 cm\(^{-1}\) (\( \lambda = 500 \text{ nm} \)) while the probe pulse has the frequency 19 250 cm\(^{-1}\) (\( \lambda = 520 \text{ nm} \)). Both pulses have a FWHM of 100 fs. The pump pulse is centered at 200 fs and the probe pulse at 800 fs. As the initial Stokes pulse we use a Gaussian pulse centered at 200 fs with the same parameters as the probe pulse. The control goal is to increase the intensity in the frequency interval 21 000 \( \pm 43 \text{ cm}^{-1} \) which is marked in Fig. 6 by vertical lines. In Fig. 6 one can see that the calculated spectrum using the shaped Stokes pulse is larger than that using the Gaussian pulse. Applying the shaped Stokes pulse the maximal amplitude is smaller but within the target frequency range the signal resulting from the shaped pulse is indeed larger and the maximum of the shaped spectrum is close to the target region. In this case the power spectrum of the Stokes pulse (not shown) features only one peak centered at approximately 210 fs and 18 700 cm\(^{-1}\). In a second example of shaping the Stokes pulse different parameters for the fixed pulses are used. This time the pump pulse has the frequency 18 675 cm\(^{-1}\) (\( \lambda = 535 \text{ nm} \)) while the probe pulse has the frequency 18 069 cm\(^{-1}\) (\( \lambda = 553 \text{ nm} \)). Again both pulses have a FWHM of 100 fs and are centered at 200 and 800 fs respectively. The initial Stokes pulse is chosen as in the previous example but now the target frequency region is lower in energy than the maximum of the spectrum with the unshaped Stokes pulse, namely 18 675 \( \pm 43 \text{ cm}^{-1} \). The result is shown in Fig. 7 and clearly a large increase of the spectrum within the target region is achieved but again an oscillatory pattern shows up as in Fig. 4. This time the shaped pulse consists of a series of three pulses as can be seen in Fig. 8.

IV. Conclusions

The aim in this paper was to shape fs-CARS spectra using the theory of optimal control. Experimentally it was shown that this control goal is feasible which might be very important
for the developing field of CARS microscopy, with a large potentiality for applications in biological systems. In most experiments the ratio between the intensities in two frequency ranges was enhanced. Here we enhanced the absolute value of the spectral amplitude in a predefined frequency range but an extension of our method to two frequency ranges should be straightforward though the equations become more involved.

To be able to develop a control algorithm for fs-CARS spectra we first reviewed the perturbative calculation of these kind of spectra. The modifications to include the influence of rotations on the dynamics were given. Using these formulas transient signals as well as CARS spectra can be determined for given probe, Stokes and pump pulses. An alternative non-perturbative determination of the CARS signal and spectra can be done at the expense of a higher numerical effort. This would also allow for arbitrarily overlapping pulses but incorporating these techniques into a control algorithm might be more cumbersome.

A coherent control mechanism for time-distributed targets in a three-pulse setup was developed by extending previous studies. As a first test a wave packet in the excited electronic state was shaped. In this case, pump and Stokes pulses were predefined while the probe pulse was successfully controlled to get a maximum control yield. However, the main goal of the paper was to develop and test an algorithm to control fs-CARS spectra. This was indeed possible by shaping either the probe or the Stokes pulse. We did not control the pump pulse since the final equations are numerically much more involved compared to the cases of controlling the probe or the Stokes pulse.

We not only developed the formalism but also tested it in the case of the iodine dimer. This is of course a very simple model in which the electronic ground and first excited state are known to a high degree of accuracy. Nevertheless this simple case can be used as a starting point to explain the spectra of more complicated systems.

A limitation of utilizing the iodine dimer as a test system is, of course, its single reaction coordinate. In more complex systems it should be possible to enhance one peak in the spectrum stemming from one reaction coordinate while suppressing some of the other peaks resulting from different reaction coordinates, i.e. an excitation of single modes should be possible. We showed that fs-CARS spectra could be enhanced in predefined frequency regions using shaped pulses. Actually in some of the resulting structures a damped oscillatory pattern arose especially when shaping the probe pulse. Allowing for longer delay times between the Stokes and probe pulses (not shown) leads to non-oscillatory spectra with quite different pulse forms.

During our investigations the shape of the laser pulse was left free, i.e. both amplitude and phase of the pulse were controlled. In most of the experimental realizations only the phase was controlled while amplitudes were kept fixed. Performing simulations with a restricted pulse form needs different control algorithms but has been done for other control scenarios.

Appendix

The aim of this Appendix is to show the modifications in some of the equations in section IIA when rotational dynamics are

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**Fig. 7** Same as in Fig. 6 but with control goal to increase the spectra in the region 18 675 ± 43 cm⁻¹.

**Fig. 8** Power spectrum of the shaped Stokes pulse used for the spectrum in Fig. 7.
included. As mentioned above, one needs to propagate more than one wave function to include rotational states in the calculations. The coupling of the wave functions is done by changing eqn (2.3) into

\[
|\psi^{(m+1)}_{k,i}(t + \Delta t)\rangle = U(\Delta t)|\psi^{(m)}_{k,i}(t)\rangle + i\Delta t \mu E_j(t + \Delta t) \\
\times (|\psi^{(m)}_{j-1,i}(t + \Delta t)\rangle + |\psi^{(m)}_{j+1,i}(t + \Delta t)\rangle)
\]

(A.41)

with the electronic states \(k\) and \(l\) \((k \neq l)\), the rotational states \(j\), \(j + 1\) and \(j - 1\) and the \(i^{th}\) laser pulse. The determination of the polarization eqn (2.5) also has to be altered to

\[
P^{(i)}(k_p - k_S + k_{pr}, t) = 2 \sum_j \sum_{j' = j \pm 1} \text{Re}(|\langle \psi^{(0)}_{j'}(t)|\psi^{(1)}_{j}(k_p - k_S + k_{pr}, t)\rangle|
\]

\[
+ |\langle \psi^{(0)}_{j}(t)|\psi^{(1)}_{j-1}(k_p - k_S + k_{pr}, t)\rangle| + |\langle \psi^{(0)}_{j}(t)|\psi^{(1)}_{j+1}(k_p - k_S + k_{pr}, t)\rangle|
\]

(A.42)

References

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Contributions to Conferences and Summer Schools


- S. Pezeshki “Simulation of Transport through OmpF Channels” (“Computer Simulation and Theory of Macromolecules” in Hünfeld, Germany, April 2008)

- S. Pezeshki, C. Chimerel, M. Winterhalter and U. Kleinekathöfer “Transport through OmpF channels simulated using molecular dynamics” (“North German Biophysical Meeting” in Borstel, Germany, January 2009)


- S. Pezeshki, M. Winterhalter and U. Kleinekathöfer “Transport through OmpF Channels Simulated using Molecular Dynamics” (“DPG-Springmeeting” in Dresden, Germany, March 2009)

- M. M. Tomozeiu, S. Pezeshki and U. Kleinekathöfer “Water Diffusion through OmpF protein using Molecular Dynamics Simulation” (“DPG-Springmeeting” in Dresden, Germany, March 2009)

Declaration

This work has not previously been submitted for any degree. This thesis is the result of my own work and investigation, except where otherwise stated. Other sources are acknowledged by explicit references.

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